European Commission

Scientific Committee on Consumer Safety

SCCS

OPINION

on the safety of Silver

(CAS/EC No. 7440-22-4/231-131-3)

used in cosmetic products

Scientific Committees
on Consumer Safety
on Health, Environmental and Emerging Risks

The SCCS adopted this document
during plenary meeting on 27 March 2024

ACKNOWLEDGMENTS

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1. ABSTRACT

The SCCS concludes the following:

(1) In light of the data provided and taking under consideration the classification as toxic for reproduction Cat. 2, does the SCCS consider micron-sized particulate Silver safe when used up to a maximum concentration of 0.2 % in rinse-off and 0.3 % in leave-on cosmetic products?

The SCCS considers micron-sized particulate Silver not safe when used in concentrations up to 0.2 % in rinse-off and 0.3 % in leave-on cosmetic products when used all together.

However, the use of micron-sized particulate Silver in eye shadow, oral exposure products and shampoo at concentration mentioned in section 3.5 is safe, either used alone or in combination.

(2) Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of micron-sized particulate Silver in cosmetic products?

/

(3) Does the SCCS have any further scientific concerns with regard to the use of micron-sized particulate Silver in cosmetic products

/

Keywords: SCCS, scientific opinion, colorant, Silver, CAS 7440-22-4, Regulation 1223/2009

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Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Silver (CAS/EC No. 7440-22-4/231-131-3) is an ingredient primarily used as a colorant (CI 77820) in cosmetics, providing a Silver hue to various cosmetic formulations. It is an authorised colorant and, therefore, listed in entry 142 of Annex IV to the Cosmetics Regulation. Silver is frequently found in makeup products such as eyeshadows, highlighters, nail polishes, and body powders, where it provides a metallic/shimmery effect. In addition, in the current dossier submission, Silver is reported as conditioning agent in rinse-off and leave on cosmetic products.

The European Risk Assessment Committee (RAC) of ECHA issued in February 2023 an opinion recommending among others a 'Toxic for Reproduction Category 2' classification for Silver[1] . Following the RAC opinion, the European Commission may propose a classification for Silver as a 'Toxic for Reproduction Category 2' (CLP Regulation Annex VI entry).

According to Article 15(1) of the Cosmetics Regulation 'the use in cosmetic products of substances classified as CMR substances, of category 2, under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited. However, a substance classified in category 2 may be used in cosmetic products where the substance has been evaluated by the SCCS and found safe for use in cosmetic products. In view of these provisions, regulatory measures must be adopted by the Commission services within 15 months of the classification as CMR 1A or 1B of the substance(s) concerned in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

In October 2023, the Commission services received a dossier to defend the safe use of micron-sized particulate Silver (CAS/EC No. 7440-22-4/231-131-3) as a conditioning agent in cosmetic products according to Article 15(1) of the Cosmetics Regulation 1223/2009. The Commission, therefore, requests the SCCS to carry out a safety assessment on this ingredient in view of the information provided.

Terms of reference

(1) In light of the data provided and taking under consideration the classification as toxic for reproduction Cat. 2, does the SCCS consider micron-sized particulate Silver safe when used up to a maximum concentration of 0.2 % in rinse-off and 0.3 % in leave-on cosmetic products?

(2) Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of micron-sized particulate Silver in cosmetic products?

(3) Does the SCCS have any further scientific concerns with regard to the use of micron-sized particulate Silver in cosmetic products

[1] https://echa.europa.eu/documents/10162/5b4397d9-7339-251a-98e6-c67774664204

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Silver

3.1.1.2 Chemical names

Silver

3.1.1.3 Trade names and abbreviations

From Applicant:
MicroSilver BG™ (referred to as 'MicroSilver BG')

3.1.1.4 CAS / EC number

From Applicant
CAS No. 7440-22-4/ EC No. 231-131-3

3.1.1.5 Structural formula

Ag

3.1.2 Physical form

(Abstracted from the Applicant's dossier)
MicroSilver BG is a natural material composed of 99.92% pure metallic Silver powder. It is manufactured from pure Silver wire via a pure physical process. Thus, it is a powder and is neither a form of nor contains colloidal Silver. It consists of highly porous, micro-sized particles of pure Silver with an average size (Laser diffraction after external dispersion in ethanol by ultrasound according to ISO 13320-1) of approximately 10 µm, a porosity of 85-90%, and a specific surface area up to 5 m^2/g. In cosmetics, it is not present in colloidal or nano form.

Sample dispersion
The test item was dispersed in ethanol using a Bandelin Sonoplus HD2200 ultrasonic homogenizer (200 Watt rated power) with the Bandelin Cup Horn BB6 following mainly the SOP 99.8%) in a 20-mL glass vial. Afterwards the sample was sonicated for 35 min. The delivered energy density is approx. 630 J/mL.

Measurement of external dimensions

All SEM images used for measurements have a size of 1280 px × 960 px (spotresolution down to 0.2 nm at an acceleration voltage of 2 kV).
All TEM images used for measurements have a size of 1685 px × 1685 px.

For the general determination of external dimension, the minimum circumscribed circle (MCC) diameter was measured for all particles.

Two groups of particles were measured, namely (I) substructures and (II) particles:

- I) The substructures were measured via SEM and TEM images. They are the smallest measurable structure within particles.
- II) The particles are clusters of substructures, which were measured via SEM images.

I/ The substructures

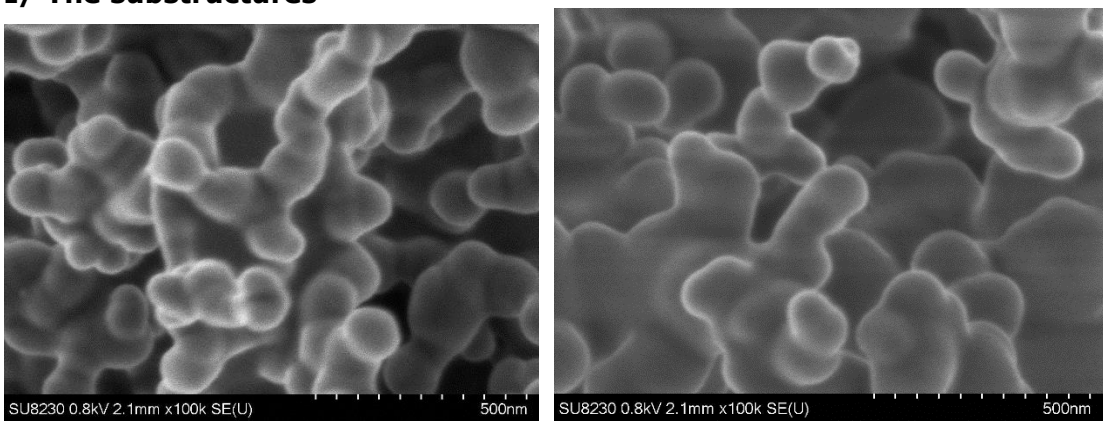

Figure 1: Image of the test item captured with a HR-SEM at 2 kV. Resolution: 1 nm/px (from KOLBENSCHLAG_PSD REPORT_2023.PDF

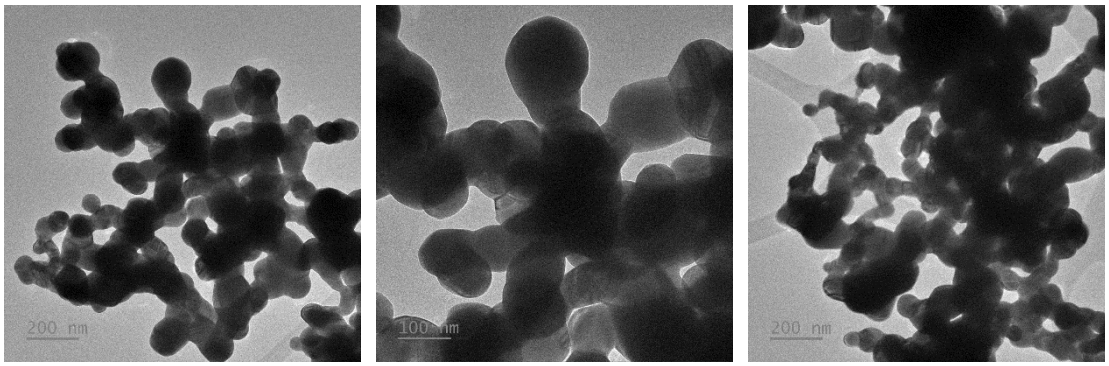

Figure 2 : Images of the test item captured with a HR-TEM at 200 keV ((from KOLBENSCHLAG_PSD REPORT_2023.PDF)

SEM imaging revealed that the substructures (not existing as individual entities but as a non-separable part of larger individual unbound units) were approximately spherical. The substructure particles in the images were measured and counted, resulting in the following histogram (Figure 3) of size distribution calculated for 1288 substructure particles.

The number-based substructure-particle size of the test substance ranged between 42.2 and 320 nm. The mean (±SD) measured substructure-particle size was 122.4 ± 37.7 nm (SD: 84.7–160.1 nm). Further characterisation of the number-based substructure-particle size distribution revealed specific percentiles: D10 measured at 80.5 nm, D50 at 116.2 nm, and D90 at 172.8 nm. Notably, 50% of the number-based measured substructure particles exhibited a size below 116.2 nm.

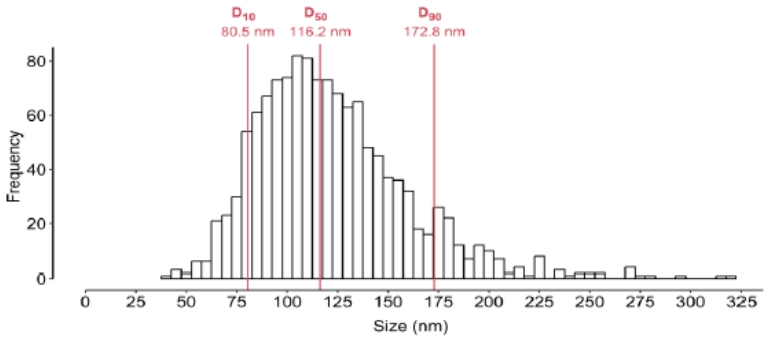

Figure 3. Histogram of the number-based particle size distribution measured using several images captured by a HR-SEM at 2 kV.

TEM analysis revealed that the number-based particle size of the test substance ranged between 29 and 314.5 nm. The number-based particle size distribution in the histogram is as follows: D10 measured at 65.5 nm, D50 at 106 nm, and D90 at 173.9 nm.

The substructures in the images were measured and counted, resulting in the following histogram (Figure 4) of size distribution calculated for 504 substructure particles. Note that these substructures are sintered together and form the observed highly porous powder which does not release nanoparticles. These substructures cannot diffuse freely as nanoparticles.

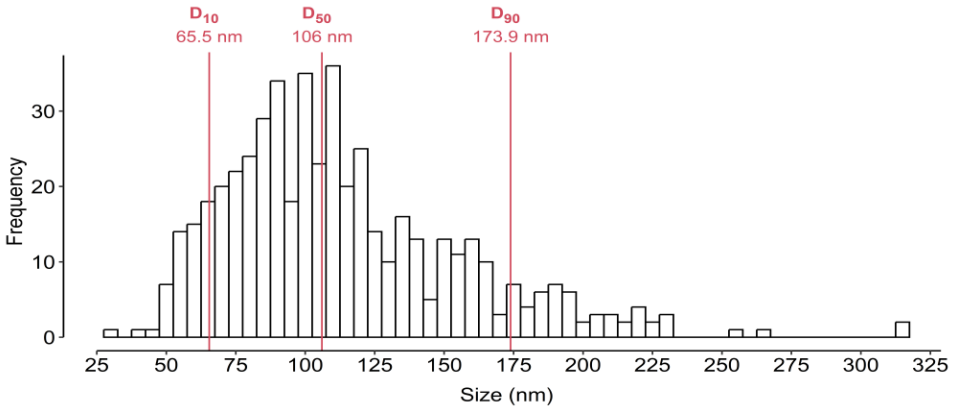

Figure 4. Histogram of the particle size distribution (number-based) measured using several images captured by HR-TEM at 200 keV.

II/ Particles

The particles could be described as porous and sintered. The pre-treatment with sonification for 35 minutes shows that the particles cannot be broken down into smaller parts. According to the manufacturer, it was not possible to chop the particles down to a smaller size. High forces only led to a compression of the particles and a reduction of the surface area.

The number-based particle size of the unbound test substance with individual existence ranged between 0.13 and 20.69 µm.
The mean (±SD) measured particle size was 2.35 ± 3.38 µm (SD: 0–5.73 µm).
The number-based particle size distribution was characterised by specific percentiles: D10 at 0.22 µm, D50 at 1.28 µm, and D90 at 5.46 µm.

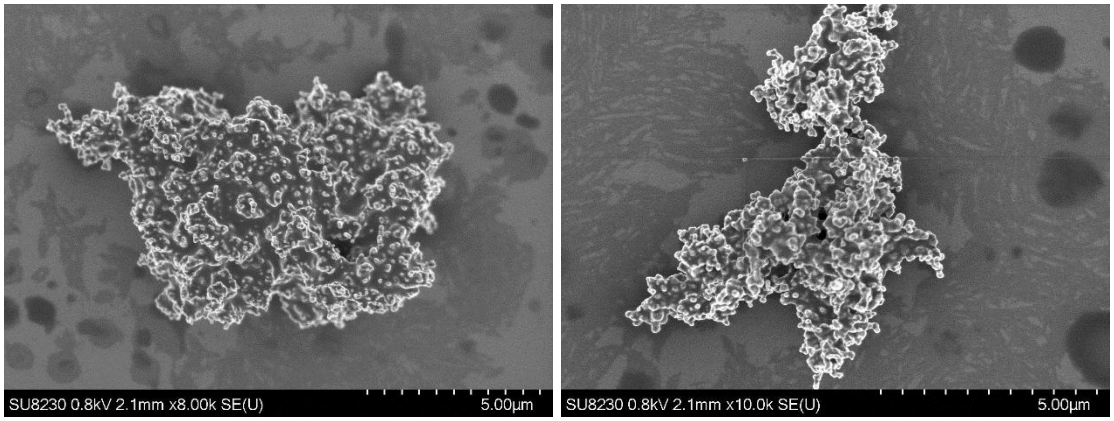

Figure 5: Images of the test item captured with a HR-SEM at 2 kV. Resolution: 12.3 nm/px (from: Kolbenschlag PSD Report 2023)

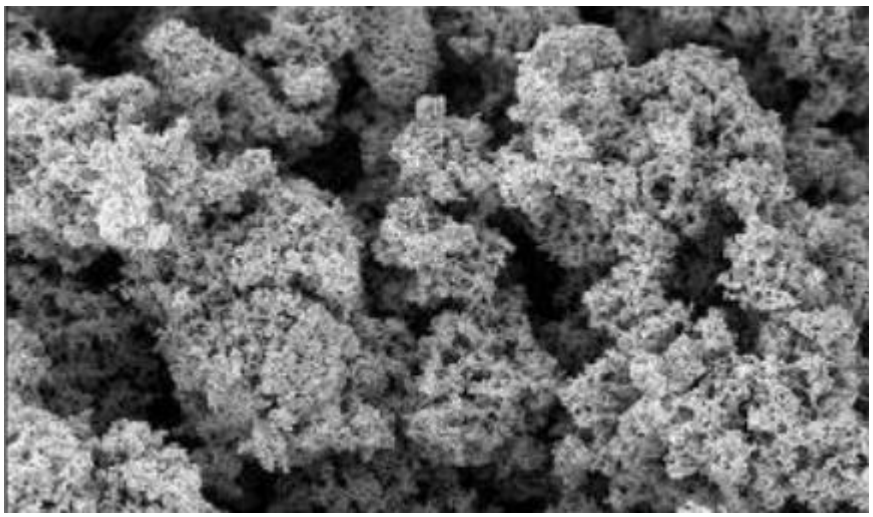

Figure 6. Scanning electron microscopy (SEM) image of MicroSilver BG (Source: Specifications of MicroSilver BG, Test report 2018, extracted from MicroSilver BG-Dossier_17Oct2023)

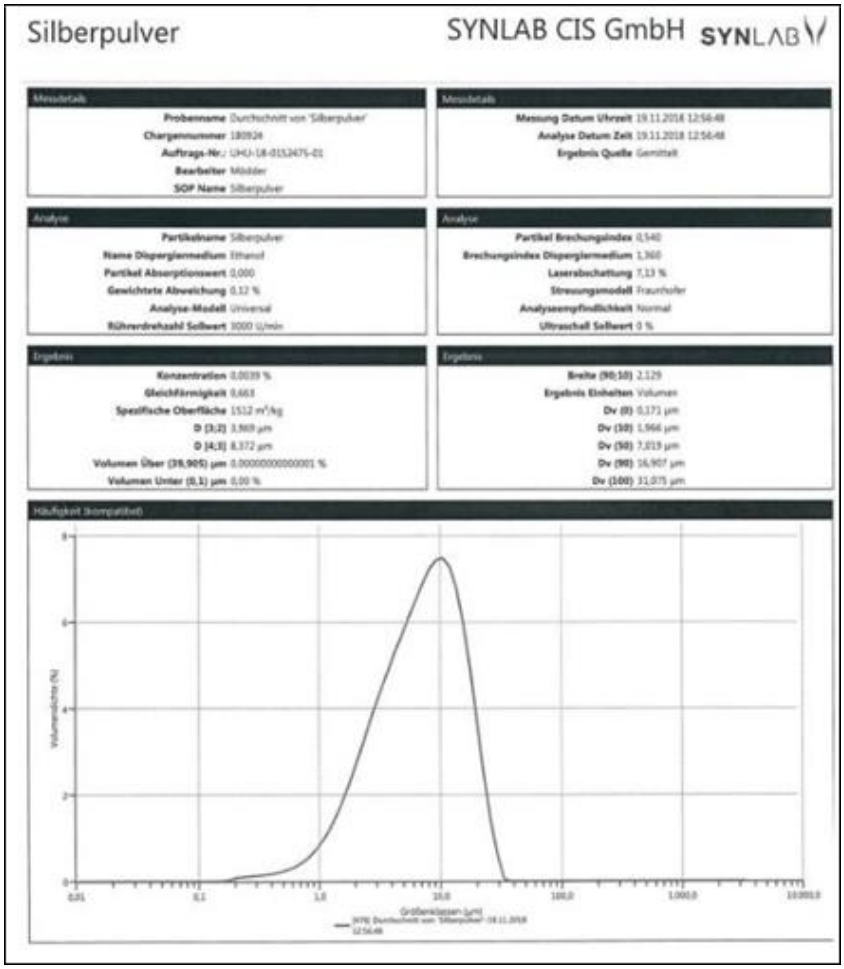

Figure 7. MicroSilver BG particle size distribution curve (Source: Specifications of MicroSilver BG, Test report, 2018 - MicroSilver BG-Dossier_17Oct2023)

Table 1. Summary of the results from both SEM and TEM analysis for the test substance and reference substance (from Kolbenschlag PSD Report 2023).

Test substance	EM	Name	Chemical name	Particle size / d0,ecd*	<100 nm	Counted particles
Test substance, dispersion, substructures (I)	SEM	MicroSilver BG	Silver	D10: 80.5 nm **D50: 116.2 nm** D90: 172.8 nm	377	1288
Test substance, dispersion, aggregates (II) (unbound basic MicroSilver BG units)	SEM	MicroSilver BG	Silver	D10: 220 nm **D50: 1280 nm** D90: 5460 nm	0	75

Test substance, dispersion, substructures (I)	TEM	MicroSilver BG	Silver	D10: 65.5 nm **D50: 106 nm** D90: 173.9 nm	222	504
Reference substance(I)	SEM	RM 8017	Polyvinylpyrrolidone coated Silver	D10: 68.1 nm **D50: 75.7 nm** D90: 81.3 nm	520	520
Reference substance	TEM	RM 8017	Polyvinylpyrrolidone coated Silver	D10: 58.1 nm **D50: 66.7 nm** D90: 73.6 nm	485	485

* Equivalent circular diameter of number-based size distribution (method used for particle size determination)

Based on the generated data, it can be concluded that according to the EC definition 2011/696 as well as the new recommendation 2022/C 229/01, MicroSilver BG is not a nanomaterial because it does not fulfil the following conditions:

• Individual existence or existing as identifiable particles in aggregates or agglomerates, where 50% or more of these particles in the numerical size distribution meet at least one of the following conditions.

a) one or more external dimensions of the particle are in the size range of 1 nm to 100 nm;
b) the particle has an elongated shape, such as a rod, fibre, or tube, in which two external dimensions are less than 1 nm and the other dimension is greater than 100 nm;
c) the particle has a plate-like shape in which one outer dimension is less than 1 nm and the other is greater than 100 nm.

The basic units of MicroSilver BG particles, which have unbound individual existence, are not nanomaterials as their external dimensions clearly exceed 100 nm.

In accordance with the definitions recommended by EC 2022/C 229/01 (see below), it is appropriate to classify MicroSilver BG unbound particles as 'particles' rather than 'aggregates.' This distinction is made because, during the production process, there are no free particles or constituent particles that bind together to form aggregates.

(From EC 2022/C 229/01, the following definitions apply: a) 'particle' means a minute piece of matter with defined physical boundaries; single molecules are not considered 'particles', b) 'aggregate' means a particle comprising strongly bound or fused particles.)

SCCS comment

Based on the submitted documentation, the SCCS agrees that micron-sized particulate Silver is not a nano material.

3.1.3 Molecular weight

107.9 g/mol

3.1.4 Purity, composition and substance codes

Solid powder. CAS nr 7440-22-4

Trade name: MicroSilver BG™ (referred to as 'MicroSilver BG')

3.1.5 Impurities / accompanying contaminants

From Applicant

Sum of impurities (ICP – OES, DIN EN ISO 11885) : ≤ 800 ppm

Trace elements Tungsten (ICP – OES, DIN EN ISO 11885) : ≤ 700 ppm

Ref.: Kolbenschlag_PSD REPORT_2023.pdf

3.1.6 Solubility

Insoluble in water; 22.8, 1.13 and 0.15 mg/L at pH 5, 7 and 9, respectively
Soluble in nitric acid (HNO_3)

3.1.7 Partition coefficient (Log Pow)

From Applicant:
Not relevant due to insolubility in octanol

3.1.8 Additional physical and chemical specifications

From Applicant

- melting point 961.93 °C
- boiling point 2187 °C
- vapour pressure 0.013 Pa at 840 °C
- Density 10.5 g/cm^3
- refractive index
- UV/visible light absorption spectrum: not submitted

3.1.9 Homogeneity and Stability: release of Silver ions

From the Applicant:
When using toxicological data from other forms of Silver to assess the toxicity of MicroSilver BG, it is of utmost importance to consider MicroSilver's characteristics which determine the release of Silver ions in comparison to other forms of Silver metal, specifically lower micron sized and nano-forms of Silver, Silver salts or SCAS. MicroSilver BG is described as highly porous, sintered, complex fine structures of approximately spherical/branched substructures. This distinctive spongy structure of MicroSilver BG promotes the physical clinging of the Silver particles to the skin when applied dermally via cosmetics. Thereby, the Silver particles remain longer on the skin surface resulting in prolonged efficacy. The special sponge-like particle structure of MicroSilver BG allows sustainable generation of Silver ions at low concentrations. This makes it different to other Silver forms used in dermal applications (*e.g.*, nano-forms of Silver, Silver salts) which, compared to MicroSilver BG, readily deliver higher concentrations of ionic Silver in daily use as well as in toxicological studies.
This was further confirmed in experiments carried out to determine the release of Silver-ion in MicroSilver BG in different formulations by Anodic Stripping Voltammetry (ASV) (See Appendix-I).

SCCS comment
In the absence of time-weighted Silver-ion release studies on representative cosmetic formulations, the SCCS will assume a 100% release of ions from the MicroSilver BG particles. In conformity with ECHA, the SCCS will base its toxicological evaluation on the exposure to Silver ions (expressed as Silver ion equivalents).

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

According to the Applicant:
In cosmetic formulations and in the presence of moisture, MicroSilver BG generates very small amounts of Silver ions. The presence of the bigger particles along with the lower specific surface area (SSA) (i.e., 5 m^2/g) and their insolubility in water triggers but also assures the generation of lower amounts of Silver ions compared to nano-forms of Silver which has an SSA of about 30-90 m^2/g. The bigger particles as well as the corral-like structure of MicroSilver BG foster its physical clinging to the skin when applied dermally via cosmetics and thus largely prevents the dermal penetration of MicroSilver BG particles and Silver ions released from it. The relationship and impact between Silver particle size, SSA and solubility on Silver ion release and reactivity towards biological targets has been shown in various investigations (Gliga *et al.*, 2014; Marambio-Jones and Hoek, 2010).

From the Applicant's dossier, with some parts abridged:

a. In vitro studies

***In vitro* dermal absorption using MicroSilver BG**
Guideline: OECD TG 428 (2004)
Test system: Pig skin
Test substance: MicroSilver BG
Test Formulation: Ointment containing 1.5% (Formulation A) and 0.5% (Formulation B) MicroSilver BG in hydrophilic cream
Batch: 23Mar06
Purity Not specified
Route: Topical application to horny layer of skin Application area: 1 cm^2
Application technique: Spreading formulation evenly on the skin with spatula and quantification of actually applied mass by weighing thickness of skin: 760 and 910 µm
Duration: Application was performed once to skin. Formulation was not washed off before termination of experiment
Washing of test formulation: 1.5 mL Tween 80® 5% 1.5 mL of deionized water; finally, by dabbing the skin dry with cellulose pad
Dose of test formulation: MicroSilver BG ointment 0.5 and 1.5%
Nominal doses: Formulation A 20 mg/cm^2 corresponding to 0.3 mg Silver/cm^2 Formulation B 20 mg/cm^2 corresponding to 0.1 mg Silver/cm^2
No of donors: 01
No of cells per donor: 03
Receptor fluid: Phosphate buffer
Sampling: Before, 2, 6 and 24 hours
Analytical method: Inductively coupled plasma mass spectrometry (ICP-MS)
Exposure time: 24 hours
GLP: Yes
Study period: 2006

The *in vitro* absorption potential of MicroSilver BG through pig skin mounted in a static Franz diffusion cell was determined in a GLP compliant OECD TG 428 study. Intact pig skin, obtained from a local farmer, was taken shortly after exsanguination. Subcutaneous fat was removed, and hair was clipped. The 1 cm^2 skin was clamped horizontally between the upper donor chamber and lower receptor chamber, with a horny layer facing the donor compartment. The test substance formulation was applied topically in a nominal quantity of 20 mg/cm^2,

corresponding to 0.3 and 0.1 mg Silver/cm² for formulation A and B respectively. 24 hours after application, the stratum corneum was removed by repeated stripping with adhesive tapes to obtain the absorbed test substance. The remainder of the skin sample (i.e., epidermis, dermis) was used to determine the absorbed test substance. The test substance was analysed by determining the Silver content with inductively coupled plasma mass spectrometry (ICP-MS).

Results

Most of the applied test substance formulation was wiped off the skin at the end of the exposure. Tape stripping removed a large amount of the test substance from the superficial layers of the skin. A sharp decrease of the Silver content in the adhesive tapes was observed with increasing number of the applied tapes, corresponding to a low Silver level (0.0014%) in the deeper layers of the stratum corneum.

The level of Silver in the receptor fluid was below the limit of quantification in experiments A and B. The intended recovery of applied Silver in various samples of 100 ± 15% was achieved with two of four formulations, with the other two formulations coming close to the tolerated level (i.e., 81.9- 88.8%).

The mean results obtained for the test formulations containing MicroSilver BG are presented in Table 2 and 3. The summary of results of % absorption of test substance through pig skin is presented in Table 4.

Table 2. *In vitro* percutaneous absorption of test substance through pig skin (% dose) (Experiment A and B)

Parameters	Experiment A (MicroSilver BG ointment 1.5) (% dose)		Experiment B (MicroSilver BG ointment 0.5) (% dose)	
	Mean	SD	Mean	SD
Receptor fluid 0 hour*	0.00012	0.00008	0.00115	0.00120
Receptor fluid 2 hour*	0.00025	0.00006	0.00052	0.00019
Receptor fluid 6 hour*	0.00028	0.00008	0.00061	0.00027
Receptor fluid 24 hour*	0.00028	0.00008	0.00058	0.00024
Rinsing receptor chamber	0.00043	0.00013	0.00080	0.00028
Dermis+ residual epidermis Skin	2.00	0.54	1.38	0.38
in flank region	2.90	0.63	2.23	1.31
Tape 1	10.215	7.262	10.733	4.751
Tape 2	2.559	1.183	3.408	2.399
Tape 3	1.155	0.540	1.626	0.842
Tape 4-8 mean	0.469	0.269	0.406	0.139
Tape, 9-15 mean	0.105	0.030	0.100	0.035
Skin rinsing, aqueous phase	0.019	0.20	0.024	0.003
Skin rinsing, pad extract	49.4	22.2	63.7	12.4
Skin rinsing, donor chamber	10.6	13.6	3.0	2.8

* Below limit of quantification (0.03 µg silver/L)

Table 3. *In vitro* percutaneous absorption of micro Silver through pig skin (µg/cm²)

Parameters	Experiment A (MicroSilver BG ointment 1.5) (% dose)		Experiment B (MicroSilver BG ointment 0.5) (% dose)	
	Mean	SD	Mean	SD
Receptor fluid 0 hour*	0.00042	0.00029	0.00131	0.00141
Receptor fluid 2 hour*	0.00084	0.00023	0.0057	0.0018
Receptor fluid 6 hour*	0.00095	0.00031	0.00067	0.00027
Receptor fluid 24 hour*	0.00093	0.00034	0.00063	0.00023
Rinsing receptor chamber	0.00144	0.00043	0.00087	0.00027
Dermis+ residual epidermis	6.53	1.39	1.52	0.40
Skin in flank region	/	/	/	/
Tape 1	35.017	27.183	11.933	5.375
Tape 2	8.675	4.670	3.788	2.667
Tape 3	3.880	1.928	1.771	0.857
Tape 4-8 mean	1.600	1.036	0.446	0.138
Tape, 9-15 mean	0.343	0.083	0.110	0.038
Skin rinsing, aqueous phase	0.062	0.063	0.024	0.003
Skin rinsing, pad extract	163.3	70.2	63.7	15.3
Skin rinsing, donor chamber	34.3	43.1	3.0	2.9

* Below limit of quantification (0.03 µg silver/L)

Table 4. *In vitro* percutaneous absorption – Results overview (% dose)

Derived data	Experiment A (MicroSilver BG ointment 1.5) (% dose)		Experiment B (MicroSilver BG ointment 0.5) (% dose)	
	Mean	SD	Mean	SD
Adsorption after 24 hour^{ads}	17.0	10.0	18.5	7.6
Absorption after 24 hour^{abs}	2.0	0.54	1.38	0.38
Penetration 0-24 hour ^{pen}	0.0007	0.0001	0.0014	0.0005
Bioavailability after 24 hour ^{bioavail}	2.00	0.54	**1.38**	0.38
Sum of rinsing of skin	60.0	12.4	66.7	13.0
Mass balance (%)#	81.9	6.0	88.8	4.3

* Below limit of quantification (0.03 µg silver/L)

#: Slight differences to the sum of the results may occur due to 1] rounding and 2] residual masses in the flange range of the penetration cell.

^{ads}: The adsorption is calculated from the amounts of the test substance silver analysed in the stratum corneum; the sum of the masses detected in the 15 tapes from the stripping.

^{abs}: The absorption is calculated from the amount of the test substance analysed in the remaining skin (residual epidermis and dermis)

^{pen}: The cumulative penetration is the mass of the test substance found in the receptor fluid at the various sampling times.

^{bioavail}: The sum of absorption and cumulative penetration is considered to be bioavailable.

Conclusion

Following the topical application of MicroSilver BG in representative cosmetic formulation to pig skin *in vitro*, the dermal absorption was determined to be 1.38 ± 0.38% and 2.00 ± 0.54% for 0.5% and 1.5% MicroSilver BG, respectively. No test substance was identified to be in the receptor fluid above the limit of quantification.
(Bornatowicz, 2006)

Note (by the Applicant)**:** The above OECD TG 428 compliant *in vitro* dermal absorption study is considered to be scientifically acceptable. On the basis of this study with 0.5% MicroSilver BG, a dermal absorption value of 1.76% (i.e., mean plus one standard deviation) has been used in the present assessment for Margin of Safety (MoS) calculations.

SCCS comment

The ICP-MS method to determine Silver content cannot distinguish between particles and ions. Therefore, as a conservative approach, the SCCS will assume that the measured Silver is in the form of ions.
Each experiment utilised a single donor with only three replicates. Three of the six individual samples did not meet the mass balance criterium of 85-115% and the mean mass balance

for experiment A was below 85%. Additionally, no information regarding the composition of the tested formulations was provided. According to the most recent SCCS Notes of Guidance (SCCS/1647/22), and assuming that the determined Silver content pertains to Silver ions, the bioavailable amount for the MoS calculation is considered to be the mean value obtained from experiment B with the 0.5% formulation ± 2SD, i.e. 1.38 ± 0.76 = 2.14%.

b. In vivo studies

1st study – *in vivo* study in human volunteers

A study aimed at determining the presence of MicroSilver BG tape strips after application of a MicroSilver BG containing ointment on the skin of human volunteers. In this study, two ointments containing two different concentrations of MicroSilver BG (i.e.,0.1 and 0.5% (w/w)) were applied in defined quantities to the forearms of ten female panellists twice a day for 28 days. At the start of the study, control samples of untreated skin were taken from each test panellists' forearms before test substance application. The forearms were washed for ten seconds with water and curd soap and each area was then stripped 60 times by a standard procedure. The tape stripes were combined to create the following pooled samples: 1-10 (n=10), 11-30 (n=20), 31-60 (n=30). After 28 days of the daily MicroSilver BG applications, the sampling procedure was repeated. The test substance pools were analysed by determining the Silver content with ICP-MS.

Results

The results show a decrease in Silver from the outermost layers (stripes 1-10, n=10) to the inner layers of the *stratum corneum* and parts of the adjacent layer of the epidermis (stripes 31-60, n=30). Similarly, the calculation of Silver content per tape strip (**Table 5**) shows that for the deeper layers (stripes 31-60) after application of the 0.1% ointment the content of Silver is below the quantification (0.094 µg Silver/L) and detection limit (0.026 µg Silver/L).

Table 5. Mean Silver content per tape sample

Sample	Mean Silver content (µg)	Standard deviation
Blank stripes 1-10	0.013	0.011
Blank stripes 11-30	0.012	0.010
Blank stripes 31-60	0.020	0.007
Sum of blank	**0.045**	-
Ointment 0.1%, stripes 1-10	0.19	0.15
Ointment 0.1%, stripes 11-30	0.17	0.14
Ointment 0.1%, stripes 31-60	0.13	0.10
Sum of ointment 0.1 %	**0.49**	-
Ointment 0.5%, stripes 1-10	0.70	0.55
Ointment 0.5%, stripes 1-10	0.58	0.50
Ointment 0.5%, stripes 1-10	0.42	0.37
Sum of ointment 0.5 %	**1.7**	

Conclusion
Under the study conditions, most of the Silver was found in the first layers of the stratum corneum, and only negligible amounts were found in the layers below.
(Von Grebe and Zweirnik, 2021)
Note (by the Applicant): This study demonstrates the very low dermal penetration potential of MicroSilver BG but does not allow for quantification of the penetrated amounts of Silver since the total amount of MicroSilver BG was not available.

SCCS comment
The ICP-MS method to determine Silver content cannot distinguish between particles and ions.

2nd study – *in vivo* study in guinea pigs
A study was conducted to determine the dermal absorption of Silver nitrate in guinea pigs (n=20). 2 mL of 0.24 molar Silver nitrate (^{100m}Ag) solution was applied occlusively to a skin surface (application site not specified) of 3.1 cm^2 for eight weeks in a depot formulation. The dermal absorption was determined by an isotope technique, by measuring the amount of radioactivity disappearing from the treated area over five hours. No further details on the study are available.
The dermal absorption was determined to be less than 1% for most animals, except for one animal, which was in the range of 3.0-3.9%. Considering all uncertainties, the dermal absorption in this study is proposed to be set based on the upper-range value of 4% to cover all the study animals.
(Skog and Wahlberg, 1965; ATDSR, 2003; ECHA RAC, 2022)
Note (according to the Applicant): The CLH (2020) review considered a dermal penetration value of 5% based on the results of this study conducted with Silver nitrate. This figure is considered overly conservative because it is based on the assumption that all radioactivity that disappeared from the test area has entered the systemic circulation through the skin.

3rd study – *in vivo* study in rats
In an *in vivo* dermal absorption study in rats, 100 mg of an antiseptic powder containing 3.7 mg metallic Silver was applied onto 2 cm^2 abraded skin in the necks of male Sprague-Dawley rats. The amount of Silver in blood, liver, kidney, testicles, spleen, femur, heart, and stomach were analysed for ^{106}Ag content and compared to the untreated controls. Tissue levels of Silver were low, and the systemic availability was estimated to be 0.01%.
(Sabioni *et al.*, 1988 in ECHA RAC, 2022)

4th study- Investigational dermal penetration study
In an exploratory study, different types of cosmetic formulations (i.e., shampoo, body lotion, deodorant) containing MicroSilver BG were applied to the normal, non-sun-exposed skin of the forearm of a human volunteer. The penetration of the MicroSilver BG particles was analysed by reflectance confocal microscopy (RCM) before, during and 2 hours after application. Before the final imaging after 2 hours, the skin was washed to remove any remaining substances from the surface.
The Silver particles were observed mainly in the skin folds, and no large aggregates were observed. Neither penetration of Silver particles into the epidermis and upper dermis, nor aggregation or occlusion of Silver in the eccrine glands was observed.
(Daniels *et al*., 2009)

SCCS comment
Although reflectance confocal microscopy detects particles, it cannot determine whether or not these particles are composed of Silver.

Applicant's summary of dermal/percutaneous absorption

Overall, available *in vitro* and *in vivo* studies confirmed the low dermal absorption of the metallic Silver. Data available on Silver salts, such as Silver nitrate, suggest a higher dermal absorption rate (up to 5%). Specifically with regard to MicroSilver BG, the data derived from the OECD TG 428 compliant *in vitro* dermal penetration study conducted with MicroSilver BG is considered to be the most appropriate study for the dermal exposure assessment of MicroSilver BG via its use in cosmetic applications. The study revealed a mean dermal absorption level of 1.38 ± 0.38% of the applied dose. Thus, a dermal absorption of 1.76% (i.e., mean plus one standard deviation) has been taken forward to be used for Margin of Safety (MoS) calculations. When also taken into account the findings of the clinical dermal penetration study, this dermal absorption level should be considered as very conservative.

SCCS overall comment on dermal absorption

The dermal penetration studies do not meet the requirements laid down in the SCCS's Notes of Guidance. From the analytical methods used, it cannot be determined whether the detected amounts of Silver relate to particles or ions.

The Applicant proposes a dermal absorption of 1.76% based on the *in vitro* study in pig skin. While the SCCS noted several shortcomings in the study (see SCCS comment above), it will use this study for an estimate of the bioavailable amount with an application of 2 standard deviations on the measured value. Thus, the dermal penetration to be used for the calculation of the MoS will be 2.14%.

3.2.2 Other studies on toxicokinetics

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3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

(From Applicant's dossier) MicroSilver BG is used as a skin conditioning agent in a range of cosmetic products, including face/hand creams, body lotions, deodorants, and oral care products. The microparticles offer a highly biocompatible depot of pure Silver that provides a sustainable generation of Silver ions. The special porous particle structure of MicroSilver BG allows sustainable generation of Silver ions at low concentrations.

3.3.2 Calculation of SED/LED

From Applicant's dossier:

The aggregate exposure assessment is generally performed when several product categories contribute, such as the preservatives and other substances that are regulated with the same maximal concentrations in all product categories. Unlike preservatives, MicroSilver BG is used as a skin conditioner in cosmetic products, and generally, it is not used in all cosmetic products. It is highly unlikely that a consumer would use all products containing MicroSilver BG daily and thus, simple adding up theoretical daily exposures stemming from all cosmetic product uses which might contain MicroSilver BG would lead to a gross overestimation of consumer exposures. In the absence of detailed information on consumers' practices with regard to the use of cosmetic products containing MicroSilver BG, allowing for a probabilistic exposure assessment, an aggregate exposure assessment was considered inappropriate and therefore not conducted (Table 6).

SCCS comment

The SCCS recalculated the Systemic Exposure Doses using dermal absorption of 2.14%.

Table 6: Systemic Exposure Doses (SED) for dermal products according to a dermal absorption of 1.74% (Applicant) and 2.14% (SCCS), and for oral exposure products.

Product category	Product sub-types	Retention	Eproduct (mg/kg bw day)	Intended use level (%)	SED	
					Calculated by the Applicant	Calculated by the SCCS
Dermal exposure						
Skin care	Face cream[1]	1	25.67	0.2	0.00090	0.00110
	Face cream[1] face tonic	1	25.67	0.1	0.00045	0.00055
	Face cream[1] anti-redness face cream	1	25.67	0.2	0.00090	0.00110
	Face cream[1] Anti-pimple face cream [4]	1	25.67	0.2	0.00090	0.00110
	Face cream[1] face refresh spray	1	25.67	0.1	0.00045	0.00055
	Hand cream[1]	1	36	0.2	0.00127	0.00154
	Body lotion[1]	1	130.33	0.05	0.00115	0.00139
Hair care	Shampoo[1]	0.01	0.18	0.2	0.00001	0.00001
Deodorant	Deodorant spray[1] *(Dermal exposure)*	1	11.5	0.3	0.00061	0.00074
	Deodorant non spray[1]	1	25	0.3	0.00131	0.00161
Foot care	Foot cream[2]	1	20	0.2	0.00070	0.00086
Make up	Eye shadow[1]	1	0.33	0.2	0.00001	0.00001
Men's cosmetics	After shave[2]	1	20	0.1	0.00035	0.00043
aggregate dermal exposure					0.00901	0.01098
Oral exposure						
Make up	Lip balm[1]	1	0.95	0.2	0.0000002	0.0000002
Oral hygiene	Toothpaste[1] adult	0.05	2.29	0.05	0.0000001	0.0000001
	Toothpaste[4] children	0.4	9.22	0.05	0.0000005	0.0000005
	Mouthwash[1]	0.1	36.03	0.05	0.0000018	0.0000018

[1] Use quantity-According to values in Tables 3A and 3B on page 24-25 of the SCCS NoG (SCCS, 2021)

[2] Use quantity According to values from RIVM Cosmetics fact sheet (Bremmer *et al.*, 2006)

[3] For children, body weight of 21.7 kg and a retention of 40% (SCCP, 2005; SCCS, 2021b)

[4] Increased dermal penetration (i.e., 3 times; worst case) is accounted considering acne condition

From the Applicant
Aggregate exposure assessment

The aggregate exposure assessment is generally performed when several product categories contribute, such as the preservatives and other substances that are regulated with the same maximal concentrations in all product categories. Unlike preservatives, MicroSilver BG is used as a skin conditioner in cosmetic products, and generally, it is not used in all cosmetic products. It is highly unlikely that a consumer would use all products containing MicroSilver BG daily and thus, simple adding up theoretical daily exposures stemming from all cosmetic product uses which might contain MicroSilver BG would lead to a gross overestimation of consumer exposures. In the absence of detailed information on consumers' practices with regard to the use of cosmetic products containing MicroSilver BG, allowing for a probabilistic exposure assessment, an aggregate exposure assessment was considered inappropriate and therefore not conducted.

SCCS comment
The SCCS calculated the aggregate exposure using dermal absorption of 2.14% (see Table 6 above)

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

The Applicant assessed the skin irritation potential of MicroSilver BG on the basis of OECD TGs 404 and the eye irritation potential on the basis of OECD 405 studies available for Silver metal powder (i.e., CAP 9). Silver metal powder was not considered to be irritating to the skin and not irritating for the eye.
ECHA-RAC considers that no classification for skin corrosion/irritation and eye irritation is warranted.

(Ref: ECHA – RAC 2022)

3.4.2 Skin sensitisation

From the Applicant:
While a dedicated skin sensitisation study with MicroSilver BG is not available, there is a substantial number of skin sensitisation studies covering various chemical forms of Silver which present or release higher amounts of Silver ions (i.e., Silver salts, SCAS, nano-size Silver metal particles) under the exposure conditions that overall support absence of a skin sensitisation potential of Silver in experimental animals. This is in line with the overall dataset in humans from case reports that even under conditions of prolonged and repeated skin contact with Silver under normal and compromised skin conditions. Most recently, ECHA's RAC (2022) concluded that there is no evidence to justify the classification of Silver metal for skin sensitisation.

SCCS comment
Following its Opinions on a Silver containing packaging material (SCCS/1577/16) and on Silver Zinc Zeolite (SCCS/1650/23), the SCCS regards the risk of sensitisation from exposure to Silver as negligible.

3.4.3 Acute toxicity

(Taken from SCCS/1577/16) 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 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.

3.4.3.1 Acute oral toxicity

3.4.3.2 Acute dermal toxicity

3.4.3.3 Acute inhalation toxicity

According to the Applicant, when taking also into account the toxicokinetic characteristics of MicroSilver BG (as discussed in the relevant section of its submission dossier), the available acute oral, dermal and inhalation toxicity data (Taken from ECHA – RAC 2022) on the different Silver entities suggest a very low acute toxicity of MicroSilver BG.

SCCS comment
The dermal and oral acute toxicity is above 2000 mg and the inhalation toxicity is in the order of 1400 - 5000 mg/m3 (for nano 0.75 mg/m3).

3.4.4 Repeated dose toxicity

Applicant's summary of repeated dose toxicity studies (for an overview see APPENDIX B)

The repeated dose toxicity of MicroSilver BG has been assessed based on a range of repeated dose toxicity studies which are available for various nanoforms of Silver metal, Silver salts and SCAS. Subacute and subchronic studies with nano-size Silver metal particles revealed changes in serum biochemistry, liver histopathology and accumulation and pigmentation in the liver and kidney at doses equal or greater than 125 mg/kg bw/day. A subacute study with Silver acetate in rats showed changes in biochemical parameters and thymus weights at doses ≥9 mg Silver/kg bw/day without histopathological correlations. One of the two subchronic studies with Silver acetate in rats showed decreased absolute heart, thymus weight and mucosal hyperplasia in the small and large intestine, as well as thymic atrophy or necrosis at doses ≥260 mg Silver/kg bw/day. Body weights were reduced at ≥65 mg Silver/ kg bw/day. Two subacute studies with Silver nitrate did not produce any adverse effects up to the highest tested dose of 95 mg Silver/kg bw/day, however two chronic drinking water studies tested at single doses produced ventricular hypertrophy and increased proteinuria at 56.5 mg Silver/kg bw/day and rapid weight loss and accumulation of Silver in the ciliary epithelium of the eyes at 141 mg Silver/kg bw/day. Subchronic repeated dose toxicity studies in rats and dogs with SCAS, changes in the haematological and clinical chemistry parameters and histopathology were observed at ≥2 mg Silver/kg bw/day. The histopathological changes included

pigmentation of pancreas, GIT, thymus, liver, kidney and the mandibular lymph node. Further, renal tubular dilation, hepatic vacuolisation and necrosis were also recorded at 20 mg Silver/kg bw/day.
With regard to the dermal route of exposure, the nano-form of Silver was tested in an OECD TG 411 compliant 13-week study in Hartley albino guinea pigs. Histopathological changes in skin, muscle, liver, spleen was evident in all the treated animals. A LOAEL was established at 0.1 mg Silver/kg bw/day.
Two OECD TG compliant subacute and subchronic repeated dose inhalation studies in rats with nano-size Silver are available. In the subacute study, there were no significant treatment related adverse effects up to the highest tested concentration of 0.0612 mg/m³. In subchronic study, histopathological changes such as minimal bile-duct hyperplasia, chronic alveolar inflammation, and macrophage accumulation in the lungs, and erythrocyte aggregation in females was observed at the highest tested concentration of 0.515 mg/m³. As a result, the NOAEC was established at 0.133 mg/m³. There was no evidence of systemic toxicity in both studies. The main adverse effect observed in a subchronic toxicity study remained local and limited to reversible or persistent lung inflammation, typically associated with nanoform of Silver but not expected with micron-sized Silver.

(From Applicant)
Relevance and conclusions of available repeated dose toxicity studies for MicroSilver BG:
Overall, the effects of repeated exposures to different forms of Silver were mainly related to the changes in haemato-biochemical parameters, histopathology and pigmentation in the liver, kidney, thymus, pancreas, and mandibular lymph nodes. Toxicokinetic studies suggest that due to its very low oral and dermal bioavailability such effects are not to be expected for micron-size Silver particles in general and MicroSilver BG specifically. In humans, Silver was observed to be deposited in numerous organs and tissues and liver was identified as the principal organ for Silver deposition. The NOAELs based on the repeated dose oral toxicity studies were significantly higher than the PoD of 0.014 mg Silver/kg bw/day which was based on 2- to 9-year period clinical study in humans with Silver arsphenamine.

SCCS comment
The SCCS has reservations regarding the applicant's choice of the PoD of 0.014 mg Silver/kg bw/day. This is further explained in 3.5 (Safety evaluation).
The SCCS will use a NOAEL (corrected for oral bioavailability) of 0.0045 mg/kg bw/d Silver-ion equivalents for the safety evaluation. This NOAEL is the most conservative value, derived from a long-term (> 12 months) study on Silver zinc zeolite in rats (Takizawa 1992, EU CAR 2021, SCCS/1650/23).

3.4.5 Reproductive toxicity

3.4.5.1 Fertility and reproduction toxicity

Applicant's summary of fertility toxicity studies (see also APPENDIX C)

The reproductive toxicity endpoint has been assessed based on a range of guideline and non-guideline compliant toxicity studies in rats available for the nanoforms of Silver, Silver salts and SCAS.
The available studies revealed effects on sexual function and fertility, such as effects on sperm counts and morphology, reduced fertility or the number of litters at doses of 0.25 mg Silver/kg bw/day and above. Reduced fertility and number of implantations were recorded at a dose of 25 mg Silver/kg bw/day. In studies with SCAS, the decreased weight of accessory sex glands and uterus, effects on sperm integrity and delayed vaginal opening in the F1 generation, increased pre-coital interval, and a lower total number of ovarian follicles were reported at and above 1.5 mg Silver/kg bw/day. The relevance of the findings with SCAS are difficult to

interpret as SCAS contains additional constituents with possible toxicological activity (*e.g.*, zeolite, zinc or zirconium ions), and the actual Silver content and release under physiological conditions is not well understood. According to EPMF, the systemic and reproductive effects observed in the EOGRTS study were attributed to a copper deficiency state. The ECHA RAC considered the information from the studies mainly with Silver nanoparticles to show some evidence for effects on testes and germ cells. As a result, the ECHA RAC proposed to classify Silver for adverse effects on sexual function and fertility as a Category 2 reproductive toxicant.

3.4.5.2 Developmental Toxicity

Applicant's summary of developmental toxicity studies. (see also APPENDIX D)

In a pre-natal developmental study with citrate-capped Silver nanoparticles, increased frequency of histopathological findings in brain and liver of dams with neuronal loss event (hippocampal sclerosis) and hepatocellular vacuolation was observed at 0.2 mg Silver/kg bw/day and above. In studies with Silver salts, increased incidence of the percent litters with late foetal deaths, decreased ceruloplasmin in dams were observed at doses of ≥19 mg Silver/kg bw/day. In foetuses, increased post-implantation deaths, cryptorchidism, hydronephrosis, decreased body weight, and viability index was observed. These effects were considered to be secondary to copper deficiency in dams which was caused by displacement of copper by Silver ceruloplasmin. In studies with SCAS, except for the skeletal abnormalities (including misshapen radii, ulnae, femurs, and wavy ribs) in one litter in the presence of maternal toxicity at the highest dose of 29 mg Silver/kg bw/day, no other treatment related teratogenic effects were observed. Further, evidence from a similar developmental study with Silver sodium zirconium hydrogenphosphate confirmed the absence of test substance-induced effects on embryo-/foetus.

Applicant's assessment of relevance and conclusions of available reproductive and developmental toxicity studies for MicroSilver BG

The relevance of the available developmental and reproductive toxicity ('DART') studies on the different Silver compounds should be seen in the context of the different toxicokinetic properties of MicroSilver BG compared to nano-forms of Silver particles, Silver salts and SCAS. Any reproductive or developmental effects were only seen at high doses of Silver and primarily attributed to the copper deficiency sequelae rather than a direct toxic effect of ionic Silver. The low and slow release of Silver ions from MicroSilver BG, due to its specific physico-chemical characteristics, suggests therefore only a very low concern for DART related effects under cosmetic use conditions, if at all. This conclusion is further supported by the findings in a toxicokinetic study conducted by Charlton *et al*. (2021) that repeated animal dosing with micron-size Silver particles did not lead to a decrease in serum copper levels, while animal dosing with Silver nitrate did.
Overall, the NOAELs established in reproductive and developmental toxicity studies were significantly higher than the PoD of 0.014 mg Silver/kg bw/day which was based on 2- to 9-year period clinical study in humans with Silver arsphenamine. Thus, using a PoD of 0.014 mg Silver/kg bw/day as done in the current assessment appropriately protects for the reproductive and developmental toxicity endpoint.

SCCS comment
For an overview and description of all the studies see ECHA – RAC 2022.

Fertility/reproduction:
While the SCCS will follow the proposal by ECHA-RAC 2022 to classify Silver for adverse effects on sexual function and fertility as a Category 2 reproductive toxicant, it will not use

the data from the studies with the nano-forms. Instead, the SCCS will set for fertility the most conservative NOAEL at 0.25 mg/kg bw/d Silver ion equivalents, derived from a study with Silver acetate (Sprando 2017, also cited in ECHA-RAC 2022). This is well above the NOAEL of 0.0045 mg/kg bw/d, derived from the long-term toxicity study (see 3.4.4: Repeated dose toxicity) that will be used for the overall risk-assessment in this Opinion.

Developmental:
ECHA-RAC 2022 is of the opinion that clear developmental toxicity has been observed with Silver salts such as Silver chloride, Silver acetate, Silver zinc zeolite (e.g., foetal/pup mortality) and to some extent with Silver sodium zirconium hydrogen phosphate. One plausible mechanism for these instances of developmental toxicity involves Silver interfering with copper binding to ceruloplasmin and thereby reducing the availability of copper, iron or perhaps both metals to the foetus (supported by the copper analysis of F2 pups in the Silver zinc zeolite study and copper and ceruloplasmin analysis in both the EOGRTS dose range-finder study (2021) and the main EOGRTS study (2022).

From the RAC evaluation of developmental toxicity, the SCCS will set for reproductive toxicity the most conservative NOAEL at 0.25 mg/kg bw/d Silver ion equivalents, derived from a study with Silver acetate (Sprango *et al.* (2016) cited in ECHA-RAC 2022).
This is well above the NOAEL of 0.0045 mg/kg bw/d, derived from the long-term toxicity study (see 3.4.4: Repeated dose toxicity) which will be used for the overall risk-assessment in this Opinion.

3.4.6 Mutagenicity / genotoxicity

Applicant's assessment of the relevance and conclusions of available genotoxicity studies for MicroSilver BG.
Taking into account the overall weight of the evidence suggesting Silver to be non-genotoxic and the poor and slow-release kinetics of Silver from MicroSilver BG under physiological conditions as a result of its specific particle characteristics, MicroSilver BG is not assessed to be genotoxic.

Taken from ECHA – RAC (2022):
While the mutagenicity database for Silver is extensive for several forms and compounds of Silver, the data are inconclusive overall because of contradictory findings and in many cases a lack of sufficient information for each study report. Some concerns remain with respect to the *in vivo* findings for both chromosomal aberrations and DNA strand breaks but the negative results generally in this case outweigh the positive ones. RAC considers Silver nanoparticles are representative of Silver bulk forms. Applying read-across to a more conservative source material (Silver nanoparticles) and applying supporting data from soluble Silver salts reinforces the need for a single conclusion for Silver metal.

SCCS comment
The SCCS concurs with ECHA – RAC (2022) that a classification for mutagenicity is not warranted.
In its Opinion on a Silver-releasing packaging material (SCCS/1577/16), the SCCS concluded that 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 mechanisms 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 process dependent on concentration levels, and since the concentrations of Silver ions present in cosmetic products are low, the SCCS has no concern with regard to human risk.

In its Opinion on Silver zinc zeolite (SCCS/1650/23), the SCCS stated that it agrees with ECHA/BPC/275/2021 that the genotoxic potential has been adequately investigated *in vitro* and *in vivo*. While the *in vitro* test in mammalian cells indicated a mutagenic potential of Silver zinc zeolite, there were no indications of genotoxicity in the *in vivo* studies conducted, which overrules the positive *in vitro* findings.

3.4.7 Carcinogenicity

Applicant's assessment of the relevance and conclusions of available carcinogenicity studies for MicroSilver BG:
Considering the lack of carcinogenic potential in combined chronic toxicity and carcinogenicity studies available with Silver zinc zeolite in rodents paired with the overall absence of genotoxicity of Silver and poor/slow Silver ion release kinetics MicroSilver BG does not present a carcinogenicity concern.

Taken from ECHA-RAC:
RAC considers that a classification in category 2 is not appropriate, but based on the poor availability of any relevant and robust data, the information presented in the CLH dossier is considered inconclusive for the assessment of carcinogenicity. No classification for carcinogenicity is proposed due to inconclusive data.

3.4.8 Photo-induced toxicity

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3.4.9 Human data

Extracted from Applicant's submission: Reference values.

Several scientific or regulatory bodies have established toxicological reference values or exposure limits for Silver. Most of the earlier assessments (WHO, 2003; US EPA, 1987; EFSA, 2004; 2005; 2006) identified argyria as the human-relevant effect caused by chronic exposure to Silver. Therefore, the recommended exposure limits mentioned below were based on this effect.

The US EPA uses a systemic lifetime (systemic) exposure of 1 g Silver from the Gaul and Staud (1935) study as a starting point, i.e., LOAEL. The LOAEL of 1 g (1000 mg; total dose) from the *i.v.* route is converted to an oral dose of 0.014 mg/kg bw/day (1 g divided by 0.04, assumed oral bioavailability factor; see Furchner *et al*., 1968 in Section 3.3.1.2) and dividing by 70 kg (adult body weight) and 25,500 days (a lifetime, or 70 years) [(1000/0.04)/

(70*25500) = 0.014]. Further, to account for differences in the individuals, an uncertainty factor (UF) of 3 is also used (0.014/3=0.0047 rounded to 0.005 mg Silver/kg bw/day) The above derived value of 0.005 mg Silver/kg bw/day has been used by the US EPA as the chronic reference dose for Silver.

In its original assessment, the WHO (1993) considered a 'total lifetime' oral intake of about 10 g of Silver as the human no observed adverse effect level (NOAEL) corresponding to 0.39 mg Silver/person/day or 0.0065 mg Silver/kg bw/day based on the toxicological dataset provided, considering argyria as sign of Silver overload. This is also based on the present epidemiological and pharmacokinetic knowledge with scientific references ranging from 1935 to 1989.
However, a recent draft background document for the development of WHO Guidelines for Silver in drinking water suggested that the above NOAEL of 10 g of Silver is inappropriate to derive the formal guidance value for Silver and considered the derivation of formal guidance value as unnecessary (WHO, 2020). In this report, the WHO considered a LOAEL of 0.6 mg Silver/kg bw/day from a case study report by Kim *et al*., 2009 to derive the bounding value for Silver.

EFSA has evaluated Silver-based preservatives for use in food-contact materials on the basis of human and animal data and derived a group restriction limit of 0.05 mg Silver/kg food. This is derived from the WHO "Guidelines for drinking water quality". According to these Guidelines a total lifetime oral intake of about 10 g of Silver (equal to 0.39 mg/day/person) can be considered on the basis of epidemiological and pharmacokinetic knowledge as the human NOAEL. Using the default food-contact material exposure scenario (European Commission, 2001), the restriction of 0.05 mg/kg of food (as Silver) limits the intake from food contact plastics to less than 13% of the human NOAEL of 0.39 mg/person/day (i.e., 0.39*0.13=0.05 mg/kg food).

From Applicant: Clinical study.

Gaul and Staud (1935) reported 70 cases of generalized argyria following organic and colloidal Silver medication, including 13 cases of generalised argyria following intravenous (*i.v.*) Silver arsphenamine injection therapy and a biospectrometric analysis of 10 cases of generalized argyria classified according to the quantity of Silver present. In this *i.v.* study, data were presented for 10 male (23-64 years old) and for two female panellists (23 and 49 years old) who were administered 31-100 intravenous injections of Silver arsphenamine (total dose was 4-20 g) over a 2- to 9.75-year period. Argyria developed after a total dose of 4, 7 or 8 g in some patients, while in others, argyria did not develop until after a total dose of 10, 15 or 20 g. In the biospectrometric analysis of skin biopsies from 10 cases of generalised argyria, the authors confirmed that the degree of the discoloration is directly dependent on the amount of Silver present. The authors concluded that argyria may become clinically apparent after a total accumulated *i.v.* dose of approximately 8 g of Silver arsphenamine. Further, the book entitled "Argyria, The Pharmacology of Silver" also reached the conclusion that a total accumulative *i.v.*
dose of 8 g Silver arsphenamine is the limit beyond which argyria may develop (Hill and Pillsbury, 1939). However, since the body accumulates Silver throughout life, it is theoretically possible that amounts less than this (for example, 4 g Silver arsphenamine) can result in argyria. Based on the findings of this study, the lowest *i.v.* dose resulting in argyria in one patient, 1 g metallic Silver (calculated as 4 g Silver arsphenamine x 0.23 (the fraction of Silver in Silver arsphenamine)), was considered as the LOAEL in humans from this study.
Note by the Applicant: US EPA has derived a chronic reference dose of 0.005 mg Silver/kg bw/day equivalent to 5 µg Silver/kg bw/day on the basis of the above Gaul and Staud (1935) study.
(Ref: Gaul and Staud, 1935. Also referenced in ECHA RAC, 2022; SCCS, 2016; US EPA, 1991).

3.4.10 Special investigations

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3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

The Applicant states that considering the available information, the chronic Reference Dose (RfD) of 0.005 mg/kg bw/day established by the US EPA (see above: 3.4.9 Human data) was chosen as a conservative POD for the risk assessment of MicroSilver BG used in cosmetic applications. This RfD is derived from the lowest LOAEL of 0.014 mg Silver/kg bw/day from a 2- to 9-year period clinical study in humans with Silver arsphenamine (see 3.4.9), corrected for an oral bioavailability of 4%. Thus, the PODsys of 0.005 mg Silver/kg bw/day was used for risk assessment purposes.
In addition, the Applicant states that, when performing an RfD-based safety assessment, the MoS should at least be 1 to conclude no safety concern for the respective ingredient (SCCS, 2021; Position paper on MoS).

SCCS comment
The SCCS does not agree with this approach. The data on which this reference dose is based are old (1935) and derived from a study describing clinical symptoms after intravenous injections of a Silver–arsenic compound as medication (Silver arsphenamine).

Instead, the SCCS will use a NOAEL (corrected for oral bioavailability) of 0.0045 mg/kg bw/d Silver-ion equivalents for the safety evaluation. This NOAEL is the most conservative value, derived from a long-term (> 12 months) study on Silver zinc zeolite in rats (Takizawa 1992, EU CAR 2021, SCCS/1650/23),
The Margins of Safety (MoS), based on this NOAEL divided by the Systemic Exposure Doses (SED) calculated by the SCCS as shown in Table 6 (section 3.3.2), are presented below in Table 7.

Table 7. Margin of Safety (MoS) for the separate product categories, based on the systemic Exposure Doses (SED) calculated by the SCCS (see section 3.3.2) and the NOAEL of 0.0045 mg/kg bw/d.

Product category	Product sub-types	Retention	Eproduct (mg/kg bw day)	Intended use level (%)	SED	MoS
Dermal exposure						
Skin care	Face cream[1]	1	25.67	0.2	0.00110	4.1
	Face cream[1] face tonic	1	25.67	0.1	0.00055	8.2
	Face cream[1] anti-redness face cream	1	25.67	0.2	0.00110	4.1
	Face cream[1] Anti-pimple face cream [4]	1	25.67	0.2	0.00110	4.1
	Face cream[1] face refresh spray	1	25.67	0.1	0.00055	8.2
	Hand cream[1]	1	36	0.2	0.00154	2.9
	Body lotion[1]	1	130.33	0.05	0.00139	3.2
Hair care	Shampoo[1]	0.01	0.18	0.2	0.00001	**584.1**
Deodorant	Deodorant spray[1] *(Dermal exposure)*	1	11.5	0.3	0.00074	6.1
	Deodorant non spray[1]	1	25	0.3	0.00161	2.8
Foot care	Foot cream[2]	1	20	0.2	0.00086	5.3
Make up	Eye shadow[1]	1	0.33	0.2	0.00001	**318.6**
Men's cosmetics	After shave[2]	1	20	0.1	0.00043	10.5
aggregate dermal exposure					0.01098	0.4
Oral exposure						
Make up	Lip balm[1]	1	0.95	0.2	0.0000002	**23684**
Oral hygiene	Toothpaste[1] adult	0.05	2.29	0.05	0.0000001	**39301**
	Toothpaste[4] children	0.4	9.22	0.05	0.0000005	**9761**
	Mouthwash[1]	0.1	36.03	0.05	0.0000018	**2498**

3.6 DISCUSSION

Physicochemical properties

The SCCS agrees that the micron-sized Silver presented in this dossier is not a nanomaterial. This Opinion is therefore related to the materials' particle specification as described in section 3.1.2.
In the absence of time-weighted Silver-ion release studies in representative cosmetic formulations, the SCCS will assume a 100% release of ions from the micron-sized particles.

Toxicokinetics

Dermal absorption:
The dermal penetration studies do not meet the requirements laid down in the SCCS's Notes of Guidance. From the analytical methods used, it cannot be determined whether the detected amounts of Silver relate to particles or ions. Therefore, as a conservative approach, the SCCS will assume that the measured Silver is in the form of ions.
The SCCS considered the *in-vivo* studies not suitable to estimate the dermal absorption. Although the SCCS noted several shortcomings in the *in vitro* study in pig skin, it will use it for an estimate of the bioavailable amount with an application of 2 standard deviations on the measured value. Thus, the dermal penetration to be used for the calculation of the MoS will be 2.14%.

Exposure

For the reason explained in section 3.3.2., the SCCS has applied a dermal absorption of 2.14% to derive a systemic exposure dose from the dermal applications of micron-sized Silver.
Considering the metallic, particulate and non-volatile nature of the micron-sized Silver, the only possibility for inhalation exposure is when it is applied through sprayable products. Therefore, the inhalation exposure from "face refresh spray" and deodorant spray was not evaluated by the SCCS in this Opinion since the MoS based on the data provided by the Applicant was not safe for these product categories.

Toxicological Evaluation

The SCCS will base its evaluation of systemic toxicity on the exposure to Silver ions. Toxicological studies from nano silver particles will not be considered because of their physical-chemical characteristics and hence their different toxicological profile (see also SCCS/1596/18).

Irritation and corrosivity

Silver metal powder was not considered to be irritating to the skin and not irritating for the eye. ECHA-RAC considers that no classification for skin corrosion/irritation and eye irritation is warranted.

Skin sensitisation

Following its Opinions on a Silver-containing packaging material and on Silver Zinc Zeolite (SCCS/1577/16, SCCS/1650/23), the SCCS regards the risk of sensitisation from exposure to Silver as negligible.

Repeated dose toxicity

The NOAEL used in this evaluation (0.0045 mg/kg bw/d) is derived from a combined chronic (up to 24 months) toxicity/carcinogenicity study in mice and rats that were fed with a Silver zinc zeolite containing 2.3% Silver and is based on pigmentation as a critical effect from Silver release. (Takizawa 1992).

Reproductive toxicity

While the SCCS will follow the proposal by ECHA-RAC 2022 to classify Silver for adverse effects on sexual function and fertility as a Category 2 reproductive toxicant, it will not use the data from the studies with the nano-forms. Instead, regarding fertility in this Opinion, the SCCS will set the most conservative NOAEL at 0.25 mg/kg bw/d Silver ion equivalents, derived from a study with Silver acetate (Sprando 2017, also cited in ECHA-RAC 2022). This is well above the NOAEL of 0.0045 mg/kg bw/d, derived from the long-term toxicity study in rats (see 3.4.4: Repeated dose toxicity), which will be used for the overall risk-assessment in this Opinion.
The NOAEL derived from the chronic oral toxicity study (which is used in the current assessment) is below the NOAEL for reproductive and developmental effects.

Mutagenicity / genotoxicity

The SCCS concurs with ECHA – RAC (2022) that a classification for mutagenicity is not warranted.
In its opinion on a Silver-releasing packaging material (SCCS/1577/16) the SCCS concluded that 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.
Moreover, in its Opinion on Silver zinc zeolite (SCCS/1650/23), the SCCS stated that it agrees with ECHA/BPC/275/2021 that the genotoxic potential has been adequately investigated *in vitro* and *in vivo*. While the *in vitro* test in mammalian cells indicated a mutagenic potential of Silver zinc zeolite, there were no indications of genotoxicity in the *in vivo* studies conducted, which overrules the positive *in vitro* findings.

Carcinogenicity

The SCCS concurs with ECHA that no classification for carcinogenicity can be proposed due to inconclusive data. The SCCS stated in its Opinion SCCS/1650/23 that it agrees with the ECHA Opinion (ECHA/BPC/275/2021) that Silver zinc zeolite is not likely to be carcinogenic.

Human data

Several regulatory agencies (WHO, EFSA, US EPA) have established human reference doses based on argyria as the human-relevant effect caused by chronic exposure to Silver via, respectively, drinking water, food / food contact material or medication.

The SCCS will not accept the Applicant's proposal to use a medication-based reference dose for its point of departure for risk-assessment. Instead, the SCCS will use the NOAEL derived from a chronic combined toxicity and carcinogenicity study on Silver zinc zeolite in rats.

Special investigations

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4. CONCLUSION

(1) In light of the data provided and taking under consideration the classification as toxic for reproduction Cat. 2, does the SCCS consider micron-sized particulate Silver safe when used up to a maximum concentration of 0.2 % in rinse-off and 0.3 % in leave-on cosmetic products?

The SCCS considers micron-sized particulate Silver not safe when used in concentrations up to 0.2 % in rinse-off and 0.3 % in leave-on cosmetic products when used all together.

However, the use of micron-sized particulate Silver in eye shadow, oral exposure products and shampoo at concentration mentioned in Section 3.5 is safe, either used alone or in combination.

(2) Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of micron-sized particulate Silver in cosmetic products?

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(3) Does the SCCS have any further scientific concerns with regard to the use of micron-sized particulate Silver in cosmetic products

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5. MINORITY OPINION

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

APPENDIX A

Applicant's Table with results showing the release of Silver-ion in MicroSilver BG in different formulations

Product	Supporting electrolyte	[Ag+] µg/g	CV%
AnimalCareMicroSilver BD-Skin+Paw ointment	0.1M KNO3	0.308	43.8
AnimalCareMicroSilverBD-soothing shampoo	0.1M KNO3	0.467	11.7
SOS MicroSilver Creme	0.1M KNO3	0.904	75.4
SOS MicroSilver Creme	0.1M KNO3	0.885	8.7
Allpresan diabetic Schaum-Creme MicroSilver	0.1M KNO3	0.001	1.01
Allpresan diabetic Schaum-Creme MicroSilver	0.1M KNO3	0.003-0005	2.3-54.9

APPENDIX B

Applicant's overview of repeated dose toxicity studies with Silver nanoforms of Silver, Silver salts and SCAS (Silver containing active substances).

Study type, Species	Doses	Key findings	NOAEL or LOAEL	Reference/ KL rating
Nano-size Silver metal				
Sub-acute studies				
28-day gavage study in Sprague-Dawley rats (10 rats/group); OECD TG 407, Silver nanoparticles (60 nm)	0, 30, 300, and 1000 mg/kg bw/day	At 300 mg/kg bw/day and above, dose-related increases in alkaline phosphatase, cholesterol and total protein levels. Increased incidences of bile duct hyperplasia around the central vein were observed in livers of male and female animals	NOAEL: 30 mg Silver/ kg bw/day	(Kim *et al.*, 2008 in ECHA, 2022)/ KL2
Sub-chronic studies				
90-day gavage study in Fisher 344 rats (10 rats/group); OECD TG 408 Silver nanoparticles (60 nm)	0, 30, 125, and 500 mg/kg bw/day	At and above 125 mg/kg bw/day, dose-related changes were found in alkaline phosphatase and cholesterol levels indicating slight liver damage. Histopathology revealed slightly higher incidences of bile-duct hyperplasia with or without necrosis, fibrosis and/or pigmentation in treated animals together with a dose- dependent accumulation of Silver in all tissues examined. Further, Right kidney weights were significantly decreased without dose-relation.	NOAEL: 30 mg Silver/ kg bw/day	(Kim *et al.*, 2010 in ECHA, 2022)/ KL2
90-day gavage study in Sprague Dawley rats (6 males); OECD TG 408, PVP capped nanoparticles	0, 50, 100, 200 mg/kg bw/day	At 200 and 100 mg/kg bw/day non-significant increase in epididymis and testis weight was observed. At 200 mg/kg bw/day, decrease sperm viability was observed. At 50 mg/kg bw/day, decrease in food intake was observed. At 50 and 100 mg/kg bw/day significant increase in sperm anomalies were observed.	LOAEL: 50 mg Silver/kg bw/day	(Lafuente *et al.*, 2016 in ECHA RAC 2022)/KL2
Silver salts				

Sub-acute studies				
28-day, gavage study in WI(Han) rats (5/sex/group); OECD TG 407; Silver nitrate	0, 20, 50, and 100 mg Silver nitrate/kg bw/day (equivalent to 0, 13, 32, and 64 mg Silver/kg bw/day)	No treatment related adverse effects at any dose level	NOAEL: 64 mg Silver/ kg bw/day	(ECHA RAC, 2022)/ KL1
28-day, gavage study in Wistar Hannover Galas rats (8 females); no guideline; Silver acetate	0, and 14 mg Silver acetate/kg bw/day (equivalent to 9 mg Silver/kg bw/day)	At 9 mg Silver/kg bw/day lower body weight gain, an increase in alkaline phosphatase and a decrease in urea concentrations in plasma and lower absolute and relative thymus weight was observed	LOAEL: 9 mg Silver/ kg bw/day	(Hadrup *et al*., 2012 in ECHA, 2022; ECHA RAC, 2022/ KL2
30-day oral swab study in Fischer 344 rats (4/sex/group); according to standard methods that comply with the guidelines of the OECD as summarised in Mosberg and Hayes (1989); antismoking mouthwash (0.5% Silver nitrate)	0, 1.5, 15, and 150 mg Silver nitrate/kg bw/day (equivalent to 0.95, 9.5, and 95 mg Silver/kg bw/day)	No treatment related adverse effects at any dose level	NOAEL: 95 mg Silver/ kg bw/day	(Tamimi *et al*., 1998 in ECHA, 2022; ECHA RAC, 2022)/ KL2
Sub-chronic studies				
90-day dietary study in Crl: WI(Han) rats (10/sex/group); OECD TG 408; Silver acetate	0, 40, 120, and 320 mg Silver acetate/kg bw/day (equivalent to 0, 26, 78, and 208 mg Silver/kg bw/day)	At 208 mg Silver/kg bw/day, reduced body weight, body weight gain and food consumption were observed during the study. However, effects on food consumption and body weight were considered adverse for males and not for females	NOAEL: 78 mg Silver/ kg bw/day for males; 208 mg Silver/ kg bw/day in females	(Study report, 2022 in ECHA, 2022)/ KL2

90-day gavage study in Sprague- Dawley rats; (10/sex/group); OECD TG 408; Silver acetate	0, 100, 200, and 400 mg Silver acetate/kg bw/day equivalent to 0, 65, 130, and 260 mg Silver/kg bw/day	At 260 mg Silver/kg bw/day, high morbidity; clinical findings, decreased absolute heart, thymus weight and mucosal hyperplasia in the small and large intestine, as well as thymic atrophy or necrosis was observed. At 65 and 130 mg Silver/kg bw/day lower overall mean body weights was observed	LOAEL: 65 mg Silver/ kg bw/day	(Boudreau *et al.,* 2016 in ECHA, 2022)/ KL1
Chronic studies				
218-day drinking water study in albino rats; no guideline specified; Silver nitrate	0, and 0.1% Silver nitrate (0, and 89 mg Silver nitrate/kg bw/day, equivalent to 56.5 mg Silver/mg bw/day)	Increase in the incidence of ventricular hypertrophy, increase proteinuria was observed	LOAEL: 56.5 mg Silver/ kg bw/day	(Olcott *et al*., 1950 in ECHA RAC, 2022)/ KL2

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9-12 months drinking water study in albino Wistar rats; no guideline specified; Silver nitrate	0, and 0.25% Silver nitrate (stated to be 222 mg/kg bw/day (equivalent to 141 mg Silver/kg bw/day) 9 months (after 10 weeks half of the animals were further exposed for 6 months, the rest for 12 months)	Rapid weight loss, massive accumulation of Silver particles in the outer aspect of the ciliary epithelium basement membrane of eyes	LOAEL: 141 mg Silver/ kg bw/day	(Matuk *et al*., 1981 in ECHA RAC, 2022)/ KL2
SCAS				
Sub-chronic studies				
14-week oral study in Sprague-Dawley rat (10/sex/group);	0, 1000, 6250, and 12500 ppm of Silver zinc	At 2 and 6 mg Silver/kg bw/day effects on behaviour/activity, pigmentation of pancreas,	NOAEL: 0.65 mg Silver/ kg bw/day	(Study report 2001 in ECHA RAC, 2022;

Similar to OECD TG 408; AgION Antimicrobial Type AK (Silver zinc zeolite)	zeolite approximately 0, 0.65, 2.0, and 6.0 mg Silver/kg bw/day	thymus, the mandibular lymph node, changes in clinical chemistry and haematology were observed		ECHA RAC, 2015)/ KL1
90-day oral gavage study in Beagle dogs (4/sex/group); Similar to OECD TG 409; Silver zinc zeolite	0, 10, 50, and 250 mg/kg bw/day of Silver zinc zeolite approximately 0, 0.2, 1.0, and 5.1 mg Silver/kg bw/day	At 5.1 mg Silver/kg bw/day changes in clinical signs, haematology and clinical chemistry was observed; Histopathological examinations revealed discoloration of the pancreas and gastro-intestinal tract and changes in the kidney	NOAEL: 1 mg Silver/kg bw/day	(Study report, 2003 in ECHA RAC, 2022; ECHA RAC, 2015; SCCS, 2023)/KL1
90-day oral study in CD rats; similar to OECD TG 408; Silver sodium zirconium hydrogen phosphate	0, 30, 300, and 1000 mg/kg bw/day of Silver sodium zirconium hydrogen phosphate equivalent to 0, 0.29, 2.9, and 9.5 mg Silver/kg bw/day	At 9.5 mg Silver/kg bw/day, relative heart weight was increased in males. At 9.5 and 2.9 mg Silver/kg bw/day, increased ALP levels, discoloration of pancreas and the Harderian gland, increase in RBCs and cholesterol (males only) and changes in organ weights. The absolute weights of testes and epididymides were reduced (for epididymides this reduction was only statistically significant for the right organ). In the absence of histopathological findings, the significance of these effects is unclear.	NOAEL: 0.29 mg Silver/kg bw/day	(ECHA RAC, 2022)/ KL2
90-day oral study in	0, 200, 400, and	At 20 mg Silver/kg bw/day one	NOAEL:	(ECHA RAC,

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dogs (strain group not specified); similar to OECD TG 409; Silver sodium zirconium hydrogen phosphate	700/1000 mg/kg bw/day of Silver sodium zirconium hydrogen phosphate equivalent to 0, 5, 10, and 18/ 20 mg Silver/kg bw/day	male and one female dog died; food consumption and reduced body weight was observed. Hepatic inflammation was accompanied with hepatic vacuolisation and necrosis, increased level of alkaline phosphatase, aspartate transaminase and alanine transaminase. Histopathological evaluation revealed renal tubular dilation and necrosis. Thymic atrophy/reduced thymus weight. At 10 mg Silver/kg bw/day pigmentation of intestine, liver, kidneys, and hepatic inflammation was observed	5 mg Silver/kg bw/day	2022)/ KL2

#The study year which was provided in regulatory references/reviews such as ECHA RAC/REACH are added. Studies where year is not mentioned, only secondary source reference (*e.g.*, ECHA RAC) is cited.

APPENDIX C

Applicant's overview of reproductive toxicity studies with Silver nanoforms, Silver salts and SCAS (Silver containing active substances).

Study type, species	Doses	Critical effects	Dose descriptor	Reference/KL rating
Nano-size Silver metal particles				
Combined repeat dose toxicity with reproductive developmental toxicity screening study, OECD TG 422; citrate-capped Silver nanoparticles (8 nm)	0, 62.5, 125 and 250 mg/kg bw/day Male rats: 14 days before mating, 14 days during the mating period and 14 days of post-mating until necropsy (daily). Female rats (maximum of 52 days): 2 weeks before mating, during the mating and gestation period, and during 4 days of lactation	No treatment related adverse effects were observed on any reproductive/ developmental parameter evaluated up to highest tested dose.	NOAEL (reproductive/ developmental) toxicity: 250 mg Silver/kg bw/day	(Hong *et al.*, 2014 in ECHA RAC, 2022; ECHA, 2022)/KL2
Silver salts				
One-generation dietary	0, 4, 40, 80, 160, and 320 mg	F0 Generation: At 208 mg Silver/kg bw/day overall body	NOAEL: Not established	(Study report 2020

Study type, species	Doses	Critical effects	Dose descriptor	Reference/KL rating
reproductive toxicity study, Wistar rats, no guideline; Silver acetate (dose range finding (DRF) study for EOGRTS)	Silver acetate/kg bw/day (equivalent to 0, 0.65, 2.6, 26, 52, 104, 208 mg Silver /kg bw/d ay)	weight gain was low; pigment was observed in Kupffer cells and to a lesser extent in hepatocytes and in the vascular walls of the liver of males administered 104 and 208 mg Silver/kg bw/day and correlated with macroscopic dark liver colour. Centrilobular hepatocellular hypertrophy was observed in males administered 104 and 208 mg/kg bw/day and correlated with increased liver weight was observed.	(DRF study) *Doses of 104 and 208 mg Silver/kg bw/day were not tolerated and were considered unsuitable for the subsequent OECD 443 main study*.	in ECHA, 2022)

Extended one generation reproductive toxicity study (EOGRTS), Sprague-Dawley, OECD TG 443; Silver acetate Trade name: AG(I) Acetate T2 HSTDP Silver(I) acetate	0, 40, 80 and 120 mg Silver acetate/kg bw/day (equivalent to 0, 26, 52 and 78 mg Silver/kg bw/day) females were treated for ten weeks before pairing, throughout pairing up to necropsy on Day 28 of lactation. The F1 generation was treated from weaning to their scheduled termination (relevant to each cohort) at the same dose levels and volume-dose as the F0 generation, with exception on animals at 120 mg Silver acetate/kg bw/day in Cohorts 1A and 1B which were terminated prematurely on	Significant decrease in cauda epididymis and testicular weight and at all dose levels, testicular and cauda epididymal total spermatid and sperm counts were low. At 78 mg Silver/kg bw/day mortality in F1, changes in neurobehavioral/sensory function, effect on sperm counts and sperm morphology was observed. At 26, 52 and 78 mg Silver /kg/ bw/day effects such as; F1 mortality at 78 mg Silver/kg bw/day; changes in F0/F1 red blood cell parameters at all dose levels; changes in F1 offspring survival at 78 mg Silver/kg bw/day was observed. At 52 mg Silver/kg bw/day in F1, changes in neurobehavioral/ sensory function, sperm morphology and effect on histopathology of brain was observed. At 26 mg Silver/kg/day, systemic toxicity in F1 adults reduced activity and rearing of males and females in the arena, reduced reactivity, abnormal motor movement/gait and brain	LOAEL (F0-systemic toxicity): 26 mg Silver/kg bw/day NOAEL (F1-systemic toxicity): 26 mg Silver /kg bw/day NOAEL (F0-mating performance and fertility): 78 mg Silver /kg bw/day NOAEL (F1-offspring survival and growth): 52 mg Silver /kg bw/day NOAEL (developmental toxicity): 52 mg Silver/kg bw/day; NOAEL (developmental neurotoxicity in F1): 26 mg Silver /kg bw/day; NOAEL (developmental immunotoxicity): 78 mg Silver/kg bw/day. [5]	(Renaut, 2022 in ECHA, 2022)/KL1
Study type, species	**Doses**	**Critical effects**	**Dose descriptor**	**Reference/KL rating**
	welfare grounds at approximately 10 weeks of age rather than 13-14 weeks of age.	morphometry was observed.		

One generation drinking water reproductive toxicity study in Sprague-Dawley rats, according to FDA CFSAN Redbook; Silver acetate (63.7-65.5% Silver), non-GLP	0, 0.4, 4 and 40 mg Silver acetate/kg bw/day equivalent to 0, 0.25, 2.5 and 25 Silver mg/kg bw/day. Parental animals were exposed 10 weeks prior to mating. The F1-pups were sacrificed on postnatal day (PND) 26	At 25 mg Silver/kg bw/day reduced fertility and the number of litters and decrease in stomach weigh and reduction in fluid consumption was observed. At 2.5 mg Silver/kg bw/day lower male and female pup weight, decrease in right kidney weight and heart, increase in right epididymal weight in female pups was observed	NOAEL (systemic) F0: 0.25 mg Silver/kg bw/day. NOAEL (fertility) F0: 2.5 mg Silver/kg bw/; NOAEL (develop-mental): 0.25 mg Silver/kg bw/day	(Sprando *et al.,* 2017 in ECHA, 2022; ECHA RAC, 2022)/ KL2
SCAS				
Two generation dietary reproductive toxicity in Sprague Dawley rats, OECD TG 416; Silver sodium zirconium hydrogen phosphate	0, 1000, 5000 and 20000 ppm correspondin g to 0, 72.5/78.2, 363/400 and 1465/1612 mg sodium zirconium hydrogen phosphate/kg bw/day or 0, 1.9, 9.9 and 40 mg Silver /kg bw/day in females	**F0 data:** At 40 mg Silver/kg bw/day increase in spleen weight, relative brain weight and a decrease in the thymus, adrenals, kidneys weight and darkened or discoloured pancreas was observed. At 9.9 mg Silver/kg bw/day increased pigmentation of pancreas and increase in spleen weight was observed. **F1 generation**: At 40 mg Silver/kg bw/day decreased body weights, further significant decrease in the number born and in live litter size on Day 1 postpartum in F1 dams and darkened or discoloured pancreas decreased absolute weight of seminal vesicles/coagulating gland and changes in semen parameters was observed. **F2 generation:** At 40 mg Silver/kg bw/day, reduced litter size, reduced group mean litter and individual weights, number of pups born, and thymus weight was recorded. At 9.9 mg Silver/kg bw/day, reduced thymus weight in females was recorded.	NOAEL (parental toxicity) (F0 and F1): 1.9 mg Silver/kg bw/day NOAEL (reproduction) (F0 and F1): 9.9 mg Silver/kg bw/day NOAEL (foetal toxicity) (F1 and F2): 1.9 mg Silver/kg bw/day	(Wood, 2002 in ECHA RAC, 2022; US EPA, 2003)/KL1

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Study type, species	Doses	Critical effects	Dose descriptor	Reference/ KL rating
Two generation dietary reproductive toxicity in Sprague Dawley rats, OECD TG 416; Silver zinc zeolite	0, 1000, 6250, 12500 ppm (equivalent to 0, 72/87, 472/548, 984/1109 mg Silver zinc zeolite/kg bw/day (premating) corresponding to approximately 0, 1.5/1.8, 9.8/11.3; and 20.3/22.9 mg Silver/kg bw/day in males and females, respectively	**F0 data**: At 20.3/22.9 and 9.8/11.3 mg Silver/kg bw/day in males and females, increase in mortality, reduced bodyweight, bodyweight gain, and food consumption increase in mortality, decrease in bodyweight, bodyweight gain, and food consumption and histopathological changes of kidney were observed. **F1 generation:** At 20.3/22.9 and 9.8/11.3 mg Silver/kg bw/day in males and females, mortality, decrease in bodyweight, bodyweight gain, histopathological changes in organs were observed. Further, there were also effects, such as a higher percentage of abnormal sperm increase on the day of the vaginal opening was observed **F2 generation**: At 20.3/22.9 mg Silver/kg bw/day, no pups due to high toxicity in parents. At 9.8/11.3 mg Silver/kg bw/day, increased stillbirth index, decreased live birth index, body weights, reduced organ weights (brain, thymus, spleen). At 72/87 mg/kg bw/day, reduced thymus weight in males	LOAEL (systemic-F0 and F1): 1.5 mg Silver/kg bw/day NOAEL (reproduction, F0 and F1): 1.5 mg Silver/kg bw/day. LOAEL (developmental toxicity (F1 and F2): 1.5 mg Silver/kg bw/day	(Schroeder, 2002 ECHA RAC, 2015; SCCS, 2023)/KL 1

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APPENDIX D

Study type, species	Doses	Critical effects	Dose descriptor	Reference/ KL rating
Nano-size Silver metal particles				
Prenatal developmental toxicity, Sprague Dawley rats, no Guideline (10 female/ group); Silver nanoparticles and Silver nitrate	0, 0.2, 2, 20 mg citrate-capped Silver nanoparticles (55 nm)	Increased frequency of histopathological findings in brain and liver of dams with neuronal loss event (hippocampal sclerosis) and hepatocellular vacuolation at all dose levels. No treatment related changes on histopathology of brain, heart, liver, kidney and lung tissues of the offspring	LOAEL (maternal): 0.2 mg Silver/kg bw/day NOAEL (developmental): 20 mg/kg bw/day	Charehsaz, *et al.,* 2016 in ECHA RAC, 2022/KL2
Silver salts				
Prenatal developmental toxicity, Sprague Dawley rats, OECD Guideline 414 (25 female/ group); Silver acetate (64.6% Silver)	0, 10, 30, 100 mg Silver acetate/kg bw/day equivalent to 6.5, 19, and 65 mg Silver/kg bw/day GD 6-19, by gavage	At 65 mg Silver/kg bw/day: Dams Piloerection, alopecia was observed in foetuses Increase percentage litters with late, foetal deaths (10%), reduced male bodyweight/litter (5%), foetal bodyweight/litter (5%). At 65 mg Silver/kg bw: increased skeletal variations	NOAEL (maternal and developmental): 19 mg Silver mg/kg bw/day	(Price *et al*., 2002 in ECHA RAC, 2022; ECHA, 2022)/ KL1-2
Prenatal developmental toxicity, albino rats, Guideline not specified (5-36 female/ group); Silver chloride	0, 50 mg/day (250 mg/kg bw/day; equivalent to 188 mg Silver/kg bw/day) (5 females were treated during GD 7-15 and 20 females during gestation days 1-20)	At 188 mg Silver/kg bw/day Dams: Decreased ceruloplasmin Foetuses: (treated during GD 1-20), increased post-implantation deaths (26%), cryptorchidism (33%), hydronephrosis (25%) decreased ceruloplasmin, bodyweight (22%) and viability index (100% deaths)	LOAEL: (maternal/ developmental): 188 mg Silver/kg bw/day	(Shavlovski *et al*.,1995 in EU CAR, 2021; ECHA RAC, 2022)/ KL2-3
Prenatal developmental toxicity, Sprague Dawley rats, no Guideline (10 female/ group);	20 mg Silver/kg bw/day as Silver nitrate	Reduced body weight in treated dams. Increased frequency of histopathological findings in brain and liver of dams with neuronal loss event (type 2 hippocampal sclerosis) and	LOAEL (maternal): 20 mg Silver/kg bw/day	Charehsaz, *et al.,* 2016 in ECHA RAC, 2022/KL2

Silver nitrate		hepatocellular vacuolation		
SCAS				
Prenatal developmental toxicity, Sprague Dawley rats, OECD Guideline 414 (30 female/ group); Silver copper zeolite	0, 200, 700, 2000 mg/kg bw/day equivalent to 0, 3, 10 and 29 mg Silver/kg bw GD 6- 15, by gavage	At 29 mg Silver/kg bw mg/kg bw: increase mortality (1/20) Decreased body weight (13%) and bodyweight gain (25%) and clinical signs: sedation, void faeces, urogenital discharge, thinness. No treatment related effects	NOAEL (maternal): 10 mg Silver/kg bw/day; NOAEL (developmental): 29 mg Silver/kg bw/day	(Study report, 1990 in ECHA RAC; US EPA, 1991)/KL1

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Study type, species	**Doses**	**Critical effects**	**Dose descriptor**	**Reference/ KL rating**
		were observed in foetuses		
Prenatal developmental toxicity- **DRF** **study**, Sprague Dawley rats, OECD Guideline 414 (8 female/group); Silver sodium zirconium hydrogenphosphate (10% Silver)	0, 100, 300, and 1000 mg/kg bw/day equivalent to 0, 2.5, 7.4 and 25 mg Silver/kg bw/day gestation day (GD) 6-15, by gavage	No treatment-related effects in either dams or foetuses up to the highest tested dose of 25 mg Silver/kg bw/day	NOAEL (maternal and developmental): >25 mg Silver/kg bw/day	(Study report, 1999 in EU CAR, 2021; ECHA RAC< 2022)/KL2
Prenatal developmental toxicity, Sprague Dawley rats, OECD Guideline 414 (25 female/ group); Silver sodium zirconium hydrogenphosphate (10% Silver)	0, 100, 300, and 1000 mg/kg bw/day equivalent to 0, 2.5, 7.4 and 25 mg Silver/kg bw/day GD 6-15, by gavage	No treatment-related effects in either dams or foetuses up to the highest tested dose of 25 mg Silver/kg bw/day	NOAEL (maternal and developmental): >25 mg Silver/ kg bw/day	(Study report, 1999 in EU CAR, 2021; ECHA RAC, 2022)/ KL2

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