Preliminary Review of Selected Nanoparticles - their Production, Consumer Applications, Potential Exposure Routes and Potential Health Implications.

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		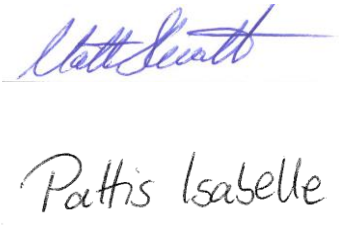
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1. INTRODUCTION

The scope of this report has been defined to cover a set of simple questions relating to the identity, nature, applications and health concerns regarding nanoparticles and some nanomaterials that may be encountered in non-occupational scenarios through contact with consumer products. The report is not intended to provide detailed analysis of health or toxicological concerns raised regarding individual nanomaterials.

The report has been prepared with the intent of being a background document, for use as a precursor to subsequent literature reviews and giving an indication of existing health concerns that are currently reported or inferred resulting from exposure to the identified nanoparticles. The selection of nanoparticles represented in this report is not exhaustive, but has been drawn from literature due to opportunity for high exposure (OECD 2010), or in the case of organic nanoparticles, because of relative novelty of the technology.

The report identifies 14 types of nanoparticles, including both established and emerging materials. The physical and chemical characteristics, method of production, relevant consumer applications or uses and the sources of non-occupational exposure are discussed for each material. Non-occupational exposures reported are for inhalation, dermal and ingestion; specific exclusions are any occupational exposure, exposure through consumption of food and medical or pharmaceutical products,

Nanoparticles can originate from natural sources (primary), artificial sources (secondary) or through intentional engineering as manufactured nanoparticles. The focus of this report is on manufactured nanoparticles.

Nanoparticles are now used extensively in the manufacture of a diverse range of consumer products[i]. The nanoparticles confer an extensive range of advantageous properties to the products in which they are used[ii]. The physicochemical properties and reactivity of nanoparticles may differ from those observed in the same materials at larger scales, this factor gives rise to concerns regarding the safety of nanomaterials in products that have unregulated exposure such as consumer products. The degree to which the nanoparticles are fixed or free within a product will determine the opportunity for their release to a receiving environment and subsequent transmission to humans, hence presenting an exposure route. Many consumer products that contain nanomaterials, such as sun protection creams, personal care products or ‘colloidal’ silver, are intended for direct application to the skin or ingestion; providing a more direct exposure route.

In this report a vignette of each of the nanoparticles identified is presented and will provide a description covering:

- Physical properties
    - Structure and forms
    - Physicochemical/surface properties.
- Method of production
- Relevant applications or uses

[i] Nanotech Project Current Products database – examples of range of products that contain nanoparticles
[ii] Knowledge Base Nanomaterials - Application of Nanomaterials

- Sources of non-occupational human exposure, and
- Specific human health concerns

2. WHAT ARE NANOPARTICLES?

2.1 UNIVERSAL CHARACTERISTICS

2.1.1 Size

Nanomaterials are defined as having one or more dimensions in the nanoscale, where the nanoscale is a range between 1 and 100 nm. For a particle to be considered as a nanoparticle it must have three external dimensions (*x*, *y* and *z* axis) in the nanoscale (ISO 2015). There are two other basic forms of nanomaterials: Nanofibres, (two external dimensions in the nanoscale, and nanosheets (one external dimension in the nanoscale).

2.1.2 Surface area of nanoparticles

One of the observed effects of producing materials at the nanoscale is the significant increase in surface area per unit mass (m^2/g). The increase in surface area has been noted to provide increased biological activity that may differ from that seen in from the same chemical or compound at larger scales. The relative ratio of surface area using a nominal 10 $\mu g/m^3$ loading of varying diameter particles has been estimated (Nel, Xia et al 2006; Oberdorster, Oberdorster et al 2005) and is shown in Table 1.

Table 1 Number of particles and surface area for 10 $\mu g/m^3$ airborne particles

Particle diameter (nm)	Particles/mL of air	Particle surface area (μm^2/ml of air)
2000	2	30
500	153	120
20	2,390,000	3000
5	153,000,000	12,000

References

ISO. 2015. Iso/ts 80004-2:2015(en)nanotechnologies - vocabulary -part 2: Nano-objects.

Nel A, Xia T, Madler L et al. 2006. Toxic potential of materials at the nanolevel. *Science* 311

Oberdorster G, Oberdorster E,Oberdorster J. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113 (7): 823-39

OECD. 2010. List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship programme for the testing of manufactured nanomaterials: Revision. *Series on the Saftey of Manufactured Nanomaterials. No.27*

2.2 FULLERENES – BUCKYBALLS

Fullerenes are any of a series of hollow carbon molecules that form either a closed cage (buckyballs) or a cylinder (nanotubes) (Kroto 2015). For the purposes of this report, the term fullerene(s) will be referring to the spherical "buckyball" structure. Carbon nanotubes are treated separately in a following section.

2.2.1 Physical and chemical properties

Fullerene molecules are typically of very low density, 1.68 g/cm^3 compared to other carbon allotropes graphite (2.1 – 2.3 g/cm^3) and diamond (3.51 g/cm^3). Unlike graphite, fullerenes are not electrically conductive. The buckyball fullerenes are typically less than 1 nm in diameter, and are described as nanoparticles through an exception to the rule stating nanoparticles should measure 1 – 100 nm in diameter. The most common fullerene is the C_{60} form, composed 60 carbon atoms joined by single or double bonds forming 12 pentagonal and 20 hexagonal faces in a 'spherical' geometry. Other forms of carbon fullerenes that contain 70, 74, 76, 78, 80, 84, 90, and 94 carbon atoms in varying geometries have also been isolated.

Fullerenes are the only carbon allotrope that is soluble in organic solvents. The fullerene cage is destroyed by exposure to ultra-violet (UV) radiation, particularly in the presence of oxygen (O_2). The C_{60} molecule demonstrates a wide range of chemical reactivity, readily accepting and donating electrons.

2.2.2 Method of production

Fullerenes can be produced at industrial scales through two techniques. The earliest derived of these techniques is through generation of an electrical arc between two graphite electrodes in an inert, helium, atmosphere. The more recent and preferred method for production is a combustion method. It is preferred as it produces a higher yield of fullerenes at lower cost and a greater range of higher carbon content fullerene molecules (Ozawa, Deotaf et al 1999; Takeara, Fujiwara et al 2004).

2.2.3 Relevant applications or uses

Fullerenes are currently applied in cosmetics and sports goods industries[iii] and as experimental additives to some automotive lubricants (Zhmud and Pasalskiy 2013). The role of fullerenes in cosmetics is that of a free-radical scavenger, using the electron-affinity of the molecule to prevent interaction of skin cells with radicals. They are thus employed in 'anti-ageing' creams. Fullerenes main role in sporting goods is as a lightweight strengthening agent for carbon structured equipment such as golf club shafts, bicycle frames and tennis or badminton racquets. The presence of fullerenes in these items enables production of thin-walled, lightweight robust carbon structures[iv]. Experimental studies suggest that C_{60} fullerene soot in lubricant significantly increases the weld load and seizure resistance of machine components (Titov 2004; Zhmud and Pasalskiy 2013). Other current uses include, fuel cells, solar cells, batteries, catalysts, polymer modifications and targeted drug delivery systems (Aschberger, Johnston et al 2010).

[iii] Fullerenes & Fullerene Nanoparticles | Knowledge Base Nanomaterials
[iv] Fullerenes & Fullerene Nanoparticles | Knowledge Base Nanomaterials

2.2.4 Sources of non-occupational human exposure

Inhalation exposure

Small quantities of fullerenes may be produced during combustion of carbonaceous material and inhaled. The passage of fullerenes across the air-blood barrier has not been demonstrated in animal models (Baker, Gupta et al 2008). In the same study the authors reported a biological half-life of 26 days for fullerenes, similar to the 29 days for carbon micro particles; suggesting a similar elimination mechanism from the lungs. It was also reported that lung deposition was 50% greater for fullerenes than carbon micro particles.

Dermal exposure

Skin exposure to fullerenes in lipophilic solution or suspension such as some skin creams allowed penetration of the nanoparticle deep into the *stratum corneum* in both *in vivo* and i*n vitro* experiments using weanling pig or biopsied skin (Xia, Monteiro-Riviere et al 2010). In a separate study carried out using human skin biopsies, fullerenes in a squalane (lipid or shark-liver oil) matrix at high concentrations (up to 223 ppm) penetrated the epidermis but not the dermis, indicating that fullerenes would not become systemically available via this exposure route (Kato, Aoshima et al 2009).

Ingestion exposure

Due to the forms that fullerene-containing products take, such as sports equipment and beauty/skincare products, it is unlikely that ingestion would be undertaken deliberately. However, the opportunity for accidental ingestion of beauty and skincare products should be considered probable. A study carried out using rats and mice showed that C_{60} was not readily absorbed, with the majority being excreted in faeces within 48 hours. However, some transmission across the gut wall was indicated by the presence of fullerenes in subjects' urine (Yamago, Tokoyuma et al 1995). Further indication of absorption via the oral route was described when oxidative damage was observed in lung and liver tissue of rats administered fullerenes by gavage in either a saline or corn-oil carrier (Folkmann, Risom et al 2009).

2.2.5 Specific human-health concerns

No data have been found showing adverse health effects of fullerenes in consumer products. Although data from use of consumer products indicates a low potential risk to human health it must be recognised that this data is generated from animal studies and no human studies or epidemiological studies are available. Therefore there are significant data gaps regarding the toxicological properties and potential exposure scenarios, with the latter growing as new applications for fullerenes are realised (Mikkelsen, Hansen et al 2011).

2.2.6 References

Aschberger K, Johnston HJ, Stone V et al. 2010. Review of fullerene toxicity and exposure--appraisal of a human health risk assessment, based on open literature. *Regulatory Toxicology and Pharmacology* 58 (3): 455-73

Baker GL, Gupta A, Clark ML et al. 2008. Inhalation toxicity and lung toxicokinetics of C_{60} fullerene nanoparticles and microparticles. *Toxicological Sciences* 101 (1): 122-31

Folkmann JK, Risom L, Jacobsen NR et al. 2009. Oxidatively damaged DNA in rats exposed by oral gavage to C_{60} fullerenes and single-walled carbon nanotubes. *Environmental Health Perspectives* 117 (5): 703-8

Kato S, Aoshima H, Saitoh Y et al. 2009. Biological safety of lipo-fullerene composed squalane and fullerene- C_{60} upon mutagenesis, photocytotoxicty, and permeability into the human skin tissue. *Basic and Clinical Pharmacology and Toxicology* 104 (6): 483-7

Kroto HW. 2015. *Fullerene*. 5 November 2015. http://www.britannica.com/science/fullerene

Mikkelsen SH, Hansen E, Christensen TB et al. 2011. *Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials. Environmental project no. 1370 2011*. 5 November 2015. http://www2.mst.dk/udgiv/publications/2011/08/978-87-92779-09-0.pdf

Ozawa M, Deotaf P,Osawa E. 1999. Production of fullerenes by combustion. *Fullerene Science and Technology* 7 (3): 387-409

Takeara H, Fujiwara M, Arikawa M et al. 2004. Experimental study of industrial scale fullerene production by combustion synthesis. *Carbon* 43 (2): 311-9

Titov A. 2004. *Effect of fullerene containing lubricants on wear resistance of machine components in boundary lubrication*. Newark, New Jersey, USA: New Jersey Institute of Technology DoME

Xia XR, Monteiro-Riviere NA,Riviere JE. 2010. Skin penetration and kinetics of pristine fullerenes (C_{60}) topically exposed in industrial organic solvents. *Toxicology and Applied Pharmacology* 242 (1): 29-37

Yamago S, Tokoyuma H, Nakamura E et al. 1995. In vivo biological behaviour of a water-miscible fullerene: 14c labelling, absorption, distribution, excretion and acute toxicity. *Chemistry and Biology* 2 (6): 385-9

Zhmud B,Pasalskiy B. 2013. Nanomaterials in lubricants: An industrial perspective on current research. *Lubricants* 1 95-101

2.3 CARBON NANOTUBES

Carbon nanotubes are a type of fullerene composed of a series of hollow carbon molecules that form a cylinder (Kroto 2015). For the purposes of this report, the term fullerene(s) refers to the spherical "buckyball" structures which are treated in a previous section. Properties and applications of carbon nanotubes are described in the following section.

2.3.1 Physical and chemical properties

Carbon nanotubes (CNTs) are rolled-up graphene layers that are, ideally, hollow on the inside. They can be open at the ends or can have a fullerene-like cap.

Carbon nanotubes are divided into two classes: single-wall carbon nanotubes (SWCNTs) and multi-wall carbon nanotubes (MWCNTs). Single-wall carbon nanotubes usually have a diameter of 0.7 to 4 nm. Due to their energy-rich surfaces, they often occur as a bundle. SWCNTs can vary in their crystal structure depending on the chirality with which the graphene layer is rolled. This differentiation between SWCNTs is important, as only the so called "armchair" structure exhibits metallic (thermal) conductivity. All other chiralities can be electrically conductive or semi-conductive, depending on the chiral angle and diameter of the CNTs.[v] MWCNTs can be imagined as tubes nested within one another (Russian doll type) or as a graphene layer rolled up multiple times (scroll type). They usually have a diameter in the range of 5–20 nm. However, large MWCNTs with diameters up to 100 nm are also known. Because of their structure, MWCNTs are always electrically conductive.

Due to their unique properties CNTs are very stable and their potential applications are of interest for research and industry. The tensile strength of a multi-walled CNT was determined to be 63 GPa, which is about 50 times that of steel, but at a much lower weight. Moreover, CNTs may also be electrically insulating, semiconducting or of metallic conductance, depending on the way they are manufactured. [vi]

2.3.2 Method of production

Various processes are used for the industrial manufacture of SWCNTs. In addition to catalytic deposition from the gas phase (catalytic chemical vapour deposition, CCVD), the arc discharge process between carbon electrodes and the laser ablation of graphite are well known; all of these processes lead to a mixture of chiralities. In contrast, MWCNTs are almost exclusively produced using the cost-effective CCVD process with high catalyst yields on an industrial scale. Various grades with different sizes and purities of the nanotubes are commercially available. Like SWCNTs, MWCNTs have a very high modulus of elasticity, as well as very high electrical and thermal conductivity. Since they are commercially available in large quantities, have a high length-to-diameter ratio, and therefore produce good electrical conductivity and high mechanical strength even in small amounts, they are frequently used today for electrically conductive coatings and in composite materials.[vii]

Due to the production process, the nanotubes are mainly present as agglomerates or aggregates. To allow their outstanding properties to fully develop, it is necessary to disperse them homogeneously and as gently as possible in the medium or in one of its components. Different technologies are used depending on the viscosity and nature of the medium, including high-pressure shear dispersion, ultrasound dispersion and three-roll mill.

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[v] Netzwerk Nanocarbon - Carbon Nanotubes accessed 09/11/15
[vi] Knowledge Base Nanomaterials - Carbon Nanotubes accessed 11/11/15
[vii] Netzwerk Nanocarbon - Carbon Nanotubes accessed 09/11/15

2.3.3 Relevant applications or uses

Due to their unique properties, CNTs might be useful for a wide range of applications.

Carbon nanotubes are used in lightweight construction, including concrete, heavy-duty components in automotive construction, incorporated in the airframe of aircraft, and for satellite components as epoxy resins with CNTs.[viii] The shipping sector utilises CNTs as a coating for ships to prevent growth of marine organisms and thus reduce sliding friction, leading to a reduction in fuel consumption. Masts and other parts of sailing vessels are also produced based on CNTs. And CNTs are applied as coating of rotor blades for wind turbines. Plastic parts and seals based on elastomers with CNTs reveal better properties with regard to friction, lubrication and wear.

There are a number of applications in the sports and leisure sector as well, mainly using CNTs to increase durability and break strength, antistatics, and weight reduction in sporting goods. These include tennis and table tennis rackets, hockey sticks, golf clubs, mountain bike frames, tires and further bike components like bars, forks and seat posts. Skis, kayaks and sports arrows can be optimised using CNTs. The main role of CNTs in sporting goods is as a lightweight strengthening agent for carbon structured equipment.[ix]

Other applications of CNTs are enhancing the performance of body armors such as vests, helmets, gloves and pants without adding extra weight,[x,xi] and fire protection for foam and coating of cables and wires. Formulations with CNTs and aluminum, in the form of sacrificial aluminum dust, are designed to protect metal surfaces in corrosive environments.

2.3.4 Sources of non-occupational human exposure

Since CNTs are produced in low amounts and since their current application is limited, the chance of direct contact of humans with unbound CNTs is low. Carbon nanotubes are used in composite materials or electronic components, which don't release nanotubes under normal circumstances.

Inhalation exposure

Up to now CNTs have not been added to foods or to cosmetic products. Therefore it is their uptake via the lung into the body that represents the most likely route for potential exposure, and this route would predominantly, if not exclusively, be an occupational exposure. Due to their long and fibre-like structure carbon nanotubes may elicit fibre-like (adverse) biological effects in the lung.[xii] During consumer use, for example. a CNT polymer composite tennis racket can release CNTs whenever the racket is scratched by abrasion on the court. While amounts released from the tennis racket will be minimal (especially when compared to releases during the manufacturing and production phases), the release of CNTs from memory devices during consumer use, such as in a cell phone, is not considered likely. Likewise, the potential for release at the end of life is orders of magnitude higher for the tennis racket than for the memory device (Jacobs, Ellenbecker et al 2014).

[viii] Netzwerk Nanocarbon - Carbon Nanotubes accessed 09/11/15
[ix] Netzwerk Nanocarbon - Carbon Nanotubes accessed 09/11/15
[x] Consumer Product Inventory - Carbon Nanotubes accessed 11/11/15
[xi] The Nanodatabase - Carbon Nanotubes accessed 11/11/15
[xii] Knowledge Base Nanomaterials - Carbon Nanotubes accessed 11/11/15

Dermal exposure

So far, it has not been investigated whether carbon nanotubes can be taken up via the skin.[xiii] Up to now CNTs have not been added to cosmetic products, thus the risk of dermal exposure in a non-occupational setting is considered to be low. Generally it is assumed that the skin is a very good barrier against nanotubes, as for example tattooing ink contains carbon particles and is deposited in the deeper layers of the skin. From there, only very few of these particles are transported into the nearby lymphatic vessels while most of them remain just where they are to stay permanently in the skin for a lifetime. Since the surface characteristics of carbon nanotubes are very similar to those of these carbon particles, it can be inferred that transport of the former is only weak. Preliminary studies on dermal and eye irritation and skin sensitization of two different SWCNTs and two different MWCNTs revealed no skin-sensitization effects and showed that one product of MWCNTs was a very weak and reversible acute irritant to the skin and eyes (Ema, Matsuda et al 2011).

Ingestion exposure

Carbon nanotubes are not permitted for use in foods, and there are no indications of their being allowed to be applied in the near future.[xiv] In theory, they can be transported into the gastrointestinal tract through unwanted hand-to-mouth transfer or when inhaled CNTs get into the gastrointestinal tract through coughing up or swallowing and that they are distributed from there further in the body. So far, only a few analyses exist on that topic. The direct administration of water-soluble CNTs into the stomachs of mice showed that for a very short time, the CNTs stayed in the gastrointestinal tract. They were excreted via the stool within 12 hours and did not pass into the bloodstream (Deng, Jia et al 2007). No biological effects were found.

2.3.5 Specific human-health concerns

So far, little research has been conducted. In order to guarantee the safety of CNT and products based on them, two comprehensive projects of the Innovation Alliance CNT, CarboSafe and CarboLifeCycle, concentrate on questions that address the potential health effects and environmental impact during production, processing, use, and recycling of products that contain CNT. The project results will consist of methods and measures to guarantee the safety of CNT throughout their lifecycle.[xv]

Studies have shown that specific CNTs, namely those that are long (10-20 µm), thin (5-10 nm), needle-shaped and insoluble (biopersistent), can promote lung diseases and show behaviour similar to that of asbestos fibres. Short or long fibres that are not needle-shaped induced no inflammatory changes (Fries, Greßler et al 2012). Further studies on mice (Ma-Hock, Treumann et al 2009; Mitchell, Gao et al 2007; Mitchell, Lauer et al 2009) have shown that carbon nanotubes can penetrate through to the deepest regions of the lung (Mitchell, Gao et al 2007; Shvedova, Kisin et al 2005). Some research groups describe stress reactions in the lung tissues, such as inflammation reactions in the lung, granuloma-formation in the epithelial tissues of the lung, cell damage, fibroses (abnormal growth of the connective tissue), and changes in the lung function. Others, however, detect only small or no changes in the lung but describe modified systemic immune reactions (for example in spleen and lymph nodes). The studies differ in several essential aspects: use of different or

[xiii] Knowledge Base Nanomaterials - Carbon Nanotubes accessed 11/11/15
[xiv] Knowledge Base Nanomaterials - Carbon Nanotubes accessed 11/11/15
[xv] Innovation CNT - Carbon Nanotubes accessed 11/11/15

differently functionalized carbon nanotubes, administration in different ways and for different lengths of time, use of different analysis methods. In conclusion, a direct comparison or final comprehensive toxicity assessment has not been possible.[xvi]

Given the range of possible applications of CNTs, the use is anticipated to increase significantly in the near future and thus it is deemed necessary to closely monitor manufacturing, use and recycling of CNT containing products.

2.3.6 References

Deng X, Jia G, Wang H et al. 2007. Translocation and fate of multi-walled carbon nanotubes in vivo. *Carbon* 45 (7): 1419-24

Ema M, Matsuda A, Kobayashi N et al. 2011. Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes. *Regulatory Toxicology and Pharmacology* 61 (3): 276-81

Fries R, Greßler S,Simkó M. 2012. *Carbon nanotubes – part ii: Risks and regulations (nanotrust dossier no. 024en – february 2012)*. 16 November 2015. http://epub.oeaw.ac.at/ita/nanotrust-dossiers/dossier024en.pdf

Jacobs MM, Ellenbecker M, Hoppin P et al. 2014. *Precarious promise: A case study of engineered carbon nanotubes*. Lowell, Massachusetts, USA: Lowell Center for Sustainable Production UoML

Kroto HW. 2015. *Fullerene*. 5 November 2015. http://www.britannica.com/science/fullerene

Ma-Hock L, Treumann S, Strauss V et al. 2009. Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. *Toxicological Sciences* 112 (2): 468-81

Mitchell LA, Gao J, Wal RV et al. 2007. Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. *Toxicological Sciences* 100 (1): 203-14

Mitchell LA, Lauer FT, Burchiel SW et al. 2009. Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. *Nature Nanotechnology* 4 (7): 451-6

Shvedova AA, Kisin ER, Mercer R et al. 2005. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *American Journal of Physiology - Lung Cellular and Molecular Physiology* 289 (5): L698-L708

[xvi] Knowledge Base Nanomaterials - Carbon Nanotubes accessed 11/11/15

2.4 SILVER NANOPARTICLES

2.4.1 Physical and chemical properties

Silver nanoparticles (Ag nanoparticles) range in size from 1 to 100 nm. The processes for production also produce particles of greater diameter, up to 250 nm, though these exceed the size range that defines a nanoparticle. The physicochemical properties such as size, shape, surface charge and coating, agglomeration and dissolution rate have significant influence in determining their biological activity (Rycenga, Cobley et al 2011).

2.4.2 Method of production

There are three main methods for production of Ag nanoparticles; physical, chemical, and biological (Abou El-Nour, Eftaiha et al 2010; Haider and Kang 2015; Iravani, Korbekandi et al 2014; Wei, Lu et al 2015). Physical production is predominantly through a thermal desorption or ablation of silver from a metallic silver parent material. The particle size produced through these methods is determined by the amount of energy put into the parent material, the closer the energy to the minimum required for desorption/ablation, the smaller the particle size produced. A significant benefit of the physical production process is the lack of additives required in production, hence in the material.

Chemical production of Ag nanoparticles is primarily through chemical reduction of silver solutions using organic and inorganic reducing agents. Additional chemical additives (poly vinylpyrrolidone for example) are required to prevent the continuing agglomeration of the Ag nanoparticles into larger flocs that exceed the nanoscale and reduce the surface area and may modify the biological activity of the material. Biological production of Ag nanoparticles has been achieved through use of bacterial and fungal strains. Different bacterial and fungal species provide a range of different particle sizes and morphologies. Biological production also provides opportunities for reduction of some of the environmental impacts of large-scale chemical production (Iravani, Korbekandi et al 2014).

2.4.3 Relevant applications or uses

A European online database of nanomaterials[xvii] and their activities identified Ag nanoparticles being used in cleaning agents, wall colour/paints, printing ink, soaps and personal care products, soft toys, textiles, toothpaste, wallpaper, colloidal silver 'therapeutic' products and wound dressings. The Project on Emerging Nanotechnologies (PEN)[xviii] identifies over 400 products containing Ag nanoparticles currently in use in the USA marketplace. In all of these applications silver is used for it's bactericidal properties (Fries, Gressler et al 2010).

The nanoparticle size and the method of inclusion in products vary significantly and are factors that determine both the efficacy of anti-microbial activity and the potential for release to the local environment. In some products, such as wound dressings it is desirable for the silver to migrate from product into the dermis of the protected area, thereby giving localised infection control.

[xvii] Knowledge Base Nanomaterials – accessed 10/11/15
[xviii] Nanotech Project - Silver - accessed 10/11/15

2.4.4 Sources of non-occupational human exposure

Inhalation exposure

Inhalation exposure is a potential route for exposure in non-occupational settings through the application of aerosols containing nanosilver, such as cleaning-agents sprays, and personal care product aerosols, such as deodorant or antiperspirant.

Dermal exposure

Dermal exposure is a significant route of exposure as many of the products are intended for use or treatment of both intact and broken skin. In many cases the transfer of the nano-silver to the skin is the intended method of action of a product (wound dressings for instance). Further exposure may be received through products not intended for dermal application or contact, such as cleaning products, paint, or printing ink.

Ingestion exposure

Intentional ingestion of nanosilver through use of colloidal silver based products may represent a significant level exposure (Hadrup and Lam 2014). Unintentional exposures are possible through mouthing activities leading to incidental transfer of products from skin to mouth; and through ingestion of aerosols.

2.4.5 Specific human-health concerns

The Scientific Committee on Emerging and Newly Identified Health Risks produced an opinion on the human health effects of nanosilver (SCENHIR 2014), identifying the following concerns. The best-described adverse chronic effects in humans is argyria, a bluish/grey discolouration of the skin or eyes. Other than this condition exposure to soluble silver may produce damage to the liver and kidney, irritation of the eyes, skin and respiratory tract. Bio-distribution studies in rodent models showed that silver nanoparticle exposure by inhalation, ingestion, or intra-peritoneal injection were subsequently detected in blood and caused toxicity in several organs including the liver and brain. Further to these findings the report stated that some studies had indicated that silver nanoparticles exerted developmental and structural malformations in model organisms (Ahamed, Alsalhi et al 2010; Christensen, Johnston et al 2010; Stensberg, Wei et al 2011; Wijnhoven, Peijnenburg et al 2009). The potential impacts on human health are difficult to describe due to the paucity of data at likely exposure levels.

2.4.6 References

Abou El-Nour KMM, Eftaiha Aa, Al-Warthan A et al. 2010. Synthesis and applications of silver nanoparticles. *Arabian Journal of Chemistry* 3 (3): 135-40

Ahamed M, Alsalhi MS,Siddiqui MK. 2010. Silver nanoparticle applications and human health. *Clinica Chimica Acta* 411 (23-24): 1841-8

Christensen FM, Johnston HJ, Stone V et al. 2010. Nano-silver - feasibility and challenges for human health risk assessment based on open literature. *Nanotoxicology* 4 (3): 284-95

Fries R, Gressler S, Simkó M et al. 2010. *Nanosilver (nanotrust dossier no. 010en)*. 16 November 2015. http://epub.oeaw.ac.at/ita/nanotrust-dossiers/dossier010en.pdf

Hadrup N,Lam HR. 2014. Oral toxicity of silver ions, silver nanoparticles and colloidal silver – a review. *Regulatory Toxicology and Pharmacology* 68 (1): 1-7

Haider A,Kang I-K. 2015. Preparation of silver nanoparticles and their industrial and biomedical applications: A comprehensive review. *Advances in Materials Science and Engineering* 2015 16

Iravani S, Korbekandi H, Mirmohammadi SV et al. 2014. Synthesis of silver nanoparticles: Chemical, physical and biological methods. *Research in Pharmaceutical Sciences* 9 (6): 385-406

Rycenga M, Cobley CM, Zeng J et al. 2011. Controlling the synthesis and assembly of silver nanostructures for plasmonic applications. *Chemical Reviews* 111 (6): 3669-712

SCENHIR. 2014. *Nanosilver: Safety, health and environmental effects and role in antimicrobial resistance*. Scientific Committee on Emerging and Newly Identified Health Risks http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_039.pdf

Stensberg MC, Wei Q, McLamore ES et al. 2011. Toxicological studies on silver nanoparticles: Challenges and opportunities in assessment, monitoring and imaging. *Nanomedicine (Lond)* 6 (5): 879-98

Wei L, Lu J, Xu H et al. 2015. Silver nanoparticles: Synthesis, properties, and therapeutic applications. *Drug Discovery Today* 20 (5): 595-601

Wijnhoven SWP, Peijnenburg WJGM, Herbert CA et al. 2009. Nanosilver - a review of available data and knowledge gaps in human and ebvirnmental risk assessment. *Nanotoxicology* 3 109-38

2.5 TITANIUM DIOXIDE (CAS NUMBER 1317-80-2)

2.5.1 Physical and chemical properties

Titanium dioxide (TiO_2) nanoparticles exist in three crystalline structures; rutile, anatase and brookite. All forms are insoluble in water[xix]. The three structures can vary in size and shape, can form agglomerates or aggregates. Brookite is less common than rutile or anatase; and samples of TiO_2 nanoparticles can contain more than one structural form. Rutile and anatase possess tetragonal crystal structure, whereas brookite has an orthorhombic crystal structure. These crystal structures are retained at the nanoscale and provide some differential thermodynamic characteristics based on particle size. Hence, at the low end of the nanoscale, with TiO_2 particles less than 14nm in diameter anatase is predominant as it becomes thermodynamically more stable than the more abundant rutile form (Zhang and Banfield 1998).

2.5.2 Method of production

Titanium dioxide mainly occurs with other types of minerals, the most common source for manufacture is through processing from ilmenite ore ($FeTiO_2$). There are two main production methods for TiO_2. The dissolution of ilmenite in sulphuric acid followed by precipitation of the iron sulphate leaving pure TiO_2 in solution (Winkler 2003); or the chloride process which is applied to naturally-occurring rutile ores[xx]. The chloride process may also use an addition of aluminium chloride to promote the development of rutile over anatase TiO_2. Further processing is required to produce nanoscale TiO_2. The so-called 'titanium alkoxylates' can be hydrolysed and subsequently treated thermally. The particles' crystal modification depends on the temperature applied during the process. Moreover, nanoscale titanium oxide particles can be obtained by reacting titanium chloride compounds with ammonia. Under the influence of heat, the titanium oxide hydrate forming during that reaction turns into rutile TiO_2. The aerosol method that was developed by Degussa in the 1940's for silicon dioxide was applied to TiO_2in the 1950's. It enables production of nanoscale TiO_2 from titanium chloride compounds through reaction of the latter with water vapour[xxi].

2.5.3 Relevant applications or uses

The uses for TiO_2 are diverse due to its various forms and sizes. The micro-scale applications in pigment production are not relevant at the nanoscale as the refractive properties of TiO_2 are lost. Nanoscale TiO_2 is found in high-factor sun protection cream. Nanoscale TiO_2 has replaced micro-scale TiO_2 in sun protection creams as it makes a more aesthetically pleasing product, that is easier to apply (especially in aerosol or pump-spray form) and provides better ultra-violet (UV) protection.

Aside from sun protection products, nanoscale TiO_2 is also noted as being present in some paints, wood preservatives and air fresheners; as well as a range of personal care products such as shampoo, hair conditioner, deodorants, lip balms and shaving foam[xxii]. In each of these products the TiO_2 may fulfil a number of functions, for instance rutile and anatase in paint both provide antibacterial, fire-retardant, self-cleaning and thermal insulation

[xix] CDC - NIOSH Pocket Guide to Chemical Hazards - Titanium dioxide accessed 21/10/15
[xx] Millennium Inorganic Chemicals - Titanium Dioxide Manufacturing Processes accessed 21/10/15
[xxi] Titanium Dioxide Nanoparticles - Knowledge Base Nanomaterials accessed 21/10/15
[xxii] Consumer Product Inventory - Project on Emerging Nanotechnologies - Titanium dioxide accessed 21/10/15

properties; rutile also provides UV protection, whereas anatase increases the ease of cleaning[xxiii].

Additionally, TiO_2 exhibits good photo-catalytic properties, hence is used in antiseptic and antibacterial compositions; it can be applied to degrade organic contaminants and microorganisms; in the manufacture of printing ink, self-cleaning ceramics and glass, coating; and in the paper industry for improving the opacity of paper.

2.5.4 Routes of non-occupational human exposure

The list of products identified contains a large number of personal care products when including sun protection creams. It is these that are likely to provide the highest concentrations and most frequent exposures.

Inhalation exposure

Inhalation provides a significant route for potential exposure. The opportunity for this exposure route to become of greater significance in non-occupational settings has grown with the increasing number of spray products such as sun protection cream, deodorant and air fresheners containing TiO_2. The non-occupational exposure may be more likely to produce a low-concentration chronic exposure rather than high-concentration, acute exposure.

Dermal exposure

Four studies carried out over the last 15 years have shown that intact skin provides an effective barrier to penetration by TiO_2 nanoparticles, due to the multiple layers of tissue present (Gamer, Leibold et al 2006; Monteiro-Riviere, Wiench et al 2011; Nohynek, Lademann et al 2007; Pflücker, Wendel et al 2001). Furthermore, the study of Monteiro-Riviere et al (2011) showed that TiO_2 did not penetrate the remaining layers of damaged skin, so application of sunscreen to damaged or (sun)burned skin did not present an enhanced risk via the dermal exposure route.

Ingestion exposure

This report is not concerned with exposures that may occur through consumption of food, a route which presents significant opportunity of increased exposure. However, we should consider other opportunities for incidental ingestion from hand to mouth ingestion of sun protection creams or other personal care products.

In vitro studies with human intestinal cells showed that high doses (20 and 80 µg/cm^2) on TiO_2 cause, after 24 h of incubation, significant membrane damage leading to a loss of vitality and may induce toxicity. After 4 h, however, the cells showed no reactions. TiO_2 particles in this study caused no DNA strand breaks or oxidative DNA damage. When different versions of TiO_2 with different surfaces were used, surface-dependent effects could not be found. The authors concluded from their *in vitro* studies using intestinal cells that ingestion of TiO_2 particles poses no risk (Gerloff, Albrecht et al 2009).

2.5.5 Specific human-health concerns

IARC have evaluated nanoscale TiO_2 as a group 2B carcinogen - possibly carcinogenic to humans. The rationale for this classification was based on sufficient evidence of carcinogenicity being found in animal studies, whereas the evidence of human carcinogenicity was inadequate (IARC 2010).

[xxiii] Nanoparticles in paints accessed 22/10/15

The role of TiO_2 nanoparticles in the promotion of a range of conditions relating to pulmonary function have been identified, predominantly in animal models where pulmonary inflammation was increased but no clinical pathologies were observed (Chen, Su et al 2006; Grassian, O'Shaughnessy P et al 2007; Han, Newsome et al 2013; Huang, Wu et al 2015; Monteiller, Tran et al 2007; Rossi, Pylkkanen et al 2010; Scarino, Noel et al 2012; Scuri, Chen et al 2010; Shi, Magaye et al 2013; Yu, Sung et al 2015); these are supported by some cohort based epidemiological reviews carried out using occupationally exposed cohorts (Kendall and Holgate 2012; Koivisto, Lyyranen et al 2012) and human cell-culture techniques[xxiv].

2.5.6 References

Chen HW, Su SF, Chien CT et al. 2006. Titanium dioxide nanoparticles induce emphysema-like lung injury in mice. *FASEB Journal* 20 (13): 2393-5

Gamer AO, Leibold E,van Ravenzwaay B. 2006. The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin. *Toxicology in Vitro* 20 (3): 301-7

Gerloff K, Albrecht C, Boots AW et al. 2009. Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal caco-2 cells. *Nanotoxicology* 3 (4): 355-64

Grassian VH, O'Shaughnessy P T, Adamcakova-Dodd A et al. 2007. Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. *Environmental Health Perspectives* 115 (3): 397-402

Han SG, Newsome B,Hennig B. 2013. Titanium dioxide nanoparticles increase inflammatory responses in vascular endothelial cells. *Toxicology* 306 1-8

Huang KT, Wu CT, Huang KH et al. 2015. Titanium nanoparticle inhalation induces renal fibrosis in mice via an oxidative stress upregulated transforming growth factor-beta pathway. *Chemical Research in Toxicology* 28 (3): 354-64

IARC. 2010. *Carbon black, titanium dioxide and talc. Iarc monographs on the evaluation of carcinogenic risks to humans*. Volume 93. Lyon, France: (IARC) IAfRoC

Kendall M,Holgate S. 2012. Health impact and toxicological effects of nanomaterials in the lung. *Respirology* 17 (5): 743-58

Koivisto AJ, Lyyranen J, Auvinen A et al. 2012. Industrial worker exposure to airborne particles during the packing of pigment and nanoscale titanium dioxide. *Inhalation Toxicology* 24 (12): 839-49

Monteiller C, Tran L, MacNee W et al. 2007. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: The role of surface area. *Occupational and Environmental Medicine* 64 (9): 609-15

Monteiro-Riviere NA, Wiench K, Landsiedel R et al. 2011. Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in uvb sunburned skin: An in vitro and in vivo study. *Toxicological Sciences* 123 (1): 264-80

Nohynek GJ, Lademann J, Ribaud C et al. 2007. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Critical Reviews in Toxicology* 37 (3): 251-77

[xxiv] NanoCare Final Report 2009 - inhalation of nanoparticles – accessed 21/10/15

Pflücker F, Wendel V, Hohenberg H et al. 2001. The human stratum corneum layer: An effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide. *Skin Pharmacology and Physiology* 14(suppl 1) (Suppl. 1): 92-7

Rossi EM, Pylkkanen L, Koivisto AJ et al. 2010. Airway exposure to silica-coated tio2 nanoparticles induces pulmonary neutrophilia in mice. *Toxicological Sciences* 113 (2): 422-33

Scarino A, Noel A, Renzi PM et al. 2012. Impact of emerging pollutants on pulmonary inflammation in asthmatic rats: Ethanol vapors and agglomerated tio2 nanoparticles. *Inhalation Toxicology* 24 (8): 528-38

Scuri M, Chen BT, Castranova V et al. 2010. Effects of titanium dioxide nanoparticle exposure on neuroimmune responses in rat airways. *Journal of Toxicology and Environmental Health. Part A* 73 (20): 1353-69

Shi H, Magaye R, Castranova V et al. 2013. Titanium dioxide nanoparticles: A review of current toxicological data. *Part Fibre Toxicol* 10 (1): 15

Winkler J. 2003. *Titanium dioxide*. Hannover: Vincentz Network

Yu KN, Sung JH, Lee S et al. 2015. Inhalation of titanium dioxide induces endoplasmic reticulum stress-mediated autophagy and inflammation in mice. *Food and Chemical Toxicology* In press Corrected proof

Zhang H,Banfield JF. 1998. Thermodynamic analysis of phase stability of nanocrystalline titania. *Journal of Materials Chemistry* 8 2073-6

2.6 ALUMINIUM OXIDE

2.6.1 Physical properties

While aluminium oxide or alumina (Al_2O_3) occurs in a number of transitional forms, two forms appear to be important as nanoparticles; α-alumina (alpha-alumina) and γ-alumina (gamma-alumina).[xxv] Alpha-alumina, also known as corundum, is characterised by its high hardness, thermal and chemical stability, thermal conductivity and electrical resistance (Hakuta, Nagai et al 2013; Piriyawong, Thongpool et al 2012; Sadabadi, Aftabtalab et al 2013). Gamma-alumina also has high mechanical strength and excellent thermal and chemical stability, but is soluble in strong acids and in bases and has high absorption capacity due to its large surface area (Peng, Zhang et al 2015).

2.6.2 Method of production

Alumina nanoparticles can be produced by either 'top down' or 'bottom up' techniques (Hakuta, Nagai et al 2013; Piriyawong, Thongpool et al 2012; Sadabadi, Aftabtalab et al 2013). While ball-milling of bulk alumina was shown to reduce the particle size to the required nanometre range, the high hardness of the alumina resulted in unacceptable contamination of the nanomaterial from the milling media (Reid, Forrester et al 2008). Bottom up production methods have included; production of soluble amorphous gels (sol-gel) from precursor chemicals (e.g. aluminium isopropoxide) (Mirjalili, Hasmaliza et al 2010), deposition following pyrolysis (Kavitha and Jayaram 2006), radio-frequency 'sputtering' (Paven-Thivet, Malibert et al 1998) or laser ablation (Piriyawong, Thongpool et al 2012) and hydrothermal phase transition (Hakuta, Nagai et al 2013).

Nanofibres can be produced from alumina nanoparticles by electrospinning (Peng, Zhang et al 2015).

2.6.3 Relevant applications or uses

Due to their high stability, alumina nanoparticles, particularly α-alumina, are used as fillers in metals, ceramics and plastics, paints, polishes, cosmetics and electric substrates (Hakuta, Nagai et al 2013). The surface properties of γ-alumina have led to use in nanofiltration and as a catalyst support (Rozita, Brydson et al 2010).

With respect to consumer products, the Consumer Products Inventory lists cosmetic, disinfectant and car polish products containing alumina nanoparticles.[xxvi] It should be noted that the disinfectant products include silver nanoparticles, as the active component, attached to 15 nm alumina-silica particles. Other products involve the incorporation of alumina nanoparticles into solid composites, such as bicycle frames and computer components.

2.6.4 Sources of non-occupational human exposure

Inhalation

There is potential for inhalation exposure to alumina nanoparticles from use of disinfectant sprays. The high thermal stability of alumina nanoparticles means they will not be easily volatilised and inhalation exposure is mainly likely from the generation of sprays or mists.

[xxv] http://www.nanopartikel.info/en/nanoinfo/materials/aluminium-oxides/material-information Accessed 30 October 2015

[xxvi] http://www.nanotechproject.org/cpi/browse/nanomaterials/aluminum-oxide/ Accessed 30 October 2015

Dermal

Dermal exposure to alumina nanoparticles from use of cosmetics or polishing products appears to be the most likely non-occupational route of exposure.

Ingestion

Ingestion exposure appears unlikely, except in the case of misuse of consumer products.

2.6.5 Specific human-health concerns

Willhite et al (2014) have reviewed the potential health risks from exposure to conventional or nanoscale forms of aluminium, its oxides and hydroxides. The review noted that there was a lack of correlation between the findings of *in vitro* and *in vivo* studies, due to the tendency of alumina nanoparticles to aggregate in the environment. Adverse effects from inhalation exposure were only observed at very high doses or in occupational settings.

Studies have demonstrated the ability of alumina nanoparticles to adversely affect human brain microvascular endothelial cells in culture, including reducing cell viability, altering mitochondrial potential, increasing cellular oxidation, and decreasing tight junction protein expression (Chen, Yokel et al 2008). Rats intravenously infused with alumina nanoparticles showed disruption of expression of tight junction proteins. However, this route of exposure is of limited relevance to human exposures.

Potential for skin irritation was examined in a Reconstructed Human Epidermis (RHE) Model (Sathya and Deepa 2014). No loss of cell viability was observed following 42 minutes of exposure and 42 hours expression, indicating that alumina nanoparticles were non-irritant. A study with aluminium nanoparticles applied to human keratinocytes found no impact on cell viability following 24 hours exposure (McCormack-Brown 2008). However, levels of inflammatory cytokine (IL-8) were significantly elevated, suggesting that aluminium nanoparticles may be skin sensitisers. Flake-like α-alumina pigments, for use in cosmetics, were tested for *in vitro* (human skin cells) and *in vivo* (rat) dermal toxicity (Kwon, Seo et al 2015). The flakes were assessed to be non-penetrative and not to induce dermal inflammation in rats or any other effects on human skin cells.

In *in vitro* and *in vivo* studies, alumina nanoparticles with higher aspect ratios (nanorods) were found to elicit stronger toxicological responses than particles with lower aspect ratios (Park, Lee et al 2015).

2.6.6 References

Chen L, Yokel RA, Hennig B et al. 2008. Manufactured aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature. *Journal of Neuroimmune Pharmacology* 3 (4): 286-95

Hakuta Y, Nagai N, Suzuki YH et al. 2013. Preparation of alpha-alumina nanoparticles with various shapes via hydrothermal phase transformation under supercritical water conditions. *IOP Conference Series: Materials Science and Engineering* 47 12045

Kavitha R,Jayaram V. 2006. Deposition and characterization of alumina films produced by combustion flame pyrolysis. *Surface and Coatings Technology* 201 (6): 2491-9

Kwon T, Seo H, Jang S et al. 2015. Dermal toxicity of flake-like alpha-alumina pigments. *Journal of Nanoscience and Nanotechnology* 15 (2): 1636-46

McCormack-Brown S. 2008. *In vitro toxicity of aluminum nanoparticles in human keratinocytes*. M Sc Thesis, Department of Systems and Engineering Management. Wright-Patterson Air Force Base, Ohio, USA: Technology AFIo

Mirjalili F, Hasmaliza M,Abdullah LC. 2010. Size-controlled synthesis of nano α-alumina particles through the sol–gel method. *Ceramics International* 36 (4): 1253-7

Park EJ, Lee GH, Shim JH et al. 2015. Comparison of the toxicity of aluminum oxide nanorods with different aspect ratio. *Archives of Toxicology* 89 (10): 1771-82

Paven-Thivet L, Malibert C, Houdy P et al. 1998. Rf-sputtering deposition of al/al2o3 multilayers. *Thin Solid Films* 336 (1–2): 373-6

Peng C, Zhang J, Xiong Z et al. 2015. Fabrication of porous hollow γ-al2o3 nanofibers by facile electrospinning and its application for water remediation. *Microporous and Mesoporous Materials* 215 133-42

Piriyawong V, Thongpool V, Asanithi P et al. 2012. Preparation and characterization of alumina nanoparticles in deionized water using laser ablation technique. *Journal of Nanomaterials* 2012 819403

Reid CB, Forrester JS, Goodshaw HJ et al. 2008. A study in the mechanical milling of alumina powder. *Ceramics International* 34 (6): 1551-6

Rozita Y, Brydson R,Scott AJ. 2010. An investigation of commercial gamma-al(2)o(3) nanoparticles. *Journal of Physics: Conference Series* 241 012096

Sadabadi H, Aftabtalab A, Zafarian S et al. 2013. High purity alpha alumina nanoparticle: Synthesis and characterization. *International Journal of Scientific & Engineering Research* 4 (4): 1593-6

Sathya TN,Deepa V. 2014. Assessment of the in vitro skin irritation potential of nanoparticles using the reconstructed human epidermis (rhe) model. *Advances in Life Sciences and Health* 1 (2): 75-83

Willhite CC, Karyakina NA, Yokel RA et al. 2014. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. *Critical Reviews in Toxicology* 44 1-80

2.7 CERIUM OXIDE

2.7.1 Physical properties

Cerium is a lanthanide metal and a member of the rare earth metals. Many of the applications of cerium oxide (CeO_2) relate to its ability to quickly transition between Ce^{3+} and Ce^{4+}, providing superior redox characteristics (Lin and Chowdhury 2010; Ta, Liu et al 2013). These characteristics have led to use of cerium oxides as catalysts and enzyme mimetics. However, the most important property of CeO_2 appears to be its ability to act as an 'oxygen buffer', with oxygen able to react at the crystal surface, permeate between crystal layers to access internal active sites, or be incorporated into oxygen vacancies in the crystal structure (Gangopadhyay, Frolov et al 2014).

Cerium oxides can form nanocrystals, which can be assembled into nanorods, nanowire or nanotubes (Lin and Chowdhury 2010).

2.7.2 Method of production

To produce CeO_2 with the required functional properties it is necessary to be able to strictly control the size of the particles produced. CeO_2 nanoparticles are produced by 'bottom-up' techniques, whereby the nanoparticles are synthesised from component chemicals. Preparation of CeO_2 nanomaterials generally involves four basic steps: synthesis of precursors, treatment of precursors before conversion to oxides, conversion of precursors to mixed oxides, and post treatment of mixed oxide material (Lin and Chowdhury 2010). Reliable production of nanoparticles in the sub-2 nm range has been achieved by simple mixing of $Ce(SO_4)_2.4H_2O$ solution and ammonia solution under ambient conditions (Xu, Li et al 2008). These seed particles can be 'tuned' to desired size ranges by hydrothermal treatment, at temperatures in the range 120 ºC to 220ºC for 5 hours.

2.7.3 Relevant applications or uses

The most economically important application of CeO_2 nanoparticles is in the petrol industry, where they act as oxygen buffers in three-way catalysts used to reduce vehicle emissions. In particular, the nanoparticles donate oxygen for combustion of waste hydrocarbons, soot and carbon monoxide (Fernandes, Alcover Neto et al 2008; Ta, Liu et al 2013). CeO_2 nanoparticles redox capacity has also led to its use in fuel cells (Murray, Tsai et al 1999), while its sharply defined UV absorption characteristics have led to its use as a UV-blocking material (Li, Yabe et al 2002). Solid CeO_2 nanoparticles are also used for the polishing of silicon chips (Kamiya, Iwase et al 2009).

While medical applications are not yet in use, CeO_2 nanoparticles are being actively investigated for use in medicine, including treatment of cancer (Gao, Chen et al 2014) and protection of cells from oxidation or radiation-induced damage (Chen, Patil et al 2006; Das, Patil et al 2007). The applicability of CeO_2 nanoparticles to heavy metal removal from water and wastewater has also been demonstrated (Hua, Zhang et al 2012).

2.7.4 Sources of non-occupational human exposure

Under the current use profile for CeO_2 nanoparticles, the most likely source of human exposure is by inhalation exposure due to emission of CeO_2 nanoparticles from their use as fuel additives (Cassee, van Balen et al 2011). Scenario-based assessment of environmental impacts suggested that CeO_2 nanoparticles discharged to the environment (soil, sediment, waterways) would contribute concentrations of cerium well below naturally-occurring levels (Johnson and Park 2012).

Introduction of a CeO_2 nanoparticle-based diesel fuel additive (Envirox) for use in bus fleets in London and Newcastle, resulted in no significant increase in the cerium content of PM_{10} particles in London air, but a rapid and sustained increase in the cerium content of PM_{10} in Newcastle air (Cassee, van Balen et al 2011). Risk assessment based on these data concluded that exposure to Envirox in ambient air was unlikely to result in adverse health effects, specifically pulmonary oxidative stress (Park, Donaldson et al 2008).

2.7.5 Specific human-health concerns

It is currently uncertain whether the use of CeO_2 nanoparticles as fuel additives should be viewed as hazardous or beneficial to human health. The reduction in exhaust particulates, with their known adverse health effects, is likely to be beneficial. Exposure of atherosclerosis-prone rats to diesel exhaust, with and without use of CeO_2 nanoparticles, demonstrated a reduction in atherosclerotic burden in rats exposed to CeO_2-treated diesel exhaust, compared to those exposed to untreated exhaust (Cassee, Campbell et al 2012). However, other rat studies indicated more adverse pulmonary effects following exposure to CeO_2-treated diesel exhaust, than following exposure to the non-treated equivalent (Snow, McGee et al 2014). A 28-day rat inhalation study with micro- and nano-sized CeO_2 found pulmonary inflammation and damage[xxvii], but no systemic effects (Gosens, Mathijssen et al 2014).

There is currently a lack of information on adverse human health effects due to CeO_2 nanoparticles exposure.

2.7.6 References

Cassee FR, van Balen EC, Singh C et al. 2011. Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. *Critical Reviews in Toxicology* 41 (3): 213-29

Cassee FR, Campbell A, Boere AJF et al. 2012. The biological effects of subacute inhalation of diesel exhaust following addition of cerium oxide nanoparticles in atherosclerosis-prone mice. *Environmental Research* 115 1-10

Chen J, Patil S, Seal S et al. 2006. Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nature Nanotechnology* 1 (2): 142-50

Das M, Patil S, Bhargava N et al. 2007. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. *Biomaterials* 28 (10): 1918-25

Fernandes DM, Alcover Neto A, Cardoso MJB et al. 2008. Commercial automotive catalysts: Chemical, structural and catalytic evaluation, before and after aging. *Catalysis Today* 133–135 574-81

Gangopadhyay S, Frolov DD, Masunov AE et al. 2014. Structure and properties of cerium oxides in bulk and nanoparticulate forms. *Journal of Alloys and Compounds* 584 199-208

[xxvii] The relative toxicity was explored in terms of three exposure metrics. When exposure levels were expressed as mass concentration, nanosized NM-211 was the most potent material, whereas when expression levels were based on surface area concentration, micro-sized NM-213 material induced the greatest extent of pulmonary inflammation/damage. Particles were equipotent based on particle number concentrations. In conclusion, similar pulmonary toxicity profiles including inflammation are observed for all three materials with little quantitative differences (Gosens, Mathijssen et al 2014).

Gao Y, Chen K, Ma J-l et al. 2014. Cerium oxide nanoparticles in cancer. *Oncotargets and Therapy* 7 835-40

Gosens I, Mathijssen L, Bokkers BGH et al. 2014. Comparative hazard identification of nano- and micro-sized cerium oxide particles based on 28-day inhalation studies in rats. *Nanotoxicology* 8 (6): 643-53

Hua M, Zhang S, Pan B et al. 2012. Heavy metal removal from water/wastewater by nanosized metal oxides: A review. *Journal of Hazardous Materials* 211–212 317-31

Johnson AC,Park B. 2012. Predicting contamination by the fuel additive cerium oxide engineered nanoparticles within the united kingdom and the associated risks. *Environmental Toxicology and Chemistry* 31 (11): 2582-7

Kamiya S, Iwase H, Kishita K et al. 2009. Study on reaction mechanism of si and pure ceo2 for chemical-mechanical-grinding process. *Journal of Vacuum Science & Technology B* 27 (3): 1496-502

Li R, Yabe S, Yamashita M et al. 2002. Synthesis and uv-shielding properties of zno- and cao-doped ceo2 via soft solution chemical process. *Solid State Ionics* 151 (1–4): 235-41

Lin K-S,Chowdhury S. 2010. Synthesis, characterization, and application of 1-d cerium oxide nanomaterials: A review. *International Journal of Molecular Sciences* 11 (9): 3226-51

Murray EP, Tsai T,Barnett SA. 1999. A direct-methane fuel cell with a ceria-based anode. *Nature* 400 649-51

Park B, Donaldson K, Duffin R et al. 2008. Hazard and risk assessment of a nanoparticulate cerium oxide-based diesel fuel additive—a case study. *Inhalation Toxicology* 20 (6): 547-66

Snow SJ, McGee J, Miller DB et al. 2014. Inhaled diesel emissions generated with cerium oxide nanoparticle fuel additive induce adverse pulmonary and systemic effects. *Toxicological Sciences* 142 (2): 403-17

Ta N, Liu J,Shen W. 2013. Tuning the shape of ceria nanomaterials for catalytic applications. *Chinese Journal of Catalysis* 34 (5): 838-50

Xu J, Li G,Li L. 2008. Ceo2 nanocrystals: Seed-mediated synthesis and size control. *Materials Research Bulletin* 43 (4): 990-5

2.8 ZINC OXIDE

2.8.1 Physical and chemical properties

Zinc oxide (ZnO) forms colourless hexagonal crystals or a white dusty powder that is insoluble in water (Greenwood 1997). It is widely used as an additive in numerous materials and products. Most applications exploit the reactivity of the oxide as a precursor to other zinc compounds. For material science applications, zinc oxide has a high refractive index, high thermal conductivity, and binding, antibacterial and UV-protection properties.

Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zincblende. The wurtzite structure is most stable under ambient conditions and thus is more common (Fierro 2006). Crystalline zinc oxide is thermochromic, changing from white to yellow when heated and in air reverting to white on cooling (Wiberg and Holleman 2001). However, nanoparticulate zinc oxide particles of 200 nm or smaller, are virtually transparent (Mitchnick, Fairhurst et al 1999).

2.8.2 Method of production

Zinc oxide occurs naturally as a coarse-grained mineral (zincite), but most zinc oxide is produced synthetically (De Liedekerke 2006). Technically, it is obtained by oxidation of zinc or zinc vapour with atmospheric oxygen (zinc white) or by calcination of different components such as zinc hydroxide, zinc carbonate or zinc nitrate. Nanostructures of ZnO can be obtained with most of these techniques, under certain conditions, and can be synthesized into a variety of morphologies including nanowires, nanorods, tetrapods, nanobelts, nanoflowers, nanoparticles.[xxviii]

Several methods for the synthesis of ZnO nanoparticles have been developed. Solid-state pyrolysis, sol–gel chemistry, magnetic sputtering, chemical vapour deposition (CVD) and molecular beam epitaxy are some of the methods reported in the literature (Ju-Nam and Lead 2008). Singh and Gopa (2008) reported the synthesis of ZnO nanoparticles by pulsed laser ablation of a zinc target in aqueous media with a simultaneous flow of pure oxygen gas to obtain smaller particles and a narrow size distribution.

2.8.3 Relevant applications or uses

Zinc oxide powder has a very versatile and broad range of applications and is added into materials and products including rubber, plastics, ceramics, cement, cosmetics, and pharmaceuticals.[xxix, xxx]

Nanostructures of ZnO have numerous potential applications, particularly in the form of thin films, nanowires, nanorods or nanoparticles (Starowicz and Stypula 2008) and can be introduced to optoelectronic and electronic devices. They are also used in the production of chemical sensors and solar cells (Singh, Thiyagarajan et al 2007). One of the most significant commercial applications of 20–100 nm ZnO nanoparticles is their use in the production of sunscreens and cosmetics, due to their property of blocking UV-B and particularly UV-A radiation from the sun (Huang, Zheng et al 2008). Physical UV filters like ZnO are mainly used in sunscreens with sun protection factors above 25. Unlike chemical UV filters, physical filters are suited for application to the sensitive skin of children and

[xxviii] Zinc Oxide Nanoparticles - Knowledge Base Nanomaterials accessed 02/11/15
[xxix] Zinc Oxide Nanoparticles - Knowledge Base Nanomaterials accessed 02/11/15
[xxx] Applications of Zinc Oxide accessed 02/11/15

allergic persons. Chemical UV filters absorb UV radiation, convert it into heat and may trigger sensitisation.

Apart from sun protection products ZnO nanoparticles are also present in some paints, cooling fluid for computer hardware, food wrapping, food supplements as well as a range of personal care products such as skin moisturisers, make-up, deodorants, and lip balms.[xxxi] In each of these products ZnO may fulfil a number of functions, for instance ZnO nanoparticles in paints provide antibacterial and anti-UV properties,[xxxii] in cosmetic products ZnO works as a mineral UV-filter, and its high conductivity enhances heat transfer rates in cooling fluids. In food wrapping, anti-UV, anti-IR, and sterilising effects as well as increasing material properties like temperature tolerance, fire-retardance and grinding capacity are the benefits of ZnO nanoparticles.[xxxiii]

2.8.4 Sources of non-occupational human exposure

The list of products identified contains a large number of personal care products, including sun protection creams and cosmetics. It is these that are likely to provide the highest concentrations and most frequent consumer exposures.

Inhalation exposure

Since ZnO nanoparticles occur in cosmetic products, textiles or plastics as bound particles, it is rather unlikely that they are taken up via the lung in a non-occupational setting. Instead, inhalation may occur during particle production and processing.[xxxiv] The increasing number of spray products such as sun protection cream and deodorant containing ZnO may lead to low-concentration exposure whose effects still need to be investigated.

The first animal model studies showed that inhalation of ZnO nanoparticles at concentrations of 50 mg/m^3 for 1 or 3 hours may effect a relatively strong but temporary inflammation of the lung (Sayes, Reed et al 2007; Warheit, Sayes et al 2009), which resembles the so-called metal fume fever, an influenza-type disease characterized by inflammation of the respiratory tract due to inhalation of metal fumes (mainly zinc fumes) during welding. Another study revealed that ZnO nanoparticles could cause eosinophilic airway inflammation (Huang, Lee et al 2015).

Dermal exposure

The size of the ZnO nanoparticles used in sun creams is in the range of 20 to 60 nm. Before being added, these very small nanoparticles are usually coated with silicon or aluminium oxide which causes clogging to form aggregates sized 200 to 500 nm.

Studies from industry and independent studies carried out within the EU project NanoDerm have shown that such particles do not get into the body through the healthy skin. To date, toxicological analyses have found that only a few of these particles enter the skin, remain in the upper layers and are transported to the surface after a few days through the growth of hair, where they are rubbed off. [xxxv] The same was true for slightly damaged skin (Campbell, Contreras-Rojas et al 2012). Health risks for consumers have not been identified. Hence, the

[xxxi] Consumer Product Inventory - Project on Emerging Nanotechnologies - Zinc oxide accessed 02/11/15
[xxxii] Nanoparticles in paints accessed 04/11/15
[xxxiii] Zinc Oxide Nanoparticles - Knowledge Base Nanomaterials accessed 02/11/15
[xxxiv] Zinc Oxide Nanoparticles - Knowledge Base Nanomaterials accessed 02/11/15
[xxxv] EU project NanoDerm accessed 04/11/15

users of such sun protection products containing ZnO nanoparticles do not incur health risks. [xxxvi]

Most of the studies carried out so far, however, were based on cell cultures, animal models or biopsies from porcine and human skin. Some more recent tests of ZnO-containing sun creams on humans under realistic conditions, indeed, revealed small quantities of stable isotopes of zinc in the blood and urine. The quantities detected only amounted to 1/1000 of the total zinc concentration naturally occurring in the blood. It remains to be determined whether zinc was taken up via the skin as ZnO particles or dissolved zinc ions (Gulson, McCall et al 2010). Leite-Silva et al. (2013) observed some limited penetration of coated and uncoated ZnO nanoparticles into the viable stratum granulosum epidermis, but found that the extent is not sufficient to affect the redox state of those viable cells. Another study, carried out to continue the work of the NanoDerm EU project, complements the previous investigations on human skin with compromised barrier function. Skin samples from two patients suffering from atopic dermatitis were treated with ultrafine zinc oxide particles in a hydrophobic basis gel with an application time of 2 days or 2 weeks. It has been shown that ZnO nanoparticles penetrate deeply into the stratum corneum in these patients, but not into the stratum spinosum to any great degree (Szikszai, Kertész et al 2011).

Ingestion exposure

For a healthy life, a human requires between 12 and 15 mg zinc on a daily basis obtained from the diet. In most cases zinc is taken up in the form of zinc oxide coming from natural food sources which then dissolves in the body releasing zinc ions. However, if absorbed in too high concentrations, zinc can be harmful to the body. Although ZnO nanoparticles are not primarily used in food, they are contained in some supplements and they may also be taken up by swallowing of other products e.g. sunscreen. Thus, opportunities for incidental ingestion and consequently overexposure should be considered.[xxxvii]

In a study of mice, increased concentrations of zinc were found in the liver, heart, spleen, stomach, and the bones after oral administration of ZnO nanoparticles (Wang, Feng et al 2008).

2.8.5 Specific human-health concerns

According to the European Union Risk Assessment Report for ZnO (2004) and taking the data available into account, currently the use of products containing ZnO nanoparticles seems to be of no health concern for consumers with respect to acute and repeated dose toxicity, skin, eye and respiratory tract irritation, corrosivity and skin sensitisation.

Sunscreens are a potentially important source of environmental contamination, due to wash off from individuals.[xxxviii]

2.8.6 References

Campbell CSJ, Contreras-Rojas LR, Delgado-Charro MB et al. 2012. Objective assessment of nanoparticle disposition in mammalian skin after topical exposure. *Journal of Controlled Release* 162 (1): 201-7

[xxxvi] BfR Opinion No. 037/2010 (18.06.2010) accessed 04/11/15
[xxxvii] Zinc Oxide Nanoparticles - Knowledge Base Nanomaterials accessed 04/11/15
[xxxviii] Zinc Oxide Nanoparticles - Knowledge Base Nanomaterials accessed 02/11/15

De Liedekerke M. 2006. *Ullmann's encyclopedia of industrial chemistry*. Wiley-VCH Verlag GmbH & Co. KGaA

Fierro JLG. 2006. *Metal oxides: Chemistry & applications*. CRC Press

Greenwood NNE, Alan. 1997. *Chemistry of the elements*. Oxford:Butterworth-Heinemann

Gulson B, McCall M, Korsch M et al. 2010. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. *Toxicological Sciences* 118 (1): 140-9

Huang K-L, Lee Y-H, Chen H-I et al. 2015. Zinc oxide nanoparticles induce eosinophilic airway inflammation in mice. *Journal of Hazardous Materials* 297 304-12

Huang Z, Zheng X, Yan D et al. 2008. Toxicological effect of zno nanoparticles based on bacteria. *Langmuir* 24 4140–4

Ju-Nam Y,Lead JR. 2008. Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. *Science of The Total Environment* 400 (1-3): 396-414

Leite-Silva VR, Lamer ML, Sanchez WY et al. 2013. The effect of formulation on the penetration of coated and uncoated zinc oxide nanoparticles into the viable epidermis of human skin in vivo. *European Journal of Pharmaceutics and Biopharmaceutics* 84 (2): 297-308

Mitchnick MA, Fairhurst D,Pinnell SR. 1999. Microfine zinc oxide (z-cote) as a photostable uva/uvb sunblock agent. *Journal of the American Academy of Dermatology* 40 (1): 85-90

Sayes CM, Reed KL,Warheit DB. 2007. Assessing toxicity of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary toxicity profiles. *Toxicological Sciences* 97 (1): 163-80

Singh S, Thiyagarajan P, Kant KM et al. 2007. Structure, microstructure and physical properties of zno based materials in various forms: Bulk, thin film and nano. *Journal of Physics D: Applied Physics* 40 (20): 6312–27

Singh SC,Gopal R. 2008. Synthesis of colloidal zinc oxide nanoparticles by pulsed laser ablation in aqueous media. *Physica E: Low-dimensional Systems and Nanostructures* 40 (4): 724-30

Starowicz M,Stypula B. 2008. Electrochemical synthesis of zno nanoparticles. *European Journal of Inorganic Chemistry* 6 869–72

Szikszai Z, Kertész Z, Bodnár E et al. 2011. Nuclear microprobe investigation of the penetration of ultrafine zinc oxide into human skin affected by atopic dermatitis. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 269 (20): 2278-80

Wang B, Feng W, Wang M et al. 2008. Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. *Journal of Nanoparticle Research* 10 (2): 263-76

Warheit DB, Sayes CM,Reed KL. 2009. Nanoscale and fine zinc oxide particles: Can in vitro assays accurately forecast lung hazards following inhalation exposures? *Environmental Science and Technology* 43 (20): 7939-45

Wiberg E,Holleman AF. 2001. *Inorganic chemistry*. Elsevier

2.9 SILICON OXIDE

2.9.1 Physical properties

Silicon dioxide (SiO_2) nanoparticles sometimes referred to as synthetic amorphous silica (SAS) may range in size from less than 10 nm to 100 nm, with a very high surface area (>500 m^2/g) and a low solubility in biological fluids (Cho, Duffin et al 2012). SiO_2 nanoparticles are very hard, resulting in their use as abrasives or in surface-protecting coatings.

2.9.2 Method of production

Despite the widespread occurrence of SiO_2 in the earth's crust, SiO_2 nanoparticles are generally produced by 'bottom up' synthetic methods, such as the Stöber method (Stöber, Fink et al 1968), involving hydrolysis and condensation of silicon alkoxides in alcoholic media, and sol-gel techniques (Alexandre and Dubois 2000). Pyrogenic SiO_2 can be produced at 1200 to 1600ºC in closed reactors from (alkyl)chlorosilanes (Fruijtier-Pollock).

SiO_2 can also be modified to create pores of various sizes in the structure and can be surface modified to change the charge characteristics of the nanoparticles (Jaganathan and Godin 2012).

2.9.3 Relevant applications or uses

SiO_2 nanoparticles have been used as fillers, to improve the technological characteristics of rubber (tyres)[xxxix], plastics (Duwe, Arlt et al 2012), paint (Mizutani, Arai et al 2006), sealants, adhesives and coatings[xxxix], surface cleaning products[xxxix], dental restoration composites[xxxix], cosmetics, medicinal products (tablets, creams, gels and suppositories) and sunscreens[xxxix] and as a component of leisure products, such as wetsuits, skis and tennis rackets[xxxix]. SiO_2 nanoparticles have also been used in dietary supplements and are approved as food additives. In New Zealand and Australia, amorphous SiO_2 (E 551) is permitted for use in salt.[xl]

SiO_2 nanoparticles are used as polishing abrasives in the electronic industry.[xli] Molecular-SiO_2 nano-hybrids have shown potential as sensitive bioimaging and biosensing agents (Jin, Li et al 2009).

Production of nanoporous and mesoporous SiO_2 nanoparticles has led to investigations into their use as nanocarriers for drugs (Jaganathan and Godin 2012; Jin, Li et al 2009; Qian and Bogner 2012).

2.9.4 Sources of non-occupational human exposure

Dermal

Humans may be dermally exposed to SiO_2 nanoparticles due to their use in cosmetics, topical medicines or sunscreens or due to spilling of SiO_2 nanoparticle-containing paints and inks onto the skin surface. While SiO_2 nanoparticles have shown cytotoxicity to dermal cell types, no evidence of adverse effects were seen in *in vivo* studies or in 3-dimensional cell

[xxxix] http://www.nanotechproject.org/cpi/browse/nanomaterials/silicon-dioxide/ Accessed 4 November 2015
[xl] https://www.comlaw.gov.au/Details/F2015C00758/Html/Volume_2 Accessed 4 November 2015
[xli] http://nanopartikel.info/en/nanoinfo/materials/silicon-dioxide/material-information Accessed 4 November 2015

models (Jaganathan and Godin 2012). The surface charge chemistry of SiO_2 nanoparticles means they are unlikely to be absorbed through the skin.

Inhalation

While inhalation exposure to SiO_2 nanoparticles is technically possible, from the breakdown of tyres or during surface application of SiO_2 nanoparticle-containing products, this is unlikely to be a major route of exposure. Potentially inhalation of SiO_2 nanoparticles should be differentiated from inhalation exposure to micro-scale crystalline SiO_2 (quartz), which may cause serious adverse health effects in humans (silicosis).

Ingestion

The most obvious routes of SiO_2 nanoparticles ingestion are through consumption of foods, supplements or medicines.

2.9.5 Specific human-health concerns

It should be noted that, while SiO_2 nanoparticles meet the definition of a nanomaterial, they are not a novel material and have been in use for approximately 60 years (Fruijtier-Pölloth 2012). Extensive reviews of animal and human toxicology and epidemiology have concluded that SiO_2 nanoparticles is essentially non-toxic via the oral, dermal/ocular or inhalation routes of exposure (ECETOC 2006; Fruijtier-Pölloth 2012; McLaughlin, Chow et al 1997; Merget, Bauer et al 2002).

While crystalline SiO_2 can cause adverse health effects following inhalation, nanoscale SiO_2 was assessed to have low potential to cause lung inflammation, compared to other nanoparticles (Cho, Duffin et al 2012). Studies on cell lines demonstrated that the genotoxic and cytotoxic potential of SiO_2 was related to its physical form, with crystalline SiO_2 being both genotoxic and cytotoxic, while amorphous (nanoscale) SiO_2 was neither genotoxic nor cytotoxic (Guidi, Nigro et al 2015).

SiO_2 nanoparticles, used in sunscreen formulations, were found to be non-irritant and non-sensitising in laboratory animal tests (Piasecka-Zelga, Zelga et al 2015). While mechanical irritation has been reported in occupationally exposed people, no human cases of contact allergy have ever been reported (ECETOC 2006).

2.9.6 References

Alexandre M,Dubois P. 2000. Polymer-layered silicate nanocomposites: Preparation, properties and uses of a new class of materials. *Materials Science and Engineering: R: Reports* 28 (1–2): 1-63

Cho WS, Duffin R, Thielbeer F et al. 2012. Zeta potential and solubility to toxic ions as mechanisms of lung inflammation caused by metal/metal oxide nanoparticles. *Toxicology Science* 126 (2): 469-77

Duwe S, Arlt C, Aranda S et al. 2012. A detailed thermal analysis of nanocomposites filled with sio2, aln or boehmite at varied contents and a review of selected rules of mixture. *Composites Science and Technology* 72 (12): 1324-30

ECETOC. 2006. *Synthetic amorphous silica (cas no. 7631-86-9)*. JACC No. 51. Bussels, Belgium: European Centre for Ecotoxicology and Toxicology of Chemicals

Fruijtier-Pölloth C. 2012. The toxicological mode of action and the safety of synthetic amorphous silica—a nanostructured material. *Toxicology* 294 (2–3): 61-79

Guidi P, Nigro M, Bernardeschi M et al. 2015. Does the crystal habit modulate the genotoxic potential of silica particles? A cytogenetic evaluation in human and murine cell lines. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 792 46-52

Jaganathan H,Godin B. 2012. Biocompatibility assessment of si-based nano- and micro-particles. *Advanced Drug Delivery Reviews* 64 (15): 1800-19

Jin Y, Li A, Hazelton SG et al. 2009. Amorphous silica nanohybrids: Synthesis, properties and applications. *Coordination Chemistry Reviews* 253 (23–24): 2998-3014

McLaughlin JK, Chow WH,Levy LS. 1997. Amorphous silica: A review of health effects from inhalation exposure with particular reference to cancer. *Journal of Toxicology and Environmental Health* 50 (6): 553-66

Merget R, Bauer T, Kupper HU et al. 2002. Health hazards due to the inhalation of amorphous silica. *Archives of Toxicology* 75 (11-12): 625-34

Mizutani T, Arai K, Miyamoto M et al. 2006. Application of silica-containing nano-composite emulsion to wall paint: A new environmentally safe paint of high performance. *Progress in Organic Coatings* 55 (3): 276-83

Piasecka-Zelga J, Zelga P, Gornicz M et al. 2015. Acute dermal toxicity and sensitization studies of novel nano-enhanced uv absorbers. *Journal of Occupational Health* 57 (3): 275-84

Qian KK,Bogner RH. 2012. Application of mesoporous silicon dioxide and silicate in oral amorphous drug delivery systems. *Journal of Pharmaceutical Sciences* 101 (2): 444-63

Stöber W, Fink A,Bohn E. 1968. Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science* 26 (1): 62-9

2.10 NANOCLAYS

2.10.1 Physical properties

The clays most commonly used in nanocomposites are 2:1 layered silicates, such as montmorillonite or saponite (Bordes, Pollet et al 2009; Chivrac, Pollet et al 2009). Each layered sheet is about 1 nm thick, but the sheet may have an aspect ratio of up to 1000:1 (Bordes, Pollet et al 2009; Mikkelsen, Hansen et al 2011). This results in nanoclay particles having very high surface areas (>750 m^2/g) (Uddin 2008).

The silicate sheets are negatively charged, but this is balanced by the presence of alkali or alkali earth cations (Na^+, K^+, Ca^{2+}, etc.) in the interlayer or 'gallery' spaces. These cations may be replaced by ammonium or phosphonium cations bearing at least one long alkyl chain, and possibly other substituted groups, to produce organomodified nanoclays. These organomodified nanoclays have increased interlayer distances compared to the native clays (Hetzer and De Kee 2008). Primary nanoparticles are made up of five to ten layers, held together by Van Der Waals forces and electrostatic attraction between positively-charged cations and the negatively-charged silicate layers (Chivrac, Pollet et al 2009).

Swelling of nanoclay particles occurs through hydration of the interlayered cations and decreases the attractive forces between the clay layers (Chivrac, Pollet et al 2009). Swelling facilitates organomodification[xlii].

Nanoclays are thermally stable and can be used to improve this characteristic in nanocomposites (Uddin 2008).

2.10.2 Method of production

Nanoclays are produced by 'top down' methods, with the parent clay (bentonite) being mined and purified, prior to milling to reduce the particle size of the resulting montmorillonite (Mikkelsen, Hansen et al 2011).

As highlighted in the previous section nanoclays are often organomodified, using wet chemical methods, before further use (Hetzer and De Kee 2008).

The major current and potential application of organomodified nanoclays is their combination with polymers, to produce nanocomposites with superior technological characteristics to the parent polymers (Mikkelsen, Hansen et al 2011). The organic modification of the clays improves the affinity between the clay and the polymer. The nanocomposites can be formed by three main methods (Bordes, Pollet et al 2009; Chivrac, Pollet et al 2009; Ojijo and Ray 2014):

- Solvent intercalation. Nanoclays are swollen in a polymer solvent to aid diffusion of the macromolecules into the clay interlayer galleries.
- *In situ* intercalation. Nanoclays are swollen in the monomer solution, prior to polymerisation.
- Melt intercalation. The polymer is processed with the nanoclay in a molten state, using processes such as extrusion.

Depending on the process conditions and the nanoclay/polymer affinity, two different categories of nanocomposite can be produced (Díez-Pascual, Gómez-Fatou et al 2015):

[xlii] Organomodification of nanoclays typically involves introduction of cationic surfactants into the interlayer zones of clay material.

- Intercalated nanocomposites. Polymer chains are inserted between the layers of the nanoclay.
- Exfoliated nanocomposites. The layers of the clay are completely separated and dispersed throughout the polymer structure.

2.10.3 Relevant applications or uses

Nanoclay-containing nanocomposites have been demonstrated to have improved gas barrier characteristics, reduced surface char and increased flame retardance in thermoplastics, and improved properties of flow injection items used in automotive construction, packaging and electronics items, such as cable and wire coatings (Mikkelsen, Hansen et al 2011).

Nanoclay has also been shown to improve the mechanical properties of cement and result in higher compressive strength and tensile strength of cement mortars (Morsy, Alsayed et al 2010).

Nanoclays are gaining increasing popularity in food packaging, mainly due to their gas barrier characteristics (Mikkelsen, Hansen et al 2011). The packaging may be in the form of a nanocomposite or have the nanoclay sandwiched between layers of another material, such as polyethylene.

Nanoclays show potential in improving the flame retardant characteristics of a range of polymers (Zhang and Horrocks 2003).

The Consumer Products Inventory lists nanoclay-containing products, including; food containers, cosmetics, automotive parts, paint and clothing.[xliii]

2.10.4 Sources of non-occupational human exposure

Inhalation

There appears to be limited potential for non-occupational inhalation exposure to nanoclays. Cosmetics and cement containing nanoclays are the most likely routes of inhalation exposure. However, use of nanoclays in these products appears to be quite rare (Mikkelsen, Hansen et al 2011). Transient inflammatory reactions were observed in rats after intratracheal instillation of Sepiolite nanoclay, but no extrapulmonary target organ effects were observed following the exposure (Warheit, Sayes et al 2010).

Dermal

Most applications of nanoclays involve inclusion in nanocomposites or their sequestration within other materials. There is some potential for dermal exposure from cosmetics, cement and paints. No information was found on the dermal toxicity, irritation or sensitisation due to nanoclays.

Ingestion

No likely routes of nanoclay ingestion are apparent from current applications.

2.10.5 Specific human-health concerns

Nanoclay has low acute toxicity in animal experiments and is normally not considered to constitute a significant health hazard except for the possible effects on the lungs from inhaling dust (Mikkelsen, Hansen et al 2011). No specific data on dermal and inhalational

[xliii] http://www.nanotechproject.org/cpi/browse/nanomaterials/clays/ Accessed 5 November 2015

exposure are available related to the nanoform of the clay. Nanoclay may contain residual crystalline quartz (up to 0.5 %), which may add to the risk from inhalation of the material. Quaternary ammonium or phosphonium functional groups on the surface of organomodified nanoclays may potentially be problematic, as ammonium and phosphonium ions in their pure form can cause asthma symptoms in some people (Mikkelsen, Hansen et al 2011). However, it should be noted that nanoclays are organomodified to improve the formation of nanocomposites and unlikely to be present in free form.

2.10.6 References

Bordes P, Pollet E,Avérous L. 2009. Nano-biocomposites: Biodegradable polyester/nanoclay systems. *Progress in Polymer Science* 34 (2): 125-55

Chivrac F, Pollet E,Avérous L. 2009. Progress in nano-biocomposites based on polysaccharides and nanoclays. *Materials Science and Engineering: R: Reports* 67 (1): 1-17

Díez-Pascual AM, Gómez-Fatou MA, Ania F et al. 2015. Nanoindentation in polymer nanocomposites. *Progress in Materials Science* 67 1-94

Hetzer M,De Kee D. 2008. Wood/polymer/nanoclay composites, environmentally friendly sustainable technology: A review. *Chemical Engineering Research and Design* 86 (10): 1083-93

Mikkelsen SH, Hansen E, Christensen TB et al. 2011. *Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials. Environmental project no. 1370 2011*. 5 November 2015. http://www2.mst.dk/udgiv/publications/2011/08/978-87-92779-09-0.pdf

Morsy MS, Alsayed SH,Aqel M. 2010. Effect of nano-clay on mechanical properties and microstructure of ordinary portland cement mortar. *International Journal of Civil & Environmental Engineering IJCEE-IJENS* 10 (1): 21-5

Ojijo V,Ray SS. 2014. Nano-biocomposites based on synthetic aliphatic polyesters and nanoclay. *Progress in Materials Science* 62 1-57

Uddin F. 2008. Clays, nanoclays, and montmorillonite minerals. *Metallurgical and Materials Transactions a-Physical Metallurgy and Materials Science* 39A (12): 2804-14

Warheit DB, Sayes CM, Frame SR et al. 2010. Pulmonary exposures to sepiolite nanoclay particulates in rats: Resolution following multinucleate giant cell formation. *Toxicology Letters* 192 (3): 286-93

Zhang S,Horrocks AR. 2003. A review of flame retardant polypropylene fibres. *Progress in Polymer Science* 28 (11): 1517-38

2.11 GOLD NANOPARTICLES

2.11.1 Physical properties

The numerous applications of gold nanoparticles (Au nanoparticles) are mainly based on the unique properties of these tiny particles. They are inert and are considered to be biocompatible and non-toxic, which makes gold interesting for biomedical applications. For example, the attenuation of X-rays by Au nanoparticles has led to their use in computed tomography imaging and as adjuvants for radiotherapy. Gold nanoparticles have numerous other applications in imaging, therapy and diagnostic systems. The absorbance and fluorescence of Au nanoparticles is much greater compared with bulk gold, and can be tuned from the visible to the near infrared (NIR) region by changing nanostructure size and morphology (Fleischer, Zhang et al 2010; Payne, Shuford et al 2006). Another highly useful feature of these nanoparticles is the electromagnetic field enhancement by sharp and spiky edges of the nanostructures, such as stars or nanorods, which can be used in surface enhanced Raman spectroscopy imaging (Kneipp, Kneipp et al 2005; Rycenga, Wang et al 2009; Stokes, Macaskill et al 2007). And the optical scattering of Au nanoparticles can be harnessed to detect them with a variety of microscopy methods (El-Sayed, Huang et al 2005). The advanced state of synthetic chemistry of Au nanoparticles offers precise control over physicochemical and optical properties (Mieszawska, Mulder et al 2013). Due to the so-called surface plasmon resonance effect the colour of the particles changes when targets such as hormones or antigens are bound.[xliv]

2.11.2 Method of production

Gold occurs on earth in primary raw materials as gold-bearing rock (gold ore) and in secondary deposits as fine metal. Significant amounts of gold are produced during the refining of other metals such as copper, nickel or other metals.[xlv]

Large amounts of gold nanoparticles are now obtained via the reduction of gold chloride solution (so-called tetra-chloro-aureate). This process uses different materials as reducing agents, such as citric acid, oxalic acid, boron hydrides or others. Alternatively, it is also possible to generate Au nanoparticles via laser radiation or UV light irradiation. In addition to the reducing agent a stabiliser is added during the process to prevent both aggregation and oxidation away from the reduced state (Turkevich, Stevenson et al 1951). The size and more importantly the shape of the nanoparticles can be controlled by the reducing agent, the capping agent and the reaction conditions used in the preparation (Grzelczak, Perez-Juste et al 2008). While spherical forms are most commonly prepared, rod-like shapes, cubes, hexagonal and even hollow forms are possible (Tréguer-Delapierre, Majimel et al 2008).

2.11.3 Relevant applications or uses

The use of gold and gold nanoparticles can be traced back many centuries: the mixing of gold metal vapours into molten glass turned the colour of the glass into a permanent purple red due to finely distributed gold nanoparticles. Such glass was used in jewellery and in many stained glass windows in churches or cathedrals. Today, Au nanoparticles have many more applications: in pregnancy test strips, in cancer diagnostics and cancer therapy (e.g. for breast cancer (Lee, Chatterjee et al 2014)). The surface of gold nanoparticles can easily be modified for a specific application and ligands for targeting, drugs or biocompatible coatings can be introduced. Gold nanoparticles can be incorporated into larger structures such as polymeric nanoparticles or liposomes that deliver large payloads for enhanced

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[xliv] Gold Nanoparticles - Knowledge Base Nanomaterials accessed 09/11/15
[xlv] Gold Nanoparticles - Knowledge Base Nanomaterials accessed 09/11/15

diagnostic applications, efficiently encapsulate drugs for concurrent therapy or add additional imaging labels (Mieszawska, Mulder et al 2013). Nanoscale gold particles also play an increasing role in electronics, for example as interconnection between carbon nanotubes in touch-screens.[xlvi]

Furthermore, nanostructures of gold are used in a wide range of personal care products and cosmetics, including face creams and fluids and hair straighteners. Gold nanoparticles can also be found surface bound to textiles in clothing and in cleaning products. [xlvii,xlviii]

2.11.4 Sources of non-occupational human exposure

For centuries the most common way to come directly into contact with gold was and still is through wearing of gold jewellery or via tooth fillings. It is possible that the abrasion of such gold surfaces may produce nanoparticles. Some cultures use gold dust or gold flakes to decorate food (e.g. the schnapps *"Danziger Goldwasser"*) and in some Asian countries, edible gold is found in fruit jelly snacks or even in coffee. If Au nanoparticles are swallowed, they will almost completely be excreted from the human body (Schleh, Semmler-Behnke et al 2012). In Europe, textiles made from threads coated with Au nanoparticles are currently in high demand for the fashion industry.[xlix]

Importantly, gold nanoparticles seem to exhibit a low cytotoxicity (Connor, Mwamuka et al 2005). However, it is essential to note that cytotoxicity is strongly dependent on the exact nature of the Au nanoparticles. Distribution and the effects in the body vary widely depending on the size of the Au nanoparticles (De Jong, Hagens et al 2008; Hirn, Semmler-Behnke et al 2011; Schleh, Semmler-Behnke et al 2012). Only the small gold particles (<10 nm) could be found in the brain of animals after injection of a certain dose. Very small Au-clusters can fit into the grooves of DNA-molecules (Schmid 2008), induce oxidative stress (Pan, Leifert et al 2009), and thereby cause cytotoxic effects. Nowadays, several gold nanoparticle-based drugs are being investigated and clinical trials are under development (Schleh, Semmler-Behnke et al 2012).

2.11.5 Specific human-health concerns

In general, gold has a high biocompatibility and shows only little toxicity. Given that gold jewellery has been worn by people for centuries, it is possible to conclude that human skin has a high tolerance for this metal. If gold nanoparticles are swallowed, they will almost completely be excreted from the human body (Schleh, Semmler-Behnke et al 2012).

The available literature reports, both *in vitro* and *in vivo*, vary widely in their methods and conclusions (Ostrowski, Martin et al 2009). Many reports indicate that gold nanoparticles are nontoxic; however, others contradict this finding. Apparently the size of the gold nanoparticles plays a big role when looking at possible effects on humans. To draw a complete conclusion, more studies are needed, which include critical nanoparticle characterization both prior to and after mixing with the biological media, with a focus on the change of the physical properties such as aggregation state, effective surface charge, degree and identity of protein adsorption, and desorption of chemicals from the surface of the nanoparticles (Alkilany and Murphy 2010). In some cases with a negative reaction to

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[xlvi] Gold Nanoparticles - Knowledge Base Nanomaterials accessed 09/11/15
[xlvii] Consumer Product Inventory - Project on Emerging Nanotechnologies - Gold Nanoparticles accessed 09/11/15
[xlviii] The Nanodatabase - Gold Nanoparticles accessed 09/11/15
[xlix] Gold Nanoparticles - Knowledge Base Nanomaterials accessed 09/11/15

gold, the effects could be attributed to a particular coating material, cetyl trimethyl ammonium bromide.[I]

2.11.6 References

Alkilany A,Murphy C. 2010. Toxicity and cellular uptake of gold nanoparticles: What we have learned so far? *Journal of Nanoparticle Research* 12 (7): 2313-33

Connor EE, Mwamuka J, Gole A et al. 2005. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1 (3): 325-7

De Jong WH, Hagens WI, Krystek P et al. 2008. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials* 29 (12): 1912-9

El-Sayed IH, Huang X,El-Sayed MA. 2005. Surface plasmon resonance scattering and absorption of anti-egfr antibody conjugated gold nanoparticles in cancer diagnostics: Applications in oral cancer. *Nano Letters* 5 (5): 829-34

Fleischer M, Zhang D, Braun K et al. 2010. Tailoring gold nanostructures for near-field optical applications. *Nanotechnology* 21 (6): 065301

Grzelczak M, Perez-Juste J, Mulvaney P et al. 2008. Shape control in gold nanoparticle synthesis. *Chemical Society Reviews* 37 (9): 1783-91

Hirn S, Semmler-Behnke M, Schleh C et al. 2011. Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration. *European Journal of Pharmaceutics and Biopharmaceutics* 77 (3): 407-16

Kneipp J, Kneipp H, Rice WL et al. 2005. Optical probes for biological applications based on surface-enhanced raman scattering from indocyanine green on gold nanoparticles. *Analytical Chemistry* 77 (8): 2381-5

Lee J, Chatterjee DK, Lee MH et al. 2014. Gold nanoparticles in breast cancer treatment: Promise and potential pitfalls. *Cancer letters* 347 (1): 46-53

Mieszawska AJ, Mulder WJM, Fayad ZA et al. 2013. Multifunctional gold nanoparticles for diagnosis and therapy of disease. *Molecular pharmaceutics* 10 (3): 831-47

Ostrowski AD, Martin T, Conti J et al. 2009. Nanotoxicology: Characterizing the scientific literature, 2000–2007. *Journal of Nanoparticle Research* 11 (2): 251-7

Pan Y, Leifert A, Ruau D et al. 2009. Gold nanoparticles of diameter 1.4 nm trigger necrosis by oxidative stress and mitochondrial damage. *Small* 5 (18): 2067-76

Payne EK, Shuford KL, Park S et al. 2006. Multipole plasmon resonances in gold nanorods. *Journal of Physical Chemistry: Part B* 110 (5): 2150-4

Rycenga M, Wang Z, Gordon E et al. 2009. Probing the photothermal effect of gold-based nanocages with surface-enhanced raman scattering (sers). *Angewandte Chemie* 48 (52): 9924-7

[I] Gold Nanoparticles - Knowledge Base Nanomaterials accessed 09/11/15

Schleh C, Semmler-Behnke M, Lipka J et al. 2012. Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration. *Nanotoxicology* 6 (1): 36-46

Schmid G. 2008. The relevance of shape and size of au55 clusters. *Chemical Society Reviews* 37 (9): 1909-30

Stokes RJ, Macaskill A, Lundahl PJ et al. 2007. Quantitative enhanced raman scattering of labeled DNA from gold and silver nanoparticles. *Small* 3 (9): 1593-601

Tréguer-Delapierre M, Majimel J, Mornet S et al. 2008. Synthesis of non-spherical gold nanoparticles. *Gold Bulletin* 41 195–207

Turkevich J, Stevenson PC,Hillier J. 1951. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discussions of the Faraday Society* 11 (0): 55-75

2.12 NANOMICELLES

2.12.1 Physical properties

Micelles share many characteristics with liposomes (see section 2.13). Micelles are amphiphilic lipids or polymers that self-assemble into spheres, with a hydrophobic core and a hydrophilic exterior 'corona' (Alai, Lin et al 2015). In contrast to liposomes, no interior 'capsule' is formed and micelles are primarily used as nanocarriers for hydrophobic compounds.

Polymeric nanomicelles are typically spherical and 10 to 100 nm in diameter (Cabral and Kataoka 2014). However, due to the polydisperse (varying in molecular weight) nature of the materials used to produce the micelles, micelle preparations will usually contain a range of particle sizes (Sakai-Kato, Nishiyama et al 2015).

2.12.2 Method of production

Micelles self-assemble in aqueous media when the polymer concentration exceeds the critical micelle concentration (Eetezadi, Ekdawi et al 2015). Consequently, production of micelles is dependent on the isolation or synthesis of polymers with appropriate properties (Ge, Li et al 2015).

Initiation of micelle formation can be triggered by processes such as sonication (Ge, Li et al 2015) or high-pressure emulsification (Sakai-Kato, Nishiyama et al 2015).

Block copolymers[li] are increasingly being used to produce micelles with increased thermodynamic and kinetic stability for particular therapeutic applications (Eetezadi, Ekdawi et al 2015). Polyethylene glycol is being increasing used as one component of the BCP, to form the hydrophilic corona of the micelle.

2.12.3 Relevant applications or uses

Micelles have been investigated as nanocarriers for drugs (Akimoto, Nakayama et al 2014; Cabral and Kataoka 2014; Jeetah, Bhaw-Luximon et al 2014), insulin (Alai, Lin et al 2015), therapeutic proteins or peptides (Baba, Itaka et al 2015), antioxidants (Ge, Li et al 2015) and nucleic acids (Jhaveri and Torchilin 2014). Several micelle-based products are in clinical evaluation, while others have been approved by USFDA for treatment of various cancer types (Cabral and Kataoka 2014; Jeetah, Bhaw-Luximon et al 2014; Jhaveri and Torchilin 2014; Lu and Park 2013).

Micelles have also been engineered to carry agents that improve contrast in various imaging technologies (Torchilin 2002). This has led to the development of multifunctional micelles that not only deliver a therapeutic payload to a particular site, but also improve imaging at that site (Jhaveri and Torchilin 2014). This approach is sometimes known as theranostics, where a diagnostic tool and a therapeutic agent are delivered by the same nanocarrier (Muthu, Leong et al 2014).

The micelle payload may be covalently bonded to the micelle polymers or attached to the micelle by non-covalent interactions (hydrogen bonding, ionic bonding or hydrophobic interactions) (Ke, Ng et al 2014).

[li] Block copolymers are formed from two different types of monomer, with the different monomers forming separate polymer 'blocks' which are joined at the end. For micelle formation, one of the polymer blocks with be hydrophobic and one hydrophilic

Nanomicelles have been used for chemical analytical purposes, such as for making functionalised indicator papers for detection of banned food dyes (Chen, Chen et al 2014), and for production of nanoporous filtration membranes (Upadhyaya, Semsarilar et al 2015).

Nanomicelles are also finding applications in the food industry, for the controlled release of labile vitamins, flavouring agents, antimicrobials and other bioactive molecules (Arunkumar, Prashanth et al 2013; Li, Peng et al 2014; Ziani, Fang et al 2012).

Consumer products claiming to incorporate nanomicelles include dietary supplements[lii,liii] colloidal cleaning products and a soil-applied plant nutrition aid.

2.12.4 Sources of non-occupational human exposure

Inhalation

There is some potential for inhalation exposure to nanomicelles during application of cleaning products and plant nutrition aids.

Dermal

There is some potential for dermal exposure to nanomicelles during application of cleaning products and plant nutrition aids.

Oral

Oral exposure to nanomicelles is the most likely route of exposure, due to their use in dietary supplements and their potential for use in food preparations.

2.12.5 Specific human-health concerns

No human health concerns have been identified with respect to nanomicelles although it is acknowledged that health impacts should be considered (Hu, Wang et al 2013; Sakai-Kato, Nishiyama et al 2015). However, it should be noted that due to a large potential variability in materials used to form the micelle nanostructure and the range of chemicals that may be used to functionalise the micelles, safety of nanomicelles will probably need to be considered on a case by case basis.

2.12.6 References

Akimoto J, Nakayama M,Okano T. 2014. Temperature-responsive polymeric micelles for optimizing drug targeting to solid tumors. *Journal of Controlled Release* 193 2-8

Alai MS, Lin WJ,Pingale SS. 2015. Application of polymeric nanoparticles and micelles in insulin oral delivery. *Journal of Food and Drug Analysis* 23 (3): 351-8

Arunkumar R, Prashanth KVH,Baskaran V. 2013. Promising interaction between nanoencapsulated lutein with low molecular weight chitosan: Characterization and bioavailability of lutein in vitro and in vivo. *Food Chemistry* 141 (1): 327-37

Baba M, Itaka K, Kondo K et al. 2015. Treatment of neurological disorders by introducing mrna in vivo using polyplex nanomicelles. *Journal of Controlled Release* 201 41-8

[lii] http://nanodb.dk/en/search-database/?keyword=micelle Accessed 12 November 2015
[liii] http://www.nanotechproject.org/cpi/browse/nanomaterials/nano-micelles/ Accessed 12 November 2015

Cabral H,Kataoka K. 2014. Progress of drug-loaded polymeric micelles into clinical studies. *Journal of Controlled Release* 190 465-76

Chen H, Chen MW, Wang XH et al. 2014. Self-assembled conjugated polymer/carboxymethyl chitosan grafted poly(p-dioxanone) nanomicelles and their use in functionalized indicator paper for fast and visual detection of a banned food dye. *Polymer Chemistry* 5 (14): 4251-8

Eetezadi S, Ekdawi SN,Allen C. 2015. The challenges facing block copolymer micelles for cancer therapy: In vivo barriers and clinical translation. *Advanced Drug Delivery Reviews* 91 7-22

Ge W, Li D, Chen M et al. 2015. Characterization and antioxidant activity of β-carotene loaded chitosan-graft-poly(lactide) nanomicelles. *Carbohydrate Polymers* 117 169-76

Hu P, Wang T, Xu Q et al. 2013. Genotoxicity evaluation of stearic acid grafted chitosan oligosaccharide nanomicelles. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 751 (2): 116-26

Jeetah R, Bhaw-Luximon A,Jhurry D. 2014. Polymeric nanomicelles for sustained delivery of anti-cancer drugs. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 768 47-59

Jhaveri AM,Torchilin VP. 2014. Multifunctional polymeric micelles for delivery of drugs and sirna. *Frontiers in Pharmacology* 5 77

Ke X, Ng VWL, Ono RJ et al. 2014. Role of non-covalent and covalent interactions in cargo loading capacity and stability of polymeric micelles. *Journal of Controlled Release* 193 9-26

Li W, Peng H, Ning F et al. 2014. Amphiphilic chitosan derivative-based core-shell micelles: Synthesis, characterisation and properties for sustained release of vitamin d-3. *Food Chemistry* 152 307-15

Lu Y,Park K. 2013. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. *International Journal of Pharmaceutics* 453 (1): 198-214

Muthu MS, Leong DT, Mei L et al. 2014. Nanotheranostics - application and further development of nanomedicine strategies for advanced theranostics. *Theranostics* 4 (6): 660-77

Sakai-Kato K, Nishiyama N, Kozaki M et al. 2015. General considerations regarding the in vitro and in vivo properties of block copolymer micelle products and their evaluation. *Journal of Controlled Release* 210 76-83

Torchilin VP. 2002. Peg-based micelles as carriers of contrast agents for different imaging modalities. *Advanced Drug Delivery Reviews* 54 (2): 235-52

Upadhyaya L, Semsarilar M, Nehache S et al. 2015. Filtration membranes from self-assembled block copolymers - a review on recent progress. *European Physical Journal-Special Topics* 224 (9): 1883-97

Ziani K, Fang Y,McClements DJ. 2012. Encapsulation of functional lipophilic components in surfactant-based colloidal delivery systems: Vitamin e, vitamin d, and lemon oil. *Food Chemistry* 134 (2): 1106-12

2.13 NANOLIPOSOMES

2.13.1 Physical properties

A liposome is a spherical vesicle having at least one lipid bilayer. They can range in size from about 30 nm to several micrometres (Akbarzadeh, Rezaei-Sadabady et al 2013). Liposomes are most often composed of phospholipids, especially phosphatidylcholine. Liposomes possess both hydrophobic and hydrophilic characteristics in aqueous solution, with hydrophobic hydrocarbon tails of phospholipids directed into the bilayer, while the polar head groups are directed to the aqueous environment of the inner and outer media (Blanco-Padilla, Soto et al 2014). Liposomes may contain a single bilayer (unilamellar) or multiple bilayers, producing an onion-like structure (multilamellar) (Patil and Jadhav 2014).

Liposomes can vary in rigidity, depending on the source of the lipid material (Akbarzadeh, Rezaei-Sadabady et al 2013). For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas saturated phospholipids with long acyl chains (for example, dipalmitoylphosphatidylcholine) form a rigid, rather impermeable bilayer structure. The addition of cholesterol can also increase liposome rigidity and decrease permeability (Cipolla, Shekunov et al 2014).

The surface of the liposomes can also be modified, using additives such as polyethyleneglycol (PEG) to target liposomes to particular cell types or to increase their *in vivo* stability (Cipolla, Shekunov et al 2014).

2.13.2 Method of production

A number of techniques are available for preparation of liposomes (McClements 2015; Mozafari 2005; Patil and Jadhav 2014).

Solvent evaporation/rehydration. Phospholipids are dissolved in an organic solvent. The solvent is then removed by evaporation, leaving a thin layer of phospholipid. Hydration results in spontaneous formation of relatively large liposomes. These can be decreased in size by application of mechanical energy (sonication, microfluidisation, high pressure homogenisation).

Solvent displacement. Phospholipids are dissolved in an amphiphilic solvent, such as ethanol. The solution is then injected into an aqueous media, resulting in the ethanol diffusing away from the lipids and the lipids self-assembling into liposomes.

Surfactant displacement. This is similar to solvent displacement, but a water-soluble surfactant is used, instead of an amphiphilic solvent.

Homogenisation. A crude lipid suspension is formed in aqueous solution, which is then homogenised by sonication, high pressure valve or membrane homogenisation or microfluidisation. This technique tends to produce small liposomes with a single bilayer.

Heating. An aqueous suspension of liposome components is heated (120ºC) in the presence of glycerol. The glycerol maintains dispersion of the liposomes, facilitates freezing for storage and is physiologically acceptable, as opposed to residual solvents and surfactants left by other methods.

Supercritical fluids can also be used in place of organic solvents or in concert with organic solvents (Patil and Jadhav 2014). Such techniques have the potential to mitigate toxicity issues due to residual solvents.

2.13.3 Relevant applications or uses

Liposomes are extensively used as nanocarriers for numerous molecules in cosmetic and pharmaceutical industries (Cipolla, Shekunov et al 2014; Fernandes, Ferreira et al 2015; Koudelka, Turanek Knotigova et al 2015; Lim, Banerjee et al 2012; Wang, Miao et al 2015). Additionally, food and farming industries have investigated the use of liposome encapsulation to deliver unstable compounds (for example, antimicrobials, antioxidants and enzymes) and shield their functionality (Blanco-Padilla, Soto et al 2014; Malheiros, Daroit et al 2012; Mozafari, Johnson et al 2008; Ruyra, Cano-Sarabia et al 2013).

Liposomes can trap both hydrophobic (in the bilayer) and hydrophilic (in the interior space) compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets (Akbarzadeh, Rezaei-Sadabady et al 2013).

Liposome-encapsulated octyl methoxycinnamate has shown superior characteristics for sunscreen formulations than currently-used carriers (Varjao Mota, Faria de Freitas et al 2013).

2.13.4 Sources of non-occupational human exposure

Inhalation

There currently appears to be little potential for inhalation exposure to nanoliposomes outside of the medical sector, where nanoliposomes are being investigated for pulmonary delivery of medication (Cipolla, Shekunov et al 2014).

Dermal

Dermal exposure is the most likely current non-occupational, non-food, non-medical route of human exposure to nanoliposomes. The Consumer Products Inventory lists four topical applications (joint and muscle pain relief cream, anti-hair loss serum, cellulite cream and baby skin care cream) as containing nanoliposomes.[liv] The Nanodatabase also lists nanoliposome products predominantly for dermal application (skin cleanser, vitamin C powder and hair growth product).[lv]

Oral

With the exception of food and medical/pharmaceutical applications, not covered by this summary, oral exposure to nanoliposomes can occur from consumption of some vitamin C preparations.[1,2]

2.13.5 Specific human-health concerns

No human health concerns have been identified with nanoliposomes. The components of the liposomes are either normally present in the human body and in human food items or closely related to substances present in the human body. Liposomes have been classified as non-toxic, biocompatible nanoparticles approved by FDA for application in human medicine (Koudelka, Turanek Knotigova et al 2015).

Cell-based cytotoxicity assays of nanoliposomes, prepared by the heating method, demonstrated no loss of cell viability (Mozafari, Reed et al 2007). Nanoliposomes prepared

[liv] http://www.nanotechproject.org/cpi/browse/nanomaterials/liposome/ Accessed 10 November 2015
[lv] http://nanodb.dk/en/search-database/?keyword=liposome Accessed 10 November 2015

by solvent evaporation show significant cell toxicity, possibly due to the presence of residual organic solvents.

The potential for nanoliposome delivery vehicles to increase the risk of sensitisation, when haptens[lvi] are delivered, has been demonstrated (Simonsson, Madsen et al 2011). However, it must be questioned why known skin sensitisers would be delivered in this manner.

2.13.6 References

Akbarzadeh A, Rezaei-Sadabady R, Davaran S et al. 2013. Liposome: Classification, preparation, and applications. *Nanoscale Research Letters* 8 102

Blanco-Padilla A, Soto KM, Hernandez Iturriaga M et al. 2014. Food antimicrobials nanocarriers. *Scientific World Journal* 2014 837215

Cipolla D, Shekunov B, Blanchard J et al. 2014. Lipid-based carriers for pulmonary products: Preclinical development and case studies in humans. *Advanced Drug Delivery Reviews* 75 53-80

Fernandes E, Ferreira JA, Andreia P et al. 2015. New trends in guided nanotherapies for digestive cancers: A systematic review. *Journal of Controlled Release* 209 288-307

Koudelka S, Turanek Knotigova P, Masek J et al. 2015. Liposomal delivery systems for anti-cancer analogues of vitamin e. *Journal of Controlled Release* 207 59-69

Lim SB, Banerjee A,Önyüksel H. 2012. Improvement of drug safety by the use of lipid-based nanocarriers. *Journal of Controlled Release* 163 (1): 34-45

Malheiros PdS, Daroit DJ,Brandelli A. 2012. Inhibition of *listeria monocytogenes* in minas frescal cheese by free and nanovesicle-encapsulated nisin. *Brazilian Journal of Microbiology* 43 (4): 1414-8

McClements DJ. 2015. Encapsulation, protection, and release of hydrophilic active components: Potential and limitations of colloidal delivery systems. *Advances in Colloid and Interface Science* 219 27-53

Mozafari MR. 2005. Liposomes: An overview of manufacturing techniques. *Cellular & Molecular Biology Letters* 10 (4): 711-9

Mozafari MR, Reed CJ,Rostron C. 2007. Cytotoxicity evaluation of anionic nanoliposomes and nanolipoplexes prepared by the heating method without employing volatile solvents and detergents. *Pharmazie* 62 (3): 205-9

Mozafari MR, Johnson C, Hatziantoniou S et al. 2008. Nanoliposomes and their applications in food nanotechnology. *Journal of Liposome Research* 18 (4): 309-27

Patil YP,Jadhav S. 2014. Novel methods for liposome preparation. *Chemistry and Physics of Lipids* 177 8-18

[lvi] Haptens are small molecules that elicit an immune response only when attached to a larger carrier such as a protein.

Ruyra A, Cano-Sarabia M, MacKenzie SA et al. 2013. A novel liposome-based nanocarrier loaded with an lps-dsrna cocktail for fish innate immune system stimulation. *Plos One* 8 (10): e76338

Simonsson C, Madsen JT, Graneli A et al. 2011. A study of the enhanced sensitizing capacity of a contact allergen in lipid vesicle formulations. *Toxicology and Applied Pharmacology* 252 (3): 221-7

Varjao Mota AdC, Faria de Freitas ZM, Ricci Junior E et al. 2013. In vivo and in vitro evaluation of octyl methoxycinnamate liposomes. *International Journal of Nanomedicine* 8 4689-701

Wang Y, Miao L, Satterlee A et al. 2015. Delivery of oligonucleotides with lipid nanoparticles. *Advanced Drug Delivery Reviews* 87 68-80

2.14 DENDRIMERS

2.14.1 Physical properties

Dendrimers are nano-sized (<100 nm), radially symmetric molecules with well-defined, homogeneous, and monodisperse structure consisting of tree-like arms or branches (Abbasi, Aval et al 2014; Duncan and Izzo 2005). They consist of symmetric branching units built around a small molecule or a linear polymer core. Dendrimers may carry a large number of chemical functional groups, either at the ends of the 'branches' or within the body of the macromolecule. These functional groups and the size of the macromolecule define the properties of the dendrimer.

Common dendrimer branch structures include, polyamidoamines (PAMAM), poly(L-lysine) (PLL), polyesters (PGLSA-OH), polypropylimines (PPI), poly(2,2-bis (hydroxymethyl) propionic acid) (bis-MPA) and aminobis(methylenephosphonic acid) dendrimer (Mignani, El Kazzouli et al 2013).

Dendrimers may be attached to a range of other molecular or atomic species, either by bonding to the peripheral functional groups or incorporation into voids within the structure of the dendrimer.

2.14.2 Method of production

Dendrimers are produced by synthetic chemical techniques. Two broad classes of methods exist; divergent (assembled from a multifunctional core, which is extended outward by a series of reactions) and convergent (reactions proceed inward and are eventually attached to a core) (Abbasi, Aval et al 2014). For divergent synthesis methods, each reaction step must be driven to completion, to avoid differing branch lengths, as these will impact on the symmetry and the associated properties of the molecule. For convergent methods, the ultimate size of the dendrimer is pre-defined and a branch of appropriate length must be synthesised.

2.14.3 Relevant applications or uses

The most important potential application of dendrimers is as 'nanocarriers', transporting other molecules, such as drugs, to their desired destination (Abbasi, Aval et al 2014; Jedrych, Borowska et al 2014). Cancer drugs can be attached to dendrimers and the functionality of the dendrimer tailored to direct it to the cancer cells (Kesharwani, Jain et al 2014). Drug-dendrimer conjugates show high solubility, reduced systemic toxicity, and selective accumulation in solid tumours. Dendrimers have also shown potential for transdermal and ocular drug delivery (Mignani, El Kazzouli et al 2013), DNA delivery in gene therapy (Luo, He et al 2014), to improve contrast in magnetic resonance imaging (MRI) and other molecular imaging techniques (Qiao and Shi 2015), and in a range of sensitive analytical chemistry and biochemistry applications (Fu and Li 2013; Hasanzadeh, Shadjou et al 2014).

The peripheral functional groups of dendrimers can also be manipulated to produce combinations which are able to form complexes with surface receptors of cells or micro-organisms, disrupting initial interactions between cells and microbial pathogens. A dendrimer-based product has been included in clinical trials as a topical microbiocide to prevent transmission of the Human Immunodeficiency Virus (HIV) (Date and Destache 2013; du Toit, Pillay et al 2010).

2.14.4 Sources of non-occupational human exposure

Currently, there are no identifiable sources of non-occupational human exposure to dendrimers. Most dendrimer applications are at the stage of laboratory experimentation or in controlled clinical trials.

2.14.5 Specific human-health concerns

No specific human health concerns have been associated with dendrimers. Depending on their chemical composition, dendrimers can exhibit toxicity in *in vitro* and *in vivo* animal studies (Duncan and Izzo 2005; Jain, Kesharwani et al 2010; Pryor, Harper et al 2014). Toxicity is believed to be mainly due to membrane disruption from interaction of cationic functional groups with negatively charged membranes and increases with increasing dendrimer size (Jain, Kesharwani et al 2010; Shcharbin, Janaszewska et al 2014). However, the proposed uses in human biomedical applications will require careful consideration of the toxicity of specific applications. A variety of strategies have been developed to mitigate the toxicity of dendrimers (Jain, Kesharwani et al 2010), including production of dendrimers with anionic or neutral functional groups, which show much lower toxicity (Kunzmann, Andersson et al 2011; Pryor, Harper et al 2014). Toxic effects of dendrimers appear to be fully reversible following cessation of dosing (Shcharbin, Janaszewska et al 2014).

2.14.6 References

Abbasi E, Aval SF, Akbarzadeh A et al. 2014. Dendrimers: Synthesis, applications, and properties. *Nanoscale Research Letters* 9 247

Date AA,Destache CJ. 2013. A review of nanotechnological approaches for the prophylaxis of hiv/aids. *Biomaterials* 34 (26): 6202-28

du Toit LC, Pillay V,Choonara YE. 2010. Nano-microbicides: Challenges in drug delivery, patient ethics and intellectual property in the war against hiv/aids. *Advanced Drug Delivery Reviews* 62 (4-5): 532-46

Duncan R,Izzo L. 2005. Dendrimer biocompatibility and toxicity. *Advanced Drug Delivery Reviews* 57 (15): 2215-37

Fu C,Li J-P. 2013. Application of dendrimers in analytical chemistry. *Chinese Journal of Analytical Chemistry* 41 (11): 1762-72

Hasanzadeh M, Shadjou N, Eskandani M et al. 2014. Dendrimer-encapsulated and cored metal nanoparticles for electrochemical nanobiosensing. *Trac-Trends in Analytical Chemistry* 53 137-49

Jain K, Kesharwani P, Gupta U et al. 2010. Dendrimer toxicity: Let's meet the challenge. *International Journal of Pharmaceutics* 394 (1-2): 122-42

Jedrych M, Borowska K, Galus R et al. 2014. The evaluation of the biomedical effectiveness of poly(amido)amine dendrimers generation 4.0 as a drug and as drug carriers: A systematic review and meta-analysis. *International Journal of Pharmaceutics* 462 (1-2): 38-43

Kesharwani P, Jain K,Jain NK. 2014. Dendrimer as nanocarrier for drug delivery. *Progress in Polymer Science* 39 (2): 268-307

Kunzmann A, Andersson B, Thurnherr T et al. 2011. Toxicology of engineered nanomaterials: Focus on biocompatibility, biodistribution and biodegradation. *Biochimica Et Biophysica Acta-General Subjects* 1810 (3): 361-73

Luo K, He B, Wu Y et al. 2014. Functional and biodegradable dendritic macromolecules with controlled architectures as nontoxic and efficient nanoscale gene vectors. *Biotechnology Advances* 32 (4): 818-30

Mignani S, El Kazzouli S, Bousmina M et al. 2013. Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview. *Advanced Drug Delivery Reviews* 65 (10): 1316-30

Pryor JB, Harper BJ,Harper SL. 2014. Comparative toxicological assessment of pamam and thiophosphoryl dendrimers using embryonic zebrafish. *International Journal of Nanomedicine* 9 1947-56

Qiao Z,Shi X. 2015. Dendrimer-based molecular imaging contrast agents. *Progress in Polymer Science* 44 1-27

Shcharbin D, Janaszewska A, Klajnert-Maculewicz B et al. 2014. How to study dendrimers and dendriplexes iii. Biodistribution, pharmacokinetics and toxicity in vivo. *Journal of Controlled Release* 181 40-52

2.15 NANOCOCHLEATES

2.15.1 Physical properties

Cochleates are structurally related to liposomes and earlier researchers suggested they were intermediates in the preparation of liposomes (Papahadjopoulos, Vail et al 1975). Cochleates consist of a solid lipid bilayer rolled into a spiral (Acevedo, Perez et al 2012). Cochleates are precipitated with a divalent cation, usually calcium, and the cation intercalates between the overlapping sheets of the negatively-charged lipid bilayer (Gould-Fogerite, Kheiri et al 1998). Hydrophilic and hydrophobic compounds can be accommodated within the lipid bilayers or between the bilayer sheets. Cochleates are more thermodynamically stable than liposomes.

If should be noted that, while nanocochleates are referred to as nanoparticles, the measured size of cochleates has been reported to range from about 100 nm (Syed, Woo et al 2008) to several hundred nanometres (Pham, Gueutin et al 2014) to several micrometres (Gil, Bracho et al 2006).

2.15.2 Method of production

Cochleates are produced from liposome preparations. The liposomes are solubilised in detergent, followed by addition of calcium ions at the same time as removal of detergent by dialysis (Acevedo, Perez et al 2012) or cross flow filtration (Bracho, Lastre et al 2006). Cochleate formation has also been demonstrated using cationic drugs or peptides to bridge the lipid sheets, rather than divalent cations (Syed, Woo et al 2008).

Size of cochleate preparations can be controlled through use of freeze-thaw cycles (Miclea, Varma et al 2007) and sonication (Gil, Bracho et al 2006).

2.15.3 Relevant applications or uses

Cochleates have been produced from the proteoliposomes of pathogenic microbes to produce stable adjuvants, used to elicit an immune response (vaccination) against the pathogen (Acevedo, Perez et al 2012; Bracho, Lastre et al 2006; Gould-Fogerite, Kheiri et al 1998). Immunogenicity can be further increased by inclusion of further molecular recognition compounds (proteins, peptides, DNA) within the cochleate structure (Gil, Bracho et al 2006). Cochleates are effective carriers for targeting mucosal tissues and present the possibility of intra-nasal or oral administration of vaccines (Gould-Fogerite, Kheiri et al 1998).

Cochleates have also shown potential as nanocarriers for therapeutics, such as the glycoprotein Factor VIII, used in the treatment of haemophilia (Miclea, Varma et al 2007) and drugs used in the treatment of visceral leishmaniasis and candidiasis (Pham, Barratt et al 2013; Pham, Gueutin et al 2014; Santangelo, Paderu et al 2000).

2.15.4 Sources of non-occupational human exposure

Applications utilising nanocochleates appear to be still at the experimental stage and no source of potential non-occupational human exposure was identified.

2.15.5 Specific human-health concerns

The safety of cochleates derived from *Neisseria meningitides* B proteoliposome has been assessed following repeated-dose intranasal administration (Infante, Sifontes et al 2012). No treatment-related adverse effects were observed after administration of four doses at five day intervals.

2.15.6 References

Acevedo R, Perez O, Zayas C et al. 2012. Cochleates derived from *vibrio cholerae* o1 proteoliposomes: The impact of structure transformation on mucosal immunisation. *Plos One* 7 (10):

Bracho G, Lastre M, Campo Jd et al. 2006. Proteoliposome derived cochleate as novel adjuvant. *Vaccine* 24, Supplement 2: S30-S1

Gil D, Bracho G, Zayas C et al. 2006. Strategy for determination of an efficient cochleate particle size. *Vaccine* 24, Supplement 2: S92-S3

Gould-Fogerite S, Kheiri MT, Zhang F et al. 1998. Targeting immune response induction with cochleate and liposome-based vaccines. *Advanced Drug Delivery Reviews* 32 (3): 273-87

Infante JF, Sifontes S, Arencibia DF et al. 2012. Assessment of repeat dose toxicity of cochleate derived from neisseria meningitidis proteoliposome in sprague dawley rats. *Vaccimonitor* 21 (3): 1-5

Miclea RD, Varma PR, Peng A et al. 2007. Development and characterization of lipidic cochleate containing recombinant factor viii. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1768 (11): 2890-8

Papahadjopoulos D, Vail WJ, Jacobson K et al. 1975. Cochleate lipid cylinders - formation by fusion of unilamellar lipid vesicles. *Biochimica Et Biophysica Acta* 394 (3): 483-91

Pham TTH, Barratt G, Michel JP et al. 2013. Interactions of antileishmanial drugs with monolayers of lipids used in the development of amphotericin b–miltefosine-loaded nanocochleates. *Colloids and Surfaces B: Biointerfaces* 106: 224-33

Pham TTH, Gueutin C, Cheron M et al. 2014. Development of antileishmanial lipid nanocomplexes. *Biochimie* 107, Part A: 143-53

Santangelo R, Paderu P, Delmas G et al. 2000. Efficacy of oral cochleate-amphotericin b in a mouse model of systemic candidiasis. *Antimicrobial Agents and Chemotherapy* 44 (9): 2356-60

Syed UM, Woo AF, Plakogiannis F et al. 2008. Cochleates bridged by drug molecules. *International Journal of Pharmaceutics* 363 (1–2): 118-25

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