2008

Relevance for Food Safety of Applications of Nanotechnology in the Food and Feed Industry

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Recommended Citation

I. Pratt, C. Adley W. Anderson G. Chambers M. Davoren, K. Dawson, R. Evans, M. Fenelon, T. Harty, L. Hyde, S. Lawrie, I. Lynch, P. O'Mahony, M. Radmoski, A. Relevance for Food Safety of Applications of Nanotechnology in the Food and Feed Industry Reilly Food Safety Authority of Ireland. Food safety authority of Ireland (FSAI) 2008.

Food Safety
AUTHORITY OF IRELAND

The Relevance for Food Safety of Applications of Nanotechnology in the Food and Feed Industries

Food Additives, Chemical Contaminants & Residues

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Published by:
Food Safety Authority of Ireland
Abbey Court
Lower Abbey Street
Dublin 1

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E-mail: info@fsai.ie
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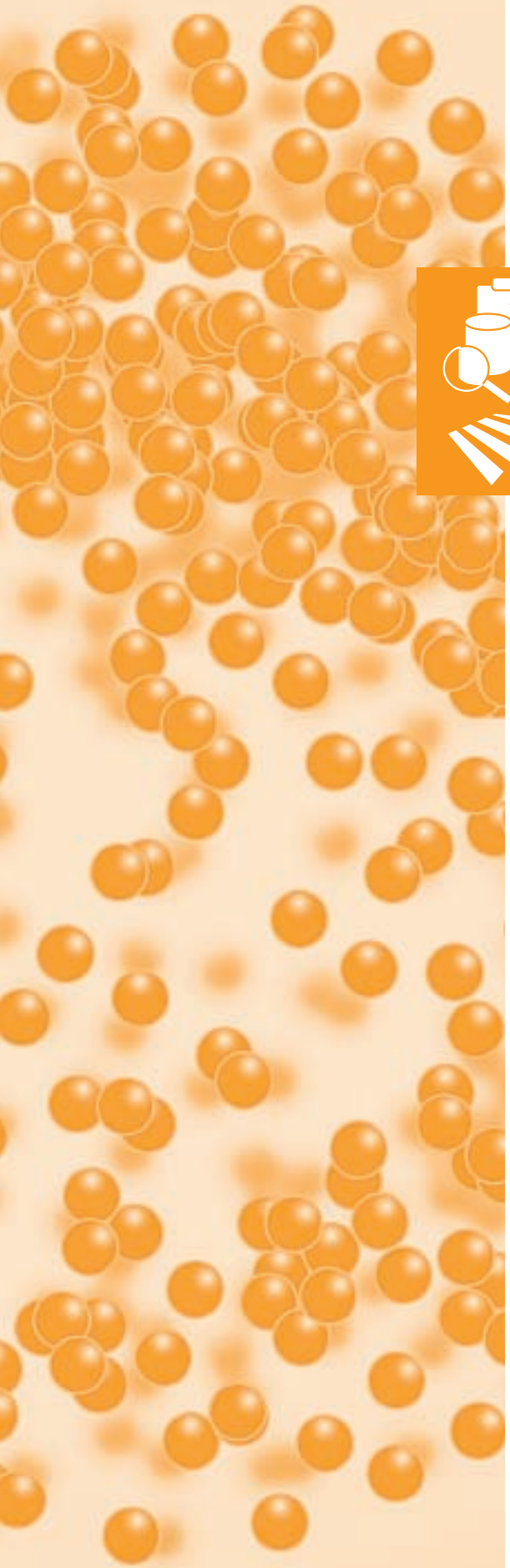
ISBN 1-904465-59-5



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DISCLAIMER

This report is intended to serve as a review of the implications for food safety of the application of nanotechnology in food production and processing as they relate to Ireland. The report does not purport to be comprehensive or to be a legal interpretation or to constitute legal or other professional advice. Advances in scientific knowledge and changes in legislation can be expected in the future that will necessitate the updating of this report.

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EXECUTIVE SUMMARY

The application of nanotechnology in the food and feed industry offers many potential benefits for both consumers and manufacturers. The ultrafine dimensions of nanoparticles, and consequently their very large surface area, enable them to function more effectively than conventional macro-scale structures in many applications. Nanotechnology is however a relatively new area of science and the benefits and risks associated with its use in the food and feed industry are not fully understood at this time. This brings with it new challenges in ensuring the safety of food and feed that has been produced with the aid of nanotechnology.

This report provides an overview of current and potential applications of nanotechnology in the food industry, which may equally be applied in the feed industry. The possible risks of nanotechnology, together with the adequacy of the existing EU regulatory framework in the control of any potential risks, are also examined with a view to determining what further legislative measures, if any, may be necessary to safeguard food safety.

This report notes that nanoparticles are already naturally present in food, given that many food and feed ingredients are comprised of endogenous proteins, carbohydrates and fats with sizes extending from large biopolymers (macromolecules) down to the nanoscale. The applications of nanotechnology in food production reviewed in this report must therefore be viewed in the context of this background exposure to natural nanoparticles in the diet.

There is currently no known use of nanoparticles in food on the Irish market, although such foods are known to be available on the global market, mainly through internet trading. This report demonstrates, however, that nanotechnology has the potential to have a major impact on food innovation over the coming decades. The application of nanotechnology to enhance the stability and texture of food and to develop "intelligent" food contact materials, including packaging, is currently of considerable interest to the food technology sector. The controlled release of nano-encapsulated food ingredients or nutrients is likely to be a primary focus in the longer term as the technology evolves.

There are some knowledge gaps regarding the potential for nanoparticles present in food and feed to enter parts of the body hitherto inaccessible to their conventional counterparts. Evidence is accumulating that engineered nanoparticles can cross natural barriers within the body, although the health implications of this, if any, are as yet unclear and have to be put in context with the background exposure to nanoparticles found naturally in food. There is an urgent need to develop predictive and validated toxicological tests that can be used to screen for potential hazards, and also to develop new methodology for the measurement of nanoparticles in biological matrices, in order to assess human exposure. Nanoscale proteins, carbohydrates and fats are unlikely to be a source of toxicity in their own right. However, little is known about possible interactions of such nanoparticles with other food components, the integrity of the nanoparticles following passage through the digestive system or how they are absorbed, distributed and excreted (cleared) from the body.

Although the basic principles of the conventional risk assessment model can be applied to nano-based food, in practice, gaps in the knowledge base make it difficult to carry out a meaningful risk assessment. Until such time as these knowledge gaps are filled and there is international agreement on the approaches to assessing the risks, each application of nanotechnology in food production, and the wider implications of nanotechnology for the food chain, will need to be assessed on a case by case basis. Hence, risk management approaches to foods resulting from nanotechnology will, because of necessity, be precautionary.

The existing EU legislative food safety framework does not contain specific provisions on nanomaterials. However, uses of nanotechnology in food are considered to fall within the scope of general food law, in that the food sector is obliged to only place safe food on the market, or are subject to specific approval processes such as those for food additives, food contact materials, novel foods, pesticide and biocide approval systems. Specific controls on the use of nanotechnology in food production are being incorporated in pending EU legislation that will see the European Food Safety Authority (EFSA) being responsible for the safety assessment of nanoscale additives and ingredients.

An important principle highlighted in this report is that the food business operator is primarily responsible for ensuring the safety of food. However, it is acknowledged that the assessment of possible risks and likely exposure patterns of nanofoods is not clear cut, given that current methodologies may be inadequate in identifying hazards and evaluating risks associated with the specific properties of nanoscale substances.

Reflecting the deficiencies in the available information on the current applications of nanotechnology in the agrifood sector and also the information needed to carry out a meaningful risk assessment; this report contains a number of recommendations, including the following:

- food business operators should conduct risk assessments on all foods involving introduction of new nanoparticles into foods and packaging or manipulation of the size distribution of natural food molecules in food matrices, in order to meet their legal obligations to produce safe food

- legal provisions should be considered at EU level to ensure that food and feed should be re-evaluated in terms of safety whenever the properties are changed/re-engineered to the nanoscale

- the FSAI should promote the establishment of a publicly available inventory of nanotechnology-based food products and food contact materials, both at European Community level and specifically available on the Irish market

- urgent consideration should be given to whether additional controls are required on the disposal and/or recycling of nanoparticle-containing food contact and other materials

- food surveillance programmes should include investigation of the potential for nanoparticles, particularly inorganic molecules such as titanium dioxide and clay particles used in packaging, to migrate into foods and also to be recycled in the environment and enter the food chain indirectly.

In addition, in order to protect and inform consumers, the report proposes that food or food packaging in contact with food and incorporating nanoparticles should be labelled, and that the FSAI should keep Irish industry informed of developments concerning the applications of nanotechnology in food and the legal requirements governing such uses. The report also recommends that the FSAI should keep under review advances in the science of nanotechnology, risk assessment approaches and the legal framework governing the application of nanotechnology in food.

Finally, the report stresses that appropriately funded research should urgently be undertaken to increase the reliability of the assessment of possible risks of nanotechnology in food, including on issues such as the fate and behaviour of nanoparticles within the human body and animals; their potential toxicity; methods for the safe and effective disposal of used or waste nanoparticles; the stability/lability of nanoparticles in various foods and their potential interactions with other food components.

Keywords: Nanoparticles, nanomaterials, nanotechnology, food, nanofood, nanotoxicology

1. INTRODUCTION

1.1 Background

The last decade has seen an explosion of interest in the applications of nanotechnology in a wide range of areas including food production. The commercial application of nanotechnology is however currently much more advanced in areas other than food, such as materials science, electronics and medicine. Many possible food applications are at a research stage in academia and industry, but at present, the full potential for such products has not been realised. Only a small number of products have actually been commercialised, mainly in countries outside the EU, the US being the leader in innovation in this field, followed by Japan and China. A recent report identified a wide range of applications in food and food technology, and suggested that the value of the worldwide nanotechology food market may total $20.4 billion (€19.4 billion) by 2010 and $1 trillion (€740bn) by 2013 (Kaiser, 2007).

The principal areas in the food sector where nanotechnology has potential for use are in encapsulation and emulsion formation, in food contact materials and sensor development, and some applications of the technology are close to utilisation. For instance, in 2007, the European Food Safety Authority (EFSA) published an opinion on the safety of a nanoscale silicon dioxide coating (less than 100 nanometre), to be used in plastic packaging behind an inner layer of polyethylene terephthalate (PET) plastic to provide gas barrier properties (EFSA, 2007). In addition, a safety evaluation for a titanium nitride-based nanoparticle to be used as a food contact material additive is currently being undertaken by EFSA. Other possible applications are explored in more detail in Chapter 2 of this report.

Application of nanotechnology in the agrifood sector, and more generally, offers potentially huge benefits for both consumers and manufacturers in a wide range of applications, and research in the area is attracting increasing investment from Governments and industry worldwide. Many of these benefits result from the ultrafine dimensions of nanoparticles, which enable them to reach new locations in the body, or from their very large surface area which enables them to function more effectively than larger scale materials, an example being the use of nanosilver as an antimicrobial agent in food contact materials.

While it is recognised that emerging nanotechnology applications are likely to bring significant benefits, it is also recognised that nanomaterials may present different hazards from those of the same material in a micro or macro form. There is concern that not enough is known about the toxicological/physiological and environmental effects of such materials, and that current risk assessment methodology may not be adequate to identify the risks for man and the environment, including animal health. This is discussed in more detail in Chapter 4 of this report.

Judging by the public response to genetically modified (GM) technology in both food and animal feed, the use of nanotechnology in food production is likely to be one of the more contentious areas of application, and the safety of consumers exposed to nanoparticles through food must be assured. In a recent survey of 1,000 German consumers commissioned by the Federal Institute for Risk Assessment (BfR, 2008), although 66% of respondents believed that the overall benefits of nanotechnology outweigh the risks and only 9% considered that the reverse was true, the majority of respondents were against the use of nanotechnology in food. Only 4% considered that the benefits of application of nanotechology in food outweighed the risks, while 84% did not want enhancement of the appearance of food by the use of nanoparticles. A UK consumer organisation conducted a survey of public perception of nanotechnology in a "Citizen's Panel" of seven men and seven women, over a period of three days. They found that overall consumers were positive about the benefits of nanotechnology, but opinions differed in relation to the application in food production and manufacturing (Which?, 2008). The UK consumer group surveyed considered that the tightest controls were required with regard to applications of nanotechnology in medicine and food, as these are areas where nanoparticles may be ingested, and were more positive about the potential for nanotechnologies in food packaging. A survey of Swiss consumers has indicated that consumers are more concerned about food produced by application of nanotechnology than about food packaging containing nanoparticles (Siegrist *et al.*, 2008)

A number of expert bodies have already issued opinions on issues related to applications of nanotechnology and its safety, including the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (SCENIHR, 2006, 2007), the EU Scientific Committee on Consumer Products (SCCP) (SCCP, 2007), the US Food and Drug Administration (US FDA) (FDA, 2007), the UK Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COT, COM, COC) (COT, 2005), the UK Royal Society and Royal Academy of Engineering (Royal Society and Royal Academy of Engineering, 2004), the German Federal Institute for Risk Assessment (BfR, 2007), the Dutch Institute of Food Safety, Wageningen UR (RIKILT) and National Institute of Public Health and the Environment; Center for substances and integrated risk assessment (RIVM) (Bouwmeester *et al.*, 2007) and the US National Institute of Occupational Health (NIOSH) (NIOSH, 2007). An EFSA opinion on the risks of nanoparticles in food is expected in 2008 and food safety agencies throughout the world are currently assessing the issue.

In April 2007, the Food Safety Authority of Ireland's (FSAI) Scientific Committee discussed the national need for a scientific assessment of the relevance/implications of the application of nanotechnology in food production and processing for food safety. An expert working group (Appendix 1) was convened in June 2007 to further consider the issues, with the aim of developing a position on nanotechnology in relation to food safety and risks to the Irish consumer.

1.2 Terms of Reference

The Terms of Reference for the Scientific Advisory Working Group on Nanotechnology were as follows:

- advise on the main applications of nanotechnology foreseen in food and animal feed
- advise on the adequacy of current risk assessment paradigms/methodology for the identification, assessment and control of any potential risks arising from the use of nanotechnologies in the agrifood sector or the presence of nanoparticles in food
- identify gaps in the regulatory framework and information needed to carry out an assessment of risk of nanoparticles in the food chain
- advise on approaches to fill such gaps
- advise the FSAI on the development of a national position on nanotechnology in relation to food safety and risks to the Irish consumer
- support the FSAI in the drafting of a report to reflect these issues, for the approval of the Scientific Committee.

1.3 Scope of the Report

The Terms of Reference apply primarily to the application of nanotechnology in food production and processing and the implications for the safety of food, the term "food" being defined in accordance with Regulation (EC) No 178/2002 on general food law (EC, 2002), as follows:

> *"Food or foodstuff[1] means any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans. For the purpose of this report, drinking water is included under the definition of food."*

The presence of nanoparticles in the food chain as a result of environmental contamination and accidental or deliberate inclusion of nanomaterials in animal feed may be an additional, indirect route of human exposure via food, in addition to the direct use of nanomaterials in the production and/or processing of food for humans. Accordingly, this report has also briefly considered the use of nanotechnology in the production of feed for food-producing animals and also potential pathways of nanoparticles from the environment into food.

Consideration of direct human exposure via the environment, other than the food chain, and via occupational exposure is outside the scope of the report.

[1] Food includes drink, chewing gum and any substance, including water, intentionally incorporated into the food during its manufacture, preparation or treatment. It includes water after the point of compliance as defined in Article 6 of Directive 98/83/EC and without prejudice to the requirements of Directives 80/778/EEC and 98/83/EC.

1.4 Definitions of Nanotechnology Applied in this Report

The term 'nanotechnology' was first applied in 1974 by Norio Taniguchi (Taniguchi, 1974) and was used to describe production technology at ultrafine dimensions, hence the use of the Greek word 'nano' - meaning dwarf. Nanotechnology has now evolved into a multidisciplinary research sector with a rapidly expanding industrial element. Likewise, the terminology has also evolved and grown to reflect the diversity of this area of science and technology with numerous broad definitions of nanotechnology emerging from a variety of professional, national and international bodies.

The International Standards Organization (ISO) recognised that there is a need to standardise terminology and provide a common language for scientific, technical, commercial and regulatory purposes. The ISO/TC229 Technical Committee on Nanotechnologies established a working group to advise on this specific issue and, although work is still ongoing, the following general definition for nanotechnology has emerged from the group:

> *'Understanding and control of matter and processes at the nanoscale, typically, but not exclusively, below 100 nanometres in one or more dimensions where the onset of size-dependent phenomena usually enables novel applications, where one nanometre is one thousand millionth of a metre.' (ISO/TC 229, 2008).*

This definition is non-specific and can be applied across the entire nanotechnology community from academia to industry. It has therefore been adopted in this report as a basic definition of nanotechnology in the absence, at the time of writing, of any internationally agreed definition. However, it is important to note that the definition makes no reference to the synthesis or use of engineered nanomaterials in food or feed products. For the purpose of clarity throughout the report, it should be taken that an engineered nanomaterial is any material which has been intentionally synthesised or incidentally produced to exploit novel functional properties exhibited on the nanoscale (i.e. typically, but not exclusively, below 100 nanometres in one or more dimensions as specified by the ISO definition).

In contrast, the generic term of 'nano-object' as defined by the EU Commission recommendation of 07/02/2008 on a code of conduct for responsible nanosciences and nanotechnologies research (EC, 2008) will include all nanomaterials, nanostructured materials, nanoparticles and their aggregation at the nanoscale, nano-systems, and nanoproducts.

2. CURRENT AND POTENTIAL APPLICATIONS OF NANOTECHNOLOGY IN THE FOOD CHAIN

2.1 Introduction

Given that most food systems are comprised of biopolymers with a range of particle sizes including some with nanoscale dimensions, nanotechnology is not a novel concept in food science. The specific applications of the technology are however rapidly increasing and are expected to have a major impact on food innovation over the coming decades. A number of reviews which describe the application of nanotechnology in the food sector have been published, e.g. Chen *et al.*, 2006a; Chaudhry, 2008; ETC group, 2004; Miller and Senjen, 2008; Morris, 2005; Morris, 2006. This chapter identifies the key areas where applications of nanotechnology are being researched or have already been introduced in the agrifood sector.

A wide range of applications are foreseen in the agrifood sector, including nanosensors, tracking devices, targeted delivery of specific components, food safety applications, new product development, precision processing and smart packaging. The potential applications of nanotechnology range from modifications of the natural (organic) protein, carbohydrate and fat molecules that form part of the normal diet, to achieve added or altered functionality, to the use of inorganic nanoparticulate materials in food packaging and food ingredients including food additives. The predominant food-related use of nanoscience in the short term is in food contact materials including packaging, while in the longer term nanoscale food research appears to be focused on controlled release of nanoscale-encapsulated food ingredients or nutrients. The use of nanoscale technologies for alteration of food stability and texture is also of particular interest.

Engineered organic nanomaterials (primarily nanoparticulate forms of natural food components) are likely to be the predominant area of interest in the agrifood sector. Many applications of nanotechnology currently under research involve modification of the normal structure and assembly of natural biopolymeric food components, e.g. modification of proteins or selected carbohydrates to make them suitable as nanostabilisers of bioactive components. Protein-carbohydrate engineering coupled with enzymatic functionalisation is being used to construct nanoscale structures which impart new functionality in food.

Nanoparticles in food will be exposed to a range of storage and use conditions, and this is an aspect that may need consideration in relation to assessment of the impact of nanotechnology in the agrifood sector. For example, nanoparticles may be used in products that should be stored at low temperatures and then heated for consumption. This may affect both the nanoparticle stability within the food, as well as changing the properties of the biomolecules in the food, and potentially how these interact with the nanoparticles. To date, there appears to be no literature dealing with this.

Figure 2.1 summarises the main areas where nanotechnology is currently used in food and where food safety issues may arise.

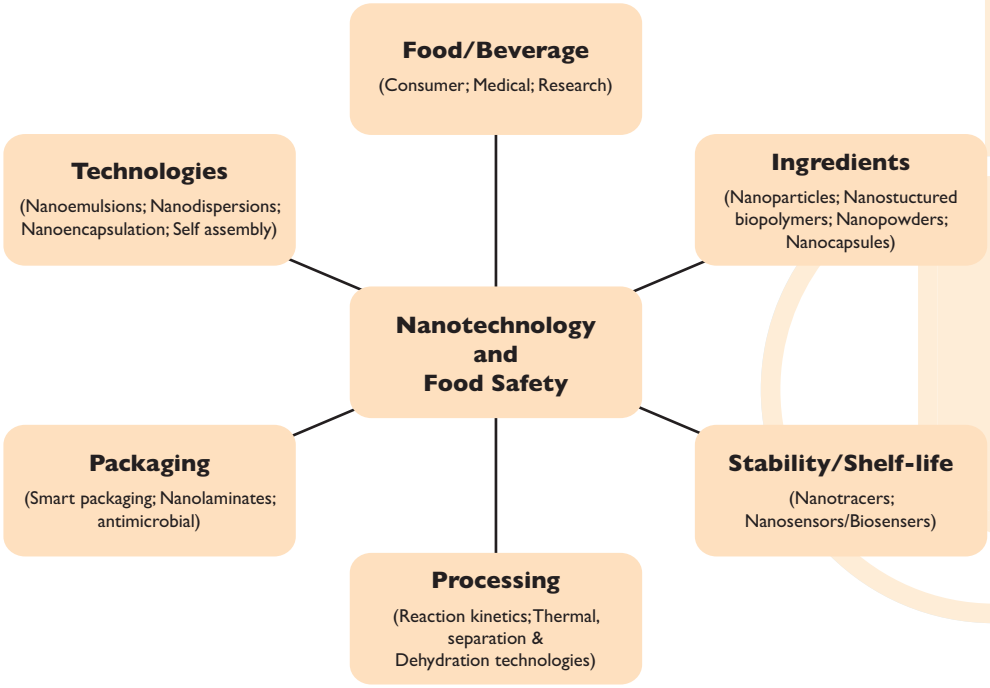

Figure 2.1 Applications of Nanotechnology in Food

2.2 Current Applications of Nanotechnology in Foods and Food Contact Materials

Some of the benefits of nanoscience have already been seen in the agrifood sector[2], others are still at the research and concept stage. They include the following global applications:

1) sensory improvements (flavour/colour enhancement, texture modification)
2) increased absorption and targeted delivery of nutrients and bioactive compounds
3) stabilisation of active ingredients such as nutraceuticals in food matrices
4) packaging and product innovation to extend shelf-life
5) sensors to improve food safety
6) antimicrobials to kill pathogenic bacteria in food.

Nanotechnology-based materials of relevance to food and food contact materials include nanoparticles, nanofibres/fibrils, nanoemulsions/dispersions and nanoclays, as summarised in Table 2.2. Each category is explained in more detail below.

2 It should be noted that the applications listed reflect international use, and there is no clear picture of which, if any, of these applications relate to food available on the European or Irish markets.

Table 2.2 provides a non-exhaustive list of examples of products currently on the international market, resulting from the applications of nanotechnology in food.

Table 2.1 Classification of Nanomaterials with Application in Food and Food Preparation

Category	Example Materials	Example Application
1. Nanoparticles		
Inorganic	Iron	Food supplement
	Silver	Food supplements, antimicrobial agent - used in food contact surfaces (cutlery, storage containers, fridges and worktops)
	Iridium	Food supplement
	Platinuim	Food supplement
	Zinc	Food supplement/colourant
Organic	Liposomes	Encapsulation and targeted delivery of food components
	Protein	Re-micellised calcium caseinate from dairy protein. Increased functionality (gelation, heat stability and other properties)
	Polymeric	Non-degradable: polystyrene Biodegradable: PGLA, gelatin, collagen
2. Nanofibres*/fibrils	Globular proteins	Thermal stability, increased shelf-life. Formation of transparent gel network for use as thickening agent
3. Nanoemulsions/ dispersions		
Emulsions	Oil in water	Stabilisation of biologically active ingredients; delivery of active compounds; extended shelf-life; flavour release; low fat products
Dispersions	Calcium Carbonate	Increased solubility of calcium carbonate – can be used at higher addition levels
4. Nanoclays	Clay composites	Used in packaging materials to extend shelf-life, durability, and thermal properties (includes nano-laminates)

* Nanofibres are not currently used in the marketplace but are being researched extensively.

2.2.1 Nanoparticles

Nanoparticles can be categorised into different types based on their ability to carry different ingredients and react to different environmental conditions. For the purposes of this review they can be divided into two broad categories, inorganic and organic, based on the chemical characteristics of the nanoparticle. Nanoparticles already reported to be incorporated into foods (although not on specifically on the EU market) include those engineered to provide encapsulation systems, e.g. micelles, liposomes, for delivery of food ingredients, and those tailored for use in food packaging such as biosensors, identification markers, shelf-life extenders and antimicrobials.

2.2.1.1 Inorganic nanoparticles

Many inorganic ingredients manufactured at the nanoscale are variations of additives already approved for use in food, e.g. titanium dioxide, a food colorant, can be used as a UV protection barrier in food packaging when used as a nanoparticle (Note: titanium dioxide loses its ability to act as a food colorant in the nano form because it is transparent). New storage containers/utensils (food contact materials) based on embedded inorganic nanoparticles have been designed for preservation of prepared foods. The most common application is the use of nanoparticles of silver (Table 2.2) as an antimicrobial. Applications for nanosilver include use in fridge panels, storage boxes, packaging lines and other surfaces which come into contact with food during manufacture. Nanosilver particles have also been added to non-food contact areas such as floor tiles to reduce the bacterial load in the surrounding manufacturing environment. Food storage bins are being produced with silver nanoparticles embedded in the plastic, killing bacteria from any food that was previously stored in the bins and minimising health risks.

Inorganic nanoceramic, solid pellet nanoparticles, have been added to oil cooking systems (Table 2.2) in restaurants in the USA to extend the life of chip oil while giving a crisper food. The technology is based on the ability of the nanoceramic particles to act as a catalyst, limiting thermal polymerisation process in the oil resulting in a crisper deep-fried food and longer shelf-life for the oil.

2.2.1.2 Organic nanoparticles

Organic nanoparticles (sometimes referred to as nanocapsules when used as vehicles for delivery of, e.g. essential nutrients or pharmaceuticals) are likely to be used to enhance the nutrient value of food systems through improvement or alteration of food functionality. Nanoparticles have been designed to deliver vitamins or other nutrients in food and beverages without affecting the taste or appearance. These nanoparticles encapsulate the nutrients and carry them via the gastrointestinal tract (GIT) into the bloodstream, increasing their bioavailability.

Micelles are organic nanoparticulates that can be assembled by the thermodynamically driven process known as self–assembly. Micelles made in this way have the ability to encapsulate non-polar molecules such as lipids, flavours, antimicrobials, antioxidants and vitamins (Chen, 2006). Compounds that are normally insoluble or only sparingly soluble in water can be made water soluble, extending their use in foods and potentially changing their bioavailability once ingested. For example, one company has developed a micelle-based technology (Table 2.2) which combines two active substances, coenzyme Q10 (CoQ10) for fat reduction and alpha-lipoic acid for satiety into a single nanocarrier (micelles of average 30nm diameter). The application is targeted at the weight management market.

Another example is a liquid droplet technology named 'Nano-sized Self-assembled Liquid Structures (NSLS)', which encapsulates and releases particles in cells. The micellar particles are used to encapsulate nutraceuticals (lycopene, beta-carotene, lutein, phytosterols, CoQ10, docosahexaenoic acid/eicosapentaenoic acid (DHA/EPA) and other compounds) into 30nm diameter self-assembled spheres. The micelles are essentially made from lipid molecules and have a unique hydrophobic interior. The NSLS particles are reported to act as vehicles for compounds to be absorbed into the bloodstream from the gut more readily, increasing their bioavailability. The technology has already been adopted and marketed by an American company to deliver an oil-based product (Table 2.2), which it claims can reduce cholesterol intake into the body by 14%, by competing for bile solubilisation.

Casein micelles (particles with diameters in the range 60 – 300nm) in fresh skim milk can be reassembled from acid casein in the presence of calcium phosphate (Mounsey *et al.*, 2005). Re-micellisation of casein in this way confers different functionality on the protein while maintaining the ability of the milk to form gels on the addition of the enzyme chymosin, commonly used in cheese making.

Liposomes are another example of micelles and can be used to encapsulate both water and lipid soluble compounds (Taylor *et al.*, 2005). The dissolution of fat-soluble nutrients in water-based drinks is one of the key applications of liposomes. Table 2.2 provides an example of one such encapsulating vehicle that can be used to encapsulate nutraceuticals for oral delivery. Examples of current research into the use of liposome technology in food is the encapsulation of enzymes, lactic acid bacteria extracts and/or antimicrobials for accelerated cheese ripening. Liposomes can be produced to differing sizes (10 – 500nm) and engineered to have different stability and/or surface charge under different environmental conditions. Development work utilising this technology is already ongoing in Ireland at Moorepark Food Research Centre, using liposomes as agents to accelerate cheese ripening. Liposome technology can be used potentially to target specific sites within a food product for enzymatic degradation.

2.2.2 Nanofibrils/Nanotubes

Recent research has demonstrated that many globular food proteins, including the whey proteins (alpha-lactalbumin, beta-lactoglobulin) and bovine serum albumin, can self-assemble into fibrils ("nano-fibrils") at high temperature and low pH. Nano-fibrils typically have a diameter of ~ 5nm and lengths of up to 15µm. Under appropriate conditions, alpha-lactalbumin can be partially hydrolysed by protease enzymes from Bacillus licheneiformis (Graveland-Bikker and de Kruif, 2006). When this partially hydrolysed protein is exposed to calcium ions, the formation of linear nanotubes is triggered. Alpha lactalbumin nanotubes have good thermal stability and can be subjected to dehydration processes such as freeze-drying for prolonged storage. The transparent gel network formed by these nanofibrils may have many uses in the agrifood sector as a thickening agent. At the time of publication of this report, the nanotubes described here have been the subject of research, but are not currently used in food.

Table 2.2 Applications of Nanotechnology in Food – Examples of Products Currently on the Market (non-exhaustive list)

Product Example	Nanomaterial or Technology Used	Application Area
MesoSilver MesoGold MesoCopper MesoPlatinum MesoPalladium MesoIridium MesoTitanium MesoZinc	Nanoparticles of silver or gold Copper Platinum Palladium Iridium Titanium Zinc	Food supplements
Colloidal Silver Cream Colloidal Silver Liquid Nano Silver dispersion	Nanosilver	Silver nanoparticles have been incorporated into different products from bandages to refrigerators for suppressing the spread of bacteria and other microbes (Nanosilver, 2004)
Synthetic Lycopene	Nanoparticles of lycopene	Nutraceutical
OilFresh	Zeolite	OilFresh is a device to keep frying oil fresh. OilFresh uses zeolite, a mineral, in the form of beads with an average diameter of 20 nanometers across, coated with an undisclosed material

Table 2.2 Continued

Product Example	Nanomaterial or Technology Used	Application Area
Aerosil	Silica Nanoparticles	Used to increase flowability of powdered ingredients
Titanium dioxide Silicon dioxide Silver	Nanoparticles	Packaging materials containing nanoparticles of titanium dioxide, silicon dioxide or silver, which increase shelf-life of food by modifying mechanical and heat resistance properties, and developing antimicrobial and antifungal surfaces
MultiSal™	Nanoparticles	Delivery system for active ingredients (water and fat soluble)
Fabuless™	Emulsion	A nanoemulsion that delays digestion until lower regions of the small intestine, stimulating satiety and reduce food intake
Nutralease	Nanoparticles	Enhances the solubilisation and bioavailability of nutrients
Canola Active Oil	Nanoparticles	Canola oil fortified with free phytosterols to reduce cholesterol levels
Nanocalcium and Magnesium		Mineral supplement with increased bioavailability
Nanoceuticals Slim Shake Chocolate	Nanoclusters	Slimming drink using nanoclusters to enhance flavour without the need for added sugar
Durethan	Nanoclay	Transparent plastic film (called Durethan) containing nanoparticles of clay to block oxygen, carbon dioxide and moisture from reaching the food
Imperm	Nanocomposites of nylon	Nanocomposites embedded in plastic beer bottles that give a six month shelf-life to beer
NovaSOL	Miceller organic nanocapsules	Delivery system for hydrophobic substances
Encapsome™	liposomes (Micellar)	Nanoparticles capable of carrying both water-soluble and oil- or fat-soluble compounds within a single particle

2.2.3 Nanoemulsions

Nanoemulsions are emulsions which are thermodynamically stable compared to conventional emulsions under a range of different conditions. This is due to their small size (typically 50 to 500nm compared to 1200nm) and monodispersivity. They can be diluted with water without changing the droplet size distribution. The type of surfactant used to formulate a nanoemulsion is critical to the stability of the final emulsion. Preparations of nano-emulsions can be used to encapsulate functional food components at oil/water interfaces, or throughout the continuous phase of the system (Weiss *et al.*, 2006).

The applications of nanoemulsions include:

- delivery of active compounds in the body
- stabilisation of biologically active ingredients
- extended shelf-life due to increased stability
- increased viscosity at lower concentrations of oil phase.

Research has shown that stabilised mono-dispersed oil-in-water (O/W) or water-in-oil (W/O) nanoemulsion systems can be used for controlled release of nutraceutical and other bioactive components in food (Weiss *et al.*, 2006). The technology has been combined with advanced processing technologies to develop novel microencapsulated products that allow controlled release of food bioactives in the gastrointestinal tract. These products may be either ready-to-drink or powdered formulations fortified with functional ingredients from a wide range of sources. Process technologies such as ultra high pressure homogenisation and micro-fluidisation have been used to create stable mono-dispersed O/W or W/O nanoemulsions for the uses described.

The physical properties of nanoemulsions such as rheological and microstructural properties, phase-separation behaviour and stability in food products is significantly different to those found in emulsions manufactured using standard emulsification techniques. Incorporation of plant-derived nutraceuticals, e.g. plant sterols, lycopene, limonenes, CoQ10, and protein-based bioactives into the oil phase (O/W emulsions) or water phase (W/O emulsions) has already been successfully carried out using this technology. One food company has produced a nanoemulsion (Table 2.2) which is not digested until it passes to the lower regions of the small intestine where it stimulates satiety signals in the brain to tell the body that food intake can be reduced. Because of their small particle size, nanoemulsions may be used in the future in the development of low fat products due to the viscosity that is imparted at low oil droplet concentrations.

Nanodispersions created by high pressure homogenisation and microfluidisation techniques have been used to reduce the size of inorganic matter to a size which can be dispersed in aqueous solutions. The technology can be used to create injectable versions of inorganic materials which can subsequently be used in medical applications. Nanodispersions can remain in a stable suspension for a longer period than larger particle based dispersions.

2.2.4 Nanoclays

Nanoclays are used to provide an impermeable barrier to gases such as oxygen or carbon dioxide in plastic bottles, cartons and packaging films. Many products containing nanoclays already exist in the market place. For example, one chemical company produces a transparent plastic film which contains nanoparticles of clay (Table 2.2). The nanoclay particles are dispersed throughout the plastic and are able to block oxygen, carbon dioxide and moisture from reaching fresh meats or other foods. The nanoclay also enables the plastic to be made thinner, lighter, stronger and more heat resistant. Similarly, there is a US patent for nanoscale films that have been used as coatings. The coatings, made from oxides of silicon or titanium, are anitmicrobial and can increase the life of many manufactured foods, even after they are opened.

2.3 Research Trends in Nanotechnology and Food

2.3.1 Food contact materials including packaging

Applications of nanotechnology under research for use in packaging include: gas/UV barriers, foils (mechanically and temperature reinforced), nanoclays, nanoparticles, nanopolymers, composites, nanocavities and strings. These and similar technologies can provide packaging solutions with functional properties of benefit to the consumer and/or the manufacturer. Promising developments have also been made in the area of antimicrobial, breathable and multifunctional packaging. Packaging which can release nanoscale antimicrobials, antioxidants and/or flavours to increase shelf-life and sensory characteristics have already been developed (LaCoste *et al.*, 2005).

Nanosensors are being developed for the purpose of detecting chemical contaminants, viruses or pathogenic bacteria in food systems. Examples of nanosensors include particles, engineered at the nano scale, to attach to pathogens or other contaminants which are then selectively identified by fluorescence or magnetic devices. The advantage of these systems is the ability to identify a wide spectrum of pathogenic organisms using just one sensor.

Currently, the research on nanosensors is focused in two areas, 1) the development of easy to use rapid biosensors for the diagnostic detection of pathogens/contaminants in the foods (including medical foods) and their surrounding environment and 2) the incorporation of nanosenors into food packaging materials for tracking, safety and biosecurity purposes. An example of the latter is triggered colour changes on the surface of a packaging materials to indicate food spoilage. Extreme condition packaging, nanocoding of plastics and paper materials for identification purposes, monitoring tagging, trade mark and fraud protection, will improve supply chain efficiency. Research on such technology is likely to continue as the need to maintain food quality during transportation increases.

2.3.2 Targeted delivery systems

One of the many benefits of nanotechnology is the ability to alter or enhance the release of molecules from food matrices. Increased bioavailability through delivery of nutraceuticals, bioactive, and inorganic materials promises to be one of the major applications of nanotechnology in the agrifood sector. In a recent report by the RIKILT Institute of Food Safety, Wageningen UR and the National Institute of Public Health and the Environment; Center for Substances and Integrated Risk Assessment in the Netherlands (Bouuwmeester *et al.*, 2007), the types of nanoparticles used as delivery systems have been classified as shown in Figure 2.2

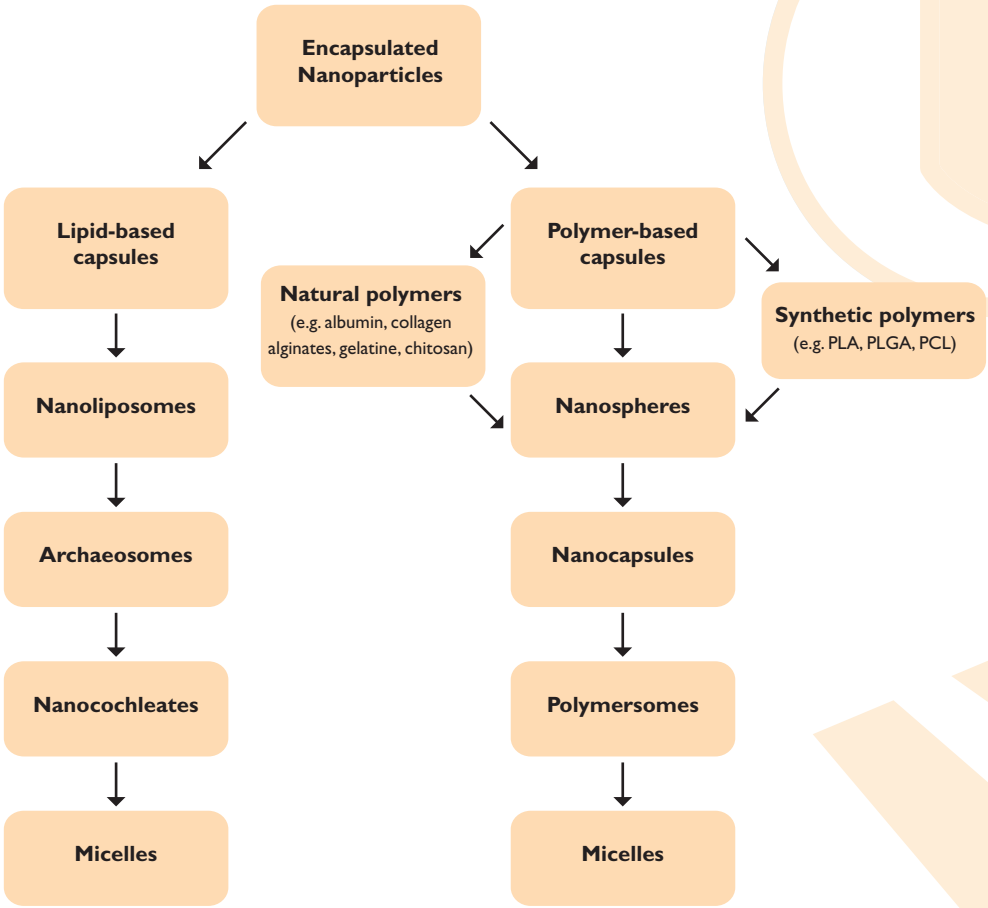

Figure 2.2 Classification of Nanoparticle Delivery Systems
(adapted from Bouuwmeester *et al.*, 2007)

Research continues in this area and for example a system designed to release ingredients consecutively has recently been developed. The manufacturers claim that the product (Table 2.2) enhances the stability and bioavailability of nutrients and other ingredients by controlling their release characteristics and prolonging their residence time in the oral cavity. The technology can be used to prolong the sensation of flavour in the mouth.

Research is also underway to explore the use of nanoparticles in so-called interactive foods. This may give consumers the ability to modify the characteristics of the food depending on their own nutritional or organoleptic requirements, by controlling the release of 'nanocapsules' containing flavour/colour modifiers and/or added nutritional elements, e.g. vitamins, which remain dormant in the food until triggered by the consumer or retailer. Nanocapsules have been developed whose walls burst at different microwave oven frequencies so the consumer can create new tastes or even colours.

2.4 Applications of Nanotechnology in Agriculture

As indicated in Chapter 1, the presence of nanoparticles in food as a result of accidental or deliberate inclusion of nanomaterials in animal feed or as a result of environmental contamination, may be an important route of human exposure. The current and potential applications of nanotechnology in the food industry may equally be applied in the feed industry and some potential applications are briefly outlined below.

2.4.1 Animal feed

In the short to medium term it is generally thought unlikely that nanotechnology will be used to a significant extent in the development of animal feed ingredients, due to cost implications. What is more likely is that nanotechnology products may enter animal feed through waste food or environmental sources and possibly as additives following on from developments in the veterinary or human medicines area. In these cases, the consequences of use of nanotechnology should already have been comprehensively evaluated in the original use.

2.4.2 Other farm applications

Farm applications of nanotechnology include more efficient and safe systems for the application of pesticides, herbicides, and fertilisers, by better control of where and when they are released. An example of this is provided by an environmentally friendly pesticide that incorporates nanomaterial as a release mechanism for pest control agents and works only when inside the host. Nanomaterials in the late stages of development include products that can detect and neutralise animal pathogens in livestock before derived food products reach the consumer. Nanotechnology applications in controlled environment agriculture include "smart" pesticide delivery, control of crops, animal health, control of microbial and chemical contamination and complete plant health monitoring.

Nanotechnology can also be used in agriculture to develop new tools for the treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients and other essential factors. Smart sensors and smart delivery systems similar to the technology described above for food can be useful to the agricultural industry to combat viruses and other crop pathogens. In the near future, nanostructured catalysts will be available which will increase the efficiency of pesticides and herbicides, allowing lower doses to be used. Nanotechnology may also protect the environment indirectly through the use of alternative (renewable) energy supplies, and filters or catalysts to reduce pollution and clean-up existing pollutants.

2.5 Nanotechnology and Water Purification

Nanotechnology can be used in water purification. A US company has developed 2nm diameter aluminium oxide nanofibres as a water filtration aid, while a product containing lanthanum nanoparticles can absorb phosphates from aqueous environments. Research at the Centre for Biological and Environmental Nanotechnology (CBEN) has shown that nanoscale iron oxide particles are extremely effective at binding and removing arsenic from groundwater.

2.6 Conclusion on Current Applications of Nanotechnology in Food

Many examples of nanoparticles with targeted release capabilities and functionalised packaging, with potential uses in the agrifood sector, have been identified in the marketplace. Of these, probably the most widespread actual or potential use is incorporation of nanoparticles into food packaging and food contact surfaces. Nanosized particles of titanium dioxide, silver and clay are being used in packaging and other food contact materials and the potential of these particles to migrate into foods should be investigated.

The report identifies that currently there is no known direct use of complex organic nanoparticles such as carbon nanotubes in foods. Furthermore, nanomaterials present in foods that are currently available on the market, mostly through internet trading, are mainly in the form of nanoscale encapsulated functional ingredients.

The potential benefits of using nanotechnology in food include the ability to monitor the quality of food, and its surrounding environment, through the use of nanosensors. Antimicrobial biosensors and controlled release technology may provide new approaches to food safety in the future. New textures and flavours may be realised with "release on demand" functionality. It is the responsibility of the manufacturer to ensure that products utilising nanotechnology in food or food contact surfaces comply with applicable legal and regulatory requirements (see Chapter 5), while Governments and the scientific community must provide reliable mechanisms for regulating the use of nanotechnology in food.

3. CHARACTERISATION OF NANOPARTICLES

3.1 Introduction

Nanoparticles encompass the full range of traditional material classes, including all forms of metals, polymers, ceramics and biomaterials. The development of ever-smaller particles presents major challenges, mainly because of difficulties in characterising the reliability, comparability and reproducibility of materials on this scale. In response to these difficulties, a new and emerging subdiscipline of metrology has begun to evolve. Nanometrology, as it has become known, is defined as the ability to conduct measurements at sub-100nm dimensions and to fully characterise the nature of these novel materials.

3.1.1 Nanometrology

Without reliable methods of measurement, it is impossible to determine human or animal exposure via food or feed (see Chapter 4.4). Of specific importance therefore in relation to the food and feed sector and to any potential regulatory framework is the determination of the physiochemical properties of nanoparticles and their measurement in food. Such properties include:

- particle size
- particle distribution
- surface area
- surface charge and topography
- composition and purity
- hydrophobicity and solubility
- chemical reactivity and bioactivity
- dispersion/aggregation state.

Particularly important among these properties are particle size and distribution, surface area and aggregation state. Normally, nanoparticle samples contain a range of particles, both larger and smaller than the mean or median value, which is often the figure actually quoted as the particle size. Figure 3.1 shows how the particle size may be distributed around the mean or median value in a Gaussian distribution. As the particle size decreases, more molecules are present at the surface giving rise to a larger surface to volume ratio or larger surface area for chemical interaction.

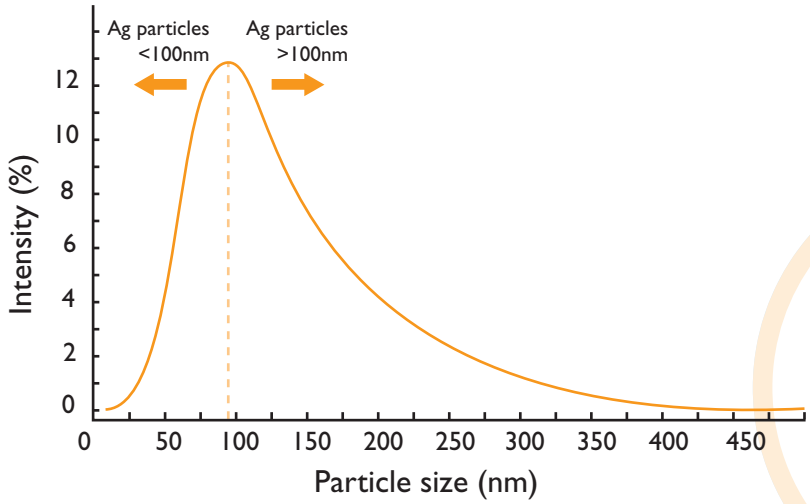

Figure 3.1 Typical Particle Size Distribution Curve for Silver (Ag) Nano-powder Dispersed in Triton X100. The Silver Nano-powder is sold as having a Particle Size of <100nm. The Curve was Obtained using a Malvern Instruments Zetasizer Nano
(courtesy of Dr G. Chambers, School of Physics, Dublin Institute of Technology)

Figure 3.2 shows a schematic of how the surface area changes as the particles become smaller.

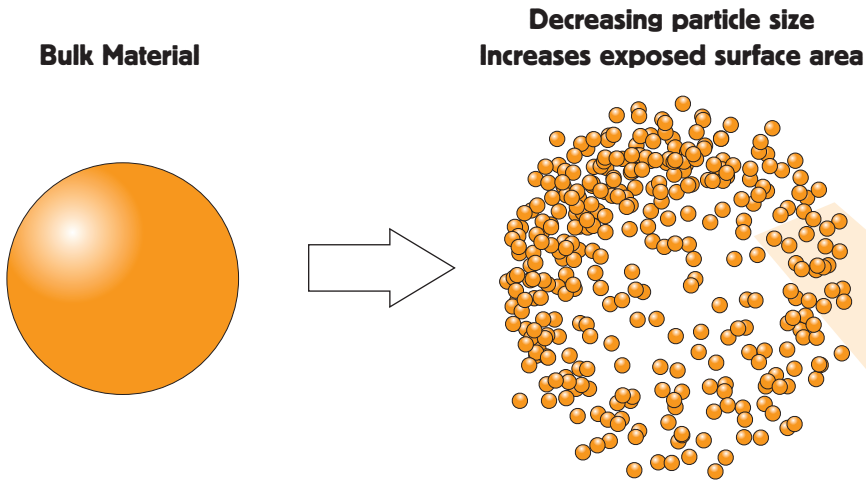

Figure 3.2 Schematic of how the Surface Area Changes as the Particles Become Smaller
(courtesy of Dr G. Chambers, School of Physics, Dublin Institute of Technology)

The tendency of nanoparticles to aggregate, often as a result of the drying stage during the synthesis process, is of particular importance to the characterisation of nanoparticles, as already indicated and as shown in Figure 3.3. Aggregation is the process whereby small molecules or particles can come together to form a secondary larger particle or cluster which is held together by one or more molecular interactions such as Van der Waals forces or hydrogen bonds. This has considerable implications for determination of the size and surface area of the nanoparticles, as well as for determination of exposure.

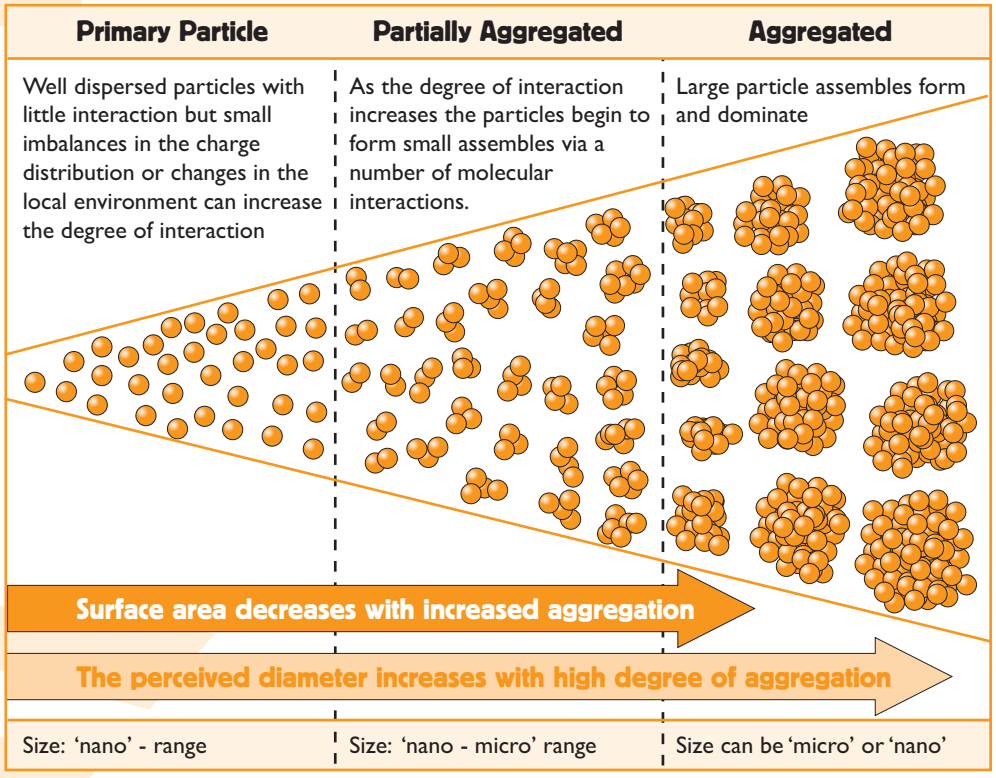

Figure 3.3 Schematic of the Process of Aggregation of Primary Nanoparticles to Form Larger Aggregated Particles
(courtesy of Dr G. Chambers, School of Physics, Dublin Institute of Technology)

Many characterisation techniques exist which can estimate or measure these properties. The reliability, precision and accuracy of these techniques on the nanoscale are however often called into question. In addition, environmental fluctuations, operator variation, and the lack of traceable standards are further difficulties facing nanometrology and regulatory bodies. There is an urgent need to establish calibration standards and protocols for the characterisation of nanoparticles. The ISO TC229 Technical Committee on Nanotechnologies was established in 2005 to address these issues and work is ongoing.

Most material characterisation techniques focus on the pristine particle (as-synthesised) in powder form, following purification and/or drying. However, many applications of nanoparticles in food involve complex environments, which can change the particle size, the aggregation behaviour, the dispersion state, and several of the physical properties. Thus, a complete characterisation of the powder form may not be relevant in terms of the nature of the particle as found in food.

Another aspect to be considered is the characterisation of the nanoparticles under a range of relevant biological conditions, such as in complex food products, where they can interact with proteins, lipids, sugars or other biomolecules. This may have consequences for the surface composition of the nanoparticles and their aggregation behaviour, as the adsorbed proteins and biomolecules may have different hydrophobicity, charge and charge distribution than the as-synthesised nanoparticles. The adsorbed proteins and other biomolecules confer a "biological identity" (the biomolecule corona; Lynch, 2006; Lynch 2007) to the nanoparticles, as it is these adsorbed molecules that are responsible for the primary interaction with living systems. Additionally, the nanoparticles can alter the functioning of the adsorbed biological molecules, e.g. enzyme activity, degradation and other properties, making them more or less active than the unbound form (Palocci *et al.* 2007). A further set of characterisation tests need to be performed on nanoparticles in biological matrices including food.[3] The rest of this chapter provides an overview of characterisation techniques for as-synthesised nanoparticles, i.e. before interaction with biological molecules, and for nanoparticle-biomolecule complexes. It also highlights the problems associated with the actual measurement of nanoparticles in biological matrices such as food and body tissues.

3.2 Physical Characterisation Techniques and Issues

The techniques and instrumentation currently used for the physical and chemical characterisation of nanoparticles, the majority of which could be adapted for use in food and feed, are summarised in Appendix II, together with the properties of the nanomaterial that they are designed to measure. It should be noted that the information provided in Appendix II does not represent a definitive list of characterisation techniques and the instruments listed often require adaptation for nanoparticle characterisation and specialist training. As a result, these instruments can often be expensive and difficult for companies to justify economically.

[3] Many of these issues are also relevant for nanomedicine, although here the point of first contact with biological material is different, often being the blood stream. However, similar approaches to determining the biological identity of the particles can be envisaged.

Particular care must be taken when measuring aspects such as particle size and surface area as nanoparticles are often highly aggregated and have very large particle size distributions, a fact that is often overlooked or ignored when characterising nanoparticles. The sizes recorded are often only a small fraction of the sample, rather than a true representation of the sample composition. A range of techniques is commonly used to disperse nanoparticles, such as ultrasound sonication, dispersing agents (common in food, e.g. emulsifiers), and milling. Even following these procedures, the dispersions are often very polydisperse, and the surface may have changed, due to exposure of "fresh" surface due to the breaking up of clusters. An important issue is to distinguish between the primary particle size, which is typically on the nanoscale, and the cluster size due to aggregation, which may be either nanoscale or micron scale (Figure 3.3).

3.3 Characterisation of Nanoparticles in Biological Matrices

In considering the characterisation of nanoparticles in a biological environment, both the effect of this environment on the nanoparticles and conversely, the effect of the nanoparticles on the environment must be explored. Engineered nanoparticles, with their very large surface areas, adsorb biomolecules to their surface immediately upon contact with natural substances (in the case of this report, food and/or biological material in the mouth and digestive system) to form a biomolecule corona (Lynch, 2006; Lynch, 2007). These biomolecules include proteins, lipids and sugars, and it is these adsorbed biomolecules that confer the biological identity of the nanoparticles, and determine how they interact with living systems.

Many of the biophysical and biological techniques available to determine protein conformation and activity can be applied to the characterisation of the nanoparticle-biomolecule complex (Lynch, 2006). A list of techniques currently being used is presented in Appendix III, many of which could be applied to/by the agrifood sector. Again, it should be noted that these tables are by no means a definitive list of characterisation techniques and the instruments listed often require adaptation for nanoparticle characterisation and specialist training.

The adsorption of biomolecules to the surface of engineered nanoparticles can affect their stability and dispersion, and it is important to also characterise the dispersion properties of nanoparticles in the relevant biological environment, e.g. in food. Many of the techniques listed in Appendix II can be applied to the characterisation of the dispersion stability of the nanoparticle-biomolecule complex.

An additional aspect of the adsorption of biomolecules to the surface of nanoparticles is the effect on the conformation of proteins such as enzymes, and also on their function, stability, activity and aggregation state, among other properties. There are a number of examples of enhanced enzyme stability and function following adsorption to nanoparticles, for example, the lifetime of the enzymes trypsin and peroxidase was shown to increase dramatically, from a few hours to weeks, by attaching them to magnetic iron nanoparticles (Sharma, 2007). This ability to enhance protein stability by interfacing them with nanomaterials may impact numerous biological processes such as digestion, metabolism and nutrient uptake. These effects of nanoparticles need to be characterised and measured, as discussed further in Chapter 4 (Section 4.2.2.3) of this report. Measurements of enzymatic activity in the presence of nanoparticles should be studied as a priority, and many of the techniques listed in Appendix III may be valuable for this purpose.

3.4 Measurement of Nanoparticles in Food and other Biological Matrices

The preceding paragraphs have shown that a wide range of physical and chemical techniques are available to characterise nanoparticles, both in the as-synthesised form and as nanoparticle-biomolecule complexes such as would occur in food matrices and upon ingestion. While these techniques may be applied to carry out measurements of nanoparticles in the pure form, the measurement and quantitation of nanoparticles in food, biological tissues and other biological matrices presents considerable challenges, since suitable equipment and measurement strategies are not yet available (SCENHIR, 2006, 2007).

Many food products also contain considerable amounts of naturally occurring nanoparticles, such as proteins, silica or traces of titanium dioxide, which makes detection of added nanoparticles difficult, as it rules out techniques such as elemental mapping where there are already significant background levels. Techniques such as elemental mapping can only be applied to nanoparticles such as gold and silver, which are not naturally present in foods or food packaging materials.

Measurement of particle mass of nanoparticles in the pristine form (as synthesised), is relatively straightforward, while measurement of the surface area of a given mass of nanoparticles is more difficult, as many of the current techniques do not distinguish fully between porosity and surface area, e.g. nitrogen adsorption. An additional complication is, as already discussed, the very ready aggregation of nanoparticles that occurs in biological media, resulting in larger particles, or even a gradual increase with size as a function of time. Such aggregation makes it almost impossible to measure either particle number or surface area in biological matrices. These technical difficulties in the measurement of nanoparticles make it correspondingly difficult to measure the actual exposure in *in vitro* and *in vivo* toxicological studies or in exposed humans for risk assessment purposes, as discussed further in Chapter 4.

3.5 Conclusions on the Problems Associated with Measurement and Characterisation of Nanoparticles

The characterisation of nanoparticles requires considerable care and there are many difficulties and uncertainties, in particular with respect to particle aggregation, size, purity, and batch variations. The characterisation is further complicated by the incorporation of nanoparticles into biological matrices. This alters their properties and requires further characterisation beyond that of the pristine nanoparticle. The adsorption of biomolecules to nanoparticle surfaces may have particular consequences for nanoparticles in food. For example, absorbed enzymes may have enhanced or reduced activity, resulting in altered efficiency of digestion and altered nutritional value of foods.

It is particularly important to distinguish between nanoparticles and nanoparticulate aggregates. Nanoparticles that have been dried during the synthesis process are typically irreversibly aggregated, and as such have dimensions on the micron scale. If the nanoparticles remain in suspension, upon contact with biological fluids, their surface properties may be altered by adsorption of proteins and other biomolecules, resulting in altered stability and aggregation. Nanoparticles need to be characterised under the conditions in which they will be utilised.

While many methods exist to characterise as-synthesised nanoparticles, such as electron microscopy and scattering methods, new methodologies are required urgently to characterise nanoparticles in situ in food matrices and food contact materials. At present, it is not possible to distinguish between background levels of nanoparticles and purposely added nanoparticles such as silica dioxide and titanium dioxide. Nevertheless, the nanometrology area is rapidly developing, with a concerted effort to develop standards and standard procedures for the characterisation of nanoparticles and nanoparticle-based systems.

4. RISK ASSESSMENT OF NANOPARTICLES IN THE FOOD CHAIN

4.1 Introduction

Typically, risk assessment consists of four components: hazard identification, hazard characterisation, exposure assessment, and risk characterisation, as illustrated in Figure 4.1. All four of these stages are essential to the process of risk assessment. A substance may be extremely hazardous, but have a small exposure potential, and the risk may be small, whereas something that is of limited hazard but to which exposure is high and/or over long periods may present a much greater risk. It is essential to characterise both the nature of the hazard and the exposure.

Risks must be considered in the early stages of any new technology. The assessment of the potential risks of nanotechnology to consumers using the conventional risk assessment model has already been the subject of a number of expert reports, e.g. SCENIHR, 2006, 2007; SCCP, 2007; FDA, 2007; COT, 2005; BfR, 2007; Bouwmeester *et al.*, 2007. The FSAI's Scientific Committee has applied the framework to the use of nanotechnology in the agrifood sector, as outlined in the following sections of this chapter. The report focuses on risk assessment of nanoparticles following exposure via the oral route of exposure since this is the relevant route in assessing the safety of nanotechnology in food. In addition, the report has attempted to identify the limitations in traditional approaches to exposure assessment and hazard characterisation of chemical risks when they are applied to nanotechnology, and to suggest areas where research may be needed.

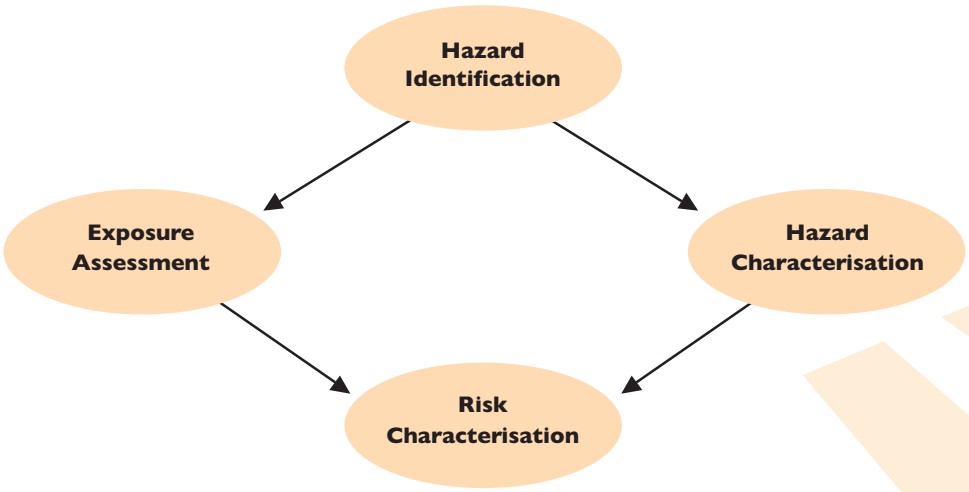

Figure 4.1 The Risk Assessment Model

As discussed in Chapter 2 there are many applications of nanotechnology in the food and animal feed industry that are either already in use or are subject to active research programmes. Prior to the incorporation of nanomaterials into new and existing food and feed applications, their potential toxicity and biological compatibility need to be investigated and the potential hazards should be identified before their use becomes widespread (Smart *et al.*, 2006). With an increasing number of consumer and industrial products containing engineered nanoparticles, the risk of human exposure increases and these materials may become a threat to human health and the environment (Oberdörster *et al.*, 2005).

4.2 Hazard Identification

As indicated in Chapter 2, the potential applications of nanotechnology in the agrifood sector range from modifications of the natural (organic) protein, carbohydrate and fat molecules that form part of the normal diet, to achieve added or altered functionality, to the use of inorganic nanoparticulate materials in food packaging and food ingredients including food additives. The molecular nature of a nanoparticle, e.g. inorganic or organic, and whether the molecule is naturally found in the body or not) are key determinants of the potential hazards of the material, in addition to its physicochemical characteristics such as shape, solubility, charge and chemical functionality. Properties such as particle size and size distribution, agglomeration state, mass, chemical composition, surface properties, crystal structure, surface area, surface charge and porosity may all be important for understanding the toxic effects of nanomaterials (Maynard *et al.*, 2006). As will be illustrated in the following paragraphs, these characteristics influence how readily nanoparticles will be absorbed from the gastrointestinal tract (GIT) and how rapidly they will be cleared from the body, in addition to influencing their toxicity.

In considering the hazard profile of a particular material in nano form, it can normally be assumed that it will have similar hazards to the same material in a micro or macro form. If the corresponding micro or macro material has an innocuous toxicological profile due to biological inertness and biodegradability, the nanomaterial may similarly be predicted to be of low hazard. For example, it can be argued that organic nanoparticles derived from substances occurring naturally in the body, such as proteins and carbohydrates, are likely to be of low hazard, although the increased bioavailability of the nano forms may have significant consequences in the body. The potential for increased allergenicity of these macromolecules via alterations in protein or carbohydrate structure may also be of concern. Essential nutrients may be rendered more bioavailable through the use of nanotechnology, with consequent implications for Safe Upper Levels (SULs) and Recommended Daily Allowances (RDAs), as discussed later in this report.

On the other hand, inherently toxic materials such as particulate metal compounds (inorganic) can be anticipated to show similar or greater toxicity if produced in a nano form from which they can solubilise (see section 4.2.1 on bioavailability and toxicokinetics). A body of evidence built up in *in silico* and/or *in vitro* models supports this premise and additionally indicates that many nanoparticles show greater reactivity in such models (Meng *et al.*, 2007; Papageorgiou *et al.*, 2007; Singh *et al.*, 2007). In contrast, some studies have suggested that the nanoform may be less toxic than the macroform. It has been reported, for example that selenium nanoparticles are less toxic to rats than selenite or high-selenium proteins (Jia, 2005).

The same physical and chemical properties that make nanomaterials potentially useful for commercial applications may be associated with potentially harmful effects on cells and tissues. Their increased reactivity and their potential for unrestricted mobility make them appealing for delivery of boactive molecules into poorly accessible compartments of the body such as the brain. There is however, limited information available on the uptake, distribution and toxicity of nanoparticles (see Section 4.2.1 below), and it is recognised that nanoparticles can be toxic even if the bulk material is not due to differences in bioavailability and reactivity (Owen, 2005).

The chemical reactivity of the surface area, including surface components, e.g. transition metals, coatings, and the inherent toxicity of the chemical in question are particularly important. Properties such as physical dimensions, surface charge and other factors determine whether a particle is able to penetrate an organ or cell and if removal by phagocytes is possible. Nanoparticles on the same scale as cellular components and larger proteins might evade the natural defences of the body. Together with the solubility, this influences the retention time of particles. The longer a particle stays in contact with cellular membranes, the greater the probability of a toxic reaction and damage (Royal Society, 2004; Smart *et al.*, 2006).

In addition, as previously indicated in Chapter 3, immediately upon contact with natural substances, e.g. biopolymers found in food, biological membranes such as those of the mouth and digestive system, engineered nanoparticles will adsorb biomolecules including proteins, lipids and sugars to their surface. It is these adsorbed biomolecules, referred to as the biomolecule corona (Lynch, 2006, 2007), that confer the biological identity and potentially the toxicity of the nanoparticles, and determine how they interact with living systems. The large surface area of nanoparticles allows a greater contact area with cellular membranes as well as greater capacity for absorption and transport of toxic substances.

Assessment of the effects of nanoparticles on food components and their bioavailability therefore needs to be considered. For example, binding of nutrients to nanoparticles could result in their digestion being altered, or result in altered absorption into the body. At present, methods to determine altered digestion in vitro could be applied, using approaches such as limited proteolysis, but such research has yet to be reported in the scientific literature, and as such represents a knowledge gap.

4.2.1 The toxicokinetics of nanoparticles (Absorption/bioavailability, distribution, metabolism, excretion (ADME))

Section 4.2 on hazard identification has suggested that substances/materials in nanoparticulate form may be as hazardous or more hazardous than the same substance/material in the micro or macro form. The likelihood that this hazard will actually present a risk to human health and the environment depends on (a) the potential for toxicity following exposure (see section 4.2.2) and (b) the potential for exposure (see section 4.4). The toxicity of any substance is influenced by a number of factors including its bioavailability, namely the amount of the substance absorbed by the body from the site of first exposure, e.g. the lungs or the GIT. A key characteristic of nanoparticles, particularly insoluble nanoparticulates, is that on a mass basis their bioavailability can be predicted to be higher than that of the equivalent micro or macro form. This has been confirmed in a number of experimental studies (Hecq *et al.*, 2006; Kotyla *et al.*, 2008).

To date, studies on exposure, absorption and bioavailability have focused on the inhalation and dermal routes, and little is known about the toxicokinetic processes following oral exposure, particularly in relation to ingestion of nanomaterials contained in food. Interest in the inhalation route of exposure in part reflects the importance of this route in the occupational situation and the concern that workers in the nanotechnology industries could be exposed to new hazards presenting long-term risks to their health, leading to the suggestion that certain nanoparticulate materials could be as toxic as asbestos (Poland *et al.*, 2008). Studies have shown the importance of particle solubility, charge and size in determining deposition and fate in the lung, as would be predicted (Kreyling *et al.*, 2002, 2004; Frampton *et al.*, 2004, Oberdörster *et al.*, 2005). Similar principles apply when considering exposure to and uptake of nanomaterials by the dermal route. Nanotechnology may have particularly important applications in the development of innovative cosmetics (SCCP, 2007) and uptake of nanomaterials via the dermal route has been demonstrated (Ryman–Rasmussen, 2006; Xin-Rui *et al.*, 2006, as reported in SCCP, 2007).

The primary route of relevance to food safety is however the oral route, and discussion of the toxicokinetics of nanoparticles in this report has therefore been largely limited to the oral route.

4.2.1.1 Absorption/uptake of nanoparticles from the gastrointestinal tract

Little information is currently available concerning the uptake of nanoparticles from the intestinal tract following oral exposure. Uptake occurs variously by passive diffusion across the mucosal cells, via active transport mechanisms and intercellularly (O'Hagan, 1996). Nanoparticles can enter the GIT in many ways, such as ingestion directly from food and water and from administration of therapeutic nano-drugs. Inhaled nanoparticles can also be swallowed and enter the GIT following clearance from the respiratory tract (Hoet *et al.*, 2004). Particle uptake in the GIT depends on diffusion and accessibility through mucus and contact with the cells of the GIT. The smaller the particle diameter, the faster is the diffusion through GIT mucus to reach the cells of the intestinal lining, followed by uptake through the GIT barrier to reach the blood.

Organic nanoparticles such as casein micelles are likely to behave similarly to their micro or macro equivalents and can be predicted to be readily absorbed and highly bioavailable. One aspect that is to date largely unresearched is whether higher molecular weight organic nanoparticles such as proteins, fats or carbohydrates will be broken down to lower molecular weight entities in the GIT in the same way as the native molecules, or whether they will be taken up intact. Insulin encapsulated in vitamin B12-dextran nanoparticles has recently been shown to be taken up from the GIT without degradation (Chalasani *et al.*, 2007).

The intestinal uptake of inorganic, insoluble nanoparticles such as titanium dioxide and gold particles, and also of insoluble polymers such as latex, has been described in several publications, e.g. Jani *et al.*, 1990; Florence, 2001; Hillyer and Albrecht, 2001; Hoet, 2004). On a mass basis, it can be predicted that these insoluble nanoparticles will be more readily taken up across the intestinal barrier and hence be more immediately bioavailable than their micro or macro equivalents. Uptake is largely determined by particle solubility, charge and size (Jani *et al.*, 1989, 1990, O'Hagan, 1996). Table 4.1 provides an overview of a number of experimental studies that have demonstrated uptake of nanoparticles across the GIT following oral administration.

Table 4.1 Uptake of Nanoparticles across the GIT following Oral Administration

Particle Type	Particle Size	Translocation/Distribution	Reference
Titanium dioxide	25 and 80nm	uptake into the blood and movement to the liver	Böckmann *et al.* 2000; Jani *et al.* 1994
Gold	4, 10, 28, and 58nm given in drinking water	4nm particles were widely distributed to lung, heart, kidney, spleen and liver, with lower levels being found in blood and brain. Levels of 10nm particles in these organs were generally 2-4 times lower than for the 4nm particles, while levels for the 28nm particles were approximately 10 times lower. There was little evidence of uptake of the 58nm particles	Hillyer and Albrecht, 2001
Polystyrene	50nm 100nm 300nm 1000nm 3000nm	34% of 50nm particles and 26% of 100nm particles were absorbed. Of the absorbed particles 7% of the 50 nm and 4% of the 100nm were found in liver, spleen, blood, bone marrow. Particles greater than 300nm did not enter the bloodstream	Jani *et al.* 1990
C60 fullerenes	Not available	73-80% of oral dose found in liver	Yamago *et al.* 1995
Nano Copper	23.5nm	severe toxicological effects in liver, kidney, and blood	Chen *et al.*, 2007
Cationic PAMAM dendrimers	Not available	three animals died after single oral administration. Liver toxicity was observed after multiple dosing (once a week for 10 weeks)	Roberts *et al.* 1996

Uptake of inert particles has been shown to occur trans-cellularly through the intestinal lining and to a lesser extent between epithelial cells (Aprahamian *et al.*, 1987; Hussain and Florence, 1997, Hussain *et al.*, 1998, 2001; Florence and Hussain, 2001).

4.2.1.2 Distribution, metabolism and excretion of nanoparticles following absorption from the gastrointestinal tract

To date, relatively few studies have investigated the uptake and disposition of nanomaterials by the GIT, and most have shown that, dependent on size, nanoparticles either pass through the GIT without uptake into the body and are eliminated rapidly (Oberdörster *et al.*, 2005), or they cross the lining of the GIT and enter the blood stream, from whence they relocate to other organs. Following uptake by the GIT, gold nanoparticles of less than 50nm translocated to the blood stream and distributed all over the body (Table 4.1; Jani *et al.*, 1990). A recent study demonstrated that tissue distribution of gold nanoparticles after intravenous (IV) administration was size dependent with the smallest (10nm) particles showing widespread organ distribution (De Jong *et al.*, 2008). As with absorption, the distribution, breakdown and excretion of nanoparticles in the body will be dependent on physicochemical characteristics such as solubility, charge and size.

Distribution/translocation of nanoparticles to body organs is expected to mirror that of their micro or macro equivalents, with small water-soluble or fat soluble organic nanomaterials such as lipid nanoparticles being widely distributed throughout the body. It can be anticipated that distribution and breakdown of such nanoparticles into constituent molecules occurs rapidly and efficiently, with subsequent clearance from the body. It is emphasised, however, that this supposition is based on theoretical considerations and there is no experimental evidence to support this. A further area about which little is known is the possibility of changes in physicochemical properties in moving to the nanoscale, e.g. fat-soluble vitamins may become water-soluble either directly or by use of nano-carriers, and vice versa. This, in turn, will lead to changes in the toxicokinetic profile of the vitamin or other biologically-active molecule and potential cascade effects on cellular homeostasis.

These anticipated changes in bioavailability of biologically-active endogenous molecules, such as vitamins and hormones, on moving to the nanoscale needs further investigation before the technology is more widely introduced, as they have profound implications for concepts such as Safe Upper Level (SUL) and Recommended Daily Allowance (RDA) for vitamins. The same applies to the Acceptable Daily Intake (ADI) established for food additives and other food chemicals potentially found in food.

Insoluble nanoparticles (primarily inorganic, but also including man-made polymeric materials such as polystyrene and carbon-based nanoparticles such as fullerenes) can be predicted to have a more restricted distribution. Following uptake from the GIT, nanoparticles can translocate via the lymph system to the liver and spleen, as demonstrated for polystyrene nanoparticles of 100nm or less (Jani *et al.*, 1989, 1990). Smaller particles that are capable of being taken up by the villus epithelium (Jani, 1990, Hillery, 1994) may directly enter the bloodstream, and are then predominantly scavenged by the liver and the spleen. A particular concern regarding the safety of nanoparticles is the possibility for these small particles to evade the protective blood-brain barrier and enter the brain. This property may be desirable and exploited in the case of delivery of therapeutic agents, but has significant health implications in the case of insoluble nanoparticles such as polymers and metal compounds that would not normally reach the brain in their micro or macro forms, as this may result in damage to this vulnerable organ.

Insoluble but ultimately biodegradable polymeric nanoparticles such as chitosan, poly(lactide-co-glycolide) (PLGA), gelatine and modified dextran are being explored as oral drug delivery systems and potentially as delivery systems for food components. These materials will ultimately be broken down into their constituent molecules and excreted, at a rate dependent on their physicochemical characteristics. Relative persistence in the body, together with their potential for ultimate total breakdown can be exploited to provide a slow release system, both for delivery of drugs and food components.

In contrast, inorganic materials and non-biodegradable polymers such as titanium dioxide and polystyrene will not be broken down. If these nanoparticles are bioavailable to any extent/are taken up from the gastrointestinal system, there is the potential for accumulation in the reticuloendothelial system of the liver and spleen and also, theoretically, translocation to other organs including the brain. In addition, the potential for local toxicity in the GIT of non-bioavailable, non-biodegradable, orally-ingested material must be considered.

4.2.2 Toxicity of nanoparticles

4.2.2.1 Toxicity of nanoparticles in vitro and underlying mechanisms

Much of the current knowledge concerning the potential toxicity of nanoparticles (nanotoxicology) has been obtained from in vitro or in silico test systems and is suggestive of a common mechanism underlying the toxicity of many nanoparticles. This may involve, in a three step process, generation of reactive oxygen species (ROS) (Xia *et al.*, 2006; Duffin, 2007), stimulation of an inflammatory response and cytotoxicity (Brown *et al.*, 2007; Foucaud *et al.*, 2007). Xia *et al.*, 2006 have suggested that measurement of ROS generation and oxidative stress provides a valid test system to compare nanoparticle toxicity. Although not all materials have electronic configurations or surface properties to allow spontaneous ROS generation, particle interactions with cellular components are capable of generating oxidative stress.

These changes are typically also produced by micro particles and fibres, both inorganic and organic (Bernstein *et al.*, 2005; Cardinali *et al.*, 2006; Donaldson and Tran, 2002). Historically, much of the nanotoxicology data have originated from data on air pollution particles (Frampton *et al.*, 2004, Oberdörster *et al.*, 2005). These particles have been studied both *in vitro* and *in vivo*, and have been clearly demonstrated to be involved in human disease processes (Augur *et al.*, 2006; Bai *et al.*, 2006, Donaldson *et al.*, 2004; Pope *et al.*, 2002).

In vitro studies with different types of nanoparticles (metal/metal oxide, titanium dioxide, carbon nanotubes and silica) on various cell lines (lung and liver) have demonstrated oxidative stress-related inflammatory reactions. The authors have postulated that this response is driven by the specific surface area of the nanoparticle and/or its chemical composition (Ghio, 1999; Hussain *et al.*, 2005; Lin *et al.*, 2006; Pulskamp *et al.*, 2007; Singh *et al.*, 2007). Typically, the biological activity of particles increases as the particle size decreases. Smaller particles occupy less volume resulting in a large number of particles with a greater surface area per unit mass and increased potential for biological interaction (Oberdörster *et al.*, 2005).

More recently, much of the fundamental data on model nanoparticles have been generated on non-biodegradable, non- deformable spheres such as polystyrene and latex or, more recently, the carbon-based nanomaterials such as fullerenes and carbon nanotubes (CNT). It should be noted however that these materials do not reflect the typical profile for nanoparticles to be used in food industry applications, as outlined in Chapter 2.

4.2.2.2 Toxicity of nanoparticles in vivo

Much of the data on particle toxicity have originated from studies on air pollution (Peters and Pope, 2002; Frampton *et al.*, 2004; Oberdörster *et al.*, 2005). The particles generated in air pollution episodes, which include both micro- and nanoparticles, have been demonstrated to produce disease in animal models exposed via inhalation, in addition to human disease. In relation to more recently characterised nanoparticles, CNT have probably been the most extensively investigated and much of the *in vivo* toxicity data in the literature relates to them (Huczko *et al.*, 2001; Lam *et al.*, 2004; Warheit *et al.*, 2004; Shvedova *et al.*, 2005). Nearly all *in vivo* studies on CNT have found histological evidence of inflammation and granuloma formation in rodent lungs. It should be noted however, that in the majority of these *in vivo* experiments, intratracheal administration of a single dose was directly introduced to the trachea or pharynx of anaesthetised animals resulting in a less realistic exposure scenario than natural inhalation. In addition it is well recognised that toxicity of CNT is hampered by their tendency to aggregate, and the observed results might be related to overload conditions.

Inhalation of ultrafine particles has been linked to thrombotic (blood clotting) effects. Radomski *et al.* have demonstrated that mixed carbon nanoparticles and nanotubes (both MWCNT and SWCNT), were able to induce platelet aggregation *in vitro* and, in addition to accelerate the rate of vascular thrombosis in the rat carotid artery (Radomski *et al.*, 2005). Carbon nanotubes have also been shown to activate the human complement system via both classical and alternative pathways (Salvador-Morales *et al.*, 2006). Recently, however, PLGA nanoparticles conjugated with alendronate were shown to have an acceptable degree of haemocompatibility following IV administration, suggesting that this material may hold promise as an IV drug delivery tool (Cenni *et al.*, 2008).

There are few *in vivo* studies involving routes of administration other than inhalation, and those that have been reported mainly involve the dermal and IV routes of administration, reflecting the potential promise of nanotechnology in cosmetic and drug delivery applications. The studies that do exist in relation to uptake of nanoparticles via the digestive tract are generally on polymer-based soft (biodegradable) micro- and nanoparticles designed for therapeutic applications such as drug delivery. However, the same general principles that influence the potential toxicity of such ploymers, such as particle size, particle shape, and particle surface (including the biomolecules adsorbed to the surface) will also apply to inorganic and engineered nanoparticles.

Experimental studies involving oral exposure to nanoparticles have been conducted using copper nanoparticles (Chen *et al.*, 2006b; Chen *et al.*, 2007) gold nanoparticles (Hillyer, 2001), PMMA nanoparticles (Araujo, 1999) and chitosan nanoparticles (Loretz, 2007). The distribution of copper nanoparticles in different organs of mice after a single dose oral exposure suggested that the main target organs are kidney, blood and, in particular, liver (Chen *et al.*, 2007). A comparison of the toxicity of copper nanoparticles (23.5nm), micro-copper particles (17µm) and cupric ions (CuCl2·2H2O) to mice exposed via oral gavage resulted in the classification of nano and ionic copper particles as class 3 (moderately toxic), and micro-copper as class 5 (practically non-toxic) on the Hodge and Sterner Scale (Chen *et al.* 2006b). Copper nanoparticles induced severe toxicological effects and organ toxicity in kidney, liver and spleen of experimental mice, but micro-copper particles did not, on a mass basis.

Reflecting the results of the *in vitro* studies, an inflammatory response to nanoparticles has frequently been observed *in vivo*, related to the tendency of nanoparticles to induce ROS (Xia *et al.*, 2006; Duffin, 2007) which is a key step in the induction of an inflammatory response. Experimental studies have shown that the extent of inflammation *in vivo* is a function of the surface area dose instilled (not of the mass dose instilled). Low-toxicity nanoparticles including inert polymeric materials such as polystyrene nevertheless have a high surface area, and are relatively inflammogenic. These approaches present the possibility of measuring the potential toxicity of nanoparticles based on the inflammatory response of a given instilled surface area dose (Duffin, 2007).

On the other hand, there are also cases reported of nanoparticles having anti-inflammatory properties, such as certain cerium oxide nanoparticles (Tsai, 2007) and silver nanoparticles (Bhol and Schechter, 2007). Nanocrystalline silver (NPI 32101) has been demonstrated to have antimicrobial and anti-inflammatory properties and was found to reduce colonic inflammation following oral administration in a rat model of ulcerative colitis, suggesting that nanosilver may have therapeutic potential for treatment of this condition (Bhol and Schechter, 2007). It would appear that no generic assumptions can be made regarding the toxicity of nanoparticles.

In considering the effects reported in the paragraphs above, indicating an enhanced toxic potential of nanoparticles compared with macroforms of the same material, it should be recognised that these data on nanoparticles identified in air pollution and derived from experimental studies on CNT nanoparticles and those derived from metals, metal oxides and polymeric materials, while providing valuable information on the potential toxicity of nanoparticles and mechanisms of toxicity, are less relevant for identifying the potential hazards of organic nanoparticles such as proteins and lipids. The latter are the primary applications of nanotechnology in food, as outlined in Chapter 2, and many/the majority are derived from endogenous compounds occurring naturally in the body.

4.2.2.3 Novel potential toxicities of nanoparticles and issues specific to food and digestion

One of the major uses of nanoparticles in food is likely to be nanoscale protein assemblies formed from re-engineered proteins, e.g. use of protein fibrils as texture modifiers or structural elements in foods (Graveland-Bikker and de Kruif, 2006). As already discussed, the common perception is that these are natural as opposed to engineered nanoparticles and not likely to be a source of toxicity. There is however emerging evidence that anomalous protein assemblies can themselves be a source of toxicity, and/or could induce other proteins to aggregate/fibrillate. Preliminary evidence indicates a potential role for nanoscale surfaces in modulating the rate of protein fibrillation (Linse, 2007), which in turn indicates a need for further research into the effects of nanoparticulates on protein assembly and aggregation. It should be emphasised that these studies were in solution, under non-physiological pH conditions, and with single protein solutions, which do not represent the physiological conditions, but rather are the standard conditions to study the mechanisms of protein fibrillation. The fact that the large surface area presented by nanoparticles can modulate the fate of fibrillation of proteins offers both huge potential for the modulation of food properties and textures, but may also raise questions as to whether nanoparticles in food could inadvertently induce protein fibrillation in the food (altering its properties) or following adsorption in the body. This is a question that requires further research.

Interaction of nanoparticles with components of food is another aspect that may need consideration and about which little is currently known. It is well recognised that the toxicity of many chemicals in the diet is markedly influenced by the food matrix, and that the toxicity profile predicted from *in vitro* tests or certain *in vivo* studies in animals will not necessarily be manifest when the same chemical is administered in the diet. The same is likely to be true of nanoparticles in the diet.

Additionally, possible change in the nanoparticles following passage through the digestive system, absorption, distribution and clearance from the body must be taken into consideration. Depending on the nature of the biomolecule corona (described in Section 4.2 as the proteins, lipids and other biomolecules which adsorb to nanoparticle surfaces immediately upon contact, thereby coating the nanoparticles and conferring a "biological identity" to the nanoparticles) and potential for enzymatic digestion, the behaviour of the nanoparticles may alter, and there is the potential for novel toxicities not anticipated from the properties of the non-coated nanoparticles or the adsorbed biological material. Much can be learned about the potential biological impacts of nanoparticles from the nature of their biomolecule corona, and a useful topic for further research would be the recovery of nanoparticles from the digestive system and characterisation of their corona using the techniques summarised in Appendices II and III.

Finally, an issue to be considered in relation to nanoparticles in food is the potential role of nanoparticles in inflammatory digestive diseases. For example, Inflammatory Bowel Disease (IBD) is a chronic disorder characterised by recurrent and serious inflammation of the GIT (Zhong, 2008). There have already been reports that microparticulates may play a role in Crohn's disease (Lomer, 2002), making a potential role for nanoparticles likely. A recent study has shown evidence of micro- and nanoparticulates (in the form of inorganic, non-biodegradable pollutants, i.e. micro- and nano-debris) in 18 samples of colon tissues affected by cancer and Crohn's disease (Gatti, 2007). Vulnerable members of the population may include those with pre-existing digestive disorders, which may potentially be impacted by the presence of nanoparticles, although on the other hand nanoparticles offer many potential routes to therapies for these same diseases.

4.3 Hazard Characterisation

The hazard characterisation step in risk assessment involves assessment of the toxicity of a particular agent, e.g. a nanoparticle, the nature of that toxicity and the information available on the dose:response relationship. Section 4.2 has provided an overview of the potential toxicities of nanoparticles, particularly of insoluble inorganic and non-biodegradable materials (which are however unlikely to be of major relevance in terms of applications of nanotechnology in food). Determination of the dose:response relationship for the potential toxicities induced by nanoparticles is however extremely difficult at the current time, due to the problems associated with measurement of dose in either *in vitro* or *in vivo* toxicological studies, as discussed in the following Section 4.4 on exposure. The hazard characterisation step is therefore hampered by gaps in the knowledge base, and modification of the traditional risk assessment model in this area may be necessary in carrying out risk assessments of nanoparticles, particularly in *in vivo* studies.

4.4 Exposure to Nanoparticles

4.4.1 Routes of exposure

As illustrated in Figure 4.2, there are various exposure routes for engineered and natural nanoparticles. The majority of the available data on exposure to nanoparticles relate to the inhalation route (via occupational and environmental exposure and potentially via new drug delivery systems involving nanotechnology) and the dermal route (occupationally, via cosmetics and skin care products). Nanoparticles may additionally be injected into the body for therapeutic purposes. While the primary focus of this report is on the oral route of exposure, via food, it is useful to summarise the state of knowledge regarding inhalation and dermal exposure.

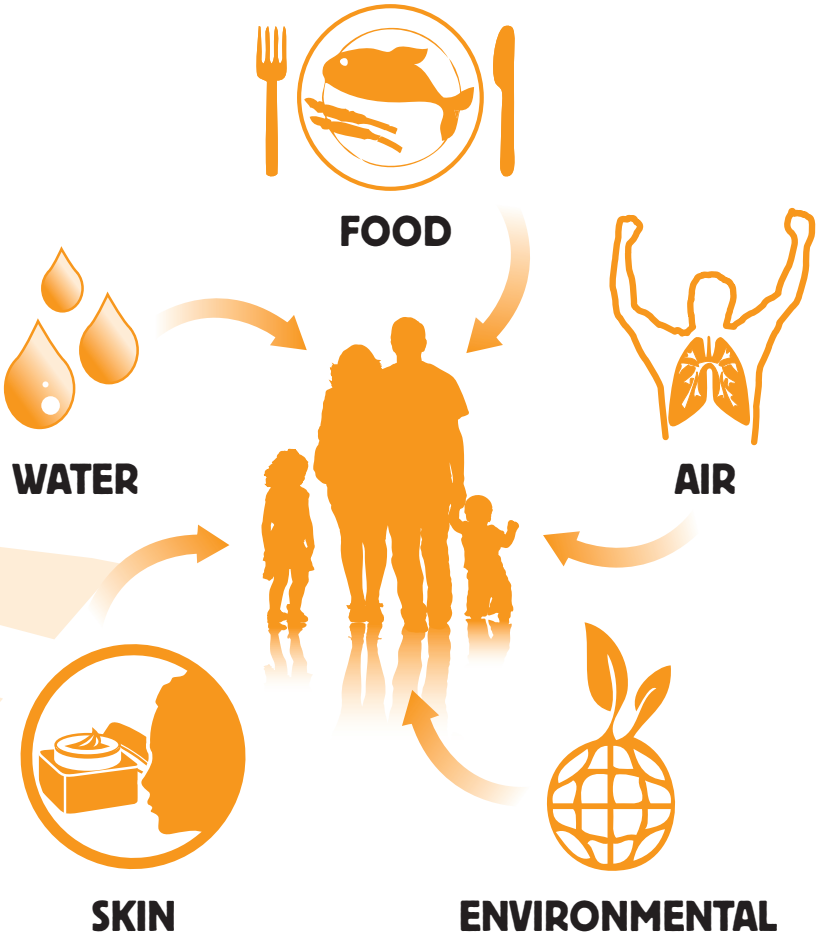

Figure 4.2 Possible Exposure Routes for Nanoparticles

In relation to inhalation exposure, nanoparticles may be inhaled in significant amounts via environmental exposure (due to air pollution) or in workplaces during manufacture of nanomaterials. Nanoparticles have been demonstrated to enter the body more readily than larger particles (Borm and Kreyling, 2004). Particles of 100nm or less have the potential to be inhaled following their suspension in air as a result of air pollution and during manufacture, distribution, use and disposal of nanomaterials. Those in the range of 20-100nm will lodge primarily in the upper airways, while those in the range of 10-20nm and below will enter the lung alveoli from where they may be translocated to other sites in the body, depending on their physical and chemical characteristics including solubility. They may interact with alveolar macrophages, resulting in clearance (removal by the immune system), or they can enter the lung interstitum where they may make contact with fibroblasts, endothelial cells and/or cells of the immune system (Oberdörster *et al.*, 2005). A build up in the lung may occur, due to macrophage toxicity and decreased clearance, with resultant inflammatory change and other lung toxicity (Oberdörster *et al.*, 2005).

The skin represents a major route of exposure to nanoparticles contained in cosmetics and skin care products. Occupational and environmental exposure to nanoparticles may also occur, particularly during manufacture of nanoproducts. The EU Scientific Committee on Consumer Products (SCCP), in their scientific opinion (SCCP, 2007), state that *"there is evidence of some skin penetration into viable tissues (mainly into the stratum spinosum in the epidermal layer, but eventually also into the dermis) for very small particles (less than 10nm), such as functionalised fullerenes and quantum dots, but that when using accepted skin penetration protocols (intact skin), there is no conclusive evidence for skin penetration into viable tissue for particles of about 20nm and larger primary particle size ….. Nanoparticles of 20nm and above penetrate deeply into hair follicles, but no penetration into viable tissue has yet been observed."* Zinc and titanium oxide particles, as used in cosmetics have already been shown to penetrate the stratum corneum of rabbit skin (Lansdown and Taylor, 1997) and highest absorption has been shown to occur from water and oily vehicles (Monteiro-Riviere *et al.*, 2005).

4.4.1.1 The oral route of exposure

Oral exposure to nanoparticles can result from ingestion of nanoparticles in food (either as additives or as re-engineered protein or lipid clusters, for example), or from food contact materials including packaging where nanoparticles are proposed to have many uses (see Chapter 2). More indirect exposure can arise from ingestion of food from animals such as fish and shellfish (i.e., molluscs and crustaceans), that have taken up nanoparticulate matter, as part of the human diet. For example, surface sediment- and filter-feeding molluscs are prime candidates for uptake of manufactured nanoparticles from environmental releases, since the molluscs are already known to accumulate suspended particle- and sediment-associated conventional pollutants (Galloway *et al.*, 2002 and Livingstone, 2001). It should also be noted that the specific use of nanotechnology in animal feed production could potentially result in exposure of humans consuming animal-based products as part of their diet. However, as already discussed in Chapter 2, there are currently no known applications of nanotechnology in the animal feed industry, and consequently, human exposure via this route is considered to be minimal at this time.

Little is known to date about actual exposure to engineered nanoparticles from food (or other routes), and even less is known about the likely future exposures. This is in part because measurement techniques do not allow quantification of nanoparticles, either naturally present or added, in food as described in Chapter 3. Also, as already discussed in Section 3.4, many food products contain considerable amounts of anthropogenic (naturally occurring) nanoparticles, such as silica (or even traces of titanium dioxide), which will make estimation of exposure to deliberately added nanoparticles difficult. There are some data relating to exposure to microparticulates in food (i.e. particles in the size range 200nm to 2000nm, which are usually considered as being too large to enter into individual cells). Lomer *et al.* estimated that 1012–1014 non-biological, e.g. engineered, particles are ingested per individual daily from the typical Western diet with an estimated uptake into the body of 0·1–1% (i.e. 109–1012 particles/d) (Lomer *et al.*, 2002). These particles are mainly titanium dioxide (TiO2) and particulate silicates (including aluminosilicates). The particles are naturally occurring as soil particles but also are added to food, pharmaceuticals and toothpaste as microparticles (defined as 0·1µm < diameter < 3µm). In practice, the microparticles observed in the intestinal mucosa are almost always <1µm diameter.

4.4.2 Measurement of exposure to nanoparticles and derivation of a dose metric

Conventionally, measurement of exposure and consequently dose[4] in toxicological studies or estimation of human exposure via the diet and/or the environment is based on mass concentration, i.e. exposure to a chemical at a level of mg/kg of food or parts per million (ppm) in air. The use of mass concentration data alone for the estimation of the dose in toxicological studies with nanoparticles or in humans following exposure to nanoparticles is regarded as inappropriate, and the number concentration and/or surface area need to be taken into account (SCENHIR, 2007). This is due to the fact that, as shown in Table 4.2 and Figure 4.3, these smaller particles occupy less volume, resulting in a large number of particles with a greater surface area per unit mass and increased potential for biological interaction (Oberdörster *et al.*, 2005).

Table 4.2 Particle Size and Surface Area (after SCCP, 2007)

Particle Diameter (nm)	Number of Particles per Gram of Material	Total Particle Surface Area cm^2/gram
1000	1.9×10^{12}	60,000
100	1.9×10^{15}	600,000
10	1.9×10^{18}	6,000,000

4 Estimation of dose is derived by measurement of the level of a "chemical" in food or the environmental milieu (air, water) followed by an estimate of the amount of food or water consumed or air inhaled over a specified time in order to derive the actual mass amount of chemical entering the body (the intake).

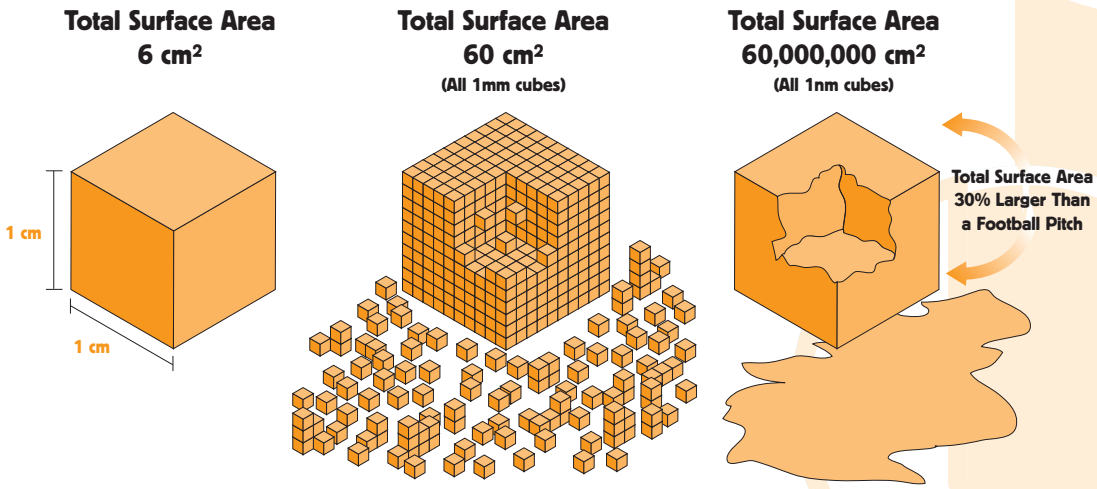

Figure 4.3 Illustration of the Small Mass: Large Surface Area Paradigm

Table 4.2 (after SCCP, 2007) shows that 1 gram of material composed of 10nm particles contains 1 million times more particles than the same material composed of 1,000nm (1µm) particles and the surface area is 100 times greater. Since the toxicity of nanoparticles, particularly inorganic engineered particles and carbon nanoparticles, appears to be primarily due to surface reactivity, obviously the larger the surface area per unit mass, the more potential there will be for toxicity.

Measurement of particle mass is relatively straightforward and, as outlined in Chapter 3, there is a wide range of physical and chemical characterisation techniques that can be employed to measure nanoparticles, most of these being suitable only for the "as-synthesised" particle in powder form. It has already been noted however that routine measurements of free nanoparticles in biological media, in order to assess exposure and hence dose, is more difficult since suitable equipment is not yet available (SCENHIR, 2006, 2007). Also, concentrations are often below the limits of detection for instrumentation such as UV, or there is no parameter that can easily be measured, e.g. fluorescence. In some cases, e.g. metallic species such as silver, there is elution of a chemical species from the nanoparticles that enables detection, but this is not always the case. Characterisation of the relationship between exposure to nanoparticles and biological response may be achieved by labelling of the nanoparticles with a fluorescent dye followed by image analysis (Yan *et al.*, 2007). Using this approach, it has been shown that silica nanoparticles are rapidly taken up by a range of different cells (Figure 4.4) and estimates of uptake can be made (Yan *et al.*, 2007, Lynch, 2008, personal communication).

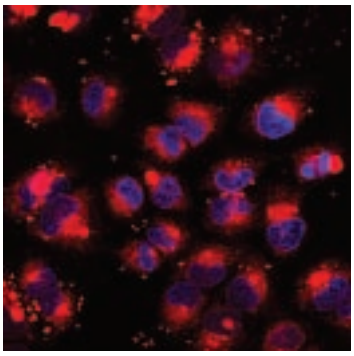

Figure 4.4 Confocal Microscopy Image of Lung Epithelial Cells Showing Significant Nanoparticle Uptake Following Exposure for 24 Hours to Fluorescently Labelled 30nm Silica Dioxide Nanoparticles (red). Individual Cell Nuclei are Stained Blue
(Courtesy of Dr I. Lynch, Department of Chemistry, University College, Dublin)

Measurement of the surface area of a given mass of nanoparticles is also difficult, as many of the current techniques, e.g. nitrogen adsorption, do not distinguish fully between porosity and surface area. An additional complication is the very ready aggregation of nanoparticles that occurs in biological media, resulting in larger particles, or even a gradual increase with size as a function of time. Such aggregation makes it almost impossible to measure either particle number or surface area and to estimate the actual exposure dose in *in vitro* and *in vivo* toxicological studies or in exposed humans.

While the most meaningful dose metric for use in assessing the risk of nanoparticles is probably (in many cases) surface area and/or particle number, in practice it is recognised that these are extremely difficult to measure on a routine basis. New approaches need to be developed to their identification and measurement or, alternatively, nanoparticles need to be specially adapted for specific studies (such as the fluorescently labelled silica particles used in the studies reported above). This is one of the major problems facing the scientific community in assessing the potential risks of nanoparticles. Additionally, effects on nanoparticle aggregation and/or release from the food matrix during digestion need to be considered, and there are no approaches to address these at present.

It should also be noted, as previously reported by the EU Scientific Committee on Consumer Products (SCCP, 2007), that there are also research reports in which the relationship between size, surface area and toxicity is not consistent and it is not always possible to predict effects on the basis of size or surface area alone (Warheit *et al.*, 2007; Yin *et al.*, 2005).

4.5 Risk Characterisation

Risk characterisation as part of the traditional risk assessment model involves a review of the identified hazards or toxicities, the dose or exposure level at which toxicity is or is not seen and a comparison of this level with the estimates of human (or environmental) exposure resulting from the exposure assessment.

A summary of what is currently known regarding the hazards posed by nanoparticles has been provided in section 4.2 of this chapter. Several of the risk assessment challenges posed by nanoparticles have been highlighted, including their large surface area, their potential to induce oxidative stress, their interactions with biomolecules such as proteins and DNA. Although *in vitro* studies with a range of particles, in particular inorganic and other non-biodegradable particles have shown that particles can be inherently toxic, few *in vivo* studies have been carried out, particularly using the oral route of administration, to confirm that the potential *in vitro* toxicity is manifest *in vivo*. Based on theoretical considerations, it is probably unlikely that the toxicity seen in *in vitro* cell systems will be expressed *in vivo* in humans or experimental animals, due primarily to the lack of bioavailability of many potentially toxic (inorganic) nanoparticles. These *in vitro* studies are also considered to have little relevance for the risk assessment of the organic nanoparticles that are the major focus of research into applications of nanotechnology in the agrifood sector.

Several models have been investigated for the identification of the hazard profile of nanoparticles, such as the ROS model (Xia *et al.*, 2007), the biomolecule corona model (Lynch, 2007) or the surface area model (Duffin, 2007). More data are needed, especially in relation to characterisation of the risk posed by nanoparticles in food. There are a range of additional factors that need to be considered in relation to nanoparticles in food that would not be detected using classical toxicity tests, since the latter measure very specific endpoints that may not be the only ones of relevance.

The specific hazard issues relating to food include (i) the increased bioavailability of nanoparticles compared with the macroforms of the same material; (ii) the potential role of nanoparticle-induced ROS in inflammatory digestive diseases such as IBS and Crohn's disease (for which there are classical tests which are also applicable to nanoparticles); (ii) the potential effects of nanoparticles on protein and enzyme stability and functionality whereby the metabolic processes may be disrupted, and/or nutrient bioavailability may be altered; (iii) the potential effects of storage, heating/and ageing on nanoparticle-biomolecule complexes in food, and perhaps others as yet unrecognised.

There is limited knowledge concerning the bioavailability and toxicokinetics of nanoparticles following exposure via the diet or the oral route. The size, shape and composition of particles will have a major effect on their bioavailablity and toxicokinetics, as will their surface modification by, for example, adsorption of proteins and other endogenous molecules, their surface charge, aggregation and rate of dissolution or degradation.

The exposure considerations outlined in 4.4 have shown that there are many gaps in knowledge regarding exposure to nanoparticles via the food chain. The exposure metric is of major importance in assessing the dose of nanoparticles producing toxicity (hazard characterisation) and hence the risk of nanoparticles in the body as well as the environment, and to date there is no clear consensus on how exposure to nanoparticles should be measured.

In relation to the possible risks of nanoparticles in food and feed, many food and feed ingredients are comprised of biopolymers, e.g. proteins, carbohydrates and fats with sizes extending down to the nanoscale. Consumers are therefore already exposed to natural nanoparticles in their diet, without any recognised adverse effect. It can be generally assumed that natural ingredients/substances in the human or animal diet such as lipids and proteins that have been produced either partially or wholly by nanotechnology will be processed normally and catabolised in the body. These natural ingredients/substances are unlikely to present a risk to health, even when the proportion of nanoparticulate material in the diet is increased by nanoengineering production methods.

The nanoforms of natural ingredients/substances may however show increased bioavailability or be able to penetrate to parts of the body normally inaccessible to the macro form. Increased bioavailability of essential nutrients such as minerals and vitamins or the transformation of a normally lipid-soluble molecule to a water-soluble form via nanoengineering may have profound effects for cellular biochemistry and consequent perturbations of physiological processes. Anticipated changes in bioavailability of biologically-active endogenous molecules such as vitamins and hormones on moving to the nanoscale needs further investigation before the technology is more widely introduced, as they have profound implications for concepts such as Safe Upper Level (SUL) and Recommended Daily Allowance (RDA) for vitamins. The same applies to the Acceptable Daily Intake (ADI) established for food additives and other food chemicals potentially found in food.

The lack of knowledge regarding the effect on pharmacokinetics and bioavailability of changes in the physicochemical properties of normally inert and non-biodegradable materials such as inorganic particles, e.g. titanium dioxide, and biological polymers in moving to the nanoscale is of particular concern, since this will inevitably lead to changes in how they are handled in the body, with potential cascade effects on cellular homeostasis. From studies conducted to date on the oral uptake and distribution of such materials, it appears that their physicochemical properties including apparent insolubility does not guarantee non-absorption of the nanoparticles from the gut. A range of studies on uptake of nanoparticles from the GIT have demonstrated increased bioavailability of such materials, but similar research does not appear to have been carried out to date for such particles in a food matrix. Biopersistence of essentially insoluble nanoparticles following repeated/long term exposure can be anticipated.

Conclusion on risk assessment on nanoparticles in the food chain

In considering the possible risks of nanotechnology in the agrifood actor, it must be recognised that the presence of nanoparticles in food is not a new phenomenon. People have always been exposed to very fine particles in their diet, without harmful effects, since many food and feed ingredients are comprised of proteins, carbohydrates and fats with sizes extending from large biopolymers (macromolecules) down to the nanoscale. Even when food is consumed predominantly as macromolecules, the natural digestive processes of the body reduce these to the nanoscale in order to utilise the energy contained in the molecules for the maintenance of physiological processes.

There are however gaps in knowledge regarding the bioavailability of nanoparticles via the oral route, their handling by the body, the methods that can be used to assess their toxicity and, in particular, the metrics that can be used to assess exposure. It is therefore difficult at this time to arrive at a fully informed assessment of the risk from nanoparticles in the food chain. Although the basic principles of the existing risk assessment model can be applied to nano-based food and feed, current methods for assessment of toxicity and quantification of exposure require some modification before they can be applied with confidence for the assessment of nanotechnology in food.

A specific risk assessment for silver nanoparticles, which are finding increasing use as anti-microbial agents, applying the traditional risk assessment model, has recently been published (Blaser, 2008). An overview of this risk assessment, which focuses on silver released into the environment from nanoparticles embedded in a polymer, is presented in Appendix IV of this report. Although this assessment is not specifically relevant to the assessment of nanotechnology in food, it is nevertheless of interest, since it does demonstrate that the basic risk assessment model can be applied to nanoparticles in the same way as other hazards.

5. LEGISLATION

5.1 Introduction

It is recognised that the application of nanotechnology may present new challenges in terms of safety, regulatory and ethical considerations, while offering many potential benefits to manufacturers and consumers (Chau *et al.* 2007).

In terms of current regulatory approaches in the European Union, it is generally considered that potential uses of nanotechnologies in the food and feed area will be covered by the existing regulatory framework, either by the principles of the general food law (EC 178/2002) or by specific approval processes. Major gaps in existing regulations were not identified in a review undertaken by the UK Food Standards Agency (FSA, 2006), but there is uncertainty in some areas as to whether a number of specific applications of nanotechnologies would be picked up consistently, for example the introduction of nanoscale preparations of existing food ingredients, or currently approved food additives. As food and feed regulations are harmonised at EU level, any action to address such uncertainties would need to be taken forward by the European Commission. A review recently completed by the Commission has indicated that the existing EU legislation is broadly adequate to cover potential risks of nanotechnology-based products, although in some areas, specific supporting instruments (guidelines, test protocols, standards) may need to be developed and some provisions clarified or adapted, in order to ensure the full effectiveness of the existing legislation in practice (EC, 2008).

Approval processes are in place for a large number of food ingredients and agents used in food and feed manufacture and processing, including novel foods and novel food processes, food additives and flavourings and food packaging materials. These agents must meet the requirements of the approval system before being permitted for use, and it is anticipated that the majority of nanomaterials in food will fall into this category. The following sections briefly outline the current applicable regulatory framework.

5.2 Provisions of the General Food Law

Under general food law, "unsafe food" (as defined in Article 14, Reg. (EC) 178 of 2002) cannot be placed on the market.

This requirement has been enacted in Ireland by three Statutory Instruments. S.I. No. 747 of 2007 European Communities (General Food Law) Regulations, 2007 covers retail food business establishments and those processing food of non-animal origin. S.I. No. 910 of 2005 European Communities (food and feed hygiene) Regulations, 2005 as amended by S.I. No. 387 of 2006, covers food business establishments requiring approval engaged in the handling and processing of products of animal origin and S.I. No. 335 of 2006 European Communities (Hygiene of Fishery Products and Fish Feed) Regulations, 2006 covers food business establishments requiring approval and engaged in the handling and processing of fish and shellfish.

Similar provisions are applicable to packaging materials and requirements are laid down in Regulation (EC) 1935/2004 which is enacted in Irish food law by the European Communities (Plastics and other materials) (Contact with food) Regulations, 2007 (S.I. No. 587 of 2007).

The general hygiene provisions which apply to most food business operations are the European Communities (Hygiene of Foodstuffs) Regulations, 2006 S.I. No. 369 of 2006 and, in the case of feed business operators, the European Communities (Food and Feed Hygiene) Regulations, 2005 S.I. No. 910 of 2005. Under these regulations, there is a general obligation on food and feed business to carry out their activities in accordance with HACCP principles. These include identifying the points in those operations where food hazards may occur, and identifying and implementing effective measures to control the hazards at these critical points. These obligations will apply to the use of nanoparticles in food production and processing as they do to any other hazard identified in the food chain. It is recognised however, that there is a need for risk managers, including food business operators, to be made more aware of their responsibilities under food law in relation to the specific applications of nanotechnology in the food and feed industries, and the difficulties that may be involved.

5.3 Novel Foods and Processes

Foods produced by the application of nanotechnology and/or containing nano-ingredients may be considered as "novel foods", falling within the scope of the novel food legislation and hence requiring prior approval before placing on the market. The Novel Food Regulation (EC) No 258/97 applies to foods or food ingredients that have not been consumed to a significant degree within the EU before 15 May 1997. In addition, Article 1. 2.(f) brings into the scope of this Regulation food and food ingredients to which a new process has been applied that gives rise to significant changes in composition or structure that in turn affect the nutritional value, metabolism or level of undesirable substances. The FSAI is the competent authority for the enforcement of novel food legislation in Ireland.

The review of the Novel Food Regulation is ongoing and in the proposed replacement legislation, nanotechnology is specifically mentioned as an example of one of a number of emerging food production processes that may require novel food authorisation prior to marketing. On February 6th, 2008, the Finnish customs authorities became the first in the EU to reject the import of a food supplement on the basis that it breached the Novel Food Regulation. The product "Lypo-spheric™ Vitamin C" from the USA was claimed, on the packaging, to provide increased bioavailability of vitamin C due its encapsulation in liposomal nano-spheres. The Finnish authorities declared it a novel process and, therefore, the product as a novel food as yet unauthorised in the EU.

The novel food authorisation process requires that a safety assessment is carried out by a Member State which is then reviewed by all other Member States. Where even one Member State objects to the marketing of the novel food, an additional assessment is requested from EFSA prior to the Commission drafting a Decision to authorise or reject the product. The safety assessment addresses composition, nutritional value, intake, toxicology and allergenicity among other issues. While particle size per se is not a criterion that could be used to determine the novelty of a food or food ingredient, any effect on the composition, nutritional value, metabolism and intended use of a product could bring particle size into consideration.

Where there is disagreement, Article 1.3 makes it possible for the Commission, in association with the Standing Committee for the Food Chain and Animal Health (SCFCAH), to decide whether a food or ingredient falls within the scope of the Novel Food Regulation.

Where new evidence comes to light, calling into question the safety of an authorised novel food, a Member State may temporarily suspend its marketing and notify the Commission and other Member States. The Commission will subsequently examine the veracity of the concerns and decide, in association with Member States, the measures to be taken, if any.

In the event that a nano-ingredient falls outside the scope of the Novel Food Regulation, the general safety articles of the EU Food Law Regulation (178/2002) would apply, which require that food placed on the market is safe.

5.4 Food and Feed Additives

The use of food and feed additives in the EU is controlled by European Parliament and Council legislation, which sets out lists of permitted additives, the foods in which they can be used, and maximum levels of use. All permitted additives have been assessed for safety by the independent Scientific Committees that advise the European Commission, a role now performed by EFSA. In addition, each additive must comply with specific purity criteria laid down in corresponding European Commission Directives. The criteria dictate the chemical structure and purity of each additive. However, minimum particle size is only specified in the case of microcrystalline cellulose (E460 (i)), while the specification for carrageenan (E407) limits the molecular weight distribution (which may indirectly limit particle size).

As only the specifications for two additives limit the size of particles, it could be argued that in principle there are potential gaps in the legislation with respect to the use of food additives in nano-form. However, individual specifications may be amended at SCFCAH. Therefore, action could be taken relatively quickly if it is deemed necessary that amendments were required to address the issue of particle size, whether as a result of an assessment undertaken by EFSA, or on the basis of information supplied by, or a request from, a Member State.

Positive lists control the use of miscellaneous food additives, colours, sweeteners and smoke flavourings in EU Member States. Any new nanomaterials would need to undergo safety assessments by EFSA before they can be included on the relevant positive list and so be permitted to be used in foods. For the majority of additives, specifications have also been elaborated for the material as used, however as noted above, these currently only specify minimum particle size for two additives.

During recent negotiations in Brussels on the package of proposals to replace and update the current legislation on food additives, flavours and enzymes it has been agreed that an additive should require re-evaluation or re-approval if it is prepared in a form that is different from that originally assessed and approved. A change in particle size (i.e. preparation in "nano" form) is given as an example of such a difference. It is anticipated that these new Regulations will be adopted by the end of 2008 and will come into force in all Member States shortly afterwards.

5.5 Food Contact Materials

This is the area where commercial applications of nanotechnology seem to be the most advanced, as evidenced by the fact that EFSA has already published an opinion on the safety of a nanoscale silicon dioxide coating (less than 100nm), to be used in plastic packaging behind an inner layer of PET plastic to provide gas barrier properties (EFSA, 2007). In addition, a safety evaluation for a titanium nitride-based nanoparticle to be used as a food contact material additive is currently being undertaken by EFSA. Current controls in the area stem mainly from Regulation (EC) 1935/2004 on materials and articles in contact with food, and those that might reasonably be expected to transfer their constituents to food. It is considered that this legislation is sufficiently wide-ranging to regulate the migration of nanoparticles into food from food contact materials.

Alternatively, where the nanoscale component might be intended to migrate into the food as part of an 'active' packaging system, it must only do so to improve the shelf-life or to maintain or improve the condition of the food. However, any change to the food must comply with EC provisions applicable to food, and the packaging must be labelled as 'active'.

Some materials and articles are subject to additional, specific measures, such as plastics, ceramics and regenerated cellulose film, and different specific requirements may apply to nanomaterials incorporated into these materials.

Whilst current legislation appears to be wide-ranging enough to cover products derived from nanotechnology in general terms, it does not specifically differentiate between conventional chemicals and those produced using nanotechnology. Draft proposals are currently being discussed that could regulate, should the need arise, particular materials, such as those derived from nanotechnology, that are not specifically covered by existing legislation.

A further potential solution would be for the Commission to amend Annex I of European Regulation (EC) 1935/2004 to require that all nanomaterials are subject to risk assessment. This would bring all such substances within scope of the Regulation, regardless of the material into which they are incorporated. This would also apply to those materials and articles not already covered by specific measures already in place.

In addition to the above mentioned controls, urgent consideration needs to be given to the control/fate of nanoparticles that are present in food contact materials intended to be recycled for further food use. A Commission Regulation on recycled food contact materials (Reg. No. 282/2008) has recently been adopted, which requires the authorisation of recycling processes following a risk assessment by EFSA. Nano-particle-containing food contact material would fall under the scope of this Regulation.

6. DISCUSSION

The presence of nanoparticles in food is not a new phenomenon. People have always been exposed to very fine particles in their diet, without harmful effects, since many food and feed ingredients are comprised of proteins, carbohydrates and fats with sizes extending from large biopolymers (macromolecules) down to the nanoscale. Even when food is consumed predominantly as macromolecules, the natural digestive processes of the body will reduce these to the nanoscale in order to utilise the energy contained in the molecules for the maintenance of physiological processes. The applications of nanotechnology in food production reviewed in this report must therefore be viewed against this background exposure to natural nanoparticles in the diet.

The potential applications of nanotechnology range from modifications of the organic (protein, carbohydrate and fat) molecules that form part of the normal diet, to achieve added or altered functionality, to the use of inorganic nanoparticulate materials in food packaging or as food ingredients, including food additives. Nanotechnology is a new area of science and the advantages and limitations of its use in the agrifood sector are not fully understood at this time. This brings with it new challenges for food and feed safety due to the diversity of the technology behind nano-enhanced food products in the market place. This report has sought to explore the applications of nanotechnology in the food and feed industry, to characterise the current state of knowledge on the possible risks of nanoparticles in food and to examine the existing regulatory framework on food safety in relation to its adequacy to protect the health of consumers from such possible risks.

The report has identified that there are many potential applications of nanotechnology in the food and feed industry. These range from minor modifications of natural food ingredients to enhance taste, palatability and other properties, to more major modifications that may result in altered handling of such ingredients in the body, including increased bioavailability of essential nutrients. Applications of nanotechnology in agriculture such as pesticides and water purification may also impact on food indirectly through residual presence in plant materials and water. It has been suggested that the distinction between nanotechnology and non-nanotechnology in the food and feed industry should be determined by whether the particular ingredient is man-made, i.e. "man-made components modified at a molecular or super-molecular level", or natural.

Organic nanomaterials (nanoparticles of natural food ingredients), whether physically or chemically constructed, are likely to be one of the main areas of interest in the agrifood sector in respect of incorporation of nanotechnology into foods. The predominant food-related area for short term future exploitability of nanoscience is food packaging, while, in the longer term, nanoscale research in foods appears to be focused on 'programmable' release of organic nanoscale encapsulated food ingredients or nutrients. The use of nanoscale technologies for alteration of food stability and texture is also of current interest.

This report has identified that currently there appears to be no direct use of complex inorganic nanoparticles such as carbon nanotubes in foods. Simple inorganic molecules like titanium dioxide and clay particles are being used in packaging and may have potential to migrate into foods. Nanomaterials are present in foods that are currently available on the market, mostly through internet trading, mainly in the form of simple nanoscale encapsulated functional ingredients.

The assessment of the borderline between nanotechnological manipulation of food and feed or conventional food engineering will present considerable difficulties. An example is enzymatic functionalisation, whereby an enzyme is used to modify a food structure to give it a new attribute. This cannot be considered to be new technology, however, if the food structure presented for modification is a nanoparticle encapsulating a non-organic compound, then this application will fall under the definition of nanotechnological manipulation. There is a need for a clear and widely accepted definition of nanotechnology that differentiates between the use of nanoscale technologies in food and feed production and the actual inclusion of nanoparticles in food. It will be essential to establish systems for the identification, categorisation and assessment of food and feed which is based on or has come into contact with nanotechnology-based technologies.

A further aspect that may need consideration in relation to applications of nanotechnology in the agrifood sector is the fact that nanoparticles in food will be exposed to a range of storage and use conditions. For example, they could be used in products that are stored at low temperatures and then heated for consumption. This may affect the nanoparticle stability within the food and also change the properties of the biomolecules in the food, and potentially how these interact with the nanoparticles. To date there is no literature dealing with this.

In relation to the possible risks of nanoparticles in food and feed, such particles are still largely an unknown quantity in terms of how they will behave in the body. As already indicated, many food and feed ingredients are comprised of biopolymers, e.g. proteins, carbohydrates and fats with sizes extending down to the nanoscale. Consumers are therefore already exposed to natural nanoparticles in their diet, without any recognised adverse effect.

There is limited knowledge concerning the bioavailablity and toxicokinetics of nanoparticles, following exposure via the diet or the oral route. The size, shape and composition of particles will have a major effect on their bioavailablity and toxicokinetics, as will their surface modification, e.g. by adsorption of proteins and other endogenous molecules, their surface charge, aggregation and rate of dissolution or degradation.

While it can be generally assumed that natural ingredients/substances (such as lipids and proteins) in the human or animal diet that have been produced either partially or wholly by nanotechnology will be processed normally and catabolised in the body, the nanoforms may show increased bioavailability or be able to penetrate to parts of the body that are normally inaccessible to the macro form. Increased bioavailability of essential nutrients such as minerals and vitamins or the transformation of a normally lipid-soluble molecule to a water-soluble form via nanoengineering may have profound effects for cellular biochemistry and consequent perturbations of physiological processes. It may not be appropriate to compare intake of such modified nutrients with existing safety- and health-based threshold values that have been developed based on the natural form of nutrients and their natural bioavailabilities.

The lack of knowledge regarding the effect on pharmacokinetics and bioavailability of changes in the physicochemical properties of normally inert and non-biodegradable materials such as inorganic particles, e.g. titanium dioxide, and biological polymers in moving to the nanoscale is of particular concern since this will inevitably lead to changes in how they are handled in the body, with potential cascade effects on cellular homeostasis. From studies conducted to date on the oral uptake and distribution of such materials, it appears that their physicochemical properties including apparent insolubility do not guarantee non-absorption of the nanoparticles from the gut. A range of studies on uptake of nanoparticles from the GIT have demonstrated increased bioavailability of such materials, but similar research does not appear to have been carried out to date for such particles in a food matrix. Biopersistence of essentially insoluble nanoparticles following repeated/long term exposure can be anticipated.

There is also limited knowledge concerning the intrinsic toxicity of nanoparticles. While a range of *in vitro* screening tests designed to predict the toxicity of nanoparticles have been developed, few *in vivo* studies in animals have been carried out, particularly via the oral route which is the only relevant route for prediction of risks in food. There is an urgent need to develop predictive and validated tests that can be used to screen for the potential hazards of nanoparticles. Nanoscale proteins formed from re-engineered macromolecules are considered unlikely to be a source of toxicity but may form anomalous protein assemblies or could induce other proteins to aggregate or fibrillate. This requires further investigation.

Little is known about interactions of nanoparticles with components of food and potential effects on toxicity, nor about the influence of possible changes in the nanoparticles following passage through the digestive system, absorption, distribution and clearance from the body. The aggregation of nanoparticles that has been observed under laboratory conditions, and which causes major problems in the characterisation of the size and surface area of the particles, is as likely or more likely to occur when nanoparticles are incorporated in a food matrix, food packaging or other applications. This has profound implications when it comes to determining likely exposure via food and/or predicting potential toxicity, based on extrapolation from *in vitro* tests or *in vivo* tests using routes other than dietary exposure.

The dose metric is of major importance in assessing the risk of nanoparticles in the body as well as the environment. The use of mass concentration data alone for the expression of dose in toxicology testing is insufficient, and the number concentration and/or surface area needs to be taken into account, as well as the aggregation state as discussed above. There is a wide range of physical and chemical characterisation techniques that can be employed to measure nanoparticles and the advent of such techniques has accelerated research and advancement of applied nanotechnology. Equipment that enables routine measurements in various media for representative exposure to free nanoparticles is however not yet available. While the most meaningful dose metric for use in assessing the risk of nanoparticles is probably (in many cases) surface area and/or particle number, in practice it is recognised that this is extremely difficult on a routine basis. This is one of the problems facing the scientific community in assessing the potential risks of nanoparticles.

This report has identified that, although the basic principles of the existing risk assessment model can be applied to nano-based food and feed, in practice there are some gaps in knowledge regarding the bioavailability of nanoparticles via the oral route, their handling by the body, the methods that can be used to assess their toxicity and in particular the metrics that can be used to assess exposure. At this point in time it may not be possible to produce a meaningful risk characterisation and therefore such exercises are more focused at highlighting the possible issues and the areas that require more research. Current methods for assessment of toxicological issues and quantifying exposure require some modification before they can be used for nanoparticles. Until such time as there is further knowledge concerning the many applications of nanotechnology and broad international agreement concerning the approaches to be used in assessing the risks, each application of nanotechnology in food and feed production and the wider implications of nanotechnology for the food chain will need to be assessed on a case-by-case basis. As a consequence, risk management approaches to foods resulting from nanotechnology will necessarily be precautionary.

In terms of the existing legal framework, current legislation does not contain specific provisions on nanomaterials. The potential uses of nanotechnology in the food and feed area are however covered either by the principles of general food law or by specific approval processes such as those for food additives, food contact materials, novel foods, pesticide and biocide approval systems. Indeed, specific controls on the use of nanotechnology in the production of food additives are expected to be adopted at EU level by the end of 2008.

A key issue is whether assessment of the risk of nanotechnology-based food and feed should be primarily the responsibility of the food business operator or whether there should be a requirement for a regulatory evaluation and approval system. The many potential applications of nanotechnology in the food, feed and other industries could have the potential to swamp current regulatory approval systems. This report considers that it would be a failure of "duty of care" for food companies intending to introduce nano-sized food ingredients and/or to change the natural balance of particulate size to achieve a new functionality, not to conduct an assessment of potential risk of the technology, given that it is a legal requirement for food business operators to only place safe food on the market and that safety must be demonstrable. At present, the lack of data regarding the safety of some nanoparticles in food would imply that a full risk assessment should be conducted.

Food ingredients produced by nanotechnology that are covered by specific approval processes at EU level, such as food additives and food contact materials, will require a re-evaluation (by the European Food Safety Authority, via the centralised application system), where there is an existing authorisation for the non-nanoform, but where presentation in the nanoform is expected to alter the behaviour of the ingredient in the human body, e.g. in terms of toxicity, or bioavailability. Where a novel food has been produced via the application of nanotechnology, this will similarly be expected to be evaluated for its possible risk to health before being placed on the market. The issue of what percentage of a food (for instance, 5%, 10% or 50%?) should be at nanolevel for it to be defined as having been produced by nanotechnology must also be taken into account. In relation to food ingredients that are considered to be natural ingredients in the human or animal diet but which have been produced either partially or wholly by nanotechnology, e.g. lipid micelles or emulsions, this report considers, as already indicated, that the responsibility for ensuring safety for consumers rests primarily with the food business operator, based on an assessment of the possible risks and likely exposure patterns.

It is recognised however that implementation of these principles will be difficult in many areas, as current methodologies for identifying hazards and evaluating risks may not necessarily fully allow the specific properties of nanoscale substances to be taken into account. Scientifically, valid new methods for assessing the use of nanotechnology in food are needed.

7. CONCLUSIONS AND RECOMMENDATIONS

The FSAI Scientific Committee has considered the current state of knowledge regarding the applications of nanotechnology in the food and feed industry, the possible risks of nanoparticles in food and feed and the existing regulatory framework on food safety in relation to its adequacy to protect the health of consumers.

In relation to current and future applications of nanotechnology in the food and feed industry, the Committee concludes that:

- nanotechnology has the potential to have a major impact on food innovation over the coming decades, with many new applications foreseen in the agrifood sector for the benefit of consumers and the environment
- nanoparticulate forms of natural food ingredients are the predominant area of current interest to the agrifood sector, in respect of incorporation of nanotechnology into foods
- in the area of food research, food contact materials, including packaging, are most likely to receive the greatest attention in the short term, while 'controlled' release of nanoscale-encapsulated food ingredients or nutrients may be the focus in the longer term. The use of nanoscale technologies for alteration of food stability and texture is also of current interest
- although the primary focus in food nanotechnology will be on endogenous organic molecules, e.g. proteins, lipids, carbohydrates, non-degradable inorganic nanoparticles such as titanium dioxide and clay particles are likely to be used in packaging and other applications and may have the potential to migrate into foods
- although the deliberate use of other engineered, non-biodegradable nanoparticles such as carbon nanoparticles, e.g. fullerenes and carbon nanotubes, in foods has not been reported, they may inadvertently enter the food chain
- foods containing nanomaterials are available on the global market, mainly through internet trading. Regulatory controls on such products for personal use are recognised to be deficient, and FSAI and other food safety bodies in Europe do not have full enforcement powers in relation to them
- nanosensors are being developed for the purpose of food surveillance, including traceability. Current research is focused on the incorporation of nanosensors into food packaging materials for tracking, safety and biosecurity purposes and on the development of rapid biosensors for the diagnostic detection of pathogens/contaminants in foods and their surrounding environment.

In relation to the assessment of possible risks of nanoparticles in food and feed, the Committee considers that a number of difficulties and gaps exist which hamper the application of current risk assessment methodologies to the use of nanotechnology in food and feed production. These include:

- the lack of robust methods for the characterisation and measurement of nanoparticles in food, feed and other biological matrices
- consequent difficulties in assessing exposure to nanoparticles and in ensuring metrological traceability
- deficiencies in the knowledge base regarding the bioavailability and possible toxicity of nanoparticles, and their behaviour at the cellular level
- an absence of standardised protocols for the assessment of the toxicological profiles of nanoparticles *in vitro* and *in vivo*.

Some modification of current risk assessment approaches and methodology is therefore necessary, in order to provide more informative characterisations of risk, although the basic model of hazard identification, hazard characterisation, exposure assessment and risk characterisation would appear to be adequate and applicable to nanotechnology.

In particular, the Committee considers that potential increases in the bioavailability of essential nutrients resulting from the application of nanotechnology will require a reassessment of the applicability of traditional threshold values developed for safety and health purposes.

The Committee also recognises that little is known concerning the potential for nanoparticles to migrate into the environment following disposal and/or recycling of articles such as food contact and other nanoparticle-containing materials. Nanoparticles released in this way may enter the food chain, with consequent unrecognised exposure of consumers. In addition, nanoparticles may migrate into foods from recycled packaging produced from material that contains nanoparticles.

The Committee concludes therefore that, until these issues are addressed and a standardised approach is developed for the assessment of the possible risks of nanoparticles in food and feed, at the level of the European Community and internationally, each application of nanotechnology in food and feed production and the wider implications of nanotechnology for the food chain will need to be assessed on a case-by-case basis.

On risk management measures, including the legal framework for ensuring food and feed safety, the Committee considers that although current legislation does not contain specific provisions on nanomaterials, potential uses of nanotechnologies in the food and feed area are broadly covered either by the principles of the general food law or by specific approval processes such as those for food additives, food contact materials and novel foods. Opportunities for strengthening the European Community legislation have however been identified in this and other reports.

The Committee also considers that, within this legal framework, it is the primary responsibility of food business operators to assess the possible risks and take all necessary steps to ensure that food is safe and healthy when applying nanotechnology in food or feed production, whether this involves introduction of new nanoparticles into foods and packaging or merely manipulation of the size distribution of natural food molecules in food matrices.

In relation to communication of information regarding the application of nanotechnology in food and feed, the Committee identifies a need for risk managers, including food business operators, to be made more aware of their responsibilities under food law in relation to the specific applications of nanotechnology in the food and feed industries, and the difficulties that may be involved. The Committee also recognises the importance of keeping consumers and the general public fully informed regarding the applications of nanotechnology in food.

The FSAI Scientific Committee therefore recommends that:

- when applying nanotechnology in food or feed production, food business operators should assess the possible risks and take all necessary steps to ensure that food is safe
- the FSAI should communicate with and advise food business operators in relation to their responsibility to conduct risk assessments on all foods utilising nanotechnology
- to this end, FSAI should keep under review advances in the science of nanotechnology, risk assessment approaches and the legal framework governing the application of nanotechnology in food and feed
- the FSAI should promote the establishment of a publicly-accessible inventory of nanotechnology-based food products and food contact materials, available both at European Community level and specifically on the Irish market
- where nanoparticles are incorporated in food or food packaging, EC labelling provisions should require that such products are labelled to provide this information
- legal provisions should be considered at EC level to ensure that food and feed ingredients produced via the application of nanotechnology are specifically controlled to ensure that where the properties are changed/re-engineered to the nanoscale, they should be re-evaluated in terms of safety
- urgent consideration should be given to whether additional controls are required on the disposal and/or recycling of nanoparticle-containing food contact and other materials
- food surveillance programmes should include investigation of the potential for nanoparticles, particularly inorganic molecules such as titanium dioxide and clay particles used in packaging, to migrate into foods and also to be recycled in the environment and enter the food chain indirectly.

The Committee also recommends that research at national level, with application of targeted funding, should urgently be undertaken to increase the reliability of the risk assessment of nanotechnology in food, including on the following issues:

- the feasibility of establishing an inventory of food and feed products and packaging involving the application of nanotechnology on the market in Ireland
- development of methods to ensure traceability of nanoparticles in the food chain and procedures for the characterisation and measurement of nanoparticles in food
- methods for the safe and effective disposal of used, unused or waste nanoparticles
- the bioavailability and fate of nanoparticles within the human body and animals
- development of methods to investigate the potential toxicity of nanoparticles *in vitro* and *in vivo* and the underlying mechanisms of nanoparticle toxicity
- the stability/lability of nanoparticles in various foods and their potential interactions with food components.

Finally, recognising the importance of a coordinated national approach between Government departments and national agencies on the evaluation of the impact of nanotechology, the Committee recommends that the FSAI should initiate contacts with other such bodies, to inform them of the FSAI's work in this area and to ascertain whether similar initiatives are ongoing elsewhere. The FSAI should also keep Irish industry informed of developments concerning the applications of nanotechnology in food and the legal requirements governing such uses.

APPENDIX I: ABBREVIATIONS

ADME	Absorption/bioavailability, Distribution, Metabolism, Excretion
AFM	Atomic Force Microscopy
CBEN	Centre for Biological and Environmental Nanotechnology
CDS	Circular Dichroism Spectroscopy
CFM	Chemical Force Microscopy
CNT	Carbon Nanotubes
COC	Committee on Carcinogenicity of Chemicals in Food Consumer Products and the Environment (UK)
COM	Committee on Mutagenicity of Chemicals in Food Consumer Products and the Environment (UK)
CoQ10	Coenzyme Q10
COT	Committee on Toxicity of Chemicals in Food Consumer Products and the Environment (UK)
DEP	Diesel Exhaust Particles
DHA	Docosahexaenoic Acid
DLS	Dynamic Light Scattering
DSC	Differential Scanning Calorimetry
EDXS	Energy Dispersive X-ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic Acid
EPR	Electron Paramagnetic Resonance
ESA	Electroacoustic Sonic Amplitude
ESR	Electron Spin Resonance
FRET	Fluorescence Resonance Energy Transfer
FFF	Field Flow Fractionation
FSAI	Food Safety Authority of Ireland
FTIR	Fourier Transform Infrared Spectroscopy
GIT	Gastrointestinal Tract
GM	Genetically Modified
HEK	Human Epidermal Keratinocytes
IBD	Inflammatory Bowel Disease
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ITC	Isothermal Titration Calorimetry
LEED	Low Energy Electron Diffraction
MALDI	Matrix-Assisted Laser Desorption Ionization
MFM	Magnetic Force Microscopy
MMP-9	Matrix Metalloproteinase 9
MWCNT	Multi-Wall Carbon Nanotubes
NIOSH	National Institute of Occupational Health (USA)

NLC	Nanostructured Lipid Carriers
NMR	Nuclear Magnetic Resonance Spectroscopy
NPs	Nanoparticles
NSLS	Nano-sized Self-assembled Liquid Structures
O/W	Oil-in-Water
PM_{10}	Particles of 10 micrometres or less
PP	Peyer's Patches
QD	Quantum Dots
USFDA	US Food and Drug Administration
IL	Interleukin
ISO	The International Standards Organization
IV	Intravenous
PCS	Photon Correlation Spectroscopy
PET	Polyethylene Terephthalate
PLGA	Polylactide-co-Glycolide
PMMA	Polymethyl Methacrylate
RFID	Radio Frequency Identification
ROS	Reactive Oxygen Species
RT-PCR	Real-Time Polymerase Chain Reaction
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-Ray Scattering
SC	Stratum Corneum
SCFCAH	Standing Committee for the Food Chain and Animal Health
SCCP	Scientific Committee on Consumer Products
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SEM	Scanning Electron Microscopy
SERS	Surface Enhanced Raman Spectroscopy
SLN	Solid Lipid Nanoparticles
SMS	Single Molecule Spectroscopy
SNOM	Scanning Near Field Optical Microscopy
STM	Scanning Tunnelling Microscopy
TEM	Transmission Electron Microscopy
TiO_2	Titanium dioxide
TIRF	Total Internal Reflectance Fluorescence
TNF alpha	Tumour Necrosis Factor alpha
ToF-MS	Time-of-Flight Mass Spectrometry
W/O	Water-in-Oil
XPS	X-ray Photo electron Spectroscopy
XRA	X-Ray Absorption
XRD	X-Ray Diffraction

APPENDIX II: GLOSSARY

ADI
The amount of a substance present in food that can be consumed daily for an entire lifespan without appreciable risk.

Aggregate
Larger particles formed through agglomeration or aggregation of primary particles (the smallest identifiable subdivision in a particulate system), in which the various components are not easily broken apart.

Agrifood Sector
A term describing industries involved in the mass production, processing, and inspection of food and animal feed products made from agricultural commodities

Antioxidant
A chemical which slows or prevents oxidation (the reaction of substances and/or cell components with oxygen in a process that can lead to their breakdown). Oxidative stress can damage cells.

Bioavailability
The amount of a substance/material absorbed by the body from the site of first exposure, e.g. the gastrointestinal tract, to reach the systemic blood circulation and the rest of the body.

Biocide
A substance used mainly as an anti-microbial agent.

Biomolecule
A chemical compound found in living organisms and which is composed predominantly of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus.

Biopolymer
Any polymer (a long repeating chain of atoms) found in nature. Examples include starch, proteins and DNA.

Biosensor
A sensor that incorporates a biologically active interface, e.g. DNA, protein etc.

Carbon Nanotubes
nanoparticles consisting of one or several graphene sheets rolled up to form a single- or multi-walled tube.

Chemical Reactivity and Bioactivity
The chemical reactivity and bioactivity of a nanoparticle that affects the particle stability and dispersion properties as well as the particle's mobility in biological systems.

Colloid
a substance consisting of particles not exceeding 1µm dispersed in a fluid.

Crohn's Disease
A chronic inflammation of the gastrointestinal tract that can lead to cancer.

Dispersion/Aggregation State (see also "aggregate")
The tendency of suspensions of nanoparticles to aggregate, the process where small molecules or particles can come together to form a secondary larger particle or cluster.

Dose
The quantity of nanomaterial absorbed and/or internalised into the cell.

Emulsion
A suspension of small globules of one liquid within a second liquid.

Endogenous
A substance, e.g. protein, carbohydrate, lipid, that originates from within an organism, tissue, or cell.

Encapsulation
A process in which particles or droplets are coated to create capsules.

Engineered Nanoparticle
A material purposely synthesised or unintentionally produced and having novel functional properties exhibited on the nanoscale.

Fullerene ('buckyball')
A closed cage pure carbon molecule. The smallest stable fullerene is the buckminsterfullerene or buckyball which is composed of 60 atoms of carbon and has a shape similar to a hollow soccer ball or a geodesic dome.

Granuloma
A small mass or nodule of chronically inflamed tissue that is usually associated with an inflammatory process or injured tissue, for example as seen in Crohn's disease, sarcoidosis etc.

Hydrophobicity and Solubility
A term describing the degree of wettability of nanoparticles, a property crucial in assessing the biological impacts of the particle.

In silico
An experiment performed via computer simulation

In vitro
An experiment performed in a test tube or culture.

In vivo
An experiment performed in a living organism.

Liposome
An oily, microscopic-sized capsule designed to package and deliver biological cargo, such as drugs, to cells in the body.

Macrophage
A large immune cell that envelopes invading pathogens and other foreign material.

Metrological Traceability
The property of a measurement which relates a result to a stated metrological reference through an unbroken chain of calibrations or comparisons, each contributing to the stated measurement uncertainty. The stated metrological reference may be a measurement procedure, or a physical standard.

Micelle
An aggregate of molecules, where in an aqueous solution the hydrophilic (water loving) head regions form a protective barrier around the oil containing hydrophobic (water hating) tail regions in the micelle centre.

Mucosa
The moist layer that lines the mouth and gastrointestinal tract.

Nano-composite
A material that is created by mixing nanoparticles into a base material.

Nanometre (nm)
One thousand millionth of a metre.

Nano-objects
The generic term of 'nano-object' includes nanoparticles and their aggregation at nanoscale, nano-systems, nano-materials, nano-structured materials and nano-products

Nanoparticle
An ultrafine particle with at least one dimension between 1 – 100 nanometers (nm).

Nanoparticle Purity
Nanoparticle samples may contain a range of impurities such as solvent residues and catalyst particles (which can also be on the nanoscale). These impurities vary considerably from batch to batch and as a result characterisation of the impurities can be time consuming and difficult.

Nanotechnology
The control of matter and processes at the nanoscale, typically, but not exclusively, below 100 nanometres in one or more dimensions where the onset of size-dependent phenomena usually enables novel applications.

Nanotubes
A molecule that resembles a cylinder with at least one dimension on the nanoscale.

Nanowires
An extremely thin wire with a diameter on the order of a few nanometers (nm) or less.

Non-degradable Particles
Persistent particles that the body is not able to break down.

Oxidative Stress
An imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage.

Particle Size
A property affecting the stability, chemical reactivity, opacity, mobility, and strength of many materials and determination.

Particle Size Distribution
The distribution or range of particle sizes found in a given sample. Normally nanoparticle samples contain a range of particles both larger and smaller then the mean value, which is often quoted as the particle size.

Pesticide
A pesticide is any chemical or biological agent used for control of plant or animal pests. Pesticides include insecticides, herbicides, fungicides, nematocides and rodenticides.

PET
Polyethylene terephthalate. A thermoplastic material used to manufacture plastic soft drink containers and rigid containers.

Polymer
A substance made of many repeating chemical units or molecules. The term polymer is often used in connection with plastic, rubber, or elastomer.

Quantum Dot
A quantum dot is a particle of matter so small that the addition or removal of an electron changes its properties in some useful way, e.g. it might glow under UV light.

RDA
Recommended daily (or dietary) allowance, the quantity of a particular nutrient which should be consumed daily in order to maintain good health.

Reactive oxygen species (ROS)
Molecules which are highly reactive due to the presence of unpaired valence shell electrons, includes oxygen ions, free radicals and peroxides.

Safe Upper Level (SUL)
The upper amount of any particular nutrient that a person could safely take for a lifetime.

Sensor
[Nanoscale] chemical, biological or physical sensory points or system used to detect and convey information about a given environment, e.g. temperature, pH, location, or the presence of diseased tissue.

Solubility
A term describing whether nanoparticles dissolve in water and biological fluids, a property crucial in assessing the biological impacts of the particle.

Submucosa
The layer of loose connective tissue that supports the mucosa and joins it to the bulk of underlying smooth muscle.

Surface Area
The surface to volume ratio of a particle or other body. As the size of a particle decreases more molecules are present at the surface, giving rise to a larger surface to volume ratio or larger surface area for chemical interaction.

Surface Charge
The electric charge present at an interface, for instance on the surface of a protein in water. The surface charge influences adsorption process at the particle's surface.

APPENDIX III: LIST OF WORKING GROUP MEMBERS

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APPENDIX IV: PHYSICAL AND CHEMICAL TECHNIQUES FOR CHARACTERISING NANOPARTICLES

Technique	Property to be Measured
Rheology	Elasticity, viscosity, shear, strain
Dynamic light scattering (DLS) photon correlation spectroscopy (PCS)	Particle size, distribution, aggregation and hydrodynamic radius, zeta potential
Size exclusion chromatography	Molecular weight, hydrodynamic properties
Absorption spectroscopy	Electronic nature of material
Fourier transform infrared spectroscopy – FTIR	Vibrational energies and composition chemical identification
Luminescence spectroscopy (electro- photo- etc.)	Defects and impurities in samples
Cyclic voltammeter, potentiometric and electrochemical techniques	Kinetic, thermodynamic and electronic parameters, surface charge
Confocal laser scanning microscopy	Topography, depth profiling, fluorescent imaging
N2 Gas adsorption	BET surface area, pore size distribution
Raman spectroscopy and surface enhanced raman (SERS)	Vibrational energies and composition and chemical identification
Atomic Force Microscopy – AFM (and associated techniques, Magnetic Force Microscopy MFM, Chemical Force Microscopy CFM etc.)	Surface topography and chemistry, grain size, electronic properties
Transmission Electron Microscopy – TEM	Morphology size shape crystallographic structure
Scanning Electron Microscopy – SEM	Topography, morphology, size distribution
Scanning Tunnelling Microscopy – STM	3-D surface topography, size, shape, defects, electronic structure.
Acoustic attenuation spectroscopy	Particle size and distribution agglomeration
Electroacoustic sonic amplitude – ESA	Particle size charge and zeta potential
Doppler micro electrophoresis	Particle size charge and zeta potential
Electro acoustic spectroscopy	Particle size charge and zeta potential

Technique	Property to be Measured
Energy Dispersive Xray Spectroscopy – EDXS	Elemental analysis
Field flow fractionation (FFF)	Composite size, molecular weight hydrodynamic radius
Nuclear magnetic resonance spectroscopy – NMR	Identification of structure and ID
Small Angle Neutron and X-Ray Scattering (SANS / SAXS)	Structural information
X-Ray diffraction (XRD)	Crystallographic information
X-Ray absorption (XRA)	Structural information
Inductively coupled plasma mass spectrometry (ICP-MS)	Composition and mass
Scanning Near Field Optical Microscopy – SNOM	Chemical specificity, orientation information
Single molecule spectroscopy – SMS	Charge separation, excited states, and fluorescence efficiencies
Ellipsometry	Surface properties, density uniformity and anisotropy
Electron paramagnetic resonance (EPR) or electron spin resonance (ESR)	Chemical state, spin orientation determination of G factor, kinetics
Auger spectroscopy	Chemical analysis and composition of surfaces
X-ray photo electron spectroscopy XPS	Chemical analysis of surface
Mössbauer	Chemical structure, and magnetic properties
Electron energy loss spectroscopy – EELS	Chemical composition bonding in crystals and at interfaces
Electron Diffraction	Geometry of gaseous molecules
Low Energy Electron Diffraction – LEED	Characterisation of surface structures

Techniques potentially in used in food sector requiring minimal adaptation for nanoparticles

Specialised techniques which should be made available to food sector as nanoparticle use increases

Highly specialised techniques with reduced relevance to food sector but which could have occasional needs as nanoparticle use increases

APPENDIX V: BIOPHYSICAL AND BIOLOGICAL TECHNIQUES FOR CHARACTERISING NANOPARTICLES IN BIOLOGICAL ENVIRONMENTS

Technique	Property to be Measured
Circular dichroism spectroscopy (CD)	Secondary and tertiary structure of adsorbed proteins
Differential scanning calorimetry (DSC)	Secondary structure of proteins, protein stability
Isothermal Titration calorimetry (ITC)	Binding isotherms, numbers of proteins bound per particle
Fourier-transform Infra-red spectroscopy (FTIR)	Secondary structure of proteins
Total Internal Reflectance Fluorescence (TIRF)	Protein orientation and spreading at surfaces
Enzymatic Assays	Catalytic activity of adsorbed proteins
Phage display Libraries	Amino acid sequences of peptides with surface affinities
1D and 2D gel electrophoresis	Identification of adsorbed biomolecules
Ellipsometry	Amount of bound protein and thickness of protein layer
Confocal laser scanning microscopy	Visualisation of nanoparticle interaction with cells
Limited proteolysis	Fragmentation patterns of proteins, accessible cleavage sites in adsorbed proteins
Raman spectroscopy and surface enhanced Raman (SERS)	Thickness and structural arrangement of adsorbed protein layer
Atomic Force Microscopy – AFM	
(and associated techniques Magnetic Force Microscopy MFM, Chemical Force Microscopy CFM etc.)	Volume and height of adsorbed proteins. Nanoparticle-cell interactions?
Immunofluorescence screening	Antibody binding to proteins as indicator of concentration of adsorbed proteins
Electron Microscopy – TEM/SEM	Cellular localisation of nanoparticles
Ribonuclease protection assays	mRNA transcripts in complex mixture – cellular response to materials
Real-time polymerase chain reaction (RT-PCR)	Gene expression changes – cellular response to materials
Site-directed mutagenesis	Identification of binding site by changing single residue in protein

Technique	Property to be Measured
Matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry (ToF-MS)	Identification of adsorbed proteins by molecular mass
Time-of-flight secondary ion mass spectrometry (ToF-SI-MS)	Peptide segments presented at surface of adsorbed layer
Fluorescence Correlation Spectroscopy	Dynamics of single molecules
Quartz crystal microbalance	
Nuclear magnetic resonance spectroscopy – NMR	Identification of structure and ID
Fluorescence Resonance Energy Transfer (FRET)	Extension of proteins upon adsorption, localisation of nanoparticles intra-cellularly
Small Angle Neutron and X-Ray Scattering (SANS / SAXS)	3D resolution of shape and arrangement of adsorbed proteins
Surface Plasmon Resonance	Amount of bound protein, association/dissociation rates

Techniques potentially in used in food sector requiring minimal adaptation for nanoparticles

Specialised techniques which should be made available to food sector as nanoparticle use increases

Highly specialised techniques with reduced relevance to food sector but which could have occasional needs as nanoparticle use increases

APPENDIX VI: RISK ASSESSMENT OF NANOSILVER CONTAINED IN A POLYMER MATRIX AND POTENTIALLY RELEASED INTO THE ENVIRONMENT

(Blaser *et al.* 2008)

Many of the uses of nanosilver involve embedding the particles into a polymer matrix, or applying it as a coating, (Marini *et al.*, 2007) meaning that the effects of nanosilver are not surface effects directly, but rather indirect – the biocidal mechanism of silver-containing products results from a long term release of silver ions (Ag+) by oxidation of metallic silver (Ag0) in contact with water (Kumar, 2005), so processing it as nanosilver results in a more efficient release of the silver ions.

In the risk assessment by Blaser *et al.* the focus is on silver released from nanoparticles embedded in the polymer with the assumption that only silver ions and not entire nanoparticles are released. The risk assessment of nanosilver is presented in four stages. First, the system boundaries are defined, mass flows of silver were quantified, and three emission scenarios were defined. Second, the behavior of silver in natural freshwater is reviewed, and a mass balance model applied to calculate predicted environmental concentrations (PECs) for freshwater and freshwater sediments. PECs are also estimated for sewage treatment plants (STPs) and sewage sludge. The uncertainty of the results is assessed and predicted concentrations compared to empirical data. Third, toxicity data for environmentally relevant silver compounds are compiled and predicted no-effect concentrations (PNECs) determined where possible.

The results of the risk assessment indicate that up to 15% of the total silver emitted into water in Europe may be released from biocidal plastics and textiles in 2010. Considering that the market for such products is expected to grow until 2015, it can be concluded that biocidal plastics and textiles will account for a substantial share of total silver emissions in the future.

BIBLIOGRAPHY

Aprahamian, M., Michel, C., Humbert, W., Devissaguet, J.P., Damge, C. (1987) Transmucosal passage of polyalkylcyanoacrylate nanocapsules as a new drug carrier in the small intestine. Biology of the Cell 61, 69–76

Araujo, L., Lobenberg, R., Kreuter. J. (1999) Influence of the surfactant concentration on the body distribution of nanoparticles. J Drug Target, 6:373-385

Auger, F., Gendron, M.G., Chamot, C ., Marano, F., Dazy, A.C. (2006) Responses of well-differentiated nasal epithelial cells exposed to particles: Role of the epithelium in airway inflammation. Toxicology and Applied Pharmacology, 215, 285-294

Bai, N., Khazaei, M., van Eeden, S.F., Laher, I. (2007) The pharmacology of particulate matter air pollution-induced cardiovascular dysfunction Pharmacology & Therapeutics, 113, 16-29

Bernstein, D., Castranova, V., Donaldson, K., Fubini, B., Hadley, J., Hesterberg, T., Kane, A., Lai, D., Mcconnell, E. E., Muhle, H., Oberdorster, G., Olin, S., and Warheit, D. B. (2005) Testing of fibrous particles: short-term assays and strategies. Inhal. Toxicol. 17(10), 497-537

BfR (2007) Nanotechnology: Health and environmental risks of nanomaterials – Research Strategy http://www.bfr.bund.de/cm/290/nanotechnology_health_and_environmental_risks_of_nanomaterials_research_strategy_final_version.pdf

BfR (2008) Wahrnehmung der Nanotechnologie in der Bevölkerung http://www.bfr.bund.de/cm/238/wahrnehmung_der_nanotechnologie_in_der_bevoelkerung.pdf

Bhol, K.C., Schechter, P.J. (2007) Effects of nanocrystalline silver (NPI 32101) in a rat model of ulcerative colitis. Dig Dis Sci. 2007 Oct;52(10):2732-42

Blaser, S.A., Scheringer, M., Macleod, M., Hungerbühler, K. (2008) Sci Total Environ.390(2-3), 396-409
Böckmann, J., Lahl, H., Eckert, T., Unterhalt. B. (2000) Blood titanium levels before and after oral administration titanium dioxide. Pharmazie 55(2), 140-3

Borm, P.J.A., Kreyling, W.A. (2004) Toxicological hazards of inhaled nanoparticles – potential implications for drug delivery. Journal of Nanoscience and Nanotechnology 4, 1-11

Brown, D.M., Kinloch, I.A., Bangert, U., Windle, A.H., Walter, D.M, Walker, G.S., Scotchford, C.A., Donaldson K., Stone, V. (2007) An *in vitro* study of the potential of carbon nanotubes and nanofibres to induce inflammatory mediators and frustrated phagocytosis. Carbon, Volume 45 1743-1756

Bouwmeester, H., Dekkers, S., Noordam, M. Hagens, W., Bulder, A, de Heer, C., ten Voorde, S., Wijnhoven, S., Sips, A. (2007) Health impact of nanotechnologies in food production. Report 2007.014. RIKILT – Institute of Food Safety, Wageningen UR and National Institute of Public Health & the Environment; Center for Substances and Integrated Risk Assessment http://www.rikilt.wur.nl/NR/rdonlyres/BDEEDD31-F58C-47EB-A0AA 23CB9956CE18/54352/R2007014.pdf

Cardinali, G., Kovacs, D., Maresca, V., Flori, E., Dell'Anna, M.L., Campopiano, A., Casciardi, S., Spagnoli, G., Torrisi, M.R., Picardo, M. (2006) Differential *in vitro* cellular response induced by exposure to synthetic vitreous fibers (SVFs) and asbestos crocidolite fibers. Exp Mol Pathol. 81(1),31-41

Cenni, E., Granchi, D., Avnet, S., Fotia, C., Salerno, M., Micieli, D., Sarpietro, M.G., Pignatello, R., Castelli, F., Baldini, N. (2008) Biocompatibility of poly(D-L- lactide-co-glycolide) nanoparticles conjugated with aldenronate. Biomaterials 29, 1400-1411

Chalasani, K.B., Russell-Jones, G.J., Jain, A.K., Diwan, P.V., Jain, S.K. (2007) Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles. J Control Release 122(2), 141-50

Chau, C-F., Wu, S-H., Yen, G-C. (2007) The development of regulations for food nanotechnology. Trends in Food Science & Technology 18, 269-280

Chaudhry, Q., Scotter, M., Blackburn, J., Ross, B., Boxall, A., Castle, L., Aitken, R, Watkins, R. (2008) Food Additives & Contaminants, Part A – Chemistry, Analysis, Control, Exposure & Risk Assessment; Applications and implications of nanotechnologies for the food sector. 25, 241-258

Chen, H., Weiss, J., Shahidi, F. (2006a) Nanotechnology in nutraceuticals and functional foods. Food Technology, 3, 30-36

Chen, Z., Meng, H., Xing, G., Chen, C., Zhao, Y., Ji, G., Wang, T., Yuan, H., Ye, C., Zhao, F., Chai, Z., Zhu, C., Fang, X., Ma, B., Wan, L. (2006b) Acute toxicological effects of copper nanoparticles *in vivo*. Toxicology Letters 163 109–120

Chen, Z., Meng, H., Yuan, H., Xing, G., Chen, C., Zhao, F., Wang, Y., Zhang, C., Zhao, Y. (2007) Identification of target organs of copper nanoparticles with ICP-MS technique. J. Radioanalytical Nuclear Chem., 272, 599-603

COT (2005) UK Committees on toxicity, mutagenicity and carcinogenicity of chemicals in food, consumer products and the environment (COT, COM, COC). Joint statement on nanomaterial toxicology. http://cot.food.gov.uk/pdfs/cotstatements2005nanomats.pdf

De Jong, W.H., Hagens, W. I., Krystek, P., Burger, M.C., Sips, A.J.A.M., Geertsma, R.E. (2008) Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials 29, 1912-1919

Donaldson, K., Stone, V., Tran, C.L., Kreyling, W., Borm, P.J. (2004) Nanotoxicology. Occup Environ Med., 61, 727-728

Donaldson, K., Tran, C.L. (2002) Inflammation caused by particles and fibers. Inhal Toxicol., 14, 5-27

Duffin, R., Tran. L., Brown, D., Stone, V., Donaldson. K. (2007) Proinflammogenic effects of low-toxicity and metal nanoparticles *in vivo* and *in vitro*: highlighting the role of particle surface area and surface reactivity. Inhal Toxicol., 19, 849-856

EC (2008) Commission Recommendation of 07/02/2008 on a Code of Conduct for Responsible Nanosciences and Nanotechnologies Research. http://ec.europa.eu/research/science-society/document_library/pdf_06/nanocode-recommendation-pe0894c08424_en.pdf

European Food Safety Authority (2007) The EFSA Journal (2007) 452 to 454, 1 of 10

ETC Group. Action Group on Erosion, Technology and Concentration (2004) Down on the Farm; the impact of Nanoscale Technologies on Food and Agriculture. http://www.etcgroup.org

Food and Drink Administration (2007) Nanotechnology. A Report of the U.S. Food and Drug Administration Nanotechnology Task Force, July 25, 2007 http://www.fda.gov/nanotechnology/taskforce/report2007.pdf

Florence, A.T., Hussain, N. (2001) Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas. Adv Drug Deliv Rev., 50:S69–S89

Foucaud, L., Wilson, M.R., Brown, D.M., Stone, V. (2007) Measurement of reactive species production by nanoparticles prepared in biologically relevant media. Toxicol .Lett. 174, 1-9

Frampton, M.W., Utell, M.J., Zareba, W., Oberdörster, G., Cox, C., Huang, L.S., Morrow, P.E., Lee, F.E., Chalupa, D., Frasier, L.M., Speers, D.M., Stewart, J. (2004) Effects of exposure to ultrafine carbon particles in healthy subjects and subjects with asthma. Res. Rep. Health Eff. Inst. 126, 1-47

Food Standards Agency (2006) Draft FSA regulatory review on nanotechnology in food: issue for comment. INT 06/04/01, 7 April 2006 http://www.food.gov.uk/multimedia/pdfs/int060401a.pdf

Galloway, T.S., Sanger, R.C., Smith, K.L., Fillmann, G., Readman, J.W., Ford, T.E. (2002) Rapid assessment of marine pollution using multiple biomarkers and chemical immunoassays, Environ Sci Technol., 36, 2219–2226

Gatti, A.M. (2004) Biocompatibility of micro- and nano-particles in the colon. Part II. Biomaterials, 25, 385-392

Ghio, A. J., Stonehuerner, J., Dailey, L. A., and Carter, J. D. (1999) Metals associated with both the water-soluble and insoluble fractions of an ambient air pollution particle catalyze an oxidative stress. Inhal. Toxicol. 11(1), 37-49

Graveland-Bikker, J.F., de Kruif, C.G. (2006) Unique milk protein based nanotubes: Food and nanotechnology meet. Trends in Food Science & Technology, 17, 196-203

Hecq, J., Deleers, M., Fanara, D., Vranckx, H., Boulanger, P., Le Lamer, S., Amighi, K. (2006) Preparation and *in vitro*/*in vivo* evaluation of nano-sized crystals for dissolution rate enhancement of ucb-35440-3, a highly dosed poorly water-soluble weak base. European Journal of Pharmaceutics and Biopharmaceutics, 64, 360-368

Hillery, A.M., Jani, P.U., Florence, A.T. (1994) Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60nm polystyrene particles. J Drug Target, 2,151-156

Hillyer, J.F., Albrecht, R.M. (2001) Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. J Pharm Sci., 90,1927–1936

Hoet, P., Bruske-Hohlfeld, I., Salata, O. (2004) Nanoparticles – known and unknown health risks. J Nanobiotechnol., 2:12

Huczko, A., Lange, H., Calko, E., Grubek-Jaworska, H., Droszez, P. (2001) Physiological testing of carbon nanotubes: are they asbestos-like? Fullerene Science and Technology, 9, 251–254

Hussain, N., Jani, P.U., Florence, A.T. (1997) Enhanced oral uptake of tomato lectin conjugated nanoparticles in the rat. Pharm. Res., 14, 613-618

Hussain, N., Florence, A.T. (1998) Utilizing bacterial mechanisms of epithelial cell entry: invasin-induced oral uptake of latex nanoparticles. Pharm. Res., 15,153-156

Hussain, N., Jaitley, V., Florence, A.T. (2001) Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv. Drug. Deliv. Rev., 50,107-142

Hussain, S.M., Hess, K.L., Gearhart, J.M., Geiss, K.T., Schlager, J.J. (2005) *In vitro* toxicity of nanoparticles in BRL3A rat liver cells. Toxicology *in vitro* 19, 975-983

Hurt, R.H., Monthioux, M., Kane, A. (2006) Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue. Carbon 44, 1028-1033

ISO TC 229 (2008) Draft standard on Nanotechnologies - Terminology and definitions for nanoparticles http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=44278

Jani, P., Halbert, G.W., Langridge, J., Florence, A.T. (1989) The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. J. Pharm. Pharmacol., 41, 809-812

Jani, P., Halbert, G.W., Langridge, J., Florence, A.T. (1990) Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J. Pharm. Pharmacol., 42, 821-826

Jani, P. U., McCarthy, D.E., Florence, A.T. (1994) Titanium dioxide (rutile) particle Uptake from the rat GI tract and translocation to systemic organs after oral administration. International Journal of Pharmaceutics 105, 157-168

Jia, X., Li, N., Chen, J. (2005) A subchronic toxicity study of elemental Nano-Se in Sprague-Dawley rats. Life Sci. 76(17),1989-2003

Kaiser (2007) Helmut Kaiser Consultancy, Nano Food 2040. Nanotechnology in Food, Food processing, Agriculture, Packaging and Consumption. State of Science, Technologies, Markets, Applications and Developments to 2015 and 2040

Kotyla, T., Kuo, F., Moolchandani, V., Wilson, T., Nicolosi, R. (2008) Increased bioavailability of a transdermal application of a nano-sized emulsion preparation. International Journal of Pharmaceutics, 347, 144-148

Kreyling, W. G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdorster, G. and A. Ziesenis, A. (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J. Toxicol. Environ. Health. A 65(20),1513-30

Kreyling, W. G., Semmler, M. and Moller, W. (2004) Dosimetry and toxicology of ultrafine particles. J. Aerosol Med., 17(2), 140-52

Kumar, R., Howdle, S., Münstedt, H. (2005) Polyamide/silver antimicrobials:effect of filler types on the silver ion release. J. Biomed. Mater. Res. Part B: Appl. Biomater.,75B, 311–319

LaCoste, A., Schaich, K., Zumbrunnen, D., Yam, K. (2005) Advanced controlled release packaging through smart blending. Packaging Technology Sci 18, 77-87

Lam, C.W., James, J.T., McCluskey, R., Hunter, R.L. (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicological Sciences 77, 126–134

Lansdown, A.B., Taylor, A. (1997) Zinc and titanium oxides: promising UV-absorbers but what influence do they have on the intact skin? Int. J .Cosmet. Sci. 19(4), 167-72

Lin, W., Huang, Y-W., Zhou, X-D, Ma, Y. (2006) *In vitro* toxicity of silica nanoparticles in human lung cancer cells. Toxicology and Applied Pharmacology 217, 252-259

Linse, S., Cabaleiro-Lago, C., Xue, W.-F., Lynch, I., Lindman, S., Thulin, E., Radford, S., Dawson, K.A. (2007) Nucleation of protein fibrillation by nanoparticles. Proceedings of the National Academy of Sciences 104, 8691-8696

Livingstone, D.R. (2001) Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms, Mar. Pollut. Bull. 42, 656–666

Lomer, M.C., Thompson, R.P., Powell, J.J. (2002) Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proc Nutr Soc 61, 123–130

Loretz, B., Bernkop-Schnürch, A. (2007) *In vitro* cytotoxicity testing of non-thiolated and thiolated chitosan nanoparticles for oral gene delivery. Nanotoxicology 1, 139 – 148

Lynch, I., Dawson, K.A., Linse, S. (2006) Detecting crytpic epitopes in proteins adsorbed onto nanoparticles. Science STKE 327, 14

Lynch, I., Cedervall, T., Lundqvist, M., Cabaleiro-Lago, C., Linse, S., Dawson, K.A. (2007) The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st Century. J. Colloid Interface Sci. 134-135, 167-174

Maynard, A.D., Aitken, R.J., Butz, T., Colvin, V., Donaldson, K., Oberdörster, G., Philbert, M.A., Ryan, J., Seaton, A., Stone, V., Tinkle, S.S., Tran, L., Walker, N.J., Warheit, D.B. (2006) Safe handling of nanotechnology. Nature, 144, 267-269

Marini, M., De Niederhausern, S., Iseppi, R., Bondi, M., Sabia, C., Toselli, M., Pilati, F. (2007) Antibacterial activity of plastics coated with silver-doped organic–inorganic hybrid coatings prepared by sol–gel processes . Biomacromolecules 8, 1246–54

Meng, H., Chen, Z., Xing, G., Yuan, H., Chen, C., Zhao, F., Zhang, C., Zhao, Y. (2007) Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles. Toxicology Letters, 175, 102-110

Miller, G., Senjen, R. (2008) Friends of the Earth Australia Nanotechnology project. http://www.nano.foe.org.au

Monteiro-Riviere, N.A., Nemanich, R.J., Inman, A.O., Wang, Y.Y., Riviere, J.E. (2005) Multi-walled carbon nanotubes interactions with human epidermal keratinocytes. Toxicology Letters 155, 377-384

Morris, V.J. (2005) Is Nanotechnology going to change the future of Food Technology? The International Review of Food Science and Technology 3, 16-18

Morris, V.J. (2006) Nanotechnology and its future in new product development. Journal of the Institute of Food Science and Technology. 20 (3)15-17 2006

Mounsey, J.S., O'Kennedy, B.T., Kelly, P. M. (2005) Comparison of re-micellised casein prepared from acid casein with micellar casein prepared by membrane filtration. Le Lait 85: 419-430

Nanosilver (2004) Technical information on nano-silver powder http://www.nano-silver.net/eng/index.php

NIOSH (2007) Progress Toward Safe Nanotechnology in the Workplace. A Report from the NIOSH Nanotechnology Research Center http://www.cdc.gov/niosh/docs/2007-123/pdfs/2007-123.pdf

Oberdörster, G., Oberdörster, E., Oberdörster, J. (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives 113, 823-839

O' Hagan, D.T. (1996) The intestinal uptake of particles and the implications for drug and antigen delivery. J Anat. 189, 477-82

Owen, R., Depledge, M. (2005) Nanotechnology and the environment: risks and rewards. Editorial, Marine Pollution Bulletin 50 609-612

Papageorgiou, I., Brown, C., Schins, R., Singh, S., Newson, R., Davis, S., Fisher, J., Ingham E., Case C.P. (2007) The effect of nano- and micron-sized particles of cobalt–chromium alloy on human fibroblasts *in vitro*. Biomaterials 28, 2946-2958

Peters, A., Pope, C.A. III (2002) Cardiopulmonary mortality and air pollution. Lancet 360, 1184–1185

Poland, C.A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W.A.H., Seaton, A., Stone, V., Brown, S., Macnee, W., Donaldson, K. (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol. 3(7), 423-8

Pope, C. A., III, Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., Thurston, G. D. (2002) Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287(9), 1132-1141

Pulskamp, K., Diabaté, S., Krug, H.F. (2007) Carbon nanotubes show no sign of acute toxicity but induce cellular reactive oxygen species in dependence on contaminants. Toxicology Letters 168, 58-74

Radomski, A., Jurasz, P., Alonso-Escolano, D., Drews, M., Morandi, M., Malinski, T., Radomski, M.W. (2005) Nanoparticle-induced platelet aggregation and vascular thrombosis. Br. J. Pharmacol. 146, 882–893

Roberts, J.C., Bhalgat, M.K., Zera, R. (1996) Preliminary biological evaluation of polyamidoamine (PAMAM) Starburst dendrimers. J Biomed Mater Res.30(1), 53-65

Royal Society and Royal Academy of Engineering (2004) Nanoscience and nanotechnologies: opportunities and uncertainties. The Royal Society. 29 July 2004 http://www.nanotec.org.uk/finalReport.htm

Ryman-Rasmussen, J.P., Riviere, J.E., Monteiro-Riviere, N.A. (2006) Penetration of Intact Skin by Quantum Dots with Diverse Physicochemical Properties. Toxicological Sciences 91, 159-165

Salvador-Morales, C., Flahaut, E., Sim, E., Sloan, J., Green, M.L., Sim, R.B. (1996) Complement activation and protein adsorption by carbon nanotubes. Mol Immunol. 43(3), 193-201. Epub 2005 Mar 16

Scientific Committee on Consumer Products (2007) Preliminary opinion on safety of nanomaterials in cosmetic products http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_099.pdf

Scientific Committee on Emerging and Newly Identified Health Risks (2006) Modified Opinion (after public consultation) on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf

Scientific Committee on Emerging and Newly Identified Health Risks (2007) Opinion on the appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_010.pdf

Sharma, A., Qiang, Y., Antony, J., Meyer, D., Kornacki, P., Paszczynski, A. (2007) Dramatic Increase in Stability and Longevity of Enzymes Attached to Monodispersive Iron Nanoparticles. Magnetics, IEEE Transactions 43, 2418-2420

Shvedova, A.A., Kisin, E.R., Mercer, R., Murray, A.R., Johnson, V.J., Potapovich, A.I., Tyurina, Y.Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A.F., Antonini, J., Evans, D.E., Ku, B.-K., Ramsey, D., Maynard, A., Kagan, V.E., Castranova, V., Baron, P. (2005) Unusual inflammatory and fibrogenic pulmonary responses to singlewalled carbon nanotubes in mice. American Journal of Physiology: Lung Cellular and Molecular Physiology 289, 698–708

Siegrist, M., Stampfli, N., Kastenholz, H., Keller, C. (2008) Perceived risks and perceived benefits of different nanotechnology foods and nanotechnology food packaging. Appetite 51(2), 283-90

Singh, S., Shi, T., Duffin, R., Albrecht, C., van Berlo, D., Höhr, D., Fubini, B., Martra, G., Fenoglio, I., Borm, P.J.A., Schins, R.P.F. (2007) Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO2: Role of the specific surface area and of surface methylation of the particles. Toxicology and Applied Pharmacology 222 141-151

Smart, S.K., Cassady, A.I., Lu, G.Q., Martin, D.J., (2006) The biocompatibility of carbon nanotubes. Carbon 44, 1034-1047

Taniguchi, N. (1974) "On the Basic Concept of 'Nano-Technology'," Proc. Intl. Conf. Prod. Eng. Tokyo, Part II, Japan Society of Precision Engineering

Taylor, T.M., Davidson, P.M., Bruce, B.D., and Weiss, J. (2005) Liposomal nanocapsules in food science and agriculture. Crit. Rev. Food Sci. Nutr. 45, 1-19

Tsai, Y.Y., Oca-Cossio, J., Agering, K., Simpson, N.E., Atkinson, M.A., Wasserfall, C.H., Constantinidis, I., Sigmund, W. (2007) Novel synthesis of cerium oxide nanoparticles for free radical scavenging. Nanomed. 2(3), 325-32

Vasir., J.K., Labhasetwar, V. (2007) Biodegradable nanoparticles for cytosolic delivery of therapeutics Advanced Drug Delivery Reviews 59, 718-728

Warheit, D.B., Laurence, B.R., Reed, K.L., Roach, D.H., Reynolds, G.A., Webb, T.R. (2004) Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. Toxicological Sciences 77, 117–125

Warheit, D.B., Webb, T.R., Reed, K.L., Frerichs, S., Sayes, C.M. (2007) Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: differential responses related to surface properties. Toxicology 230(1), 90-104. Epub 2006 Nov 10

Weiss, J., Takhistov, P., McClements. J. (2006) Functional materials in food nanotechnology. J Food Sci 71(9), R107-R116

Which? (2008) Report on the Citizens' Panel examining nanotechnologies. Prepared by Opinion Leader http://www.which.co.uk/files/application/pdf/Which%20Nanotechnology%20Citizens%20Panel_Opinion%20Leader_Report%20Autumn%20071-445-133279.pdf

Xia, T., Kovochich, M., Brant, J., Hotze, M., Sempf, J., Oberley, T., Sioutas, C., Yeh, J.I., Wiesner, M.R., Nel, A.E. (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett. 6, 1794-1807

Xin-Rui. X., Monteiro-Riviere, N.A., Riviere, J.E. (2006) Trace analysis of fullerenes in biological samples by simplified liquid–liquid extraction and high-performance liquid chromatography. J. Chromat. A, 1129, 216–222

Yamago, S., Tokuyama, H., Nakamura, E., Kikuchi, K., Kananishi, S., Sueki, K., Nakahara, H., Enomoto, S., Ambe, F. (1995) *In vivo* biological behavior of a water-miscible fullerene: 14C labeling, absorption, distribution, excretion and acute toxicity. Chem Biol. 2(6), 385-9

Yan, J., Estévez, M.C, Smith, J.E., Wang, K., He, X., Wang, L., Tan, W. (2007) Dye-doped nanoparticles for bioanalysis. NanoToday, 2, 44-50

Zhong, W., Kolls, J.K., Chen, H., McAllister, F., Oliver, P.D., Zhang, Z. (2008) Chemokines orchestrate leukocyte trafficking in inflammatory bowel disease. Front Biosci. 2008, 13, 1654-1664

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