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Scientific Committee on Consumer Safety

SCCS

OPINION ON

preservative EcoG+

The SCCS adopted this opinion at its 2nd plenary meeting

on 6 October 2016

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SCCS

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1. BACKGROUND

Submission I for the preservative with the name EcoG+ was submitted by June 2012.

The proposed new preservative 'EcoG+' is silver-containing phosphate glass, a powder consisting of small glass beads. 'EcoG+' is intended to be mixed with glass beads and an appropriate polymer and used to manufacture composite materials for cosmetic product packaging. The substance intended to be released into the cosmetic product is the silver ion. 'EcoG+' therefore acts as an inert carrier, into which the silver ion is dispersed.
'EcoG+' is mixed with glass beads and a suitable polymer to form a composite packaging material, a proportion of the glass matrix remains at the surface of the material. The achieved level of the active component (i.e. silver) in 'EcoG+' is 2%; the proportion of 'EcoG+' envisaged for use in the packaging material is 3%. On use of the composite material as cosmetic product packaging, small amounts of silver ions are released into the cosmetic product, where it is intended to have a preservative function.

The typical use of 'EcoG+' is envisaged to be a level of 1.4-2.0% in cosmetics packaging. The maximum achieved level of silver ion in 'EcoG+' is 2%, therefore the maximum achieved level of silver present in the cosmetic packaging material is 420-600 ppm.

The information is subject of the attached Submission I.

Submission II that updates the previously submitted dossier (submission I) was submitted by the applicant in February 2014, in response to specific questions for clarifications from the SCCS.

2. TERMS OF REFERENCE

1. *Does SCCS consider* release of silver ions from *"EcoG+" as component in* packaging *material safe for use as preservative with a concentration of maximum 2.0 % in the cosmetic packaging material, taking into account the scientific data provided?*

2. *And/or does the SCCS recommend any further restrictions with regard to the use of "EcoG+" as preservative in cosmetics packaging?*

3. OPINION

This draft contains information as provided in submission I (of 2012) and the update (submission II of 2014).

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

EcoG+ is a composite material consisting of three components, i.e. (1) silver-containing phosphate glass powder, (2) glass beads and (3) an appropriate polymer (for example polypropylene, PP), as detailed in Table 1, below. The inclusion of glass beads is not required for the antimicrobial activity of EcoG+ and it is envisaged that some packaging may contain components (1) and (3) only.

Table 1 Components of EcoG+

Component	Function	Typical composition
Silver glass powder containing 1.4-2.0% Ag+	Active component	3%
Glass beads	Inert carrier	≤52%
Polymer (polypropylene)	Inert carrier	≥45%
Silver ion	Active component	420-600 ppm

The silver glass powder (Million Guard PG721ST) contains a level of 1.4-2.0% silver ion and is mixed with the other components of EcoG+ (glass beads and polymer) to a maximum level of 3%. The level of silver ion achieved in the EcoG+ packaging is therefore 420-600 ppm.
As described above, EcoG+ contains three components; i.e. silver-containing phosphate glass powder, glass beads and an appropriate polymer. EcoG+ therefore acts as a carrier for the active component, the silver ion, which is intended to be released in small amounts into the cosmetic product contained within the EcoG+ packaging. The species ultimately released into the cosmetic product is the silver ion; the silver released into the cosmetic product is not in the form of colloidal silver or nanosilver. Additionally, although silver nitrate is used as the source of silver, it is important to note that the silver ion is released into the cosmetic product without the nitrate counterion. This is because any nitrate present is destroyed by the very high temperatures used in the manufacture of EcoG+ glass and is liberated during the manufacturing process as nitrogen gas.

3.1.1.1. Primary name and/or INCI name

Phosphate glass / Silver ion

3.1.1.2. Chemical names

Phosphate glass containing 1.4 to 2 % Ag / Silver

3.1.1.3. Trade names and abbreviations

‘EcoG+’

3.1.1.4. CAS / EC number (silver)

CAS: / 7440-22-4

EC: / 231-131-3

3.1.1.5. Structural formula

/ Ag^+

3.1.1.6. Empirical formula (silver glass powder)

subm II: 1/6 $Ag_2O \cdot (P_2O_5 \cdot ZnO)m \cdot (9CaO \cdot 2Al_2O_3 \cdot B_2O_3 \cdot Na_2O)n$

m=10, n=0.2～0.4

3.1.2. Physical form

EcoG+ is a solid composite material containing silver glass powder (Million Guard PG721ST), glass beads and a suitable polymer. The silver ions present in the silver glass powder (Million Guard PG721ST) will disperse throughout the structure of EcoG+. The dispersed silver ions will subsequently be released from EcoG+ and will be present (dissolved) in the aqueous component of the cosmetic product contained within the EcoG+ packaging.

3.1.3. Molecular weight

Calculation of molecular weight is not possible for ‘EcoG+’. The molecular weight of the silver ion (active component of the product) is 107.88

3.1.4. Purity, composition and substance codes

‘EcoG+’ is manufactured from the following components:

Calcium dihydrogen phosphate
Phosphoric acid
Zinc oxide
Zinc phosphate
Boric acid
Cerium oxide
Aluminium hydroxide
Sodium nitrate
Silicon dioxide
Silver nitrate [achieved level of silver: 2%]

The silver nitrate used in the manufacture of silver glass is of high purity (>99%). In use (i.e. following incorporation into the cosmetic product packaging), silver ions are released from the glass matrix into the cosmetic product.

3.1.5. Impurities / accompanying contaminants

The specification of the silver nitrate used for the manufacture of the silver glass is as follows:

Purity	>99%
Sulphates	<20 ppm
Chlorides	<5 ppm
Copper	<2 ppm
Iron	<2 ppm
Lead	<10 ppm

Impurities in silver nitrates (the source of silver present in 'EcoG+') are present at very low levels and will therefore not influence the toxicity of the product.

3.1.6. Solubility

EcoG+ is a solid composite material containing silver glass powder (Million Guard PG721ST), glass beads and a suitable polymer. The silver ions present in the silver glass powder (Million Guard PG721ST) will disperse throughout the structure of EcoG+. The dispersed silver ions will subsequently be released from EcoG+ and will be present (dissolved) in the aqueous component of the cosmetic product contained within the EcoG+ packaging.

3.1.7. Partition coefficient (Log Pow)

Calculation of the Log Pow is not possible for the composite material 'EcoG+' or for the silver glass component Million Guard PG721ST. Calculation of Log Pow for the silver ion is also not relevant due to its insolubility in octanol.

3.1.8. Additional physical and chemical specifications

Table 2.5 Summary of additional relevant properties of EcoG+ and Million Guard PG721ST

Physical state	Solid (EcoG+) White powder (Million Guard PG721ST)
Organoleptic properties	Odourless; colour may vary due to the incorporation of colorant agents into the composite material (EcoG+) Odourless white powder (Million Guard PG721ST)
Flash point/Flammability	Not relevant (EcoG+; Million Guard PG721ST)
Melting temperature	961.93°C (Million Guard PG721ST)
Oxidation/Reduction	Stable to oxidation, contact with strong reducing agents should be avoided. (Million Guard PG721ST)
Explosivity	Contains no functional groups that would confer explosive potential (EcoG+), (Million Guard PG721ST)
pH	Not relevant (EcoG+) The pH of a 1% solution of Million Guard PG721ST in water is 4.97 at 21°C
Density	Not relevant (EcoG+) The pour density of Million Guard PG721ST is 1.020 g/mL The tap density of Million Guard PG721ST is 1.331 g/mL

3.1.9. Homogeneity and Stability

'EcoG+' is chemically inert and is therefore stable following storage. Due to the nature of the active component (the silver ion), studies of stability in cosmetic products are not relevant.

3.2. Function and uses

EcoG+ is a composite packaging material consisting of three components; silver glass powder, glass beads and a suitable polymer (see above 3.1.1). A proportion of the glass matrix remains at the surface of the material. The proportion of silver glass (Million Guard PG721ST) envisaged for use in the EcoG+ packaging material is 3%. The achieved level of the active component (i.e. the silver ion) in EcoG+ packaging is 420-600 ppm. During the proposed use of the composite material as cosmetic product packaging, small amounts of silver ions are released into the cosmetic product, where a preservative function is intended. EcoG+ packaging may be used with a variety of cosmetic product types.

EcoG+ glass containing silver ions will be used only in the internal parts of any packaging (i.e. the parts of the packaging in direct contact with the cosmetic product) and will not be used on external parts potentially coming into contact with the hands of the consumer. The external parts of the cosmetic product packaging will be made of other materials that will prevent the migration of silver from the internal layer to the external parts of the packaging.

3.3. Toxicological Evaluation

Due to the nature of the product, its intended use and the low levels of silver to which consumers are predicted to be exposed, no new toxicity data have been generated using 'EcoG+'. Reference is made to existing public domain data on the toxicity of silver. Extensive toxicity data on silver, silver salts and silver compounds are available in the public domain and these data have been reviewed by various national and international bodies as part of the safety assessment of silver and silver compounds. An overview of the available data is presented in this dossier.
The applicant has stated that the silver in EcoG+ is not present in the form of nanosilver.
Several investigations have demonstrated the continuous release of silver ions from the surface of silver nanoparticles and metallic silver (Hadrup and Lam, 2014; Chernousova & Epple, 2013) and several studies showed similar responses of Ag+ ions and AgNPs on cells, suggesting that the toxicity can be due to release to free silver ions though there are also nano-specific responses (Beer et al., 2012; SCENIHR 2014; Huk et al. 2014, 2015; Butler et al. 2015). Thus, some information available from recent studies with nanosilver is also included when suitable, and the Opinion of SCENIHR on nanosilver was also consulted with regard to toxicity studies (SCENIHR 2014).

The safety assessment of EcoG+ is based on the release of silver ions from the packaging material.

3.3.1. Acute toxicity

No data have been generated for 'EcoG+'; adequate public domain data are available to address the acute toxicity of the silver ion to which consumers will be exposed.

Acute oral LD50 values for silver salts in mice are reported to be in the range 50-100 mg/kg bw (Faust, 1992; WHO, 2003). Acute oral LD50 values in the mouse of 100 mg/kg bw for colloidal silver and 129 mg/kg bw for silver nitrate; and acute oral LD50 values in the rat of 125 mg/kg bw for silver cyanide and >2820 mg/kg bw for the insoluble silver oxide are also reported (Faust, 1992). The US EPA (1992) stated that sufficient data are available to conclude that the acute toxicity of silver is relatively low.

A recent guideline- and GLP-compliant study of acute oral toxicity performed in the rat with nanosilver reports an LD50 value of >2000 mg/kg bw; no mortality or signs of toxicity were observed at the limit dose in this study (Kim et al., 2013). Juberg (1997) states that acute oral LD50 values of silver compounds including silver nitrate, silver oxide, silver fluoride and silver chloride are indicative of slight to moderate toxicity.

Nanosilver is of low acute dermal toxicity in the guinea pig (Korani et al., 2011; Maneewattapinyo et al., 2011) and the rat (Kim et al., 2013), with no mortality or signs of toxicity reported in the rat at the limit dose of 2000 mg/kg bw. No additional data are identified for the acute dermal toxicity of silver or silver salts. However in light of the negligible systemic availability of silver following dermal exposure, the acute dermal toxicity of 'EcoG+' can be predicted to be very low.

No reliable data have been identified for the acute inhalation toxicity of silver or silver salts; however, inhalation exposure is not relevant to the proposed use of 'EcoG+'.

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Some salts of silver (for example silver nitrate) are known to be corrosive or irritating (Juberg, 1997); however due to the chemical nature of 'EcoG+' and the low silver content, skin irritation is not predicted.
Kim et al. (2013) report an absence of dermal irritation in a study performed with nanosilver in rabbits and in compliance with GLP and the OECD guideline 404. Samberg et al. (2010) studied effects of silver nanoparticles on porcine skin after topical dosing for 14 days: Macroscopic observations showed no gross irritation in pig skin, whereas microscopic and ultrastructural observations showed areas of focal inflammation and localisation of nanoparticles on the surface and in the upper stratum corneum layers of the skin.
The US EPA (EPA, 1992) conclude, based on animal data and experience of medical use of preparations containing silver, that sufficient data are available to conclude that silver itself is not a skin irritant.

3.3.2.2. Mucous membrane irritation

Some salts of silver (for example silver nitrate) are known to be corrosive or irritating (Juberg, 1997); however due to the chemical nature of 'EcoG+' and the low silver content, eye irritation is not predicted.
Kim et al. (2013) report an absence of ocular irritation in a study performed with nanosilver and in compliance with GLP and the OECD guideline 405. In another study with instillation of colloidal silver particles in the eyes of guinea pigs, some (1 of 4) animals of the high dose group (5000 ppm) showed conjunctivae irritation grade 1 during the first 24 h observation time whilst no ocular reaction was found in any animal 48 hrs post exposure (Maneewattapinyo et al., 2011).

The US EPA (1989) concluded, based on animal data and experience of medical use of preparations containing silver, that sufficient data are available to conclude that silver itself is not an eye irritant.

3.3.3. Skin sensitisation

Despite the widespread and historic use of silver jewellery and dermal exposure to silver and the widespread silver salts in cosmetics, medical and personal care products, reports of skin sensitisation are infrequent and often associated with contaminants (e.g. nickel in silver jewellery). The EPA (1989) concluded that sufficient data are available to conclude that silver itself is not a skin sensitiser.

When citrate-coated silver nanoparticles (AgNP, 10 nm) were applied in the guinea pig maximization test (GPMT), a skin sensitisation test based on OECD TG 406 using 20 guinea pigs, a single animal showed discrete or patchy erythema (Kim et al., 2013). According to the OECD test guideline, substances that induce a positive response in 30% or more animals in the GPMT are considered as skin sensitisers. Hence, under the conditions of the test, silver nanoparticles did not have a skin-sensitising potential.

3.3.4. Dermal / percutaneous absorption

As an inorganic metal ion, the dermal absorption of silver is predicted to be very low based on its physicochemical properties (lipid solubility and molecular weight) and interaction with dermal proteins (Hostynek, 2003). This prediction is confirmed by observations of Skog & Wahlberg (1964) who report the dermal absorption of silver nitrate through intact guinea pig skin to be less than 1% after 5 hours of exposure (based on the limits of detection), and Snyder (1975), who reports a similar finding for human skin. Clinical and experimental studies indicate that the dermal absorption of silver is 'exceedingly low' (Lansdown, 2010); this observation is attributable to the barrier properties of epidermal phospholipids and the irreversible binding of free silver ions to keratin sulphydryl groups. More recent investigations of the dermal absorption of silver from impregnated nanosilver dressings in burns patients report a level of dermal absorption of 0.1% or less, even in severely damaged skin [US EPA assessment of data reported by Moiemen et al., 2011; US EPA slides 19 and 20]. The authors indicate that this level of absorption is orders of magnitude higher than that measured in intact skin.

Larese et al. (2009) studied penetration of silver nanoparticles (AgNP) *in vitro* using full thickness abdominal human skin. AgNP (25 nm in size) coated with polyvinylpyrrolidone dispersed in ethanol and diluted in synthetic sweat were applied for 24 hours. The experiments were conducted using both intact and abraded skin. Low, but detectable, AgNP absorption through intact skin was seen, with 0.0057% passing within one day (according to Bachler et al. 2013). Penetration through damaged skin was five times greater than that through intact skin. Using transmission electron microscopy, AgNP could be detected (in the stratum corneum and upper layers of the epidermis (Larese et al., 2009).

SCCS comment
The level of 0.1% dermal absorption is considered to be relevant to 'EcoG+' and is used for calculations of systemic exposure to silver resulting from the use of this product as cosmetic packaging material.

3.3.5. Repeated dose toxicity

The principle effect of repeated exposure to silver in humans and experimental animal species is argyria, where the rate of absorption of silver exceeds the rate of biliary or urinary excretion. Argyria is characterised by the deposition of inert precipitates of silver selenide and silver sulphide and may be observed in numerous organs and tissues, but is not associated with toxicity (Greene & Su, 1987; ATSDR, 1990; Faust, 1992; EPA, 1996; Juberg, 1997; WHO, 2003; Lansdown, 2010). Silver is widely distributed and consequently deposition may occur in any organs, however the liver is widely identified as the principle organ of silver deposition due to its role in the excretion of silver. In patients with high levels of hepatocellular deposition, silver is precipitated as inert, lysosomally bound deposits; findings are not associated with any evidence of liver malfunction or toxicity.

Sub-acute (28-day) toxicity tests in the rat performed with nanoparticulate silver report an absence of overt toxicity even at a dose level of 1000 mg/kg bw/d (Kim et al, 2008). Elevated serum alkaline phosphatase activity and cholesterol levels observed in this study at dose levels of 300 mg/kg bw/d and above are indicative of mild hepatotoxicity. Renal deposits of silver are similarly not associated with marked organ toxicity; in animal studies silver deposits are reported on the glomerular basement membrane, arteriolar endothelia and laminae, without apparent structural damage. Renal toxicity was not observed in a sub-chronic toxicity study in mice administered 65 mg/kg bw/d silver nitrate (Faust, 1992).

A slight greyish pigmentation of the eyes was observed in albino rats receiving approximately 60 mg/kg bw/d silver for 218 days (WHO, 2003); pigmentation of additional organs was observed in these animals following a lifetime exposure. Rats exposed to 222 mg/kg bw/d silver for 37 weeks showed growth depression and granular silver deposits in the eyes (EPA, 1989; ATSDR, 1990).

Faust (1992) also notes a study in which rats were administered silver nitrate or silver chloride over a lifetime at dose levels equivalent to 635-660 mg silver/day. Enlargement of the left vertical of the heart was reported in addition to slight thickening of the basement membranes of the kidney glomeruli; renal effects were observed in the absence of overt organ toxicity. Deposition of silver was also observed in the skin, eyes and several internal organs. A study in which rats were administered 0.1% silver nitrate in the drinking water to rats for 218 days (equivalent to approximately 89 mg/kg bw/d silver) reported effects on the cardiovascular and hepatic systems. A statistically significant increase in the incidence of ventricular hypertrophy was reported; advanced pigmentation was observed in body organs. The ventricular hypertrophy observed in this study was not attributed to silver deposition (EPA, 1989; ATSDR, 1990).

More recent studies on the toxicity of silver nanoparticles have been reviewed elsewhere in more detail (e.g. Fetrell 2014; Hadrup & Lim 2014; SCENIHR 2014). Here only oral and dermal investigations where also ionic silver was studied in parallel are summarised.

Van der Zande et al. (2012) found no hepatotoxicity or immunotoxicity in a 28 day feeding study in rats exposed to silver nanoparticle (< 20 nm non-coated; <15 nm PVP coated) at 90 mg/kg of bw or ionic silver (silver nitrate) at 9 mg/kg of bw/day. In another 28 day study of with oral administration of nanoparticulate or ionic silver at doses up to 9 mg/kg of bw/day, a decreased thymus weight was recorded following the administration of 9 mg/kg of bw/day of ionic silver (Hadrup et al., 2012b). In a 90-day study with oral administration of silver nanoparticles (60 nm) to rats, decreased body weight was observed at 500 mg/kg of bw/day for males only (Kim YS et al., 2010). The authors of a recent review (Hadrup &

Lam 2014) noted that in several other oral studies with nanosilver compounds, no effects on body weight were reported.

Korani et al (2011) conducted a sub-chronic dermal toxicity study in guinea pigs where the shaved skin (10% of the body surface area) of the animals was rubbed five times a week for 13 weeks with solutions containing silver nanoparticles (AgNP, 1000 or 10,000 µg/ml) or silver nitrate ($AgNO_3$ at 100 µg/ml). The observed skin responses (inflammation, decreased thickness of epidermis and dermis) were dose-dependent and the impact of $AgNO_3$ was similar to that from the same dose of AgNP. Negative impacts from AgNP were also seen on the liver and spleen, including overproduction of Kupffer cells and degeneration of hepatocytes in the liver. The authors comment that, based on their results, exposure to >0.1 mg/kg (100 µg/ml dose) of AgNP may result in slight liver, spleen and skin damage.

There are reports on possible silver-induced neurotoxic effects in rodents: One study found that 0.015% silver nitrate in the drinking water for 125 days (14 mg Ag/kg of bw/day) induced hypoactivity in argyric mice after a 10 day silver withdrawal period (Rungby & Danscher, 1984). An earlier study by these authors found an increase in liveliness observed in rats given 0.01 % silver nitrate in the drinking water (6 mg Ag/kg bw/day) for 4 months (Rungby & Danscher, 1983). Hadrup et al. (2012c) investigated the effects of oral administration of ionic and 14 nm nanoparticulate silver for 28 days on neurotransmitters in rats: Alterations in noradrenaline, dopamine and 5-hydroxytryptamine concentrations were found in the brain at doses of 2.25 mg/kg bw/day of nanoparticulate silver and 9 mg/kg of bw/day of ionic silver (as Ag-acetate). In another study, with intraperitoneal injection of silver nanoparticles (25 nm, non-coated) at doses of 10, 25, and 50 mg/kg bw for 7 days, the exposure did not affect cognitive outcome or hippocampal neurogenesis in adult male mice (Liu P et al., 2013).

3.3.6. Mutagenicity / Genotoxicity:

ATSDR (2003) concluded that the mutagenicity data for silver were inconsistent, but indicated the ability of the silver ion to bind to isolated DNA *in vitro*, causing strand breaks and affecting the fidelity of DNA replication.

Studies of reverse mutation in *S. typhimurium* and *E. coli* are negative. WHO reported a negative rec-assay with *B. subtilis* and a positive result in a DNA repair assay at cytotoxic concentrations in cultured primary rat hepatocytes. Kim *et al.* (2013) reported an absence of genotoxicity in an Ames test and a study of chromosomal aberration in mammalian cells *in vitro*, both performed in compliance with GLP and current OECD guidelines. Nishioka (1975) showed that silver chloride induced no mutagenicity in bacteria, and Eliopoulos and Mourelatos (1998) found no mutagenicity of silver iodide by the Ames test. Recent tests with ionic silver ($AgNO_3$) in another Ames test with five bacterial strains were also negative (Butler *et al.* 2015). It should, however, be noted that silver is strongly bactericidal, and consequently Ames test data are of limited value to conclude on silver mutagenicity.

Eliopoulos and Mourelatos (1998) have shown that silver iodide induces sister chromatic exchanges in human lymphocytes in concentrations 0.1 µg/ml and above (when dissolved in acetone) and in concentrations 1 µg/ml and above (when dissolved in polyacrylamide suspension). In the same study, $AgNO_3$ was used as positive control and induced sister chromatid exchanges (concentration above 1 µg/g bw) in P388 lymphocyte leukemia cells that were cultured in the mouse peritoneal cavity. Concentration-dependent chromosomal damaging effects were also observed in human T-cell and monocyte cell lines (Jurkat and THP-1 cells) after exposure to silver nitrate (Butler *et al.* 2015).

Both silver ions (Ag^+) as well as silver nanoparticles (AgNPs) induced DNA damage (strand breaks, DNA adducts, DNA oxidation) (8-oxodG) *in vitro (*Foldbjerg et al., 2011; Huk et al., 2014 and 2015) and micronucleus formation in CHO cells in a concentration-dependent manner (Jiang *et al.,* 2013). Micronucleus induction has been detected also in the TK6 lymphoblastoid cell line following incubation with 5 nm AgNPs, but only at a cytotoxic concentration of 25 µg/mL (Hadrup *et al.,* 2012c; Li et al., 2012). AgNPs (50 nm, 80 nm and 200 nm) induced mammalian gene mutation in *hprt* gene in V79 cells in very low concentrations (Huk *et al.,* 2014). These authors also showed that small (8 nm) nanosilver particles with different charges and surface compositions (six different capping agents) induced gene mutations in V79 cells (Huk *et al.,* 2014).

In an *in vivo* study with oral dosing, no micronucleus induction was found following 28 days of 60 nm AgNP administration to rats (Kim *et al.,* 2008). Also genotoxicity testing by an *in vivo* micronucleus assay in an inhalation study with rats exposed to AgNP for 90 days did not reveal genotoxicity (Kim *et al.* 2011). Additionally, Li *et al.* (2014) studied PVP- and silicon-coated AgNPs for micronucleus, in a *pig-A* gene mutation assay and the comet assay. Both the PVP- and silicon-coated AgNPs induced oxidative DNA damage in mouse liver. However, none of the treatments resulted in a significant increase of either mutant frequencies in the *pig-A* gene or the percent of micronucleated reticulocyte over the concurrent controls, indicating no mutagenicity and clastogenicity.

Silver ions are released from silver nanoparticles. Several studies showed similar responses of Ag^+ ions and AgNPs on cells, suggesting that the toxicity can be due to release to free silver ions though there are also nano-specific responses (Beer *et al.,* 2012; SCENIHR 2014; Huk *et al*. 2014, 2015; Butler *et al.* 2015). However, the rate of silver ion dissolution from AgNPs differs depending on surface properties (silver charge and capping agent) and storage conditions as shown in the recent study of Izak-Nau *et al.* (2015). Thus storage conditions, surface properties as well attachment of Ag^+ ions to container walls can be a significant contributor to the contradictory toxicity results observed in the literature for identical nanoparticles (Izak-Nau *et al.* 2015). As the amount of silver released differs depending on particle surface properties and storage conditions, both positive and negative data on AgNPs must be regarded as inconclusive and not generalisable.

SCCS Conclusion

The genotoxicity of Ag^+ ions was investigated for all the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy, although results from mammalian cell gene mutation tests were not provided. The available tests were not always performed according to present standards and the data obtained are generally inconclusive. Ames test data are of limited value due to strong bactericidal properties of Ag^+ ions. Gene mutation tests in mammalian cells are not provided. Results on chromosomal damage show negative and positive results.

As Ag^+ ions are released from silver nanoparticles and as one of the toxicity mechanism of silver nanoparticles (AGNPs) is via Ag^+ ions, the genotoxicity of AgNPs was considered as well. Genotoxicity/ mutagenicity data on AgNPs are also inconclusive, showing both positive and negative effects. Due to different amounts of Ag^+ ions released from different AgNPs, these data can only be tentatively considered.

As the main mechanism of genotoxicity of silver ions is via ROS production, which is an indirect and concentration dependent process, and since the concentrations of silver ions present in cosmetic products are low, the SCCS has no concern with regard to human risk. Also due to the low dermal penetration of silver ions, systemic genotoxicity is not expected.

3.3.7. Carcinogenicity

There is some indication from old reports that the subcutaneous implantation of silver metal foils in rodents may cause the induction of local fibrosarcomas. However, such tumours are attributable to solid-state carcinogenesis and are not of relevance to the human risk assessment. This applies also to contradictory findings of tumours following subcutaneous and intramuscular injection of colloidal silver in rats, *i.e.* routes of exposure with no established human relevance. ATSDR (1990) conclude that animal toxicity and human occupational studies using more relevant routes of exposure have not provided indications of carcinogenicity, and silver is not expected to be carcinogenic in humans. The EPA (1989) concludes that silver is not classifiable with respect to carcinogenicity since animal carcinogenicity data on silver are inadequate due to the phenomenon of solid-state carcinogenesis.
The weight of evidence therefore indicates that silver is not carcinogenic.

3.3.8. Reproductive toxicity

Data on the reproductive toxicity of silver are limited; however the ATSDR concludes that 'existing evidence' does not point to a strong effect of the silver ion on reproduction and no historical evidence to indicate an effect of silver on reproduction in humans. While subcutaneous injection of silver nitrate has been reported to cause reversible damage to the testes in rats (Hoey, 1966), no effects on spermatozoa or fertility were observed in a drinking water study.
A high-quality developmental toxicity study performed in the rat with silver acetate (NTP, 2002) concludes a NOAEL for developmental toxicity of 100 mg/kg bw/d, the highest dose level tested. Indications of slight maternal toxicity were observed at this dose level; more severe maternal toxicity was observed at a dose level of 160 mg/kg bw/d in a screening study. Published data (Shavlovsky et al., 1995) indicate an effect of silver on development at maternally toxic dose levels and secondary to copper deficiency caused by the displacement of copper by silver from ceruloplasmin.

3.3.9. Toxicokinetics

3.3.9.1. Absorption

Studies in humans and animals indicate that silver compounds are absorbed by the inhalation and oral routes and poorly by the dermal route. Absorbed silver is distributed widely throughout the body. Data on the extent of oral absorption is variable but is generally accepted to be relatively low. Juberg (1997) reports oral absorption values for silver in animal studies of less than 10%; WHO (2003) states that up to 5% of colloidal silver can be absorbed after oral exposure. Important factors in the absorption of silver include the presence and extent of silver-binding proteins and the solubility of the silver species (Juberg 1997). Lansdown (2010) states that passive gastrointestinal absorption of the free silver ion is unlikely to be significant due to its reactivity with sulphydryl, carboxyl, hydroxy and protein ligands on mucosal surfaces and cell debris. Silver readily reacts with organic constituents of food, which further limits its absorption from the gastrointestinal tract. The extent of oral absorption is therefore variable but is estimated not to exceed 5-10% and is thought likely to be influenced by factors such as the presence of food and gastrointestinal transit time (ATSDR, 1990).

3.3.9.2. Distribution

The systemic distribution of orally absorbed silver is limited by extensive biliary excretion (first-pass effect) (Juberg, 1997). Absorbed silver is extensively bound to serum proteins including albumin and ceruloplasmin. Soluble silver ions may be deposited in tissues as insoluble salts such the silver chloride or silver phosphate (ATSDR, 1990); these insoluble salts are subsequently transformed to silver selenide and silver sulphide. Dermal silver deposits in one case of argyria were identified as being primarily composed of silver sulphide (Buckley et al, 1965). The characteristic blue or grey discoloration of skin exposed to sunlight in humans with argyria may be caused by the photoreduction of deposited insoluble silver salts to metallic silver (ATSDR, 1990). Studies in the rat have also characterised silver deposits in internal organs as the silver sulphide (Berry & Galle 1982).

While deposition of silver has been observed in many organs and tissues, the liver and skin are identified as the main storage centres for silver in the body (ATSDR, 1990; Faust, 1992). Exposed experimental animals and humans show granular deposits containing silver in both pigmented and non-pigmented skin.

In studies using injection of radiolabelled metallic silver and silver nitrate in the rat, the highest concentrations of silver were identified in the gastrointestinal tract, liver, blood, kidney, muscle, bone and then skin. The proportion of silver distributed to the tissues is positively correlated with the dose administered (ATSDR, 1990). Lansdown (2010) states that there is no convincing evidence for the passage of silver across the blood-brain-barrier or blood-CSF-barrier.

3.3.9.3. Metabolism

Systemically absorbed silver may bind to plasma proteins and is deposited in tissues as the insoluble silver chloride and phosphate, which are subsequently reduced to silver sulphide or silver selenide (ATSDR, 1990). Silver chloride deposited in exposed areas of skin may be photochemically reduced to metallic silver and subsequently oxidised by the tissue to form the black silver sulphide, resulting in skin discoloration.

3.3.9.4. Excretion

Excretion of silver in experimental animals is mainly via the bile, with little urinary excretion (Juberg, 1997; WHO, 2003). Biliary excretion is also the major route in humans, with urinary excretion of minor importance (Lansdown, 2010). Silver binds to and induces the expression of metallothioneins in the liver, thereby facilitating its biliary excretion. Cumulative excretion of silver in experimental animals (mice, rats, monkeys and dogs) ranged from 90-99%, with retention of up to 10%. Similar retention is observed in humans. Half-lives of approximately 50 days are reported for silver (Faust, 992; Juberg, 1997; WHO, 2003). Whole-body retention studies in mice and monkeys following oral dosing with radiolabelled silver nitrate indicate that silver excretion in these species follows a biexponential profile with biological half-lives of 0.1 and 1.6 days in mice and 0.3 and 3 days in monkeys. In similarly exposed rats and dogs, silver excretion followed a triexponential profile with biological half-lives of 0.1, 0.7, and 5.9 days in rats and 0.1, 7.6, and 33.8 days in dogs (Furchner et al, 1968). Saturation of the elimination pathway in the liver occurring with chronic or high level acute exposure leads to excretion in the faeces decreasing and deposition in tissues increasing. Faust (1992) and ATSDR (1990) reported a study in dogs using intratracheal administration of metallic silver, the lung clearance of the metallic silver was accompanied by increased silver in the stomach and liver due to the mucociliary escalator and consequent ingestion. Dissolution of the silver into the blood was the main route of clearance from the lungs. Within 30 days, approximately 90% of the dose was excreted in the faeces.

3.3.10. Photo-induced toxicity

The assessment of phototoxicity is appropriate for substances absorbing light in the range 290-700 nm and is relevant for organic substances rather than simple inorganic ions such as silver.
Previously, the SCCS assessed studies with topical application of a silver citrate containing formulation (FAT 81'034/B) followed by UV irradiation (SCCS/1274/09): Treatment did not induce any photosensitising reactions in guinea pigs and revealed no photoirritation when the formulation was applied at concentrations up to 1% (corresponding to 0.01 % silver).

3.3.11. Human data

Silver has no known physiological function (Faust, 1992). The WHO (1977) estimates the oral intake of silver for humans from dietary sources to range from 27-88 µg/day; a small but measurable amount of silver is accumulated by individuals over their lifetime.

The US EPA (1996) considers the critical effect in humans exposed to silver to be argyria, a medically benign but probably permanent blue-grey discoloration of the skin resulting from the deposition of silver in the dermis and possibly also from the silver-induced production of melanin. Silver is uniformly deposited in the skin but increased pigmentation is pronounced in areas exposed to sunlight due to photo-activated reduction of the metal. Although the deposition of silver is permanent, it is not associated with systemic health effects (EPA, 1996) and no pathologic change or inflammatory reactions have been shown to result from silver deposition (Greene & Su, 1987). Lansdown (2010) also notes that argyria is not associated with cellular or tissue damage and is widely considered not to be of toxicological significance. Following prolonged dermal contact or exposure, argyria may be dermal and localised; widespread argyria (including the skin) has been observed following the prolonged use of high levels of colloidal silver by humans and in experimental animals administered silver compounds. Silver deposits observed in argyria are extracellular or intracellular (lysosomal) and may be more marked in light-exposed areas of the skin due to the stimulation of melanin production by silver (Greene & Su, 1987; Wadhera & Fung, 2005). Argyrosis, pigmentation of the cornea and conjunctivae resulting from the deposition of insoluble inert silver complexes has been reported following industrial (ocular) exposure to silver compounds (for example in soldering) and following the historical use of colloidal silver to treat ocular infection. Similarly to argyria, deposits of silver selenide and silver sulphide are associated with proteins of the conjunctivae and cornea but are not associated with cellular damage. Following systemic absorption, the deposition of silver is apparent in internal organs; however the evidence indicates that silver does not cross the blood-brain-barrier or the blood-CSF-barrier (Lansdown, 2010). Relatively high concentrations of silver are reported in the liver and may be associated with transient changes in clinical chemistry parameters but not with any evidence of pathological damage in patients with severe argyria or in animal models.

Gaul & Staud (1935) reported 70 cases of argyria following the medical use of organic and colloidal silver, including 13 cases of generalised argyria following the intravenous injection of silver arsphenamine. Argyria was found to develop after a total dose of 4-8 g in some patients, while in others, argyria did not develop until after a total dose of 10-20 g. The authors confirmed that the degree of the discoloration in skin biopsies was directly

proportional to the amount of silver present. They concluded that argyria may become clinically apparent after a total accumulated (intravenous dose) of approximately 8 g of silver arsphenamine. Based on cases presented in this study, the EPA use the lowest dose resulting in argyria in one patient of 4 g silver arsphenamine (equivalent to 1 g silver) as the minimal effect level for argyria. East et al (1980) report a case of argyria in a 47-year-old woman who had taken excessively large oral doses of silver acetate over a period of 2.5 years. Symptoms of argyria appeared after the first 6 months of exposure. The total body burden of silver was estimated to be 6.4 g; the total body burden and the concentration of silver in the skin were estimated to be 8000 times higher than normal.
Workers exposed to silver as a consequence of work in the manufacturing and packaging of silver nitrate; mining, smelting, polishing and hammering of silver have been reported to show generalised argyria. Localised argyria may also be manifested as isolated areas of pigmentation occurring at the tracheobronchial junction the smaller bronchi (Faust, 1992).
The inhalation of silver at concentrations that produce argyria can also cause respiratory tract irritation.
Large oral doses of silver nitrate have been reported to cause abdominal pain, diarrhoea, vomiting, shock, convulsions and death (Faust, 1992); however effects are attributable to the corrosive properties of this form of silver. The estimated fatal dose in humans is 10 g.

Juberg (1997) notes that acute occupational exposure to high airborne concentrations of silver nitrate has been associated with local irritant reactions, however findings are attributable to the corrosive properties of this salt rather than silver *per se*. The author also states that the results of several occupational epidemiology studies have not revealed any adverse health effects in workers exposed by inhalation to metallic silver, soluble and insoluble salts of silver for periods of 5-20 years. Studies demonstrated pigmentation of the skin, eyes (conjunctivae) and mucous membranes and elevated silver concentrations in the blood, faeces and hair but no evidence of haematological change or clinical chemistry indications of organ damage.

Workers exposed to silver nitrate and silver oxide dusts for up to 10 years were reported to have experienced sneezing, stuffiness and running nose or sore throat, coughing, wheezing, chest tightness and abdominal pain (Drake and Hazelwood, 2005). Granular silver-containing deposits correlated with the duration of employment and some people experienced decreased night vision. Another group of workers manufacturing metal silver powder also reported a decrease in night vision. Conjunctival argyria was seen in 21% of silver reclamation workers exposed to silver and insoluble silver compounds, while 25% exhibited corneal argyria and many of the workers manifested pigmentation of the nasal septum.
While isolated cases of immediate or delayed contact dermatitis have been reported, silver is not considered to be highly allergenic (ATSDR, 1990; Faust, 1992). Following the accidental inhalation of radiolabelled silver by a worker, silver was found mainly in the liver; ciliary action removed the silver from the lungs followed by ingestion, with elimination mainly in the faeces and limited urinary excretion (Faust, 1992; ATSDR, 1990).

The dermal absorption of silver compounds in intact human skin is low, but may be greater in burns patients (ATSDR, 1990). Silver was detected in the urine, blood, and body tissue of humans with seriously burned skin following treatment with topical preparations containing silver nitrate. Distribution into muscle, liver, spleen, kidney, heart and bones has been demonstrated following the topical application of silver nitrate for the treatment of burns.

3.4. Calculation of exposure

3.4.1. Silver ion migration from containers

Achieved levels of silver resulting from the use of 'EcoG+'

The levels of silver likely to be attained in cosmetic products stored in EcoG+ packaging have been investigated in two preliminary studies and a more detailed study.

Study 1

A study of migration of silver from a cosmetic container manufactured using 'EcoG+' was performed; the container was filled with distilled water and silver concentrations measured by ICP atomic emission spectroscopy. The achieved levels of silver were 56 ppb after 2 days and 73 ppb after 7 days.

Table 4.1 Migration of silver ions from EcoG+ into distilled water

Time point	Silver concentration (ppb)
2 days	56 ppb
7 days	73 ppb

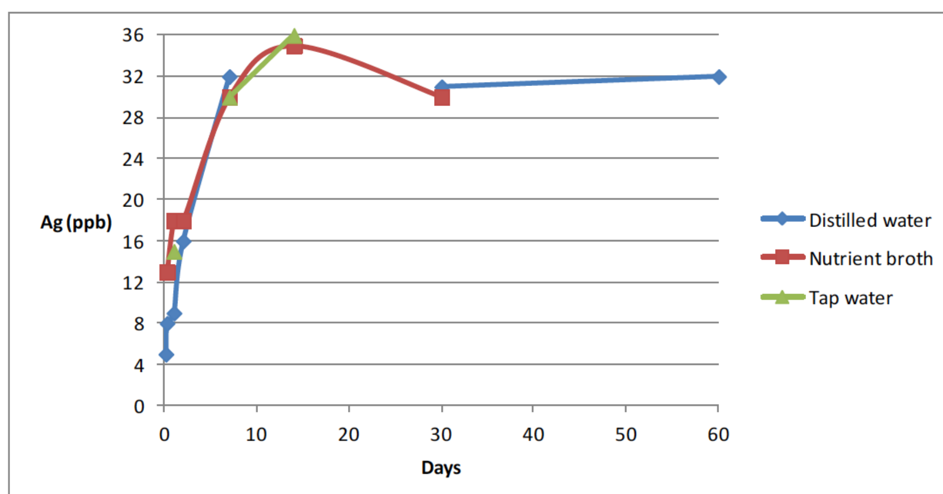
NOTE: The composition of the container used in this study is not given.

Study 2

A further study (Taiki 2012) was performed over a period of two months using cosmetic containers filled with distilled water, tap water or an aqueous dilution (1/500) of nutrient broth. Silver concentrations were measured at various time points using inductively coupled plasma emission spectroscopy.

Table 4.2 Achieved levels of silver

Time point	Silver concentration (ppb)		
	Distilled water	Nutrient broth	Tap water
3 hours	5 ppb	–	–
6 hours	8 ppb	13 ppb	–
24 hours	9 ppb	18 ppb	15 ppb
48 hours	16 ppb	18 ppb	–
7 days	32 ppb	30 ppb	30 ppb
14 days	–	35 ppb	36 ppb
1 month	31 ppb	30 ppb	-
2 months	32 ppb	–	–



It can be seen from the results of this study that the kinetics of silver migration were comparable for the three simulants investigated. Maximal silver concentrations (~ 35 ppb) were seen up to approximately Day 14. Silver levels subsequently remained relatively constant at a level of approximately 32 ppb. Ref. Taiki 2012

NOTE: The liquid dispenser used in this study consisted of polypropylene (58%), glass bead (40%) and silver glass (1.5%).
Containers manufactured with EcoG+ can contain higher amounts of silver glass (3%). The test was apparently conducted at neutral pH.

Study 3
A further study of achieved silver concentrations (TAIKI Corporation, 2014) was performed in order to assess the influence of product pH on the level of silver release from cosmetic containers manufactured from 90% polypropylene, 9% glass and 1% silver glass. Containers were filled with an aqueous buffer solution containing 0.1% sodium chloride and 0.05% citrate; the pH of the buffer solution was adjusted to 4.0, 5.0 or 6.0 by the addition of sodium citrate solution. Containers were maintained either at room temperature or at elevated temperature (40°C and 75% relative humidity) for a period of four weeks. Silver levels in the buffer were measured by ICP optical emission spectrophotometry. The results of these analyses are shown below.

Table 4.3 Achieved levels of silver

Conditions	pH	Silver concentration (µg/mL)
Ambient	4.0	0.02
	5.0	0.01
	6.0	0.02
40°C 75% RH	4.0	0.03
	5.0	0.03
	6.0	0.03

Silver concentrations in the buffer solution after 4 weeks were slightly higher for the jars stored at elevated temperature. There was no clear effect of pH on silver concentration.

NOTE: The cosmetic jar used in this study consisted of polypropylene (90%) with 10% EcoG+ resin that contained 10 % of silver glass.

Overview of migration data

The silver ions present in the silver glass component (Million Guard PG721ST) of the EcoG+ packaging will migrate within the packaging and will subsequently also migrate into the aqueous phase of any cosmetic product contained within the packaging. Migration of silver ions into the cosmetic product may potentially be influenced by the pH or presence of other ionic species in the aqueous phase of the product. The available data show that the concentration of silver achieved in the product is relatively low, and that a maximum concentration is achieved within approximately 30 days. The silver concentration achieved in the cosmetic product is governed by an equilibrium. Reduction of the product content in the container through usage will not therefore result in a higher level of silver in the residual product.

NOTE: The migration of silver ions from 'EcoG+' packaging material has not been tested with typical cosmetic formulations (w/o or o/w), however it is assumed not to be higher in those cosmetic formulations.

The following calculations are based on a silver ion concentration of 0.03 µg/mL in cosmetic products stored in EcoG+ packaging material.

Systemic Exposure Dose

Systemic exposure to silver resulting from the proposed use of the product is calculated using the following parameters:

- The global daily exposure value for preservatives of 17.4 g/day (269 mg/kg bw/d) for all cosmetic products which may be applied on the skin (SCCS, 2016)
- An estimate of the level of silver attained in cosmetics of 0.03 µg/mL (TAIKI data)
- Dermal absorption of 0.1% (Moiemen *et al.*, 2011 and US EPA 2011)

Using these parameters results in an estimated systemic exposure of approximately 0.000008 µg/kg bw/d silver following the use of 'EcoG+'.

The value of 0.1% for dermal absorption of silver is derived from serum levels in persons with severely damaged skin (burn patients) during treatment with silver-rich wound dressings (Moiemen et al., 2011; US EPA 2011). This value is a conservative upper-bound estimate and orders of magnitude higher than those measured *in vitro* with intact or mildly damaged human skin (Larese et al. 2009).

Reference values

All published reviews concur that the relevant (critical) effect of silver exposure is argyria. Toxicological reference values for silver have been derived by the EPA, WHO and EFSA.

The US EPA uses a systemic lifetime (systemic) exposure of 1 g silver as starting point[1];

[1] The EPA (1989) used the study of Gaul & Staud to derive a minimal (lifetime exposure) NOAEL of 1 g silver (systemic). This level of systemic exposure is calculated to be equivalent to an oral reference dose (RfD) of 0.00056 mg/kg bw/d (0.56 µg/kg bw/d) following correction for the extent of oral absorption (5 %), bodyweight (70 kg), exposure over a lifetime of 70 years (25500 days) and an uncertainty factor of 3

this is calculated to be equivalent to a systemic exposure level of 0.00056 mg/kg bw/d (0.56 µg/kg bw/d) assuming a lifetime of 70 years and a bodyweight of 70 kg.

Based on the epidemiological and pharmacokinetic data, WHO (2003) derives a human NOAEL (lifetime oral intake) for silver of 10 g, corresponding to 0.39 mg Ag/person/day or 0.0065 mg/kg bw/d. Assuming a gastrointestinal absorption of silver of 5%, this is equivalent to a systemic reference value of 0.000325 mg/kg bw/d (0.325 µg/kg bw/d).

EFSA has evaluated silver-based preservatives for use in food-contact materials on the basis of human and animal data and has derived a group restriction limit of 0.05 mg Ag/kg food, which corresponds to a worst-case exposure of 0.05 mg Ag/person/day or 0.00083 mg/kg bw/day. Assuming a gastrointestinal absorption of silver of 5%, this is equivalent to a systemic reference value of 0.0000415 mg/kg bw/d (0.0415 µg/kg bw/d).

The (oral and systemic) reference values for silver are listed in a table (below) for a comparison to the systemic silver exposure dose resulting from use of EcoG+ as packaging material and related MOS calculations.

Table 4.4 Exposure and Safety Calculations

Cosmetics product usage	17.4 g/d [269 mg/kg bw/d]		
Silver level in cosmetic product	0.03 µg/mL [0.03 µg/g]		
External exposure to silver	0.008 µg/kg bw/d		
Dermal absorption of silver	0.1%		
SED silver	0.000008 µg/kg bw/d		
Reference value source	**WHO**	**EFSA**	**EPA**
Silver oral reference value	0.0065 mg/kg bw/d	0.00083 mg/kg bw/d	NA
Oral absorption of silver	5%		
Silver systemic reference value	0.00325 mg/kg bw/d [0.325 µg/kg bw/d]	0.0000415 mg/kg bw/d [0.0415 µg/kg bw/d]	0.00056 mg/kg bw/d [0.56 µg/kg bw/d]
Margin of Safety	40273	5143	69393

Comparison of the SED for silver resulting from the proposed use of EcoG+ to the toxicological reference values derived by the EPA, WHO and EFSA shows Margin of Safety (MoS) values well in excess of 100. Therefore the proposed use of EcoG+ is not considered to result in adverse health effects in consumers using cosmetic products stored in EcoG+ packaging.

3.4.2. Discussion

Physicochemical properties

EcoG+ is a solid composite material containing silver glass powder (Million Guard PG721ST), glass beads and a suitable polymer. The silver ions present in the silver glass powder (Million Guard PG721ST) will disperse throughout the structure of EcoG+. The dispersed silver ions will subsequently be released from EcoG+ and will be present (dissolved) in the aqueous component of the cosmetic product contained within the EcoG+ packaging.

When containers made with EcoG+ were used to study migration of silver ions into simulants (aqueous buffer solutions), the achieved levels were about 30 ppb (0.03 µg/mL).

Toxicity of silver (local and systemic)

Some salts of silver (for example silver nitrate) are known to be corrosive or irritating [Juberg, 1997]; however only at very high concentrations (>1000 ppm). The very low levels of ionic silver attained in cosmetic products stored in 'EcoG+' packaging materials will not result in skin irritation or in mucous membrane irritation.
Overall, the acute and chronic toxicity of oral or parenteral administered silver and silver salts is relatively low, although accidental and self-inflicted poisonings can occur with ionic or colloidal silver [Lansdown, 2010; Hadrup & Lam 2014].
The principle effect of repeated exposure to silver in humans and experimental animal species is argyria, where the rate of absorption of silver exceeds the rate of biliary or urinary excretion resulting in blue-grey discoloration of the skin. *Argyria* is characterised by the deposition of inert precipitates of silver selenide and silver sulphide and may be observed in numerous organs and tissues, but is not associated with toxicity. *Argyrosis*, pigmentation of the cornea and conjunctivae resulting from the deposition of insoluble inert silver complexes has been reported following industrial (ocular) exposure to silver compounds (for example in soldering) and following the historical use of colloidal silver to treat ocular infection [Drake & Hazlewood 2005; Lansdown, 2010].
Available data in animals do not point to a specific effect of the silver ion on development and reproduction (only at maternally toxic doses), and there is no historical evidence to indicate an effect of silver on reproduction in humans.

All published reviews concur that the relevant (critical) effect of silver exposure in humans is argyria, a medically benign but probably permanent blue-grey discoloration of the skin resulting from the deposition of silver in the dermis and possibly also from the silver-induced production of melanin [ATSDR, 1990; Faust, 1992; EPA 1996; Juberg, 1997; WHO, 2003; Lansdown, 2010].

Mutagenicity and genotoxicity

The genotoxicity of Ag^+ ions was investigated for all the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy, although results from mammalian cell gene mutation tests were not provided. The available tests were not always performed according to present standards and the data obtained are generally inconclusive. Ames test data are of limited value due to strong bactericidal properties of Ag^+ ions. Gene mutation tests in mammalian cells are not provided. Results on chromosomal damage show negative and positive results.
As Ag^+ ions are released from silver nanoparticles and as one of the toxicity mechanism of silver nanoparticles (AGNPs) is via Ag^+ ions, the genotoxicity of AgNPs was considered as well. Genotoxicity/ mutagenicity data on AgNPs are also inconclusive showing both positive and negative effects. Due to different amounts of Ag^+ ions released from different AgNPs, these data can only be tentatively considered.
As the main mechanism of genotoxicity of silver ions is via ROS production, which is an indirect and concentration dependent process, and since the concentrations of silver ions

present in cosmetic products is low, the SCCS has no concern with regard to human risk. Also due to the low dermal penetration of silver ions, systemic genotoxicity is not expected.

Carcinogenicity

Animal toxicity and human occupational studies using relevant routes of exposure have not provided indications of carcinogenicity, and silver is not expected to be carcinogenic in humans.

Dermal absorption

As an inorganic metal ion, the dermal absorption of silver is predicted to be very low based on its physicochemical properties (lipid solubility and molecular weight) and interaction with dermal proteins [Hostynek, 2003]. This prediction is confirmed by an *in vivo* study [Skog & Wahlberg 1964] reporting the dermal absorption of silver nitrate through intact guinea pig skin to be less than 1% (based on the limits of detection) as well as clinical and experimental studies indicating that the dermal absorption of silver is 'exceedingly low' [Lansdown, 2010]. More recent investigations of the dermal absorption of silver from impregnated nanosilver dressings in burns patients report a level of dermal absorption of less than 0.1%, even in severely damaged skin [US EPA assessment of data by Moiemen et al., 2011]. This level of absorption is orders of magnitude higher than that measured *in vitro* with intact or mildly damaged human skin [Larese et al. 2009]. The value of 0.1% is a conservative upper-bound estimate, which will be used in the calculation of systemic exposure resulting from dermal exposure to silver and margins of safety.

Risk assessment

All published reviews concur that the relevant (critical) effect of silver exposure is argyria.
Toxicological reference values for silver have been derived by the EPA, WHO and EFSA in other regulatory contexts (drinking water regulation, release of silver ions from food-contact materials). The systemic silver reference values are a suitable basis for calculating margins of safety for exposure resulting from dermal exposure to silver ions used as preservative in cosmetic products.

4. CONCLUSION

1. *Does SCCS consider* release of silver ions from *"EcoG+" as component in packaging material safe for use as preservative with a concentration of maximum 2.0 % in the cosmetic packaging material, taking into account the scientific data provided?*

   Safety assessment is based on the release of silver ion from the packaging material.

   SCCS considers the release of silver ions from "EcoG+" as a component in packaging material safe for use as preservative with a concentration of maximum 2.0 % in the cosmetic packaging material.

2. *And/or does the SCCS recommend any further restrictions with regard to the use of "EcoG+" as preservative in cosmetics packaging?*

   /

5. MINORITY OPINION

None

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