CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Silver nitrate

EC Number: 231-853-9

CAS Number: 7761-88-8

Index Number: 047-001-00-2

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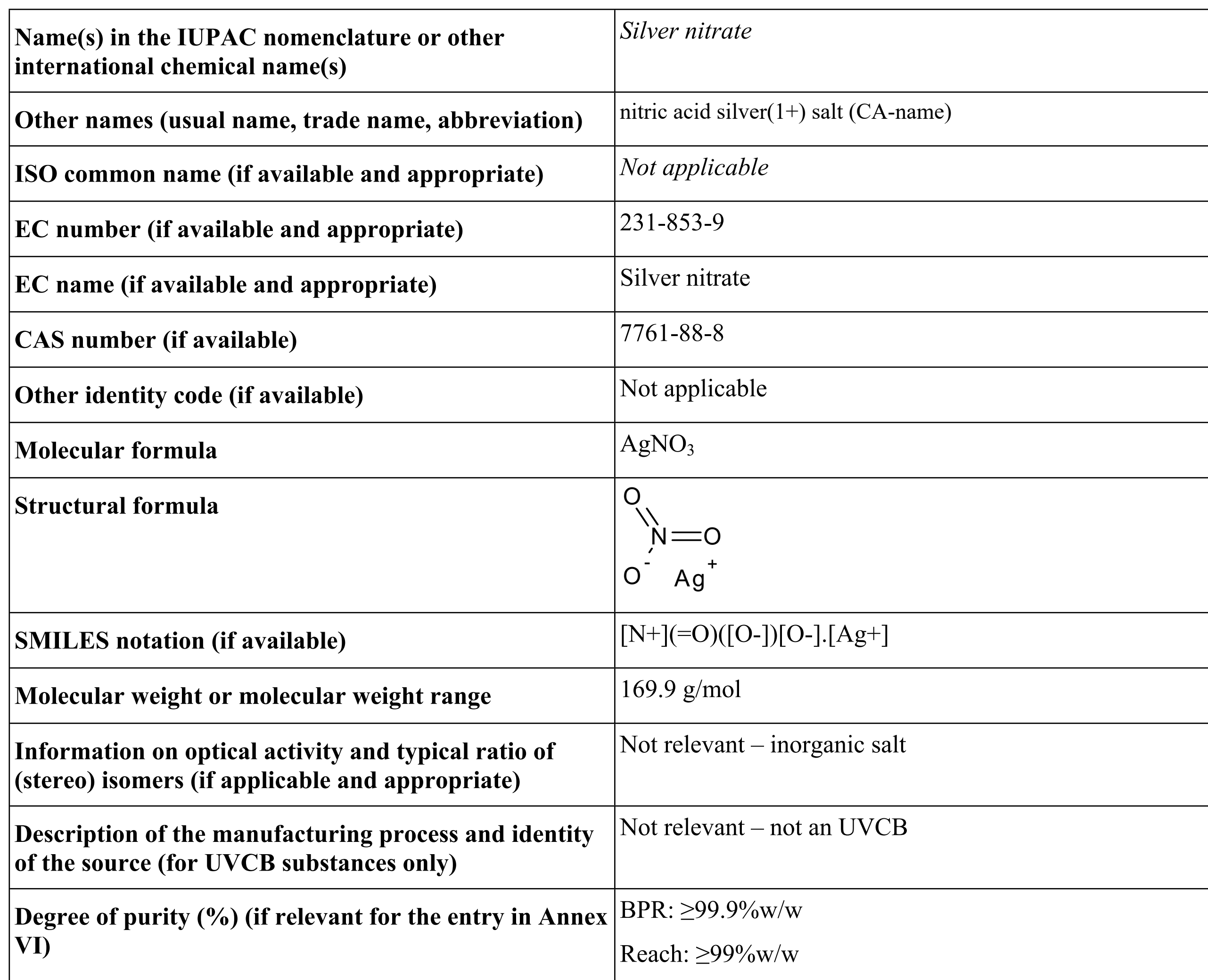
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance



Name(s) in the IUPAC nomenclature or other international chemical name(s)	*Silver nitrate*
Other names (usual name, trade name, abbreviation)	nitric acid silver(1+) salt (CA-name)
ISO common name (if available and appropriate)	*Not applicable*
EC number (if available and appropriate)	231-853-9
EC name (if available and appropriate)	Silver nitrate
CAS number (if available)	7761-88-8
Other identity code (if available)	Not applicable
Molecular formula	$AgNO_3$
Structural formula	O N=O O^- Ag^+
SMILES notation (if available)	[N+](=O)([O-])[O-].[Ag+]
Molecular weight or molecular weight range	169.9 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant – inorganic salt
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant – not an UVCB
Degree of purity (%) (if relevant for the entry in Annex VI)	BPR: ≥99.9%w/w Reach: ≥99%w/w

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Silver nitrate EC-No: 231-853-9 CAS-No: 7761-88-8	≥99%w/w	Ox. Sol. 2; H272 Skin Corr. 1B; H314 Aquatic Acute 1; H400 Aquatic Chronic 1; H310	See ECHA C&L Inventory[1]

[1] https://echa.europa.eu/regulations/clp/cl-inventory

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
None of the impurities are relevant for the classification.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity of test substance and % of Ag of the test substance (w/w)	Silver ion release (% of total silver content)	Other information	The study(ies) in which the test substance is used	Estimated doses of silver ion equivalents tested mg/kg bw/d
Silver nitrate	Purity: No information available Ag: 65%	Assumed to be 100%		Toxicokinetic studies Chronic toxicity studies	0.25 2.5 38.1, 56,5 (in IIIA 6.7(01)) 141 (in IIIA 6.5(03))
Silver nitrate	Purity: 99.98% Ag: 65%	Assumed to be 100%		*in vitro* Skin Corrosion	
Silver nitrate	Purity: 99.98% Ag: 65%	Assumed to be 100%		28-day study	12.7 31.8 63.5

Identification of test substance	Purity of test substance and % of Ag of the test substance (w/w)	Silver ion release (% of total silver content)	Other information	The study(ies) in which the test substance is used	Estimated doses of silver ion equivalents tested mg/kg bw/d
Nanoparticles of silver		No information available.	Different types of nanoparticles used in various published studies. For details such as shape, particle size and surface area, zeta-potential etc; please refer to the study summaries attached.	Developmental toxicity studies Skin sensitisation study Subchronic studies Genotoxicity studies	No information available (see annex 3)
Silver acetate	Purity: 99% Ag: 64.6%	No information available		Developmental toxicity study	6.5, 19, and 65 mg
Silver acetate	Purity: 99% KSCN Ag: 63.7-65.5%	No information available		One-generation fertility study	0.25, 2.5, 25
Silver Acetate	Purity: >100%			90-Day Study	0, 26, 78 ,208 mg/kg bw/d (0, 40, 120 and 320 AgOAc)
Silver Acetate:	Purity: >99.5%			Extended One Generation Reproductive Toxicity Study in	0, 26, 52, 78 mg/kg bw/d (0, 40, 80, 120 mg/kg bw/d AgOAc)
Silver Acetate:	Purity: >99.5%			Preliminary Reproductive Performance Study	0, 2.6, 26, 104 ,208 mg/kg bw/d (0, , 40, 160 and 320 AgOAc)

Identification of test substance	Purity of test substance and % of Ag of the test substance (w/w)	Silver ion release (% of total silver content)	Other information	The study(ies) in which the test substance is used	Estimated doses of silver ion equivalents tested mg/kg bw/d
Silver chloride	Purity: No information available Ag: 75%	No information available		Developmental toxicity study	No information available ~50 mg AgCl/mg/kg bw
Axenohl (silver citrate/laurate solution)	2438 ppm Ag+	No information available		Skin sensitisation study	
Zeomic/Agion Antimicrobial Type AD (silver zeolite)	Purity: ≥99% (dry weight basis) Ag: 22% (18-26%)	42[2],[3] No data available regarding if silver ions are only released in the GI tract prior to absorption or if parent substance also is absorbed and releases silver ions.	The exact composition in %w/w for the other constituents is confidential.	Skin sensitisation study	
Alphasan RC 2000 (silver sodium zirconium hydrogenphosphate)	Purity: ≥96.7% Ag: 9.7-10.2%	25[2] No data available regarding if silver ions are only released in the GI tract prior to absorption or if parent substance also is absorbed and releases silver ions.		Two-generation fertility study Subchronic study in dog	1.9, 9.9, 40 5, 10, 18/20
Alphasan RC 5000 (silver sodium zirconium hydrogenphosphate)- - Acute oral toxicity	Purity: ≥96.7% Silver: 3.6-3.9%			Genotoxicity studies Subchronic study in rat	 0.29, 2.9, 9.5

[2] maximum % release up to 12 hours – simulated rat stomach conditions 150 mM phosphate buffer at pH4 and 37°C). Chloride is likely to be the predominant anion in the mammalian digestive tract, however the low solubility of silver chloride was thought to present significant analytical difficulties for the experiment.

[3] Assuming a similar rate of release as Type AK.

Identification of test substance	Purity of test substance and % of Ag of the test substance (w/w)	Silver ion release (% of total silver content)	Other information	The study(ies) in which the test substance is used	Estimated doses of silver ion equivalents tested mg/kg bw/d
Zeomic/Agion Antimicrobial Type AK (silver zinc zeolite)	Purity: ≥99% (dry weight basis) Ag: 4.9% (4.3-5.3%)	42[2] No data available regarding if silver ions are only released in the GI tract prior to absorption or if parent substance also is absorbed and releases silver ions.	This specific silver zinc zeolite contains 12.9% w/w zinc (11.4-13.8%) The exact composition in %w/w for the other constituents is confidential.	Subchronic studies in rats and dogs Two-generation fertility study	Rat:0.65, 2.0 and 6.0 Dog: 0.2, 1.0, 5.1 1.5/1.8, 9.8/11 and 20/23
Zeomic/Agion Antimicrobial Type AJ (silver zinc zeolite)	Purity: ≥99% (dry weight basis) Ag: 2.5% (2.4-2.6%)	Assumed to be 42	This specific silver zinc zeolite contains 14.4% w/w zinc (13.7-15.1%) The exact composition in %w/w for the other constituents is confidential.	Chronic/carcinogenicity study	Rat: 0.03, 0.09, 0.3, 0.9 Mouse: 0.67, 2.0 and 6.9

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	047-001-00-2	Silver Nitrate	231-853-9	7761-88-8	Ox. Sol. 2 Skin Corr. 1B Aquatic acute 1 Aquatic Chronic 1	H272 H314 H400 H410	GHS03 GHS05 GHS09 Dgr	H272 H314 H410			

Dossier submitters proposal	047-001-00-2	Silver Nitrate	231-853-9	7761-88-8	**Add** Repr. 1B Acute Tox 2 Skin sens. 1 STOT RE 2 Muta. 2 **Modify** Ox. Sol. 1 Skin Corr. 1A **Retain:** Aquatic Acute 1 Aquatic Chronic 1	**Add:** H360FD H300 H317 H373 (nervous system) H341 **Modify** H271 **Retain:** H314 H400 H410	**Add:** GHS06 GHS07 **Retain** GHS03 GHS05 GHS09	**Add:** H360FD H300 H317 H373 (nervous system) **Modify** H271 **Retain:** H314 H410	**Add:** EUH071	**Add:** ATE 29 mg/kg bw M = 1000 M = 100	
Resulting Annex VI entry if agreed by RAC and COM	047-001-00-2	Silver Nitrate	231-853-9	7761-88-8	Ox. Sol. 1 Repr. 1B STOT RE 2 Acute Tox 2 Skin Corr. 1A Skin sens. 1 Muta. 2 Aquatic Acute 1 Aquatic Chronic 1	H271 H360FD H373 H300 H314 H317 H341 H400 H410	GHS03 GHS06 GHS05 GHS07 GHS09 Dgr	H271 H360FD H373 H300 H314 H317 H410	EUH071	ATE 29 mg/kg bw M = 1000 M = 100	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Hazard class not applicable	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Data lacking	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Harmonised classification proposed	Yes
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Inconclusive data	Yes
Acute toxicity via inhalation route	Inconclusive data[4]	Yes
Skin corrosion/irritation	Harmonised classification proposed	Yes
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	Inconclusive data	Yes
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Harmonised classification proposed	Yes
Carcinogenicity	Inconclusive data	Yes
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	Yes
Aspiration hazard	Hazard class not applicable	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not applicable	No

[4] EUH071 proposed.

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Silver nitrate is included in Annex VI and currently classified Ox. Sol. 2; H272, Skin Corr. 1B; H314, Aquatic Acute 1; H400, Aquatic Chronic 1; H410. A proposal for classification and labelling of silver was submitted by Swedish Chemicals Agency (KEMI) to ECHA in 2019 and discussed during RAC 58, 59, 60 and 61 in 2021 and 2022. Based on the data available in the CLH dossier, the outcome of the consultation, including additional data submitted and the discussions in RAC, silver (all forms) was proposed by RAC to be classified for STOT RE 2; H335 (nervous system) and Repr. 2; H361f. As regards the environmental hazards, the following classifications were adopted by RAC:

Silver powder (particle diameter > 100 nm < 1 mm):

Very toxic to aquatic life (Aquatic Acute 1; H400, M=10)

Very toxic to aquatic life with long lasting effects (Aquatic Chronic 1; H410, M=10)

Silver powder (particle diameter > 1 nm ≤ 100 nm): as above for silver powder with particle diameter > 100 nm < 1 mm, but with acute and chronic M-factors of 1,000

Silver ≥ 1 mm: no classification for environmental hazards.

The underlying data for the RAC opinion on silver is mainly the same as the data included in this proposal for classification and labelling of silver nitrate. However, in the absence of data for silver in other forms, the general principle of RAC when assessing the different hazard classes for human health was to base decisions on classification and labelling on data available for the nanoform. Data available for salts of silver were only considered as supportive information since the forms of silver included in the proposal were considered to release low amounts of silver ions compared to salts. The approach taken by RAC thus considers bioavailability and considerations of risk in the classification. It was justified by the following line of reasoning *" The silver ion, Ag+, is recognised as the toxicophore, but it was necessary to consider the potential toxicity of silver metal in reduced and aggregated form such as nanoparticles or massive forms compared to that from exposure to silver salts, such as AgNO3, or ion exchange matrices such as silver zeolites. A specific evaluation and assessment of bioavailability was thus warranted for silver and different silver compounds from which read-across to silver was proposed in the CLH report.*

[…]

According to Art. 12(b) of CLP, as a result of the evaluation carried out pursuant to Art. 9(5) (according to which available hazard information on the forms or physical states in which the substance is placed on the market and in which it can reasonably be expected to be used shall be considered for the purposes of classification), where conclusive scientific experimental data is identified that shows that the substance is not biologically available, then this property of the substance shall be taken into account for classification purposes. Also, under Art. 9(5) of CLP, hazard information on the physical states and forms of a particular substance placed on the market must be considered for the classification of a substance, and furthermore, under Art. 12(b), the lack of bioavailability of the substance, where identified, must also be taken into account for the purposes of classification. CLP states that bioavailability shall only be considered when conclusive data show that the substance is not biologically available, which is not the case here. However, it is not explained in CLP if/how different degrees of bioavailability for different forms of the same substance shall be considered for classification purposes and this is problematic for silver. It is noted that bioavailability per se is not considered an intrinsic property of a substance, it is however dependent on several factors, many of which may be considered as specific intrinsic properties in their own right, e.g., solubility, silver ion surface release.

[…]

The degree to which silver metal atoms and ions are available to cause toxicity to mammalian systems may be determined by site specific, i.e. local conditions controlling the speciation/precipitation and/or complexation of the metal. For example, relevant biological fluids such as gastric fluid, intestinal fluid, lung interstitial/alveolar fluid, sweat and blood all present different environments that may impact on the ultimate

bioavailability of silver ions. The bioavailability of silver ions (and counter ions) may be key to the toxicities observed with different silver salts (of which only a few are soluble in aqueous systems) and other silver-containing active substances. The main problem is extrapolating these toxicities to the different forms of silver metal (the target substance), when the physical and chemical properties of the source substances are hugely variable and not comparable and when there is a lack of specific data for the target substance itself. Bioavailability is then determined by the initial degree of dissolution of silver combined with the final nature of the resultant silver species that is presented to a biological membrane for absorption. Comparing silver ion bioavailability from the silver forms with that from silver salts and other silver-containing active substances is difficult simply because of how silver compounds interact differently with the relevant biological environments as a consequence of their specific physicochemical characteristics. Soluble salts such as AgNO3 and Ag+2[CH3COO-]2 (also reported as AgOAc) will release more silver ions than the different silver metal forms in such environments. On this basis the read-across proposed by the DS from several different silver compounds is not supported by RAC when there is a lack of co-existing key data on silver."

Silver nitrate is a highly soluble salt thus the approach taken by RAC for elemental silver will not be followed in this assessment (see section 6). Therefore, although the underlying data in the proposals for classification and labelling of silver and silver nitrate is almost identical, the use of data for the comparison with criteria differs.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Silver nitrate is an active substance in the meaning of Regulation (EU) No 528/2012 and shall thus be subject to harmonised classification and labelling.

5 IDENTIFIED USES

Silver nitrate is used in biocidal products. The active chemical entity is the silver ion released during use and claimed to be effective against a broad spectrum of microorganisms (e.g. Gram-positive and Gram-negative bacteria, moulds/fungi and yeasts).

Silver nitrate is used in products categorised into the following product types (according to Regulation (EU) No 528/2012):

1 Human hygiene

2 Disinfectants and algaecides not intended for direct application to humans or animals

3 Veterinary hygiene

4 Food and feed area disinfection

5 Drinking water disinfection

7 Film preservatives

9 Fibre, leather, rubber and polymerised materials preservatives

11 Preservatives for liquid-cooling and processing systems

Some of these uses may potentially result in a vast range of consumer applications.

6 DATA SOURCES

This CLH report is mainly based on the data included in the dossier submitted by the applicant for the assessment of silver containing active substances (SCAS) within the review programme for biocides under Regulation (EU) No 528/2012 (Biocides Products Regulation). In addition to this data, the report includes published studies available in the open literature (including the registration dossier submitted under Regulation (EC) No 1907/2006 (REACH)) and four new studies submitted by the European Precious Metal Federation (EPMF); a toxicokinetic study (including silver nitrate, silver acetate, micron-sized silver and nanoparticulate silver), a dose-range finding study, an extended one generation reproductive toxicity study and a 90.day study,

all three performed with silver acetate. Summaries of studies used for the human health hazard assessment are included in an annex marked as confidential as they were prepared under the former Biocides Directive (98/8/EC) and thus not expected to become publicly available. These study summaries include all toxicological data submitted in the original dossier and are also included in the Competent Authority Reports and CLH reports for silver zinc zeolite and the other silver containing active substances reviewed under the BPR. Study summaries of new studies with silver nitrate, silver acetate and published studies performed with nanosilver are available in the non-confidential annex I to this report.

Use of data for other silver containing active substances (SCAS) to address hazard classes for human health:

There are only a few studies available specifically investigating the toxicity of silver nitrate and the quality of these studies is rather poor. The substance has corrosive properties which complicates animal testing thus a key issue for this type of substance is to identify the limit concentration at which the corrosive properties determine the toxicity of the substance. Below this threshold, testing would be possible, and any toxic effects are expected to be caused by the silver ion. This means that data obtained with other silver substances may provide information on the intrinsic properties of the silver ion at concentrations below the threshold for corrosion since the silver ion is released from the SCAS studied and thereby indirectly tested in these studies. Nevertheless, in the absence of information on the critical concentration for corrosion, all data considered relevant for the silver ion is taken into account, regardless of dose level.

The use of data obtained with SCAS is yet complicated by the content of additional constituents as well as by the content and release of silver (and other metal) ions from the active substance. Due to the possible contribution to toxicity from the additional constituents, data for some substances is considered to take precedence over other data. This is further discussed in section 10, where relevant. The rate of release may have a significant impact on the actual silver ion exposure in the toxicological studies. If assuming complete silver ion release from the substance when in fact the fraction released is lower, the true effect level for the silver ion may be underestimated since the internal exposure to the silver ion would be lower than the level if all silver was released. To avoid this, effect levels for the silver ion are estimated based on a correction for silver content and the percentage of release during conditions assumed to mimic the gastrointestinal tract of the rat (table 5). Recently, bioavailability data for silver following exposure to silver acetate, silver nitrate or nano- or micron-sized silver powder became available through a comparative toxicokinetic study performed in accordance with OECD TG 417 and GLP. The results indicate that silver is bioavailable from all test items but to highest extent from silver salts, followed by silver nanoparticles and least from micron-sized silver (≈ 300nm). The bioavailability of silver from silver acetate and silver nitrate was similar.

There is, however, no data available to clarify if the other silver releasing substances used in toxicological tests are absorbed in the gastrointestinal tract in the form of the parent or if the substances dissociates and the silver ions released from the parent are absorbed. In the absence of information, it is assumed that 5% of the silver ions released during conditions mimicking the gastrointestinal tract of the rat are absorbed. This figure is based on data for silver nitrate which is a highly soluble substance. Considering that chloride ions are in excess of silver ions in the stomach, it is likely that the silver ions released form different silver chloride complexes prior to absorption.

In similarity with the different SCAS, nanoparticles release silver ions. However, the data available in the open literature do not give a definite answer to the question if effects observed result from the silver ions released or if they are caused by the nanoparticles per se since studies rarely include ionic silver as a concurrent control. Overall, effects (e.g., oxidative stress, pigmentation of organs and tissues, increases in ALP) seem to be similar between silver ions and silver nanoparticles. Although induction of oxidative stress is often reported as an effect specific to nanoparticles, this effect is also observed with ionic silver , e.g., Charehsaz et al (IIIA, 6.8.2-10) stating *"It can be concluded that the oxidative response/damage of Ag-NPs reported in previous studies depends not only on the NPs, but also on the amount of Ag ions released from the surface of the NPs."*. Consequently, effects discussed in this assessment that may result from a mode of action involving oxidative stress cannot be disregarded as being specific to nanoparticles since it is clearly also an intrinsic property of the silver ion. However, nanoparticles may have a higher potency with respect to reactive oxygen species (ROS) production due to their oxidative potential. It may be argued that the concentration/distribution of silver ions released from silver nanoparticles differs from ionic silver released from a salt as there could be a local

accumulation/localisation of nanoparticles in specific tissues and organs. Silver ions can then be released from these deposits resulting in higher local concentrations of silver ions and/or nanoparticles as compared to silver ions released from the salt and absorbed from the GI tract or respiratory tract. This seems to be the case following inhalation when nanoparticles distribute to the brain via the olfactory nerve thus circumventing the blood-brain barrier. However, for the oral route, published data on nanosilver indicate accelerated dissolution in gastric acid and uptake of soluble silver through ion and nutrient uptake channels and transport in blood probably as thiol complexes[5]. The article also states that the ability of particles to cross the gut epithelium is limited and ion uptake is thus likely the main route to systemic circulation. Furthermore, results from a 28-day study in rats indicate a similar distribution pattern between silver from silver nitrate and nanoparticles of silver following oral exposure with a higher uptake of silver in animals treated with silver nitrate (Van der Zande, M., et al, doc IIIB, 6.8.2-12). In this study, the proportions of silver uptake from the fraction of soluble silver were similar between the Ag nanoparticles (< 20 nm) and the silver nitrate treated animals indicating that silver is probably bioavailable mainly in the ionic form. However, since all measured silver could not be accounted for in blood, testis, and spleen solely by the fraction of soluble silver alone, the authors state that a small fraction of particles might still be bioavailable. Since silver-containing nanoparticles were detected in liver, spleen, and lungs also in $AgNO_3$ exposed animals, nanoparticles form in vivo from silver ions. A similar distribution pattern between nano and micron-sized forms of silver is also indicated from the recent bioavailability study although, as discussed in section 9, there are quantitative differences between substances. Taken together, this information is considered to demonstrate that results obtained with nanosilver are relevant for the assessment of the intrinsic properties of silver ions released from silver nitrate at non-corrosive concentrations.

Unfortunately, there is rarely quantitative information with respect to the amount of silver ions released and/or the relative amounts of silver ions versus nanosilver absorbed in published studies. It is well known that surface coatings stabilise the nanoparticles and thereby impact on the release of silver ions[6]. However, most articles do not specify the type of coating used hence it is not possible to analyse the possible impact on the results. As shown in the read-across matrices attached to this report (annex 3), information available regarding the nanoparticles in published studies is very limited compared to the information requested in the template for read-across presented in the guidance document[7]. Nevertheless, it would not be scientifically justified to exclude the studies and ignore the effects noted on that basis. Although factors such as the surface coating could impact on the silver ion release and explain differences in effects observed between studies, classification is based on the intrinsic properties thus effects with nanosilver referred to in this report are considered to represent an intrinsic property of the silver ion. At least for the nanoparticles (and coating) used in the bioavailability study, the results indicate a higher bioavailability of silver from silver nitrate and silver acetate than from nanoparticulate silver. Therefore, while results from studies with nanosilver is considered relevant for the assessment of the silver ion, it should be noted that the severity of effects may be milder than if studies were performed with a salt.

Data used to address hazard classes for the environment:

Silver nitrate is a metal compound and falls under the classification scheme for metals and metal compounds in the CLP-guidance on the Application of the CLP Criteria Annex IV, chapter 5 (ECHA, 2017). The environmental classification strategy is based on the short- and long-term hazards posed by metals and metal compounds when they are available, i.e., exist as dissolved metal ions. In the present CLH dossier the ecotoxicity tests presented have been performed with highly soluble silver salts of which most is silver nitrate. Some studies for chronic toxicity to algae were performed with silver chloride. Chloride in test medium has both shown to lead to decreased silver toxicity for some

[5] Liu et al, ACS Nano, 2012, 6 (11)

[6] E.g. Reidy. B. et al (2013), Materials, Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications

[7] Appendix 2 of Appendix R. 6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals.

fish species like rainbow trout and less protection or even higher silver toxicity for other species. This indicates that the protection against silver toxicity by chloride is species dependent and not simply due to water chemistry and silver speciation. The mechanisms behind the differences in protection between species remain unclear (Bielmyer et al. 2008). The key studies for acute and chronic toxicity are however performed with silver nitrate.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Crystalline solid	BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press Reach dossier GESTIS-database on hazardous substances, 2009 www.dguv.de/bgia/stoffdatenbank	
Melting/freezing point	212°C	BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press The Merck Index, 13th Edition, 2001 Reach dossier RÖMPP Online, Version 3.1, 2008	
Boiling point	Not applicable – decomposition at 440°C before boiling.	BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press The Merck Index, 13th Edition, 2001 RÖMPP Online, Version 3.1, 2008	
	Not applicable – decomposition before boiling (>250-440°C; decomposition complete at 440°C).	Reach dossier GESTIS-database on hazardous substances, 2009 www.dguv.de/bgia/stoffdatenbank	
Relative density	4.35 g/cm^3 at 20°C	BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press The Merck Index, 13th Edition, 2001 Reach dossier RÖMPP Online, Version 3.1, 2008	

Property	Value	Reference	Comment (e.g. measured or estimated)
	4.35 g/cm³ at 19°C	Reach dossier GESTIS-database on hazardous substances, 2009 www.dguv.de/bgia/stoffdatenbank	
Vapour pressure	Not relevant – it is a high melting inorganic salt and the vapour pressure is anticipated to be negligible.	Waivers in BPR- and Reach dossiers	
Surface tension	Not relevant – silver nitrate contains no surface active functionality and in water will dissociate completely resulting in silver ions and nitrate ions. Thus surface activity is not anticipated.	Waivers in BPR- and Reach dossiers	
Water solubility	2340 g/l at 25°C	BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press	
	2500 g/l	BPR ESTF The Merck Index, 13th Edition, 2001	
	548 g/l at 0°C 710 g/l at 25°C 815 g/l at 60°C 880 g/l at 100°C	Reach dossier RÖMPP Online, Version 3.1, 2008	Results are not in line with the others and thus treated as of lower reliability.
	2160 g/l at 20°C	Reach dossier GESTIS-database on hazardous substances, 2009 www.dguv.de/bgia/stoffdatenbank	
	2150 g/l at 20°C 9100 g/l at 100°C	Reach dossier Lehrbuch der anorganischen Chemie, Holleman-Wiberg (ed.) Walter de Gruyter, 101st ed., 1346, 1995	
Partition coefficient n-octanol/water	Not applicable to an inorganic substance as silver which is highly soluble in water. In accordance with column 2 of REACH Annex VII, the partition coefficient study does not need to be conducted as this substance is inorganic.	Waivers in BPR- and Reach dossiers	

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	Not applicable as the melting point is >40°C	Waivers in BPR- and Reach dossiers	
Flammability	Silver nitrate is not anticipated to be flammable (also claimed to be non-flammable from experience in use).	Waiver in BPR-dossier	In accordance with CLP, the substance should in any case not be considered for classification as a flammable solid as it is a oxidising solid (see below).
	According to “Weiss G, ed. 1986. Hazardous chemicals data book. 2nd ed. Park Ridge, NJ: Noyes Data Corporation, 887-893 Weiss”, silver nitrate is non-flammable. Further, as a purely inorganic material (silver cations and nitrate anions in a crystalline matrix), silver nitrate can be considered as intrinsically not combustible, as it does not contain any components which are capable of reaction with oxygen. Long-term industrial handling experience does not indicate any concern for flammability of silver nitrate. Thus, waiving for this study is applied, because a study is scientifically unjustified.	Waiver in Reach dossier	
Explosive properties	Based on experience in use it can be concluded that silver nitrate is not explosive as a single substance. Contact with certain organic substances may result in spontaneous ignition (wood impregnated with silver nitrate is recognised as a hazard).	Waiver in BPR-dossier	

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
	Siver nitrate is devoid of any chemical structures commonly associated with explosive properties, such as metal peroxides, peroxo-acid-anions, azides, and halogen oxides.) Whereas silver nitrate exhibits oxidising properties it is not combustible itself. Further, it is noted that despite long-term industrial use of the substance, it is not classified for explosive properties according to UN transport regulations. In consequence, no experimental verification is required and silver nitrate is considered to be non-explosive.	Waiver in Reach dossier	

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Self-ignition temperature	Self-ignition is not considered to be of relevance for this substance, since this would require heat to be developed either by reaction of this substance with oxygen or by exothermic decomposition and which is not lost rapidly enough to the surroundings. Silver nitrate is an inorganic material, consisting of silver cations and nitrate anions in a crystalline matrix. Based on this structure, any reaction with oxygen or exothermic decomposition is not scientifically plausible. Long-term industrial experience supports the assessment that silver nitrate is neither flammable nor auto-flammable. In conclusion, the conduct of experimental verification is not considered to be required.	Waiver in Reach dossier	
Oxidising properties	Silver nitrate (sieved to < 0.500 mm and dried): Category 1 oxidising solid according to UN-Test O.1	Reach dossier Möller, 2009	
	Silver nitrate (d10=153.9 µm, d50=294.9 µm, d90=497.8 µm): Category 1 oxidising solid according to UN-Test O.1 Silver nitrate (d10=272.3µm, d50=532.5 µm, d90=957.9 µm): Category 2 oxidising solid according to UN-Test O.1	Reach dossier Bremble, 2010	

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
	Silver nitrate (d10=240 µm, d50=360 µm, d90=470 µm): Category 1 oxidising solid according to UN-Test O.1	Reach dossier Michael-Schulz, 2010 (non-GLP)	
Granulometry	Data relevant to classification – provided for oxidizing properties above. No information available in BPR-dossier		
	Information in Reach-dossier for the registrants shows d10 in the range of 75-329 µm, d50 in the range of 266-520 µm and d90 in the range of 459-816 µm	Reach dossier	
Stability in organic solvents and identity of relevant degradation products	This study does not need to be conducted for inorganic substances (cf. Annex IX section 7.15 Column 2 of regulation (EC) 1907/2006).	Waiver in Reach dossier	
Dissociation constant	Not relevant. Silver nitrate contains no acid or base functionality and in water will dissociate completely resulting in silver ions and nitrate ions.	Waiver in BPR-dossier	

Property	Value	Reference	Comment (e.g. measured or estimated)
	Silver nitrate dissolves readily in water, yielding only silver cations and nitrate anions. The substance does not contain relevant functional groups for which an assessment of the dissociation behaviour would provide information for risk assessment purposes. Therefore, the determination of a dissociaion constant is not considered to be required (Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance, section R.7.1.17.1).	Waiver in Reach dossier	
Viscosity	Not applicable as silver nitrate relevant is a solid.	Waiver in BPR-dossier	

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
-	Based on experience in use it can be concluded that silver nitrate is not explosive as a single substance. Contact with certain organic substances may result in spontaneous ignition (wood impregnated with silver nitrate is recognised as a hazard).		Waiver in BPR-dossier

Method	Results	Remarks	Reference
-	Siver nitrate is devoid of any chemical structures commonly associated with explosive properties, such as metal peroxides, peroxo-acid-anions, azides, and halogen oxides.) Whereas silver nitrate exhibits oxidising properties it is not combustible itself. Further, it is noted that despite long-term industrial use of the substance, it is not classified for explosive properties according to UN transport regulations. In consequence, no experimental verification is required and silver nitrate is considered to be non-explosive.	Argument in relation to absence of functional groups associated with explosive properties not valid since it contains the nitrate group.	Waiver in Reach-dossier

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Two waiving arguments are available, stating that based on experience in use, silver nitrate as a single substance does not possess explosive properties. It is further outlined that it is an oxidizer and in combination with organic material it can be hazardous.

8.1.2 Comparison with the CLP criteria

It is noted that the waiving criteria with respect to chemical groups is not met since it is a nitrate-salt. Experience in use is also not mentioned as a valid ground for waiving in the Guidance on the Application of the CLP Criteria. However, it seems that strong oxidizers like inorganic nitrate salts are normally not explosive as such but in combination with combustible organic materials. Moreover, the DS notes that indeed silver nitrate is included in the Dangerous Goods list of the "UN Model Regulations on the Transport of Dangerous Goods"[8] as follows:

UN No.	Name and description	Class or division	Subsidiary hazard	UN packaging group	Special provisions	Limited and excepted quantities		Packaging and IBCs		Portable tanks and containers	
								Packaging instruction	Special packaging provisions	Instructions	Special provisions
(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7a)**	**(7b)**	**(8)**	**(9)**	**(10)**	**(11)**
-	**3.1.2**	**2.0**	**2.0**	**2.0.1.3**	**3.3**	**3.4**	**3.5**	**4.1.4**	**4.1.4**	**4.2.5/4.3.2**	**4.2.5**
1493	Silver nitrate	5.1		II		1 kg	E2	P002 IBC08	B2,B4	T3	TP33

Since the UNTGs does not classify silver nitrate as explosive, no classification is warranted under CLP.

[8] Recommendations on the Transport of Dangerous Goods, Model Regulations, Volume I, Twenty-second revised edition, United Nations, 2021

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed (data conclusive but not sufficient for classification).

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable – the substance is not a gas.

Table 10: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference

8.3 Oxidising gases

Hazard class not applicable – the substance is not a gas.

Table 11: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference

8.4 Gases under pressure

Hazard class not applicable relevant – the substance is not a gas.

Table 12: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference

8.5 Flammable liquids

Hazard class not applicable – the substance is not a liquid.

Table 13: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference

8.6 Flammable solids

Table 14: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
-	Silver nitrate is not anticipated to be flammable (also claimed to be non-flammable from experience in use).	-	Waiver in BPR-dossier
	According to "Weiss G, ed. 1986. Hazardous chemicals data book. 2nd ed. Park Ridge, NJ: Noyes Data Corporation, 887-893 Weiss", silver nitrate is non-flammable. Further, as a purely inorganic material (silver cations and nitrate anions in a crystalline matrix), silver nitrate can be considered as intrinsically not combustible, as it does not contain any components which are capable of reaction with oxygen. Long-term industrial handling experience does not indicate any concern for flammability of silver nitrate. Thus, waiving for this study is applied, because a study is scientifically unjustified.	-	Waiver in Reach dossier

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Two waiving arguments are available, stating that based on handbook information and experience in use, silver nitrate is not flammable. Experimental testing is thus considered unjustified.

8.6.2 Comparison with the CLP criteria

In the Guidance on the Application of the CLP Criteria (section 2.7.3) it is stated that *"Explosives, organic peroxides, self-reactive substances and mixtures as well as pyrophoric or oxidising solids should not be considered for classification as flammable solids since flammability is an intrinsic hazard in these classes."* Since silver nitrate is an oxidising solid classification for flammability should not be considered.

8.6.3 Conclusion on classification and labelling for flammable solids

No classification proposed (hazard class not applicable since it is an oxidising solid).

8.7 Self-reactive substances

Data/waiving information lacking.

Table 15: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No information addressing this hazard class is available.

8.7.2 Comparison with the CLP criteria

In the Guidance on the Application of the CLP Criteria (section 2.8.4.2) it is stated that substances and mixtures must be considered for classification in this hazard class as a self-reactive substance or mixture unless:

"*(b) they are oxidising liquids or solids, according to the criteria given in 2.13 or 2.14, except that mixtures of oxidising substances, which contain 5 % or more of combustible organic substances shall be classified as self-reactive substances according to the procedure defined in 2.8.2.2".* Since silver nitrate is an oxidising solid, classification for self-reactive substances should not be considered.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification proposed (hazard class not applicable since it is an oxidising solid).

8.8 Pyrophoric liquids

Hazard class not applicable – the substance is not a liquid.

Table 16: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference

8.9 Pyrophoric solids

Table 17: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
-	Testing for pyrophoric properties of solids is not considered to be required, since this substance is stable at ambient temperature. This substance also does not contain any chemical groups that might lead to spontaneous ignition a short time after coming in contact with air at room temperature (circa 20°C). Furthermore, long-term industrial experience in handling shows that the substance does not ignite in contact with air.		Waiver in Reach dossier

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

One waiving argument is available stating that the substance is not pyrophoric as it is stable at room temperature, does not contain any functional groups known to spontaneously ignite in air at room temperature and as experience in handling and use show that it does not ignite in contact with air.

8.9.2 Comparison with the CLP criteria

Experience in use shows that the substance is not pyrophoric (i.e. valid waiver according to CLP). However, as stated in 2.14.3 of the Guidance on the Application of the CLP Criteria, the hazard class pyrophoric solids is not applicable since the substance is as an inorganic oxidising solid.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed (hazard class not applicable since it is an oxidising solid).

8.10 Self-heating substances

Table 18: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
-	Self-ignition is not considered to be of relevance for this substance, since this would require heat to be developed either by reaction of this substance with oxygen or by exothermic decomposition and which is not lost rapidly enough to the surroundings. Silver nitrate is an inorganic material, consisting of silver cations and nitrate anions in a crystalline matrix. Based on this structure, any reaction with oxygen or exothermic decomposition is not scientifically plausible. Long-term industrial experience supports the assessment that silver nitrate is neither flammable nor auto-flammable. In conclusion, the conduct of experimental verification is not considered to be required.		Waiver in Reach dossier
Unknown	Melting point: 212°C		BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press The Merck Index, 13th Edition, 2001 Reach dossier RÖMPP Online, Version 3.1, 2008
Unknown	Decompostion at 440°C before boiling.		BPR ESTF CRC Handbook of Chemistry and Physics, 83th Edition, 2003, CRC Press

Method	Results	Remarks	Reference
			The Merck Index, 13th Edition, 2001 RÖMPP Online, Version 3.1, 2008
Unknown	Decomposition before boiling (>250-440°C; decomposition complete at 440°C).		Reach dossier GESTIS-database on hazardous substances, 2009 www.dguv.de/bgia/stoffdatenbank

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

One waiving argument is available for general auto-flammability, stating that it is not auto-flammable based on structural properties and experience in use. In addition, data on the melting point and thermal decomposition is available which is relevant to this hazard class.

8.10.2 Comparison with the CLP criteria

According to the Guidance on the Application of the CLP Criteria only results from experimental screening procedures are described as valid grounds for waiving. Since no such data is available a comparison against the CLP criteria is not possible.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed (data lacking).

8.11 Substances which in contact with water emit flammable gases

Table 19: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
-	Testing of flammability in contact with water is not considered to be required, because the substance does not contain groups that might lead to a reaction with water or damp air, leading to the development of dangerous amounts of gas or gases which may be highly flammable. In contrast, silver nitrate, which is an inorganic substance, dissolved readily in water, yielding only dissolved ions.		Waiver in Reach dossier

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

One waiving argument is available, stating that testing for this physical hazard is not required since the substance does not contain functional groups that can react with water or moist air. It is also evident that silver nitrate does not react with water as it is placed on the market as aqueous solutions.

8.11.2 Comparison with the CLP criteria

Experience in use shows that the substance does not emit flammable gases in contact with water (i.e. valid waiver according to CLP).

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification is proposed (data conclusive but not sufficient for classification).

8.12 Oxidising liquids

Hazard class not applicable – the substance is not a liquid.

Table 20: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference

8.13 Oxidising solids

Table 21: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
UN-Test O.1	Burning time silver nitrate (sieved to < 0.500 mm and dried)/cellulose mixtures: 4:1 15 s 1:1 100 s Burning time reference (potassium bromate)/cellulose mixtures: 3:7 128 s 2:3 66 s 3:2 21 s **Test item determined to be a Category 1 oxidising solid.**		Reach dossier Möller, 2009
UN-Test O.1	Burning time silver nitrate (d10=153.9 µm, d50=294.9 µm, d90=497.8 µm)/cellulose mixtures: 4:1 12.7 s 1:1 61.6 s Burning time reference (potassium bromate)/cellulose mixtures: 3:7		Reach dossier Bremble, 2010

Method	Results	Remarks	Reference
	97.3 s 2:3 38.1 s 3:2 14.1 s **Test item determined to be a Category 1 oxidising solid.**		
	Burning time silver nitrate (d10=272.3µm, d50=532.5 µm, d90=957.9 µm)/cellulose mixtures: 4:1 24.3 s 1:1 89.5 s Burning time reference (potassium bromate)/cellulose mixtures: 3:7 97.3 s 2:3 38.1 s 3:2 14.1 s **Test item determined to be a Category 2 oxidising solid.**		
UN-Test O.1	Burning time silver nitrate (d10=240 µm, d50=360 µm, d90=470 µm)/cellulose mixtures: 4:1 12.8 s 1:1 16.0 s Burning time reference (potassium bromate)/cellulose mixtures: 3:7 Not determined 2:3 49.8 s 3:2 13.3 s **Test item determined to be a Category 1 oxidising solid.**	Non-GLP	Reach dossier Michael-Schulz, 2010

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

In the Reach registration dossier robust study summaries are available for three studies on silver nitrate with different particle size distribution in accordance with UN-Test O.1 (recommended test method in CLP). The results of all studies indicate that the test materials are oxidising solids.

It should be noted that in the Reach registration dossier a table is shown, giving results for seven test materials with different particle size distributions. The DS has been able to assign the above-mentioned

robust study summaries to five of these test materials (i.e. the Bremble study includes testing of two test items and the Michael-Schulz study is reported in the table with results for the test item before and after grinding but this cannot be verified by the study summary). The additional two test items not linked to the robust study summaries were reported as Category 2 (d10=329 µm, d50=491 µm, d90=727 µm) and Category 1 (d10=254 µm, d50=421 µm, d90=677 µm) respectively. It is also noted that the the particle size distribution of the test item used in the Michael-Schulz study is differently reported in the referred table and the study summary (i.e the particle size distribution reported in the table above is taken from the study summary).

In addition in the reach registration dossier it is also referred to the studies that formed the basis for the existing classification of silver nitrate as oxidising solid Category 2. Neither the registrant nor the DS has been able to retrieve these.

8.13.2 Comparison with the CLP criteria

Three out of the four test items in the available robust study summaries comply to the criteria for oxidising solids Category 1 according to CLP since the test item/cellulose mixtures (4:1) burned to completion in shorter time than the reference/cellulose mixture (3:2). The burning time for the last test item/cellulose mixture (4:1) was shorter than the reference/cellulose mixture (2:3) but longer than the reference/cellulose mixture (3:2). Thus it complies to the criteria for oxidising solid Category 2.

In the Reach dossier, it is argued that the particle size is an important factor for the oxidising properties and based on the results included in the table therein (see 8.1.3.1 above) they have proposed a cut-off particle size (a d10 finer than 250 µm) when it should be classified as Category 1 instead of Category 2. However, it is highlighted that this d10 is based on very few data points.

The DS recognises that particle size may have a large impact on the oxidising properties as also stated in the Application of the CLP Criteria (Section 2.1.4.2), i.e. the smaller the particles are , the higher the oxidising capability of the solid. However, as the DS has not been able to assess robust study summaries for all the results reported in the Reach dossier and for the results which formed the basis of the existing classification in Category 2 the DS finds the data points as being too few to propose a particle size specific classification. Instead, based on a weight of evidence approach for the data provided in the available robust study summaries, the DS proposes that silver nitrate should be classified as oxidising solid Category 1.

8.13.3 Conclusion on classification and labelling for oxidising solids

Silver nitrate should be classified as Ox. Sol. 1 (H271).

8.14 Organic peroxides

Hazard class not applicable – the substance is not an organic peroxide.

Table 22: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference

8.15 Corrosive to metals

No data is available.

Table 23: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference

Method	Results	Remarks	Reference

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

In the Reach registration dossier. no information or data is presented either than that a classification as Met. Corr. 1 (H290) is given.

8.15.2 Comparison with the CLP criteria

No data is available to compare against the CLP-criteria. Since silver nitrate has an existing classification as skin corrosive, according to the Guidance on the Application of the CLP Criteria (section 2.16.6.1), it should be considered for testing for corrosive against metal.

As noted in 8.1.5.1 above, the Reach registration dossier contains a proposed classification for corrosive to metal. In addition. in the C&L Inventory many notified classification and labelling proposals as Met. Corr. 1 are given.

However, the DS recognises that silver nitrate is a solid with a melting point >55 °C which means that according to current practice no classification can be proposed (i.e. refer to RAC Guidance note[9] for members on: assessing physical hazards as part of CLP).

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed (data conclusive but not sufficient for classification).

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 24: Summary table of toxicokinetic studies

Summary table of toxicokinetic studies					
Method **Guideline, GLP status,** **Reliability**	**Species,** **Strain,** **Sex,** **No/Group**	**Test substance,** **Dose levels** **Duration of exposure**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
No guideline No GLP	Rats Sprague-Dawley Male Six week old Average weight 245g 5/group	AgNO3 Silver nanoparticles: Non-coated AgNPs <20nm PVP-coated AgNPs <15nm	Main target organs for AgNPs and AgNO3: liver and spleen, followed by the testis, kidney, brain, and lungs, without differences in the distribution pattern between the two different	Fraction of soluble silver rather similar between the Ag < 20 nm and AgNO3 animals in blood and in organs with	IIIA, 6.8.2-12 Van der Zande, M., Vandebriel, R.J., van Doren, E., Kramer, E., Herrera Riviera, Z., Serrano-Rojero, C.S., Gremmer, E.R.,

[9] RAC Guidance note for members on: assessing physical hazards as part of CLP, 62ND MEETING OF THE COMMITTEE FOR RISK ASSESSMENT, Agenda point 8.1.2, RAC/62/2022/04

	2 control groups, 3 expermental groups	Non-coated – matrix = 4% polyoxyethylene glycerol trioleate and 4% Tween 20 in H2O Coated - suspended in water Oral gavage exposure 28-day exposure Post-exposure: wash out until days 36 and 84 Silver exposure: 90 mg/kg bw for the Ag < 20 and Ag < 15-PVP groups 9 mg/kg bw for the AgNO3 group	AgNPs, or the AgNO3 exposed animals. Higher uptake of silver in blood and organs of AgNO3 exposed rats. Elimination of silver occurred at an extremely slow rate in brain and testis, which still contained high concentrations of silver two months after the final exposure.	the exception of testis and spleen (see text). This indicates that silver is probably mainly bioavailable in the ionic form (see text) Nanoparticles are formed in vivo from silver ions and they are probably composed of silver salts.	Mast, J., Peters, R.J.B., Hollman, P.C.H., Hendricksen, P.J.M., Marvin, H.J.P., Peijenberg, A.A.C.M., and Bouwmeester, H. (2012): Distribution, Elimination, and Toxicity of Silver Nanoparticles and Silver Ions in Rats after 28-day Oral Exposure. ACS Nano. 28;6(8):7427-42 Study summary in Annex I
Oral Summary of published data available in the open literature. Articles referred to as original sources of information: Shavlovski et al. (1995) Linder (1991) Linder (2002) and ATSDR (1990) citing the following published research:		Silver nitrate	10-20% absorption of “silver” in mammals Silver is excreted in the bile by a first-pass route and to a large extent as a glutathione conjugate	Reliability 3	IIIA 6.2(01) Leeming, N.M. (2007)
Oral Furchner, J.E, Richmond, C.R. and Drake, G.A. (1968) Evaluated in IIIA 06 Silver Addendum 1 Reliability 2-3	mouse/rat/monkey/ dog	Silver nitrate, dose unknown single exposure	Mouse and monkey: biexponential excretion profile with biological half-lives of 0.1 and 1.6 days in mouse and 0.3 and 3 days in monkey. 100 and 94% of oral dose cleared at two days in mouse and monkey respectively. Rat and dog: triexponential excretion	Reliability 2-3	Furchner et al. 1968; *This study is evaluated in a non-confidential addendum to section 6*

			profile with biological half-lives of 0.1, 0.7, and 5.9 days in rat and 0.1, 7.6, and 33.8 days in dog 98 and 90% of oral dose cleared at two days in rat and dog respectively.		
Intravenous Furchner, J.E, Richmond, C.R. and Drake, G.A. (1968) Evaluated in IIIA 06 Silver Addendum 1 Reliability 2-3	mouse/rat /monkey/dog	Silver nitrate	Triexponential excretion profile Slower clearance rate compared with clearance after oral administration. Increased difference between species (from 15 in dog to 82% in mouse at 2 days)		
Intraperitoneal Furchner, J.E, Richmond, C.R. and Drake, G.A. (1968) Evaluated in IIIA 06 Silver Addendum 1 Reliability 2-3	Mouse/rat /monkey/dog	Silver nitrate	Retention in all tissues resembles whole-body retention except for brain and spleen that seem to retain silver longer.		
Intramuscular Scott, K.G. and Hamilton, J.G. Reliability 2	Rat	Silver nitrate 0.4, 4.0 mg/kg/day	Biliary excretion involved Low dose: ~89% of radioactivity absorbed from the low dose excreted via feces, ~2.2% retention in liver and 4.2% in GI tract. Highest concentrations in % per organ: GI tract followed by liver, blood, kidney, skin, muscle, bone, heart and lungs and spleen. in % per gram: kidney, followed by liver, GI tract, spleen blood, heart and lungs, bone, skin and muscle.	Reliability 3	Scott and Hamilton 1950 *This study is evaluated in a non-confidential addendum to section 6*

			High dose: ~37% of radioactivity absorbed from the high dose excreted via feces, ~34% retention in liver and 8% in GI tract. Highest concentrations in % per organ: liver followed by GI tract, skin, blood, spleen, muscle, bone, kidney, heart and lungs. in % per gram: liver followed by spleen, GI tract, kidney, heart and lungs, skin, blood, bone and muscle.		
Intravenous Scott, K.G. and Hamilton, J.G. Reliability 2	Rat	Silver nitrate 0.4, 4.0 mg/kg/day	~93% of radioactivity absorbed excreted via feces after 4 days. Highest concentrations in % per organ: large intestine followed by blood, muscle ,skin, liver, bone, small intestine, kidney, testes, brain, adrenals, spleen, heart, pancreas, stomach, fat, lungs, eye. in % per gram: adrenals followed by, pancreas, large intestine, kidney, fat spleen, heart, brain, blood, liver, lungs, small intestine,eyes , testes, stomach, skin, bone, muscle.		
Dermal Published research	guinea pig/human		Refers to the ATSDR report (1990) citing Snyder et al., 1975 and Wahlberg et al., 1965	Reliability 3-4	IIIA 6.2(02) Summary by Plautz, J. and Trendelenburg, C.F. (2005)
Oral/iv Published report			The toxicokinetic discussion in the document mainly refers to the results of Furchner et al (see IIIA 6.2-01)	Reliability 3	IIIA 6.2(03) US EPA (1998) Integrated Risk

					Information System.
Oral Handbook on the Toxicology of Metals.			This document is one of the references included in 6.2(01). Some of the results discussed are therefore already included in this table. Further articles referred to:	Reliability 3	IIIA 6.2(04) Fowler, B.A. and Nordberg, G.F. (1986)
Intraperitoneal	Rat	Silver nitrate	Clearance: Half-lives: 40 hours for clearance from blood, plasma, kidneys and liver. Circa 70 hours for the spleen and 84 hours for the brain.	Original publication not evaluated	Matuk (1983)
Inhalation	Rabbit	Monodispersed silver-coated Teflon particles (MMAD: 4 µm)	30% of deposited silver particles cleared from the lungs within a day and a further 30% in the following week.	Original publication not evaluated	Camner et al (1974)
Inhalation	Dog	Silver-110m (30 µCi of silver-110m per mg silver)	Biological clearance half-lives in lungs: 1.7, 8.4 and 40 (accounting for 59, 39 and 2% of administered dose). Biological clearance half-lives in liver: 9 and 40 days (accounting for 97, and 3% of administered dose).	Reliability 2-3	Phalen and Morrow (1973) *This study is evaluated in a addendum to section 6*
Inhalation	Human	Silver-110m (no further information available)	Inhaled silver is distributed to the liver. Biological half-lives of 1 and 52 days are assumed to represent rapid lung clearance by ciliary action and liver clearance respectively.	Reliability 3-4	Newton and Holmes (1966) *This study is evaluated in a non-confidential addendum to section 6*
Oral	Human (single case)	Silver acetate	18% absorption	Original publication not evaluated	East et al. (1980
Subcutaneous	Rat Sprague-Dawley 4 males	Silver zinc zeolite in 1% carboxymethyl cellulose	Peak tissue levels observed 24 hours ≤ 1% and 56.8% excretion via urine and faeces at 7 days Half-life in blood: 61.6 ± 9.4 hours.	Reliability 2-3	IIIA 6.2(05) (1992)

			2.4% maximum dermal absorption		
Percutaneous		Silver zinc zeolite (10%) cream	Damaged skin: 0.24 and 5.38% excretion in urine and faeces at 7 days. Half-life in blood: 49.5 ± 3.5 hours Normal skin: blood levels too low for analysis 0.12 and 1.1% excretion in urine and faeces at 7 days.		
Oral	Chicken Published research	1 ppm CuSO4x5H2O, 0, 10, 25, 50, 100, 200 ppm Ag2SO4	No specific information on ADME. Results indicate that silver may function as a copper antagonist. Silver was considered to be antagonistic to copper as all the symptoms of copper deficiency were apparently accentuated in the presence of silver and the absence of copper. However when copper was present, silver had no effect. *"The proposed method of antagonism was conversion of the univalent cation of silver as the sulphate salt to a divalent ionic species by redox systems in the intestinal tract. Copper exists in functional cytochrome oxidase as both Cu+ and Cu++ and therefore metal ions that can attain either 4 coordinationtetrahedral or 4 coordination-square planar configurations could function as copper antagonists. Silver as Ag++ would be expected to antagonise Cu ++ since it has the potential to attain the copper complex configuration."*	Reliability 3	IIIA 6.2(06) Hill, C.H., Starcher, B. and Matrone, G. (1964)

In vitro	Rat hepatocytes Published research	Silver nitrate silver lactate (10-70 µM final concentration of Ag+)	No specific information on ADME. Results show a decrease in intracellular thiols and lipid peroxidation, in treated hepatocytes. It is postulated that this may lead to the depletion of the intracellular GSH pool and thus be involved in silver cytotoxicity.	Reliability 3	IIIA 6.2(07) Baldi, C., Minoia, C., Di Nucci, A., Capodaglio, E. ad Manzo, L. (1988)
	Published report from ATSDR		This document serves as one of the main references to the summary in 6.2(01). The articles referred to in this document are already included in this table.		IIIA 6.2(08) Agency for Toxic Substances and Disease Registry (ATSDR). (1990)
	Published report prepared for the Oak Ridge Reservation Environmental Restoration Program		This document is partly based on the ATSDR report. The results discussed are thus already included in this table. Further articles referred to:	Reliability 3	IIIA 6.2(09) Faust, R. (1992)
Intratracheal instillation	Dog	Metallic silver silver-110m MMAD: 0.42 to 0.54 µm (GSD 1.5). Each anaesthetised dog inhaled 10-20L of aerosol tagged with silver-110m via tracheal intubation during a 7-15 minute exposure period	96.9 % deposited in lungs, 2.4% in liver and 0.35% in blood after six hours with remaining silver detected in gall bladder and bile, intestines and stomach. The distribution in tissue type (if not considering silver in the lung) remained similar after 225 days with most silver found in liver (77%).	Original publication not evaluated	*Phalen and Morrow (1976)*
Oral	Rat	Silver nitrate and silver chloride	Wide distribution with high concentrations found in the reticuloendothelial tissues.		Olcott (1948) *This study is evaluated in a non-confidential addendum to section 6*
In vitro skin absorption	Human (full thickness female abdominal skin)	1% JMAC Cream R10 containing the reaction mass of titanium dioxide	Dermal absorption is <0.31% Dermal absorption of this formulation is not	Reliability 2	IIIA 6.2(10)

		and silver chloride	considered relevant for the risk assessment of the silver containing active substance.		Walters, K.A. and James, V.J. (1994)
Intraperitoneal Percutaneous	Guinea Pig Published research	Silver nitrate, 0.239M (along with 7 other metal compounds)	Dermal absorption was not investigated in the study. The absorption rate reported (< 1% per five hour period) was determined in a previous in vivo study.	Reliability 4	IIIA 6.2(11) Wahlberg, J.E. (1965)
Percutaneous	Guinea Pig Published research	Silver nitrate, (along with 5 other metal compounds) 0.00048, 0.005, 0.08, 0.118, 0.239, 0.398, 0.753, 4.87M	Dermal absorption less than 4% based on the disappearance of radioactive compound from the cutaneous surface of the living guinea pig	Reliability 3-4	Skog, E, Wahlberg, J.E. (1963) This study is evaluated in a non-confidential addendum to section 6
Comparative Toxicokinetic Study in Rats by Single and Repeat Administration	CD Sprague Dawley rats were 4/sex single intravenous dose: 20 mg/kg single oral dose: 36, 1000 mg/kg repeated daily oral doses for 4 weeks: 36, 180, 1000 mg/kg	**Micron-sized Silver (referenced as AgMP** throughout the study report) Alternative names: AgMP; Bulk Silver; Bulk Ag; Micron-sized silver particulate; Silver powder and silver flake Composition: Uncoated silver particulate (crystalline Ag powder of highly uniform spheroidal shape) Particulate size ~ 0.4 µm Purity: > 99%	The findings in this study have demonstrated that after single oral doses of the test items, the systemic exposure to silver can be ranked in the following order $AgNO_3$ > AgAc > AgNP > AgMP.		Anonymous et al. (2021) Silver Acetate, Silver Nitrate, Micron-sized Silver and Nanoparticulate Silver: A Comparative Toxicokinetic Study in Rats by Single and Repeat Administration
	single intravenous dose: 2.5 mg/kg single oral dose: 36, 360 mg/kg repeated daily oral doses for 4	**Nanoparticulate Silver** (referenced as AgNP throughout the study report) Alternative names: AgNP, nanoAg, nanosilver			

	weeks:3.6, 36, 360 mg/kg	Composition: Surfactant stabilized aqueous dispersion of nanoparticles Particle size: 15 nm (mean) Particulate size range: PSD: d99 20 nm Purity: Specification is for an elemental silver concentration of 10.0 ± 0.50% wt (100 mg/mL).			
	single intravenous dose: 1.5 mg/kg single oral dose: 5, 175 mg/kg repeated daily oral doses for 4 weeks: 5, 55, 175 mg/kg	**Silver acetate** **AgAc** Solubility Aqueous (room temperature): 10.2 mg/mL Purity: ≥ 99.5%			
	single intravenous dose: 1.5 mg/kg single oral dose: 5, 125 mg/kg repeated daily oral doses for 4 weeks: 5, 55, 125 mg/kg	**Silver Nitrate** AgNO3 Solubility Aqueous (room temperature): 2560 mg/mL Purity: 99.97 %			
Comparative Toxicokinetic Study in Rats by Repeat Administration	CD Sprague Dawley rats were 4/sex	**Micron-sized Silver (referenced as AgMP** throughout the study report) Alternative names: AgMP; Bulk Silver; Bulk Ag; Micron-sized silver particulate; Silver powder and silver flake Composition: Uncoated silver particulate (crystalline Ag	The data suggest that following administration of AgMP (range 36 to 1000 mg/kg/day), AgNP (range 3.6 to 360 mg/kg/day) and AgNO3 (range 5 to 125 mg/kg/day) over a 28-day period, the test items were well tolerated. Systemic exposure, as assessed by silver mean Cmax and AUC0-t values, increased with the increase in AgMP dose level from 36 to 1000 mg/kg/day, the increase in AgNP dose level from		Anonymous et al. (2021) Silver Nitrate, Micron-sized Silver and Nanoparticulate Silver: A Comparative Toxicokinetic Study in Rats by Repeat Administration

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<td>powder of highly uniform spheroidal shape)
Particulate size ~ 0.4 µm
Purity: > 99%
repeated daily oral doses for 4 weeks (gavage):
0, 36, 180, 1000 mg/kg</td>
<td rowspan="3">3.6 to 36 mg/kg/day and the increase in AgNO3 dose level from 5 to 125 mg/kg/day. These increases in mean Cmax and AUC0-t were less than dose proportional and this particularly notable for AgMP. Concentrations of silver in tissues generally increased with dose, however, there was no consistent relationship and the dose response was not a simple monotonic characteristic. Systemic exposure and concentrations of silver in tissues were slightly higher in females than in males. After repeated oral doses, measurable concentrations of silver were found in all tissues, with the exception of the liver in males after low doses of AgMP and AgNP and in the uterus in females at the low dose of AgMP. The highest concentrations following doses of AgMP and AgNP were found in the gastro-intestinal tract, accounting for 40-70 % of the total amount of silver. Highest concentrations following oral doses of AgNO3 were generally found in the spleen. Lowest concentrations were generally found in the brain, liver and uterus. The findings in this study have demonstrated that after repeated oral doses of the test items the systemic exposure to silver can be ranked in the following order AgNO3 > AgNP > AgMP. There was no treatment-related effect on the concentrations of copper in serum following repeated oral doses of AgMP,</td>
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<td>CD Sprague Dawley rats were 4/sex</td>
<td>Nanoparticulate Silver (referenced as AgNP throughout the study report)
Alternative names: AgNP, nanoAg, nanosilver
Composition: Surfactant stabilized aqueous dispersion of nanoparticles
Particle size: 15 nm (mean)
Particulate size range: PSD: d99 20 nm
Purity: Specification is for an elemental silver concentration of 10.0 ± 0.50% wt (100 mg/mL).
repeated daily oral doses for 4 weeks (gavage):
0, 3.6, 36, 360 mg/kg</td>
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<td>CD Sprague Dawley rats were 4/sex</td>
<td>Silver nitrate repeated daily oral doses for 4 weeks (gavage):
0, 5, 55, 125 mg/kg</td>
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			however, after repeated oral doses of AgNO3, the serum concentrations of copper decreased with increasing dose.		

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The data available in open literature and considered relevant for silver nitrate include summary reports prepared by the consultant company engaged by the Silver Task Force (applicant under BPR), by the United States Environmental Protection Agency (US EPA), the Agency for Toxic Substances and Disease Registry (ATSDR) and the Oak Ridge Reservation Environmental Restoration Program. In addition, Doc IIIA of the BPR assessment report also includes a textbook chapter on silver toxicity, an in vitro mechanistic study and two studies on percutaneous absorption of a cream containing the reaction mass of titaniumdioxide and silver chloride and a cream containing silver zinc zeolite, respectively. Despite a number of summaries, the amount of information in these documents is limited as some of the documents (e.g. 6.2(01) and 6.2(09)) are principally based on the summary report prepared by the ATSDR (6.2(08)). These reviews summarise case reports and published research performed with silver nitrate/lactate or metallic silver. This information is rather old and the majority of studies are poorly reported but the most robust data for silver nitrate indicate an oral absorption of 5% in mammals (see below). Recently new bioavailability data for silver and soluble silver salts became available in a comparative study investigating the toxicokinetics of silver acetate, silver nitrate or silver in nano- or micron-form following single or repeated exposure. The toxicokinetic study was performed in Sprague-Dawley rats and in accordance with OECD TG 417 and the principles of GLP. The results indicate that silver is bioavailable from all substances tested but to highest extent from silver salts, followed by silver nanoparticles and to least extent from micron-sized silver (≈ 300nm). The bioavailability of silver from silver acetate and silver nitrate was similar.

Oral absorption/Excretion: according to published summaries, the general understanding is that only a small amount of silver (<10 %) is absorbed by mammals following oral administration. This figure is based mainly on data from a study by Furchner et al. which is summarised in an addendum to Doc IIIA, section 6. The study investigates the excretion of silver in mice, rats, dogs and monkeys following oral or intravenous administration of silver nitrate. The research by Furchner et al. shows a biexponentional excretion profile in mice and monkeys upon oral administration whereas a triexponential excretion profile is observed in dogs and rats. Since only dogs were assayed for a sufficiently long period, it was assumed that the long component would have been detected if excretion had been assayed longer also in the other species. The two-day clearance via urine and faeces ranged between 90 % and 99 % in the different species following oral administration and between 15 and 82 % following an intravenous dose. Only a minor fraction was excreted in urine. The interspecies difference in clearance rate was explained to as the differences in time taken for passage through the gut.

This study was not performed according to any guideline or GLP and there is no detailed information on the test substance (with respect to purity and other physical data), test animals (housing and feeding conditions) and residues in bile, tissues and carcass were not measured. However, the strength of the study is that results are based on a large data set including four different species and between 4 and 28 animals in each experiment. Based on the cumulative Day 2 excretion data in the four species, the oral absorption of silver ions is estimated at 5 %. This figure is expected to be conservative since the excretion data may include residues that were absorbed and then excreted in bile. Published data on nanosilver show accelerated dissolution in gastric acid and uptake of soluble silver through ion and nutrient uptake channels and transport in blood probably as thiol complexes[10]This information has until now served as the key data for the toxicokinetic assessments of different silver assessments made under EU regulations.

[10] Liu et al, ACS Nano, 2012, 6 (11)

However, more robust and reliable information is now available from the recent bioavailability study investigating silver levels following single or repeated exposure to silver nitrate, silver acetate or massive silver in nano- or micron-form. According to the study report*"an analytical cross-contamination event called into question the integrity of data for two of the investigated tissues (GI tract and liver) relating to the AgMP, AgNP and AgNO3 test items. All other tissues obtained were not affected by this technical issue. However, to assure the absolute integrity of the overall TK program, a decision was made to repeat all repeat dose phase work (i.e., Ag in tissue and Ag in blood) for these test items."* Therefore, a second study was performed and it was claimed that findings obtained for the different forms of silver (AgNP and AgMP) and soluble silver salts across the two study segments could be compared as both segments were performed according to an exactly equivalent experimental design, under the same conditions and at the same contract laboratories (for both the in-life and bioanalytics phases). Blood samples were taken at pre-dose and 6 hours post-dose on Day 15 and up to 96 hours post-dose on Day 28 of daily repeat oral doses. Selected tissues (bone marrow, brain, gastro-intestinal tract, liver, spleen, ovaries, testis and uterus) were collected at the end of the 4-week repeated dose phases and, at termination, the serum total copper concentration was analysed in animals administered AgMP and AgNO3. Investigations of clinical condition, detailed physical examinations, bodyweight, organ weight, and macropathology were made during the study. Based on the mean Cmax and AUC0-t values, systemic exposure increased with the increase in AgNO3, AgMP and AgNP. These increases in mean Cmax and AUC0-t were less than dose proportional and this was particularly notable for AgMP. The findings in this study have demonstrated that after repeated oral doses of the test items the systemic exposure to silver can be ranked in the followingg order AgNO3 > AgNP > AgMP. The results showed no treatment-related effect on the concentrations of copper in serum following repeated oral doses of AgMP, however, after repeated oral doses of AgNO3, the serum concentrations of copper decreased with increasing dose (this is further discussed in section 10.10).

Toxicokinetic investigations were also included in a recent 90-day study in Wistar Han rats administered silver acetate in diet at doses of 40, 120 and 320 mg/kg body weight/day (see section 10.10.12). The analyses of whole blood samples collected on Day 1 and in Week 13 showed absorption of silver from silver acetate with exposure, expressed as AUC/Dose, increasing less than dose proportionally over the used dose range of 40 to 320 mg/kg/day on Day 1 and Week 13 in both sexes. There were no clear sex differences in exposure observed during the study.

Distribution/excretion: According to information available in the open literature, the silver absorbed from silver nitrate undergoes a first-pass effect in the liver and is excreted into bile after being conjugated to glutathione. The biliary excretion appears to vary between species and the mechanism seems to be saturated at higher doses, at least in the rat (Scott and Hamilton 1950). The silver absorbed from silver nitrate appears to be widely distributed in the rat. Scott and Hamilton (in addendum to the toxicological section of Doc IIIA) observed that the highest amount of silver after an intramuscular dose of silver nitrate was found in the GI tract followed by liver, blood, kidney, skin, muscle, bone, heart, lungs and spleen. Microscopic analyses of tissues from rats orally exposed to silver nitrate and silver chloride in sodium thiosulphate is presented in a publication by Olcott (1948). Silver was regularly found in histiocytes of lymph nodes and liver, in association with the reticulum fibrils of the sinuses of the lymph nodes and the periphery of the malpihian bodies of the spleen and in close approximation to blood vessels (between endothelium and epithelium of thyroid, choroid of the brain and the glomeruli and tubules of the kidney). It was also found near or in fine blood vessels of pancreas, adrenal medulla, pituitary body (in pars nervosa), choroid of the eye and in striated muscle. According to Olcott (1948), a few black granules were observed in the bone marrow but it was not possible to determine whether or not this was silver and the bone marrow of rats exposed to either silver or water appeared the same. Consequently, it is not possible to conclude whether or not the substance is distributed to the bone marrow which is useful information for the interpretation of genotoxicity data in section 10.8.

The recent bioavailability study shows a general increase of the concentrations of silver in tissues with dose, however, there was no consistent relationship, and the dose response was not a simple monotonic characteristic. After repeated oral doses, measurable concentrations of silver were found in all tissues, except for the liver in males after low doses of AgMP and AgNP and in the uterus in females at the low dose of AgMP. Following oral doses of AgNO3, the highest concentrations were generally found in the spleen and bone marrow whereas the lowest concentrations were generally found in the brain and testis. The highest

concentrations following doses of AgMP and AgNP were found in the gastro-intestinal tract, accounting for 40-70 % of the total amount of silver. However, it should be noted that for each organ analysed there was a large variation in tissue concentrations between the four individual animals in each group. Systemic exposure and concentrations of silver in tissues were slightly higher in females than in males but generally less than two-fold. For all forms of silver at mid and high doses, a major part of the administered silver was detected in the ovaries of female rats.

Accumulation: Silver accumulates in tissues and organs. Visible deposition of silver in human skin is a condition known as argyria and is further discussed in section 10.12. After repeated oral doses of AgAc, there was evidence of accumulation in the systemic exposure and comparison of pre-dose and 6-hour post-dose concentrations on Days 15 and 28 indicate steady state had been reached by Day 15. This effect was most pronounced at a target dose level of 320 mg/kg/day.

Dermal absorption: Silver ions have been detected in body fluids of humans treated for serious burn damage with a topical formulation containing silver nitrate. However, silver can also penetrate intact skin as demonstrated by a case report describing a photochemical worker with silver deposits in the dermis following six month exposure to silver thiosulphate (in ATSDR (1990)). There is no robust information available for silver nitrate but a dermal absorption of 1% is commonly reported in literature and is proposed by the applicant under the BPR. The figure is based on the results from a study estimating the uptake of silver nitrate through intact skin of guinea pigs (E. Skog and J.E Wahlberg (1963)) and is further supported by a different study on intact human skin (Snyder et al. (1975)). The original publication by E. Skog and J.E Wahlberg is presented in an addendum to the toxicological section of Doc IIIA. The study which was performed before guidelines and principles of GLP were established is poorly reported with limited information regarding test substance, methodology and results. Concentrations of 0.00048, 0.005, 0.080, 0.118, 0.239, 0.398, 0.753 and 4.87 M, thus covering the intended use-concentrations of the representative formulations, were applied on an unknown area of the skin on living guinea pigs. The dermal absorption was determined as the amount of radioactivity disappearing from the treated area during five hours. A dermal absorption in the range 3.0-3.9 was observed in one animal but for the majority of animals it was less than 1%. Considering all uncertainties, the dermal absorption in this study is proposed to be set based on the upper-range value (i.e. 4%) in order to cover all animals in the study. Although common practice is to use a default value of 100% in the absence of robust data, it is considered appropriate to refine the default value to 5% based on the results of this study. This figure is expected to be conservative taking into account that it is based on the assumption that all radioactivity that disappeared from the test area entered the systemic circulation through the skin.

The applicant in the biocides review considers a figure of 1% supported by information in a review by Hostynek (2003) stating that the electrophilic nature of metals such as silver leads to a reaction with proteins in the skin inhibiting further diffusion. While this may be correct, the information on dermal absorption in the review is restricted to the sentence “experiments to determine the penetration of human skin by water-soluble silver salts have not given measurable results”. The basis for this statement is unclear and it is also unclear if the silver compound, dose levels or test conditions used are relevant for the risk assessment of silver nitrate and/or silver ion equivalents. The article is thus considered to be of limited use for this assessment. Another publication referred to by the applicant is a study by Nadworny et al. (2010) investigating systemic anti-inflammatory effects of nanocrystalline silver. The analyses made by ToF-SIMS (time-of flight secondary ion mass spectrometry) showed silver species in the epidermis and the upper dermis but the information on dermal absorption of silver nitrate or nanocrystalline silver was not specified further than the term “minimal”. In the absence of an exact figure, the article is considered to be of limited use for this assessment. A third study referred to by the applicant was conducted by the Joint Research Centre and reported on behalf of the Commission (Sabioni et al 1988). In this study, 100 mg of an antiseptic powder (3.7 mg Ag) was applied onto 2 cm2 abraded skin in the necks of male Sprague-Dawley rats. The amount of silver in blood, liver, kidney, testicles, spleen, femur, heart and stomach was analysed for ^{106}Ag content and compared to untreated controls. Tissue levels of silver were low, and the minimum retention factor estimated was 0.01%. The study was not performed according to GLP or any guideline and is briefly reported. It is not clear from the report if results are based on pooled tissues but there is no individual data. The two major deviations from OECD TG 427 (skin

absorption in vivo method) include the amount of product applied per cm^2 and the lack of analysis of excreta. To enable a reliable calculation, OECD TG 427 recommends application of 1-5 mg per cm^2 on an area of at least 10 cm^2 for rats of 200-250 g bodyweight. In this study, the amount applied was 100 mg on an area of 2 cm^2 (50 mg/cm^2) which is an overload far above the recommendations. Moreover, the report states that ^{106}Ag contaminations were found on the walls of the cages. This finding and the fact that rats often lick themselves were interpreted as an indication that tissue residues partly originate from oral ingestion. However, the contamination observed on walls could also indicate that the amount available for dermal absorption was less than intended.

The three articles referred to are considered to support a refinement of the default value for dermal absorption from 100% to 5% but further refinement of this value requires reliable and quantitative information. Therefore, study summaries of the three articles referred to by the applicant have not been requested. In the absence of substance-specific data it is not possible to set an exact figure for dermal absorption but the results from the study by E. Skog and J.E Wahlberg (1963) is considered to support a refinement of the default value of 100% to 5%. This value is supported also by the general conception that oral absorption rarely exceeds dermal absorption.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 25: Summary table of animal studies on acute oral toxicity

Summary table of animal studies on acute oral toxicity						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance Dose levels, Type of administration** *(gavage, in diet, other)*	**Signs of toxicity** *(nature, onset, duration, severity, reversibility)*	**Value LD$_{50}$**	**Remarks** *(e.g. major deviations)*	**Reference**
	Human Mouse Rat Rabbit Guinea Pig	Dose unknown	Ingestion of the caustic silver nitrate results in acute toxicity involving gastroenteritis, diarrhoea, lower blood pressure, decreased respiration, spasms and paralysis first affecting the diaphragm musculature	LD50 /LD100/ MLD (minimum lethal dose) (mg/kg bw)	In the absence of original test data, the results obtained cannot be properly assessed by the DS thus confidence in data is low.	IIIA 6.1.1(02) Venugopal B., Luckey T. D. Extract from chapter in textbook
		Silver (metal colloid)		100 mg/kg bw (LD50 oral, mouse)		
		$KAg(CN)_2$		21 mg/kg bw (oral, rat)		
		Ag_2O		2820 mg/kg bw (MLD, oral, rat)		
		AgNO3		129 (LD50 oral mouse) 140 mg/kg bw (LD100 oral, human)		
		AgCN		123 mg/kg bw (oral, rat)		
		AgF		300 mg/kg bw (MLD oral, guinea pig)		
Other studies						
Non guideline/ GLP.	Albino rat Cpb:WU, Wistar random strain 10/sex	Colloidal silver solution (8g/L) 240 mg/kg bw Oral Single dose 14 day observation period		>240 mg/kg bw ↑black discoloration of faeces, severe diarrhoea	Study of low reliability (3)	IIIB 6.1.1(13) (1980)

Table 26: Summary table of human data on acute oral toxicity

Summary table of human data on acute oral toxicity				
Type of data/ report **Reliability**	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
	$AgNO3$		The lethal oral dose for humans 140 mg/kg	IIIA 6.1.1(02) Venugopal B., Luckey T. D. Extract from chapter in textbook

Table 27: Summary table of other studies relevant for acute oral toxicity

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
See tables 25 and 26				

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

There is no robust data available for silver nitrate. The applicant for the review under Regulation (EU) No 528/2012 (Biocides Products Regulation) refers to the results from a study performed with colloidal silver but taking into account that silver nitrate is a corrosive substance (currently classified Skin Corr. 1B; H314 and herein proposed for classification Skin Corr. 1A; H314), it seems reasonable to expect the acute oral toxicity of silver nitrate to be higher than colloidal silver

There is no robust information available in the open literature allowing for a comparison of the acute oral toxicities of the two compounds. The oral LD_{50} values reported for mice (i.e. 129 and 100 mg/kg bw for silver nitrate and the colloidal form of silver metal, respectively) seem to indicate a similar toxicity (Venugopal, B. and Luckey, T.D. in (6.1.1(02)). Nevertheless, the only oral dose tested in the study with colloidal silver is 240 mg/kg bw which is a dose far below the limit dose for acute toxicity testing. Therefore, this information is not sufficient to allow for a comparison with criteria irrespective of the read across to silver nitrate would be justified or not.

Besides the study with colloidal silver, the only data available to assess this endpoint is information from published research. Since these studies were not performed for regulatory purposes, the information on study design and test conditions is often limited. Moreover, data is fairly old and original publications are difficult to trace.

The following information is available in different reviews of silver:

- The textbook chapter by Venugopal, B. and Luckey, T.D, reports acute symptoms of toxicity following ingestion of silver nitrate including gastroenteritis, diarrhoea, reduced blood pressure, decreased respiration, spasms and paralysis first affecting the diaphragm musculature. The lethal oral dose for humans is stated to be 140 mg/kg bw.
- Several material safety data sheets (MSDS) for silver nitrate available on the internet report oral LD50 values of 1173 and 50 mg/kg bw in rats and mice, respectively. Another value reported is an estimated lethal oral dose of 28.6 mg/kg in humans[11]. Since the reference to the underlying data could not be traced, the reliability of this information cannot be assessed.
- The website for the European chemical Substances Information System (ESIS) refers to an acute oral toxicity value of 50 mg/kg bw in mouse (with reference to Registry of Toxic Effects of

[11] https://fscimage.fishersci.com/msds/20810.htm

Chemical Substance (RTECS) 1995), a value of 473 mg/kg bw in guinea pigs and 1173 mg/kg bw in rat (with reference to Mezhdunarodnaya K (1957) "as quoted in RTECS, 1995").

The registration dossier for silver nitrate submitted under Reach contains acute toxicity data for nanoparticles of silver. Taking into account that silver nitrate has corrosive properties, this data is not considered representative for the acute toxicity of silver nitrate.

Conclusion: There is no robust data to assess the acute oral toxicity of silver nitrate. Despite the lack of data, further testing would be unethical due to the corrosive properties of the substance and would go against the recommendations in the guidance document for BPR "Guidance on information requirements" stating (section 8.7 of version 1.1, November 2014: *"The study/ies do(es) not generally need to be conducted if: The substance is classified as corrosive to the skin."*

It would also go against the recommendations in OECD TG 401: "*Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out"*. Although an exact LD_{50} value cannot be set, the acute oral toxicity can be estimated based on the information available in the open literature. The animal data generally referred to in different reviews indicate oral LD_{50} values between 50 and 1173 mg silver nitrate/kg bw whereas an oral dose of 10g (approximately 140 mg/kg bw in a 70 kg person) is stated to be lethal for humans (WHO, IPCS, ATSDR etc Lansdown, 2010). For humans, a lethal oral dose as low as 2 g in a 70 kg person (approximately 28.6 mg/kg bw) has also been reported in material safety data sheets but the original reference could not be found. Considering the uncertainties in the data base and the corrosive properties of the substance, it is not considered safe to disregard the low dose reported to be lethal in humans and 29 mg/kg bw is thus proposed as an estimate of the acute oral toxicity of silver nitrate.

10.1.2 Comparison with the CLP criteria

The criteria reads *"Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the numeric criteria shown in Table 3.1.1. Acute toxicity values are expressed as (approximate) LD50 (oral, dermal) or LC50 (inhalation) values or as acute toxicity estimates (ATE)."*

The acute oral toxicity categories and acute toxicity estimates (ATE) of each category (mg/kg body-weight):

Category 1: ATE≤ 5

Category 2: 5 < ATE≤50

Category 3: 50 < ATE≤ 300

Category 4: 300 < ATE ≤ 2 000

Based on the information available, animal data would place silver nitrate in either category 2 (mice) or 4 (rat) whereas the human data would place silver nitrate in either category 2 or 3.

Test substance	LD50 (mg/kg bw)	Reference
Silver nitrate (oral, mouse) Silver nitrate (oral, human)	129 140 (LD_{100})	IIIA 6.1.1(02) Venugopal, B. and Luckey, T.D.
Silver nitrate (oral, mouse) Silver nitrate (oral, rat) Silver nitrate (oral, human)	50 1173 28.6 (LD_{100})	Various material safety datasheets

Despite the uncertainties in the data base and the few human cases known it is considered appropriate to place the substance in category 2 taking into account the corrosive properties of the substance and the oral LD_{50} value reported for mice. In accordance with this, silver nitrate should be classified for acute oral toxicity based on the lowest dose reported to be lethal in humans (29 mg/kg bw). This is in line with section 3.1.2.3.1 of the CLP guidance[12] stating *"The minimum dose or concentration or range shown or expected to cause mortality after a single human exposure can be used to derive the human ATE directly, without any adjustments or uncertainty factors."*

10.1.3 Conclusion on classification and labelling for acute oral toxicity

In a weight of evidence approach based on secondary information found in different reviews, silver nitrate is proposed to fulfil criteria for classification in category 2. Although the quality and reliability of the original data cannot be properly assessed, further animal testing for the purpose of establishing a LD_{50} for silver nitrate would be unethical due to the corrosive properties of the substance. Existing data indicate an LD_{50} between 50 and 1173 mg/kg bw in animals and human data indicate a lethal dose of 29 mg/kg bw. Based on table 3.1.2 in Annex I, this corresponds to converted ATEs between 5 and 500 for animal data and 5 for the human data. Although there is no robust data justifying the lethal dose for humans, i.e. 29 mg/kg bw, this value is proposed to serve as ATE taking into account the animal data indicating converted ATEs between 5 and 500 mg/kg bw.

10.2 Acute toxicity - dermal route

Table 28: Summary table of animal studies on acute dermal toxicity

Summary table of animal studies on acute dermal toxicity					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, Vehicle, Dose levels, Surface area,**	**Value LD_{50}**	**Remarks** *(e.g. major deviations)*	**Reference**
Published study	Guinea pigs 20	Percutaneous 2ml of 0.239M solution silver nitrate (approximately 217 mg /kg bw). 3 week observation period	>217 mg/kg bw	↓Bodyweight	IIIA 6.1.2 (05)

Table 29: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 30: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

[12] Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 5.0 July 2017.

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

There is no robust data on acute dermal toxicity available. The information is limited to a published non guideline/non GLP study submitted for the section on toxicokinetics investigating the percutaneous toxicity of silver nitrate (and eight other metal salts) in guinea pigs. The study design deviates from the current OECD TG 402, most importantly by the use of a single dose level and the lack of information on exposure time. Moreover, the study is only briefly described with no information on sex, housing conditions, exposure time, clinical observations (including bodyweights) and pathological examinations. According to the report all 20 guinea pigs administered 2ml of a 0.239 M silver nitrate solution (approximately 217 mg/kg bw) survived treatment but a reduced bodyweight gain was observed in test animals. The magnitude of change was not clear from the report as it was only shown graphically and the quality of the publication from 1965 was poor. The weight gain appears to cease during the first week and does not significantly increase during the remaining weeks. This is a clear indication of toxicity. However, the absence of mortality among the 20 animals is yet a strong indication that the LD_{50} is above 217 mg/kg bw. Since the only dose tested in the study was far below the limit dose in OECD TG 402, the dermal LD_{50} cannot be established. , Nevertheless, testing for acute dermal toxicity up to the limit dose would go against the recommendations in the document "Guidance on information requirements" as well as in OECD TG 402 since silver nitrate is a corrosive substance.

10.2.2 Comparison with the CLP criteria

The criteria read "Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the numeric criteria shown in Table 3.1.1. Acute toxicity values are expressed as (approximate) LD50 (oral, dermal) or LC50 (inhalation) values or as acute toxicity estimates (ATE)".

The acute dermal toxicity categories and acute toxicity estimates (ATE) of each category (mg/kg bw):

Category 1: ATE≤ 50

Category 2: 50 < ATE≤ 200

Category 3: 200 < ATE≤ 1000

Category 4: 1000 < ATE ≤ 2000

The results from the dermal study indicate that the substance causes significant toxicity at doses above 217 mg/kg bw. However, this information is not considered sufficient stand-alone data for classification. Due to the limited amount of information available on the acute toxicity via the oral or inhalation route, extrapolation between routes to compensate for the lack of robust acute dermal toxicity data is not possible. No appropriate human exposure data was identified and considering the corrosive properties of the substance, read across to data from other silver substances is not justified.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

The data available is inconclusive to assess acute dermal toxicity. Consequently, classification is not proposed.

10.3 Acute toxicity - inhalation route

Table 31: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
No data available					

Table 32: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 33: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

There is no robust study investigating the acute inhalation toxicity of silver nitrate. Data is available for different formulations containing silver nitrate but these are not considered representative due to the low content of the active substance and are thus not included here.

Brief information is also found in different published reports (6.1.1(05) and 6.2(08)) describing respiratory effects in humans following acute exposure to an unknown level of silver nitrate dust. The first summary (6.1.1(05)) refers to a study reporting acute irritation of the respiratory tract upon inhalation of silver nitrate dust. Irritation was only observed at doses also causing argyria. The other summary (6.2(08)) refers to a study of workers exposed to silver nitrate or silver oxide. Among workers, upper respiratory irritation was observed in 25 of 30 persons and cough, wheezing or chest tightness in 20 of 30 persons. There is no information on exposure levels and exposure duration but according to the summary, the eight-hour time weighted average concentration analysed four months prior to the study indicated levels from 0.039 to 0.378 mg silver/m^3. From this limited information, it is only possible to conclude that silver nitrate causes respiratory tract irritation and respiratory tract effects above a certain dose level. Furthermore, it can be concluded that a dose of 0.378 mg silver/m^3 (0.378µg/L) is not lethal for humans.

10.3.2 Comparison with the CLP criteria

The criteria read *"Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route according to the numeric criteria shown in Table 3.1.1. Acute toxicity values are expressed as (approximate) LD50 (oral, dermal) or LC50 (inhalation) values or as acute toxicity estimates (ATE)."*

The acute inhalation toxicity categories and acute toxicity estimates (ATE) of each category for dusts and mists (mg/l):

Category 1: ATE≤ 0.05

Category 2: 0.05 < ATE≤0.5

Category 3: 0.5< ATE≤ 1.0

Category 4: 1.0 < ATE ≤ 5.0

It is not possible to set an LC_{50} value for silver nitrate based on the existing data. However, the CLP states*: "In addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity was corrosivity, the substance or mixture shall also be labelled as 'corrosive to the respiratory tract' (see note 1 in 3.1.4.1)."*

This is discussed further in the CLP guidance.

"Corrosive substances and mixtures may be acutely toxic after inhalation to a varying degree, although this is only occasionally proved by testing. In case no acute inhalation study is available for a corrosive substance or mixture, and such substance or mixture may be inhaled, a hazard of respiratory tract corrosion may exist. As a consequence, substances and mixtures have to be supplementary labelled with EUH071, if there is a possibility of exposure via inhalation taking into consideration the saturated vapour concentration and the possibility of exposure to particles or droplets of inhalable size as appropriate (see also chapter 3.8.2.5 of this Guidance. It is strongly recommended to apply the precautionary statement P260: Do not breathe dust/fume/gas/mist/vapours/spray."

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The data on acute inhalation toxicity is limited to case reports of workers exposed to silver oxide or silver nitrate. Since respiratory irritation was observed already at a low dose (assumed to be 0.378 µg/L), the acute toxicity via inhalation can be expected to be high. However, in the absence of any information on effects at doses above an air concentration of 0.378 µg/L, it is not possible to assess acute inhalation toxicity of silver nitrate. Due to its corrosive properties (section 10.4), silver nitrate should be classified EUH071: *'corrosive to the respiratory tract'* in order to protect the user from inhalation exposure.

10.4 Skin corrosion/irritation

Table 34: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
No data available					

Table 35: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 36: Summary table of other studies relevant for skin corrosion/irritation

Summary table of in vitro studies on skin corrosion/irritation					
Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
in vitro Skin Corrosion using EpiDerm™ OECD TG 431 GLP Reliability 2	Silver nitrate 1%, 2.5%, 5%, 10% and 25% w/v in vehicle	60 minute exposure	Skin viability: 1%: 90% 2.5%: 93% 5%: 79% 10%: 66% 25%: 7% corrosive at a concentration of 25% w/v (UN GHS classification system)	Subcategorisation of classification into 1A, B or C is not possible since viability was not measured after 3 minutes.	IIIA 6.1.4-21
in vitro Skin Corrosion (Human Skin Model Test) Claimed to be performed according to OECD TG 431 and in compliance with the principles of GLP	Silver nitrate 25 mg of the test material applied to the tissues and wetted with 25 µL deionised water	Duration of treatment / exposure 3 minutes/ 1 hour	The relative absorbance values were decreased to 8.4% after 3 minutes. After the 1-hour exposure relative absorbance values were reduced to 25.1%. This fact distorted the measurement of the absorbance values. In addition, the cells of the 1-hour treatment tissues were not able to reduce the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) at all (no blue coloration at all) indicating complete death of the cells due to exposure to the test item.	The test item precipitated and could not be washed of the tissues completely after the 1 hour exposure. The poorly soluble precipitate was probably silver chloride, formed during the washing step with PBS.	REACH registration dossier (The original study report not available to the DS)

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Silver nitrate is presently classified for corrosive properties; Skin Corr. 1B, H314 (causes severe skin burns and eye damage) according to CLP but no specific concentration limits (SCL) have been set. Since the data upon which the current harmonised classification is based could not be traced, the threshold concentrations for skin irritation and skin corrosion are unknown and hence the basis for the

subcategorization in 1B is unclear. Following an agreement made during an early working group for biocides in 2015, the applicant conducted a study investigating the skin irritation potential of silver nitrate, mainly to find appropriate doses for a subsequent 28-day oral study in rat. The skin viability was assessed after 60-minute exposure to silver nitrate at concentrations of 1%, 2.5%, 5%, 10%, and 25% w/v using the in vitro skin model EpiDerm™. The viability was below 50% at the top dose (i.e., 7%) indicating corrosive properties of the substance. However, since skin viability was not measured after 3 minutes, as recommended in OECD TG 431, the results cannot be used for categorization as corrosive and thus not for subcategorization according to table 5 of this guideline.

The corrosive properties of silver nitrate were confirmed in an in vitro Skin Corrosion test (Human Skin Model Test) described in the REACH registration dossier which is available via the ECHA dissemination site[13]. The study was conducted in 2009 and in accordance with OECD TG 431 and the principles of GLP (original study report not accessible). The type of reconstructed human epidermis (RhE) model used was EST-1000 (now named epiCS®). The registration dossier states that the relative absorbance value was decreased to 8.4% after 3 minutes, a value well below the threshold for corrosivity (<50% at the 3 minutes treatment). At the 60 minutes reading, the relative absorbance values were reduced to 25.1%. Although this value is above the threshold of 15% for the 1-hour exposure, the viability at the 3-minute reading means that criteria for classification as corrosive to skin are met. Moreover, precipitation of the test substance was considered to have distorted the measurement of the absorbance values at the 1-hour exposure. Additionally, the cells of the 1-hour treatment tissues were not able to reduce the MTT at all (no blue coloration at all) indicating complete death of the cells due to exposure to silver nitrate.

10.4.2 Comparison with the CLP criteria

The CLP states *"On the basis of the results of animal testing a substance is classified as corrosive, as shown in Table 3.2.1. A corrosive substance is a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4-hour duration. Corrosive reactions are typified by ulcers, bleeding, bloody scabs and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia and scars. Histopathology shall be considered to discern questionable lesions."*

"Three subcategories are provided within the corrosive category: subcategory 1A —where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B — where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C — where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days."

According to OECD TG 431, a reduction of viability to 50 and 25% after 3 min exposure fulfils Step 1 and Step 2 of the prediction model for EpiDerm™, respectively and thereby criteria for classification in subcategory 1A.

The results from the study presented in the registration dossier clearly indicate that silver nitrate meet the criteria for classification (i.e., the relative absorbance value was decreased to 8.4% after 3 minutes).

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Silver nitrate is considered to fulfil criteria for classification as Skin corr. 1A.

[13] https://echa.europa.eu/registration-dossier/-/registered-dossier/14995/7/4/2

10.5 Serious eye damage/eye irritation

Table 37: Summary table of animal studies on serious eye damage/eye irritation

Summary table of animal studies on serious eye damage and eye irritation					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance** **Dose levels, Duration of exposure**	**Results** *Average score (24, 48, 72 h), observations and time point of onset, reversibility*	**Remarks** *(e.g. major deviations)*	**Reference**
Instillation	Young albino rabbits from different sources (1.5-3 kg) 1/5-1 drop Controls: Distilled water Sodium chloride 1%: 6 1.5%: 3 2%: 10 4%: 4 6%: 5 12%: 4	Silver nitrate	1% Hyperemia (2-4), swelling (1-2), Exudation (1-2), coagulation (0-1), scar tissue (none) corneal injury (clear to hazy), duration (2-4 days) 1.5% Hyperemia (2-4), swelling (1-3), Exudation (2-3), coagulation (none), scar tissue (none), corneal injury (clear), duration (2-3 days) 2% Hyperemia (2-4), swelling (1-4), Exudation (1-3), coagulation (0-1), scar tissue (none), corneal injury (clear to cloudy), duration (2-9 days). Eye of one animal healed slowly and the majority in five days. 4% Hyperemia (2-4), swelling (2-3), Exudation (1-2), coagulation (0-1), scar tissue (none), corneal injury (clear), duration (4-6 days) 6% Hyperemia (3-4), swelling (3), Exudation (2-3), coagulation (1-2), scar tissue (none), corneal injury (clear to cloudy), duration (4-6 days) 12% Hyperemia (3-4), swelling (4), Exudation (3-4), coagulation (2-4), scar tissue (2-3), corneal injury (hazy to opaque), duration (7-9days). Blindness (1), silver deposits (1)	**Evaluation criteria:** 0= no observed changes 1= mild changes or a small amount 2= moderate changes or moderate amount 3= severe changes or severe amount 4= intense change or enormous amount	Calvery, H. O., et al , Ph.D.; Arch Ophthalmo. 1941;25(5):839-847.

Table 38: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 39: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

There is no robust data on the eye damage/eye irritation potential of silver nitrate in the dossier and the underlying data for the current harmonised classification (Skin Corr. 1B, H314 (causes severe skin burns and eye damage) has not been found. However, severe effects on the rabbit eye are described in a published study (Calvery et al (1941)). The study investigates effects of silver nitrate (as well as silver ammonium nitrate and/or silver ammonium sulphate) on the rabbit eye. One drop of the substance (in concentrations of 1-12%) was instilled in the lower cul-de-sac of the left eye and the right eye served as control. Two to five minutes after instillation, the eye was rinsed with 1% boric acid. The eyes were examined every 24 hours except for higher concentrations that were examined twice daily. All concentrations caused irritation of the rabbit eye and resulted in black deposits on the eyeball. The author states that in most cases it was mainly the protein of the conjunctival membrane that was injured but when higher concentrations were used, the conjunctival membrane was entirely removed. Often heavy scar tissue was formed on the eyelids and eyeballs.

10.5.2 Comparison with the CLP criteria

According to section 3.3.2.3 of the CLP, skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1). (

For completeness, the information from the rabbit study is yet compared with criteria below:

The criteria for classification in category 1 reads:

"If, when applied to the eye of an animal, a substance produces:– at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or – at least in 2 of 3 tested animals, a positive response of:– corneal opacity ≥ 3 and/or– iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material."

In the study by Calvery et al, severe effects in the eye, including blindness in one animal, were observed in animals administered 12% silver nitrate. The substance thus fulfils the criteria for classification in category 1 for irreversible effects on the eye. However, if the substance is classified for skin corrosion, the hazard statement H318 'Causes serious eye damage' becomes redundant:

CLP guidance*: "A skin corrosive substance is also classified for serious eye damage which is indicated in the hazard statement for skin corrosion (H 314: Causes severe skin burns and eye damage). However, although classification for both endpoints (Skin Corr. 1 and Eye Dam. 1) is required and has to be addressed in the safety data sheet, the hazard statement H318 'Causes serious eye damage' is not indicated on the label because of redundancy (CLP Article 27)."*

The current harmonised classification does not include specific concentration limits (SCL). Article 10 of the CLP reads:

”Specific concentration limits shall be set by the manufacturer, importer or downstream user where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I.”

This is elaborated further in the CLP guidance: *“ However, if there are adequate, reliable, relevant and conclusive existing data from other already performed animal studies with a sufficient number of animals tested to ensure a high degree of certainty, and with information of dose-response relationships, such data may be considered for setting a lower or, in exceptional cases, a higher SCL on a case-by-case basis.”*

In the study by Calvery et al. a range of doses were tested, and results indicate an eye irritation potential of silver nitrate also at a concentration of 1%. This could be considered to indicate the need for setting of a SCL as the current GCLs for substances classified Eye Dam. 1 trigger classification in category 1 if the substance is present in a mixture at or above 3% and in category 2 if present at or above 1 % and below 3%, levels that may thus not be sufficiently protective. The effects among the six rabbits treated with 1% were categorised as “mild to moderate (1-2)” with “no visible change” or “no visible change to hazy” effects on the cornea and were reversed within 2-4 days. However, without access to primary information and individual results for different time points (i.e. 24, 48 and 72 hours), it is difficult to assess the severity of these effects and the reliability of the results. Therefore, the information from this study is not considered to represent *“adequate and reliable scientific information showing that the hazard of a substance is evident when the substance is present at a level below the concentrations set”* and thus to justify setting of specific concentration limits.

In the absence of SCLs, the generic concentration limits (GCL) apply.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Silver nitrate is presently classified for corrosive properties (Skin Corr. 1B, H314 (causes severe skin burns and eye damage) according to CLP but is proposed to be changed to Skin Corr. 1A. Skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1). In addition, the study by Calvery et al (1941) supports classification of silver nitrate in Eye Dam. 1. No specific concentration limits can be proposed based on the data available.

10.6 Respiratory sensitisation

Table 40: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No data available					

Table 41: Summary table of human data on respiratory sensitisation

Summary table of human data on respiratory sensitisation				
Type of data/report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Case report described in textbook.	Colloidal silver "Argyrol"	Young lady treated twice weekly with nasal drops to treat chronic purulent otitis media	Swelling of face, generalised urticarial, near collapse state. Cleared after treatment. (Howard, R.C., 1930)	A. B. G. Lansdown (2010)
Case report described in textbook.	Colloidal silver "Argyrol"	26-year old man treated with nasal spray for pharangytis and hay fever	Asthma and increased discomfort. Regressed after treatment. (Criep, L. H, 1943)	A. B. G. Lansdown (2010)

Table 42: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There are no studies available on respiratory sensitisation. However, a compilation of allergic reactions in humans is included in a textbook entitled "Silver in healthcare" (A. B. G. Lansdown (2010)). Among these, two cases of allergic reactions to colloidal silver involves the respiratory system; an allergic reaction to nasal drops and a case of an allergic reaction to a nasal spray. Following exposure to the nasal drops, one patient developed swelling of the face, generalised urticaria and a state of near collapse whereas the patient exposed to the nasal spray developed severe asthma. The reactions regressed in both patients when treatment was withdrawn. According to the author of the article in which the latter case was originally described (Criep, L. H., 1943) the patient exhibited a dermal reaction to the formulation with colloidal silver when tested but the patient showed no dermal reaction to silver nitrate. Based on these observations, the author concluded that the patient was either allergic to the protein vehicle, the formulation with colloidal silver or both.

10.6.2 Comparison with the CLP criteria

The criteria reads: *"Substances shall be classified as respiratory sensitisers (Category 1) in accordance with the following criteria:*

(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or

(b) if there are positive results from an appropriate animal test.

The CLP further states *"Evidence that a substance can induce specific respiratory hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated."*

The symptoms described in the two patients referred to above indicate that the substance induces respiratory sensitisation. Since a colloidal silver solution contains silver particles and silver ions, silver nitrate could be expected to have similar properties. However, the data is considered weak evidence

of respiratory sensitisation since at least for one of the cases described, the reaction seemed related to other components of the solution than the silver ion.

Additionally, the CLP states:

"When considering the human evidence, it is necessary for a decision on classification to take into account, in addition to the evidence from the cases:

(a) the size of the population exposed;

(b) the extent of exposure. " (section 3.4.2.1.1.2)

In this case, the human evidence consists of a single case with little information on health status thus data does not meet the recommendations of the CLP (section 3.4.2.1.1.4):

"Clinical history shall include both medical and occupational history to determine a relationship between exposure to a specific substance and development of respiratory hypersensitivity. Relevant information includes aggravating factors both in the home and workplace, the onset and progress of the disease, family history and medical history of the patient in question. The medical history shall also include a note of other allergic or airway disorders from childhood, and smoking history."

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Two cases of allergic reactions to colloidal silver involving the respiratory system have been described. For at least one of the cases, the reaction seemed associated with the protein vehicle or components of the formulation rather than the silver ion as no reaction was observed when the patient at a later stage was dermally exposed to silver nitrate. The data available is thus insufficient for the assessment of respiratory sensitisation and no classification is proposed due to inconclusive data.

10.7 Skin sensitisation

Table 43: Summary table of animal studies on skin sensitisation

Summary table of animal studies on skin sensitisation					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, Vehicle,** **Dose levels, Route of exposure** *(topical/intradermal, if relevant)*, **Duration of exposure**	**Results** *(EC3-value or amount of sensitised animals at induction dose)*	**Remarks** *(e.g. major deviations)*	**Reference**
Study described in textbook Selective induction of ant-nucleolar antifibriallarin antibodies after 5 weeks (Hultman, P., et al 1994) Non guideline, non GLP	Mouse	0.05% silver nitrate			A. B. G. Lansdown (2010)

Table 44: Summary table of human data on skin sensitisation

Summary table of human data on skin sensitisation				
Type of data/report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Case report described in textbook.	"10% silver nitrate"		Vesicular reaction. (Gaul, L.E.and Underwood G. B, 1948)	A. B. G. Lansdown (2010)
Case report described in textbook.	"silver nitrate"	Test substance applied to an area of eczema on heel	Increased pain and dermatitis. (Gaul, L.E.and Underwood G. B, 1948)	A. B. G. Lansdown (2010)
Case report described in textbook.	0.5% silver nitrate	30-year old man exposed to metals and precious stones for many years	Positive allergic reaction in test stated to be consistent with European standards. (Agarwal, S, and Gawkrodger D, J, 2002)	A. B. G. Lansdown (2010)
Case report described in textbook.	silver (no further details)	Survey of 93 workers	No reactions	A. B. G. Lansdown (2010)
Case report described in textbook.	silver dye with up to 5% silver nitrate	Used in cosmetic salons for colouring eyebrows and eyelashes.	Skin reactions when challenged at 1% silver nitrate. (Fisher, A. A, 1987)	A. B. G. Lansdown (2010)
Case report described in textbook.	silver nitrate	Female radiographer exposed to silver chloride	Eczema underneath watch. Positive skin reactions in patch test when challenged at 1% silver nitrate and the fixing fluid. (Fisher, A. A, 1987)	A. B. G. Lansdown (2010)
Case report described in textbook. (Howard, R.C., 1930)	Colloidal silver "Argyrol	Human Young lady treated twice weekly with nasal drops to treat chronic purulent otitis media	Swelling of face, generalised urticarial, near collapse state. Cleared after treatment.	A. B. G. Lansdown (2010)
Case report described in textbook.	Colloidal silver "Argyrol"	Human 26-year old man treated with nasal spray for pharangytis and hay fever	Asthma and increased discomfort. Regressed after treatment. (Criep, L. H, 1943)	A. B. G. Lansdown (2010)

Table 45: Summary table of other studies relevant for skin sensitisation

Summary table of animal studies on skin sensitisation					
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/group**	**Test substance, Vehicle,** **Dose levels, Route of exposure** *(topical/intradermal, if relevant)*, **Duration of exposure**	**Results** *(EC3-value or amount of sensitised animals at induction dose)*	**Remarks** *(e.g. major deviations)*	**Reference**

OECD TG 406 (1992) No information on GLP Reliability 2	Guinea Pig (20 males 10 control males according to supplementary material for the the publication)	Ag-NP dispersed in 1% citric acid, Average particle size: 10.0 nm. **Induction:** Day 1:, three pairs of 0.1 mL injections into the shoulder region on each side 1st inj: 1:1 mixture of Freund's Complete Adjuvant (FCA) and physiological saline. 2nd inj: test substance 3rd inj: a mixture of FCA/saline and the test substance concentration Day 5: test site painted with sodium dodecyl sulphate in Vaseline 0.5 mL of test substance was applied to the shaved test site. Day 6: topical appl of test substance (102.4 mg). Occlusive dressing for 48 hrs. **Challenge:** Day 21 after inj: topical appl of test substance. Occlusive dressing for 48 hrs.	48 and 72 h post-challenge: discrete and patchy erythema in 1/20 animals No visible changes were observed in the negative controls.	No information if any 'reliability check' (the sensitivity and reliability of the experimental technique) was performed. The information available in the published study is not as detailed as that would be in a GLP study report for e.g., individual animal data, and detailed pathological findings, if any.	Doc IIIA 6.1.5-11 and REACH Registration dossier
Buehler OPPTS 870.2600 (1998) GLP Reliability 2	Guinea pigs 10/sex 10 (4f, 6m) control animals for challenge (naïve group)	Axenohl (2438 ppm Ag^+, citric acid) Induction: 75% w/w test solution in distilled water 6 hours (1/week) x 3 Challenge: 50% w/w test solution in distilled water 27 days post first application Evaluation 24 and 48 hours post challenge	Positive Frequency of reactions graded ≥0.5 at 24 hours after induction injections 1, 2 and 3 respectively: 18/20, 20/20, 20/20 Frequency of reactions graded 0.5 at 24 hours after challenge: 16/20 Naïve control: 6/10	A score of 0.5 is not a positive response according to the criteria used in the study report. However, the scoring system (incidence/severity index) used differs from OECD TG 406 and OPPTS 870.2600 (see discussion below).	IIIA 6.1.5-02 and REACH Registration dossier
Buehler US EPA 870.2600 GLP Reliability 2	Guinea pigs 20 males 10 naïve control males	Silver zeolite Agion Antimicrobial Type AD Induction: 55% w/w test solution in distilled water Challenge: 41%w/w test solution in distilled water Induction: 6 hours (1/week) x 3 Challenge: 27 days post first application Evaluation 24 and 75 hours post challenge	Positive Frequency of reactions graded 0.5 at 24 hours after injections 1, 2 and 3 respectively: 4/20, 0/20 and 4/20 The frequency of reactions graded 0.5* at 24 hours: 7/20 Naïve controls: 2/10	See above	IIIA 6.1.5-08

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

There is no robust data available in which the sensitisation potential of silver nitrate has been investigated. According to information in a summary document on silver nitrate embedded in the study summary Doc IIIB, section 6.5(01), hypersensitivity to silver nitrate can occur through exposure to silver compounds derived from exposure to dental amalgam. The applicant for the review under the BPR argues that other components of amalgam are responsible for the sensitization reactions observed and refers to an article by McCullough, M.J. and Tyas, M.J (2008) stating "*The allergens thought to be responsible are usually mercury or mercury compounds, and rarely tin, zinc, copper, silver, gold or palladium.*" The article does not contain any references to data supporting this statement thus the information is not considered to present convincing evidence to exclude a sensitising potential of silver nitrate. There are also several reports describing silver (nitrate) allergy compiled in the textbook "Silver in healthcare" by A. B. G. Lansdown (2010). According to the author, many people tolerate metals when in their solid state but may acquire allergic dermatitis when exposed to ions in solution. The book refers to research (published in 1948 and 1954) stating that allergy and skin reactions to silver nitrate are direct proportional to the silver ion concentration. This is based on observations where freshly prepared solutions kept in the dark (with less ionized silver) were shown to be less allergenic than "aged" solutions. Cases discussed include patients who developed sensitization reactions following dermal applications or following occupational exposure to silver nitrate, silver cyanide, silver fulminate and silver chloride.

A sensitising potential of silver ions is also indicated from the results of two studies performed in guinea pigs with a type of silver zeolite denoted Agion Antimicrobial type AD and a silver-ion containing solution denoted Axenohl inducing reactions of grade 0.5. However, in both studies, animals were challenged at concentrations inducing reactions of grade 0.5 (see below) in 2/4 of the animals during the preliminary irritation tests. According to the guideline used for the study (OPPTS. 870.2600) as well as OECD TG 406, the concentration used for challenge should be the highest non-irritating dose. Considering that dermal reactions were observed in 50% of animals during the preliminary irritation tests with silver zeolite (Agion Antimicrobial type AD) and Axenohl, the doses used for challenge were less than ideal and this reduces the reliability of the study results. According to the test lab criteria, reactions of grade 0.5 are not regarded as a positive response. This is questioned by the dossier submitter since grade 0.5 was defined in the scoring system used by the laboratory as "very faint erythema, usually non-confluent" which is quite similar to grade 1 in OECD TG 406 which is defined as "discrete or patchy erythema". Grade 1 in the scoring system used by the laboratory is defined as "faint erythema, usually confluent" which is considered to require more severe reactions than score 1 in OECD TG 406 " discrete or patchy erythema". Consequently, reactions of grade 0.5 are therefore taken into consideration by the dossier submitter in this assessment. The applicant under the BPR argues that the skin reactions observed were due to minor skin abrasion during the clipping process and handling of the animals. This is not supported by any such remarks in the study report and if this would be true, the frequency would not differ between treated and untreated animals and the reaction would not be expected to last until 24 hours post challenge. Another argument made by the applicant is that the skin reactions observed are reactions to the bandage (despite best practice use of hypoallergenic dressings) as it is common for guinea pigs to react to periods of wearing occlusive dressings by developing a slightly reddened skin which typically resolves over the following 24 hours. Again, if this was the sole explanation, the frequency of reactions could be expected to be similar between treated and untreated animals. Regardless of the score, some kind of reaction to treatment did occur in 80 and 35 % of animals treated with Axenohl and silver zeolite, respectively. Although reactions graded 0.5 were noted also in 60 % and 20 % of naïve animals, the frequencies were yet 20 and 15% higher in animals treated with Axenohl and silver zeolite compared to controls. Therefore, in contrast to the study author, the dossier submitter does not find it safe to exclude that these substances have sensitizing properties. In similarity with silver nitrate, silver zeolite releases silver ions and Axenohl is a solution containing silver ions thus the results from these two studies are considered in the weight of evidence presented in section 10.7.2.

In contrast to these results, dermal reaction to treatment was observed only in 1/20 (5%) of animals exposed to nanoparticles of silver in a guinea pig maximisation test. The amount of released silver ions during topical application of nanoparticles during induction and challenge is not known and thus the exposure level to silver ions is not known. Therefore, the negative result is interpreted with some caution. Axenohl is stated to contain 2438 ppm Ag+ produced by electrolysis in a citric acid/water solution. The exposure to silver ions from silver zeolite is not known but taking into account that the result was considered positive and that no data available for other silver zeolites containing less silver (i.e., silver zinc zeolite and silver copper zeolite, see study summaries in confidential annex) indicate sensitizing properties it seems realistic to assume that the reactions observed with silver zeolite is associated with silver ions.

The skin sensitising potential of nanoparticulate silver dispersed in citric acid was investigated in male guinea pigs. A dose of 102.4 mg was used for the topical induction and challenge phases. This dose was considered to be the maximum amount that could be applied to the surface. Twenty animals were allocated to the test group and ten to the negative control group but there is no information regarding a positive control study or any preliminary dose-setting investigations. Treated animals received three paired intradermal injections (FCA; test substance or test substance in FCA) and controls received the same injections but without the test substance. A week later the same area was treated with a slight irritant and subsequently topically with the test substance under an occlusive dressing for 48 hours. Animals were challenged on day 21 and dermal reactions were assessed 48 and 72 hours after the initial application. Upon challenge one of the test group animals developed discrete erythema 24 hours after dressing removal that improved over the following 24 hours but a patchy erythema persisted at the 72 hour assessment. There were no reactions observed in the control group at any assessment timepoint. The positive reaction rate of 5% (1 animal out of 20 animals tested) was graded as a weak sensitiser according to the Kligman scale. However according to CLP criteria, in a GPMT a rate of ≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose is needed to fulfil criteria for classification. Therefore, this result does not warrant classification.

Following the consultation of the CLH report for elemental silver, additional data on skin sensitisation were taken into consideration for the assessment of silver and the silver ion. The REACH dossier includes a GPMT conducted in accordance with OECD TG 406 (1992) and GLP performed with sodium silver thiosulphate which was considered negative for skin sensitisation, Prinsen (1995). It should be noted that the silver content in sodium silver thiosulphate is only 1% and the study was performed with a 10% test dilution of sodium silver thiosulphate thus the actual silver tested was low. Moreover, an OECD TG 429 (2002) and GLP compliant LLNA performed on a low concentration colloidal silver nano-form preparation (AgPURE W, 0.008%, 80 ppm Ag0, report from 2007) was also negative. A summary of another LLNA test was described in the SCCP Opinion on citric acid (and) silver citrate, (2009) SCCP/1196/08. This test was also negative. According to discussions in RAC, there has been debate in the literature regarding the suitability of the LLNA for metals and their salts. There is no definitive conclusion on this point, some publications show acceptable application of the assay, but one feature of note is that the sensitivity of the assay in these cases may be reduced necessitating the use of higher concentrations of test substance. In this context it should be noted that although citric acid (and) silver citrate was tested up to a concentration of 25%, silver citrate only contains 2400 ppm Ag+ (0.24%). Despite the very low silver content tested in these studies, more emphasis was placed on the negative LLNAs.

10.7.2 Comparison with the CLP criteria

The criteria states:

"Substances shall be classified as skin sensitisers (Category 1) in accordance with the following criteria:

(i) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or

(ii) if there are positive results from an appropriate animal test (see specific criteria in paragraph 3.4.2.2.4.1).”

According to information available in the open literature, hypersensitivity to silver nitrate can occur following exposure to dental amalgam and several cases of silver (nitrate) allergy are described in a textbook “Silver in healthcare” from (2010). However, due to the number of cases and the limited information available regarding test substance and the human cases, this information is not considered to fulfil the first criterion (i) *“evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons”*.

There are no robust animal studies available in which the sensitising potential of silver nitrate has been investigated. Silver nitrate is a corrosive substance but a sensitising potential at concentrations below the threshold for corrosivity cannot be excluded. Therefore, data available for silver substances releasing silver ions could be considered relevant also for silver ions released from silver nitrate at sub-corrosive concentrations.

The results from studies performed with a silver citrate/laurate solution and a silver zeolite, respectively, indicate a 15 and 20% higher frequency of dermal reactions at challenge concentrations of 41 and 50% respectively compared to the frequencies at induction concentrations. Since criteria for classification in category 1B are fulfilled by *“≥ 15 % responding at > 20 % topical induction dose”* this means that these substances would fulfil criteria for classification. However, there are two uncertainties that need to be taken into consideration; first whether the effects can be unequivocally assigned the silver ion (and not the citrate/laurate and zeolite parts respectively) and secondly, the deficiencies in the studies with respect to the challenge concentrations used and the definitions of the grades in the scoring system (see section 10.7.1). Therefore, this data is not considered sufficient to fulfil the second criterion (if there are positive results from an appropriate animal test) but the results are considered in combination with the case reports in a weight of evidence approach.

With respect to weight of evidence, the CLP 3.4.2.2.4.1. in Annex I states:

“For classification of a substance as a skin sensitiser, evidence should include any or all of the following using a weight of evidence approach:

(a) positive data from patch testing, normally obtained in more than one dermatology clinic;

(b) epidemiological studies showing allergic contact dermatitis caused by the substance. Situations in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small;

(c) positive data from appropriate animal studies;

(d) positive data from experimental studies on humans (see section 1.3.2.4.7);

(e) well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic;

(f) severity of reaction may also be considered.”

Criterion (c) could be considered fulfilled by the positive results from the animal tests while the information in the textbook could be considered to meet criterion (e) although it may be questioned whether the data is of sufficient quality to fulfil “well documented”.

However, the CLP also states:

“If none of the above mentioned conditions are met the substance need not be classified as a skin sensitiser. However, a combination of two or more indicators of skin sensitisation as listed below may alter the decision, which shall be considered on a case-by-case basis:

(a) isolated episodes of allergic contact dermatitis;

(b) epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence;

(c) data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in paragraph 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant;

(d) positive data from non-standard methods;

(e) positive results from close structural analogues."

There are several cases of reactions to silver nitrate described in literature but they seem to be isolated episodes. Therefore criterion (a) rather than (b) is considered fulfilled. The information which is summarised in table 44 is very brief with insufficient information on exposure concentration, duration, exposed area and reaction grade complicating for an assessment of human data in the way it is outlined in the guidance document and it is not possible to assign the exposure index (Table 3.3.). However, the number of cases reported is below 100 (indicating low/moderate frequency of occurrence of skin sensitisation) despite a widespread use of silver in different forms. Therefore, based on sub-categorisation decision Table 3.4 of the CLP guidance, category 1 is proposed. Furthermore, a sensitising potential of silver ions was indicated also in two different Buehler studies performed with a type of silver zeolite and with a formulation containing 2438 ppm silver ions, respectively. Based on this, indication (e) could be considered fulfilled and thereby two criteria are met and classification Skin Sens. 1 is proposed.

Following the consultation of the CLH report for elemental silver, additional data on skin sensitisation were taken into consideration for the assessment of silver and the silver ion by RAC. Following discussions, more emphasis was placed on the negative LLNAs than indications from other studies with silver compounds and the limited human cases reported. Therefore, criteria for classification were not considered fulfilled. However, as discussed above, it should be noted that the silver content was very low in these studies thus it may be questioned if the skin sensitising potential of the silver ion was adequately investigated.

10.7.3 Conclusion on classification and labelling for skin sensitisation

There are no guideline studies available in which the sensitising potential of silver nitrate has been adequately investigated. Further testing is complicated by the corrosive properties of the substance. However, sensitising reactions to silver nitrate and other ionic silver solutions in humans are described in a textbook on silver and in different reviews. Moreover, a sensitising potential of silver ions was indicated in two different Buehler studies performed with silver zeolite and a formulation containing 2438 ppm silver ions respectively. Nanoparticles of silver tested in an OECD TG 406 study did not show any sensitising properties, but it is not clear to the dossier submitter if and to what extent silver ions are released from these particles when topically applied. The negative response is thus interpreted with some caution and is not considered to dismiss the concern raised from the other two studies. Consequently, two of the indicators for skin sensitisation are fulfilled and in such cases, the CLP indicates that classification could be considered. Due to the low silver concentration tested in the additional studies taken into account for the classification and labelling of elemental silver, the dossier submitter does not consider that these should be given more weight than the other studies in the assessment of the intrinsic skin sensitising potential of the silver ion that is proposed to mediate the skin sensitising effects of silver nitrate. Consequently, classification Skin Sens. 1 is proposed for silver nitrate.

10.8 Germ cell mutagenicity

Table 46: Summary tables of mutagenicity/genotoxicity tests in vitro

Summary table of in vitro genotoxicity studies					
Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study** *(e.g. cell type, strains)*	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
Un-scheduled DNA synthesis Published research	Silver nitrate (and other metals) 4.6, 9.3 and 18.5 µM		Ag caused a small but statistically significant increase in DNA labelling at the highest concentration. Ag can accumulate in the nucleus of rat hepatocytes in culture.		Denizeau F and Marion M (1989)
DNA fidelity assay	Silver nitrate (and other metals)		Decreased fidelity of DNA synthesis was observed with salts of Ag.		Loeb, L. A et al (1977)
CHO/ HGPRT	Silver nitrate	Silver nitrate tested among many other metal salts in CHOK1-cells in absence/presence of S9	No further information but a conclusion that the substance was mutagenic in the study.		Hsie, A. W et al (1979)
Rec Assay Reversion Assay	Silver nitrate	Silver nitrate tested among 127 metal compounds in Bacillus subtilis	Concluded in the study to be negative (with no further information). The samples are stated to be highly toxic to bacteria.		Kanematsu, N.et al (1980)
Back-mutations from streptomycin dependence	Silver nitrate	127 metal compounds tested in two strains of Escherichia Coli	Negative result. However, the author states “the result does not exclude the possibility of having mutagenic activity at the lowest survival levels because it was extremely difficult to get survivals below 1.0% and yet not so low as to fall beyond the range of sensitivity of the method.”		Demerec, M (1951)
Salmonella assay	“Silver oxidation state +1”	Unknown silver salt tested (among seven metallic cations) in Salmonella Typhimurium strain TA 100.	Concluded to be negative at 10^1M No metabolic activation.		Tso, WW and Fung WP (1981)

UV induced mutagenesis	Silver nitrate	21 metal salts tested in two strains of Escherichia Coli	Test of silver nitrate was limited by its toxicity. Non mutagenic at 0.1M		Rossman T.G. and Molina. M (1986)
Rec assay	Silver chloride	56 metal compounds tested in two strains of Bacillus subtilis	Concluded to be negative at 0.05M		Nishioka, H (1975)
Ames test	Silver sulfadiazine	Tested in Salmonella Typhimurium strains TA100, TA1535, TA1537, TA1538.	Concluded to be negative.		McCoy E.C and Rosenkranz H.S (1978)

Summary table of in vitro genotoxicity studies					
Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study** *(e.g. cell type, strains)*	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
Silver zinc zeolite Type AK					
Ames/Salmonella Mutagenesis Assay EC: A6.6.2 US EPA: 84-2, 870.5100 GLP Reliability: 1-2	Silver Zinc Zeolite Type AK 0.15, 0.5, 1.5, 5, 15, 50, 150 and 500 µg/plate with and without S9	S. typhimurium and E. coli	Negative	Bacterial toxicity evident at dose concentrations of 500 µg/plate and higher	IIIA 6.6.1-11
Mammalian cell mutation Forward mutation at TK locus EU: 2000/32/EC Annex 4E- B17 USA EPA: 870.5300 GLP Reliability: 1	Silver Zinc Zeolite Type AK 0 to-25 µg/ml without S-9 and 0 to 175 µg/ml with S-9	Mouse lymphoma L5278Y cells	Positive	Cytotoxicity at 10 µg/mL and higher without S9. Cytotoxicity at 100 µg/mL and higher with S9 Positive response within cytotoxic dose ranges with or without S9 Tendency towards an increase in % small mutant colonies, indicating a possible clastogenic effect.	IIIA 6.6.3-03
Silver zinc zeolite					
Ames/Salmonella Mutagenesis Assay EPA FIFRA Guideline 84-2 GLP Reliability: 2	Silver zinc zeolite (unspecified form assumed to be equivalent to Irgaguard 8000) 4% silver Without S9: 0.0005, 0.001, 0.0015, 0.003, 0.005, 0.01 and 0.015 mg/plate. With S9: 0.003, 005, 0.01, 0.015, 0.03, 0.05 and 0.15 mg/plate	The ability to detect DNA cross-linking mutagens was not investigated.	Negative	In the non activated assay, bacterial toxicity was evident at concentrations in excess of 0.015 mg/plate (noted as decreased mean no of revertants compared to water control) and at concentrations greater than 0.15 mg/plate in the activated assay.	IIIA 6.6.1-03

Mammalian cell mutation Forward mutation at TK locus OECD 476 GLP Reliability: 1	Silver zinc zeolite type Irgaguard B 8000 Dose levels, selected on the basis of preliminary test results: Assay 1, without S9: 3.1, 6.3, 12.5, 25.0 and 50 µg/mL in Assay 1 with S9: 13.1, 26.3, 52.5, 105.0 and 210.0 µg/mL in Assay 2 without S9: 6.3, 12.5, 25.0 and 50 µg/mL in	Mouse lymphoma L5278Y cells	Negative (+S9) Positive (-S9)	An increase in the number of small colonies observed indicating a possible clastogenic activity.	IIIA 6.6.3-05
In vitro chromosome aberration test OECD 473 GLP Reliability: 1	Silver zinc zeolite type Irgaguard B 8000 Without S9: **0.9, 1.9, 3.8, 7.5,** 15,30 µg/mL With S9: **6.3, 12.5, 25.0, 50.0,** 75.0, 100 (evaluated concentrations in bold)	Chinese Hamster V79 cells	Negative (+S9) Positive (-S9)		IIIA 6.6.2-07
Silver copper zeolite					
Ames/Salmonella Mutagenesis Assay EPA Guideline 84-2 GLP Reliability: 2	Silver copper zeolite (unspecified) With S9: 0.005, 0.015, 0.05, 0.15, 0.5 and 1.5 mg/plate Without S9: 0.0005, 0.01, 0.015, 0.03, 0.05, 0.1 and 0.15 mg/plate.		The test material was non-mutagenic at all concentrations tested in the two assays.	The ability of silver copper zeolite to cross-link DNA was not investigated in this study.	IIIA 6.6.1-06
In vitro chromosomal aberration assay in CHO cells EPA FIFRA 84-2 GLP: Yes Reliability: 2-3	Silver copper zeolite (unspecified) For non activated assay: 0.5, 1.0, 1.5, 3, 5, 10, 15, 30, 50 and 100 µg/mL Activated assay 1: 10 hr - 1, 1.5, 3, 5, 10, 15, 30, 50, 100, 150 and 500 µg/mL 20 hr - 0.15, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/mL Activated assay 2: 10 hr - 10, 25, 50, 75, 100, 125 and 150 µg/mL 20 hr - 10, 25, 50, 75, 100, 125 and 150 µg/mL		+S9: Weakly positive at 100 µg/mL -S9: Negative	Toxicity was observed in the 10 h non-activated assay at 30, 50 and 100 µg/mL and in the 20 h non-activated assay at 100 µg/mL. In the 10 h activated assay, toxicity was observed at 150 and 500 µg/mL in the initial assay and at 150 µg/mL in the replicate. For the 20 h activated assay, toxicity was apparent at concentrations of 150, 500, 1500 and 5000 µg/mL and at 150 µg/mL in the replicate assay.	IIIA 6.6.2-05

Silver sodium zirconium hydrogen phosphate					
Ames/Salmonella Mutagenesis Assay with Salmonella typhimurium and Escherichia coli.	AlphaSan RC2000 0.78, 1.56, 3.13, 6.25, 12.50, 25.0 and 50 µg/plate without S9 7.81, 15.63, 31.25, 62.50, 125, 250, 500. 1000 and 2000 µg/plate with S9 Assay 2: 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 µg/plate without S9. 7.81, 15.63, 31.25, 62.50, 125, 250, 500 µg/plate with S9 Assay 3: 7.81, 15.63, 31.25, 62.50, 125, 250, 500 µg/plate with S9		Negative The three replicate assays failed to show reproducible increases in revertants.		IIIA 6.6.1-07
Ames/Salmonella Mutagenesis Assay with Salmonella typhimurium.	AlphaSan RC5000 1.5, 5.0, 15.0, 50, 150 and 500 µg/plate with and without S9 in both assays		Negative There were no significant increases in the number of revertants per plate in the non-activated or metabolically activated assays. Bacterial toxicity evident at dose concentrations of 50 µg/plate and higher	In the main mutagenicity assays, Novaron AG 300 was tested as a suspension in DMSO. The ability of Novaron AG 300 to cross-link DNA was not investigated in this study. According to the criteria used by the test laboratory, a minimum of three non-toxic dose levels are required to determine the validity of the test. However, according to OECD TG 471 (adopted July 21st, 1997), at least five different analysable concentrations of the test substance should be used. Due to the toxicity of Novaron 330, this was not achieved in the assays performed without activation.	IIIA 6.6.1-08

Ames/Salmonella Mutagenesis Assay with Salmonella typhimurium and Escherichia coli	AlphaSan RC7000 Assay 1: 7.81, 15.63, 31.25, 62.50, 125.0, 250 and 500, 1000 and 2000 µg/plate with and without S9 Assay 2: 3.91, 7.81, 15.63, 31.25, 62.50, 125, 250, 500 and 1000 µg/plate with and without S9 Assay 3: 7.81, 15.63, 31.25, 62.50, 125, 250, 500, 1000 and 2000 µg/plate with S9		Negative	In the main mutagenicity assays, Novaron AGZ330 was tested as a suspension in DMSO. Due to the toxicity of Novaron AGZ330, only four concentrations could be analysed in assays performed without without activation.	IIIA 6.6.1-09

Chromosome aberration test in human lymphocytes	AlphaSan RC2000 Experiment 1 ca 78 to 938 µg/mL without S9 ca 78 to 635 µg/mL with S9 Experiment 2 ca 39 to 469 µg/mL with and without S9		Negative	Assessment of the slides indicated metaphase cells were present in cultures treated at up to 625 µg/mL with or without S9 in the 4 (20)-hour cultures. The maximum dose with sufficient scorable metaphases, present in the 24 hour continuous exposure cultures, was 312.5 µg/mL.	Doc IIIA 6.6.2-06
Mammalian cell mutation – Mouse lymphoma L5278Y cells. Forward mutation at TK locus	AlphaSan RC2000 Assay 1 12.5 to 15 µg/mL without S9 and 10 to 80 µg/mL with S9. Assay 2 2.5 to 30 µg/mL		Positive in presence and absence of S9	Small dose-related increase in mutant frequency with and without S9. The result was reproducible without S9. Positive evidence for weak mutagenic effect at toxic concentrations. The threshold for absence of mutagenic response was also the level of incipient toxicity.	IIIA 6.6.3-08
Mammalian cell mutation – Mouse lymphoma L5278Y cells. Forward mutation at TK locus	AlphaSan RC5000 Mutation test 1 (-S9): 0.5, 1, 2.5, 5, 10, 20, 25 µg/mL Mutation test 1 (+S9): 5, 10, 15, 20, 25, 50, 75, 100 µg/mL Mutation test 2 (-S9): 1, 5, 10, 15, 20, 25, 37.5, 50 µg/mL Mutation test 2 (+S9): 10, 15, 20, 25, 37.5, 50, 75, 100 µg/mL.		Positive	AlphaSan RC5000 did produce a significant increase in mutant frequency, especially in the absence of metabolic activation.	IIIA 6.6.3-09

Table 47: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance,</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr>
<td>OECD TG 474 (1997 version)

Reliability: 2
(no positive control included, no individual animal data)</td>
<td>AgNPs (NAMATECh Co., Ltd, Korea).
52.7-70.99 nm (average 60 nm)
99.98%
Release of silver ions as the % of total silver content is not known</td>
<td>Rat
Sprague-Dawley
10/sex/group
30, 300 and 1000 mg/kg bw/d</td>
<td>Dose-dependent deposition of the test material in the blood, stomach, brain, liver, kidneys, lungs and testes.
Weak dose-related increase in MNE frequency in male rats.
<table>
<tr><th>Dose (mg/kg bw/d)</th><th colspan="2">Frequency of MNEs in 2000 PCEs (mean ± SE)</th><th colspan="2">PCE(PCE + NCE) (mean ± SE)</th></tr>
<tr><td>Sex</td><td>male</td><td>female</td><td>male</td><td>female</td></tr>
<tr><td>0</td><td>5.20 ± 0.63</td><td>2.50 ± 0.43</td><td>0.30 ± 0.024</td><td>0.37 ± 0.022</td></tr>
<tr><td>30</td><td>6.00 ± 0.93</td><td>3.50 ± 0.50</td><td>0.25 ± 0.019</td><td>0.32 ± 0.019</td></tr>
<tr><td>300</td><td>6.60 ± 0.64</td><td>2.40 ± 0.52</td><td>0.28 ± 0.036</td><td>0.34 ± 0.023</td></tr>
<tr><td>1000</td><td>7.40 ± 0.54</td><td>3.40 ± 0.64</td><td>0.26 ± 0.023</td><td>0.30 ± 0.019</td></tr>
</table>
</td>
<td>Kim, Y.S. et al (2008): Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhalation Toxicology 20, 575-583.</td>
</tr>
</table>

<table>
<tr>
<td>OECD TG 474 (1997 version)

Reliability: 2 (no positive control included, no individual animal data)</td>
<td>AgNPs

Particle size: 1.98 to 64.9 nm.

No further details</td>
<td>Rat

Sprague-Dawley

10/sex/group

Concentration in the low-, mid- and high-doses was 49 µg/m^3, 133 µg/m^3 and 515 µg/m^3, respectively</td>
<td>Dose-dependent deposition of silver nanoparticles in the blood, stomach, brain, liver, kidneys, lungs and testes.

No dose-related increase in MNE frequency.
<table>
<tr><th>Dose</th><th>No. of rats (male)</th><th>Males: Frequency of MN PCEs in every 2000 PCEs</th><th>Females: Frequency of MN PCEs in every 2000 PCEs</th></tr>
<tr><td>0</td><td>10</td><td>0.14</td><td>0.14</td></tr>
<tr><td>30</td><td>10</td><td>0.13</td><td>0.09</td></tr>
<tr><td>300</td><td>10</td><td>0.21</td><td>0.08</td></tr>
<tr><td>1000</td><td>10</td><td>0.18</td><td>0.13</td></tr>
</table>
</td>
<td>Kim, J. S., et al (2011): In vivo Genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure. Saf Health Work 2: 34-8.</td>
</tr>
<tr>
<td>Published literature Guideline/GLP not reported</td>
<td>AgNPs are coated with 0.2% PVP for easy dispersion in aqueous solutions, trace metal levels below 0.5%

Oral Gavage

500 mg/kg</td>
<td>Mice C57BL/6J p^{un}/p^{un}

Wildtype: 6-7
$Myh^{-/-}$: 9-10

Pregnant dams were treated from 9.5 to 13.5 days post coitum</td>
<td></td>
<td>Kovvuru, P.; Mancilla, P. E.; Shirode, A. B.; Murray, T. M.; Begley T. J.; Reliene, R. (2015): Oral ingestion of silver nanoparticles induces genomic instability and DNA damage in multiple tissues</td>
</tr>
</table>

Summary table of in vivo genotoxicity studies					
Method, Guideline, GLP status, Reliability	**Test substance, Doses**	**Relevant information about the study** *(e.g. species and strain, duration of exposure)*	**Observations**	**Remarks** *(e.g. major deviations)*	**Reference**
In vivo chromosome aberration assay in rats EPA FIFRA 84-2 GLP: Yes Reliability: 2-3	silver zinc zeolite (unspecified) 500, 1500 and 5000 mg/kg	Rats Sprague-Dawley 5/sex Single oral dose (gavage,) 6h, 18h, 24h post exposure	Negative	Unclear exposure of target tissue; no signs of toxicity at doses up to dose of 5000 mg/kg bw. The sampling time was not optimal. Only 50 metaphase cells were scored per animal. According to OECD guideline, at least 100 metaphase cells should be scored	IIIA 6.6.4-01
In vivo chromosome aberration assay in rats EPA FIFRA 84-2 GLP: Yes Reliability: 2	silver copper zeolite (unspecified) Single oral dose (gavage) 500, 1500 and 5000 mg/kg	Sprague-Dawley rats 5/sex Sampling time: 6h, 18h, 24h post exposure	Negative	No signs of toxicity in the target tissue at any dose level.	IIIA 6.6.4-02

Rat Alkaline Comet Assay OECD 489 (2014) GLP Reliability 1	Hygentic 8000 Silver zinc zeolite 0, 500, 1000 and 2000 mg/kg bw Administered as 2 doses separated by 21 hours	Han Wistar Crl:WI males 6 animals/dose 3 controls	No evidence of genotoxicity in tissues analysed (liver, stomach or duodenum)	This result is considered relevant to assess the genotoxic potential of the silver and zeolite in silver zeolite	IIIA 6.6.5-02 (separate document)
Silver sodium hydrogen zirconium phosphate					
Unscheduled DNA synthesis Single dose i.p	Alphasan RC2000 666.7 and 2000 mg/kg assay 1 and assay 2	Rat Spague-Dawley 4/group (both sexes) 16 hours and 2 hours in separate experiments	Negative		IIIA 6.6.3-07
Micronucleus test Single dose i.p	Alphasan RC2000 500, 1000 and 2000 mg/kg	Mice Crl:CD®-1(ICR)BR 7 males per group 24 and 48 hours	Negative	The test article did not produce any signs of toxicity in the target tissue, even though the highest dose was 2000 mg/kg bw. Hence, it is difficult to draw conclusions, since it remains uncertain if the test article reached the target tissue.	IIIA 6.6.4-04

Micronucleus test Single oral dose	Alphasan RC5000) 1250, 2500 and 5000 mg/kg (males and females)	Mice CD-1/ 5 animals per group (both sexes) 24, 48 and 72 hours.	Negative	The PCE/NCE ratio at 48 hours was slightly but significantly decreased, probably indicative of a slight transient bone marrow depression induced by the test substance.	IIIA 6.6.4-05

Table 48: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
See above				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro: The bactericidal activity of silver involves damage of several cellular structures. According to published research, the silver ion may cause the cytoplasm membrane to separate from the cell wall and when inside the cell it can bind free thiol groups and structurally alter enzymes. The bacteria appear to have defence systems protecting the genetic material from the silver ion. However, at dose levels exceeding the defence capacity, the silver ion can interact with DNA leading to condensation and thus prevention of replication (Feng et al (2000), Jung et al (2008)). This mechanism may protect the genetic material from being propagated with mutations.

There is no substance specific robust data on the mutagenicity of silver nitrate in the dossier. Upon request, the applicant submitted full-text versions of the articles referred to in Doc IIIA, section 6.6.5-01. Two of the articles did not include any silver substance in the experiment (Amacher, D. E and Paillet S. C (1980), DiPaolo, J. A. and Casto B. C (1979)) and are thus not relevant for this section. All remaining articles were performed in vitro, and the majority used bacteria as test organism. Only one of the articles was performed with focus on a silver compound (i.e., Elena C et al (1978)), in all other studies the silver compound was one among many metal compounds tested.

There were no indications of mutagenicity observed in four of the studies in bacteria and the results of two studies were considered equivocal as it is unclear if the absence of mutagenicity was due to the cytotoxicity observed (Kanematsu et al (1980), Demered et al (1951)).

In contrast, genotoxic effects were observed in mammalian cells; in a study performed with rat hepatocytes (Denizeau F and Marion M (1989), in Chinese hamster ovary cells (Hsie, A. W et al. (1979)) and in a study investigating the fidelity of DNA synthesis (Loeb, L. A et al (1977)). Although these studies are fairly old and the reliability of the information varies the results are in agreement with results obtained for other silver containing active substances (see tables 46 and 47) generally showing

negative results in bacteria but positive findings in tests investigating mutagenicity and/or chromosome aberrations in mammalian cells.

In vivo:

There are no studies investigating the in vivo germ cell mutagenicity of silver nitrate. The in vivo data available in the core dossier submitted for the review under BPR include studies with four other SCAS. Although data on chemically related compounds can be considered in a weight-of-evidence approach, the data on these types of SCAS is of limited use here since the amount of silver ion equivalents actually tested in the studies is fairly low compared to the recommendations in OECD guidelines. Based on release data, the maximum dose silver ion equivalents exposed to in studies with different SCAS is only 68 mg/kg/bw. Considering that silver nitrate contains both a higher amount of silver and is a readily soluble substance this data is not sufficient to exclude mutagenic properties at non-corrosive concentrations. The threshold for corrosivity is not known but available data indicates that it is above 100 mg/kg bw/d (corresponding to 64 mg/kg bw/day silver ions, see section 10.12). Moreover, three of five in vivo studies in the core dossier failed to demonstrate convincing evidence that the test substances reached the target tissue in quantities sufficient to be able to detect any genotoxic effects.

The information available to assess in vivo germ cell mutagenicity is thus limited to published studies performed with nanoparticles of silver. Since nanoparticles at least partly dissolve in gastric juice and release silver ions following oral intake[14], this information is considered relevant to assess effects of silver ions dissolved from silver nitrate at non-corrosive concentrations. Although the actual amount of silver ions released is not known, it seems reasonable to assume that effects of AgNP, at least in vivo, are mainly caused by the silver ion[15] (see section 6). Depending on size and surface treatment some nanoparticles may be absorbed and transported in the blood as such, but they may also agglomerate reducing bioavailability. As discussed in section 6, the distribution is expected to be similar between nanoparticles and ionic silver although it is not possible to exclude that they may also distribute to different organs and release silver ions on site. However, classification is based on intrinsic properties and the genotoxic effects observed are considered to represent the intrinsic property of the silver ion released.

The silver nanoparticles were investigated in a 28-day study performed via the oral route and a 90-day study performed via inhalation. Both studies were performed according to the principles of GLP and OECD TG 474 to investigate the frequency of micronuclei in bone marrow cells. In the oral 28-day study a weak dose-dependent increase was observed in males but the result from the 90-day inhalation study was considered negative. Although there was no bone marrow toxicity observed in any of the studies (based on the PCE/NCE ratio), the test material apparently reached systemic circulation as there was dose-dependent deposition of the test material in the blood, stomach, brain, liver, kidneys, lungs and testes.

The in vivo genotoxicity of silver nanoparticles was further investigated in a series of assays performed in Myh-/- and wild type mice. The ability of nanoparticles to induce DNA deletions, micronucleus formation, γ-H2AX foci, 8-oxoG and to modulate the expression of DNA repair genes was investigated and recombinant mice (deficient in a type of DNA repair) were included to test the hypothesis that AgNP exert effects via oxidative DNA damage.

The results of the assay performed to detect deletions during or shortly after exposure to nanosilver i.e., during embryonic development, showed a significantly higher frequency of DNA deletions in both

[14] Jingyu Liu., et al (2012), Chemical Transformations of Nanosilver in Biological Environments, ACS Nano. Also discussed e.g. in a review by Behra R, et al. (2013). Bioavailability of silver nanoparticles and ions: from a chemical and biochemical perspective. J R Soc Interface 10: 20130396. http://dx.doi.org/10.1098/rsif.2013.0396

[15] Discussed e.g. in Yan, Neng & Zhong Tang, Ben & Wang, Wen-Xiong. (2018). In Vivo Bioimaging of Silver Nanoparticle Dissolution in the Gut Environment of Zooplankton. ACS Nano. 10.1021/acsnano.8b06003; Jonathan L. Falconer a, Jeremiah A., David W. Grainger (2018) Comparing ex vivo and in vitro translocation of silver nanoparticles and ions through human nasal epithelium, Biomaterials, Vol 171

wild type mice and Myh-/- mice compared to their respective controls indicating that intake of AgNPs during gestation induces large-scale genome rearrangements in the developing embryos.

The number of micronucleated erythrocytes was increased about 4-fold in both wild type mice and Myh-/- mice following one day of ingestion and by 5- and 7-fold in wild type and Myh-/- mice, respectively following 5 days. This indicates that oral ingestion of AgNPs results in cumulative permanent chromosomal damage in the bone marrow and that Myh-/- mice are hypersensitive to AgNP-induced chromosomal damage.

AgNP ingestion for one day increased the percentage of γ-H2AX foci positive cells in peripheral blood by about 2.5-fold in both wild type mice and Myh-/- mice and a trend toward a further increase was seen following 5 days exposure. In addition, AgNPs induced γ -H2AX foci formation in the bone marrow by 2- and 6-fold in wild type mice and Myh-/- mice, respectively. This indicates that AgNP intake results in markedly increased double strand breaks in mononuclear cells in the bone marrow and leukocytes in peripheral blood suggesting that double strand breaks may have contributed to the observed permanent genomic changes (rearrangements) observed.

Furthermore, AgNPs increased 8-oxoG levels by 3.4-fold in wild type mice and by 2.2-fold in $Myh^{-/-}$ mice indicating that oxidative damage can play a role in the genetic damage observed.

An investigation of the impact on gene expression indicated that 36 of 84 genes were altered (24 genes downregulated and 12 genes upregulated) in response to AgNP exposure. Some of the downregulated genes and some of the upregulated genes were involved in DNA repair.

In conclusion, the study is considered to indicate that maternal ingestion of AgNPs results in large DNA deletions in developing embryos and that AgNPs induces irreversible chromosomal damage in the bone marrow.

During the discussions of the CLH proposal for silver, which is largely based on the same data, RAC noted one published study that was not included in the CLH report (Sycheva et al., 2016)[16] investigating effects of nanosilver particles with a diameter of 14 nm and silver sulphate on germ cells in vivo. Male CBAB6F1 mice were exposed for 14 days to silver in drinking water over a range of concentrations: 0.1, 50, and 500 mg/L. There was no information with respect to any guideline or GLP status and the study is categorised as supplementary. In brief, the only data considered useful is the indication that silver from silver nanoparticles and silver sulphate reach the testes. An increase in the frequency of micronucleated spermatids was observed for silver in nanoform. However, the effects were not consistent, showed no dose response and differed between the nanoforms and the salt.

Another study available in the open literature (Narciso et al., 2020), investigated the in vivo genotoxicity of unstabilised AgNP 20 nm in CD-1 mice using both a Comet and a Micronucleus assay. Studies were performed in accordance with OECD TG 489 and OECD TG 474 under GLP conditions. Mice were treated once a day orally by gavage for 3 consecutive days at doses of 50, 150 and 300 mg AgNPs /kg bw/day. There was no statistically significant increased DNA damage observed in blood, liver, kidney, spleen and duodenum of male and female mice exposed to AgNPs in comparison to controls. The oral exposure to AgNPs did not increase the percentage of micronuclei in lymphocytes of spleen in male and female mice, although a slight, dose-related increased frequency was present in females administered 150 and 300 mg/kg bw/day. Exposure was confirmed by mass spectrometry in several tissues including the spleen and TEM analysis showed the presence of AgNPs into the cells of liver and duodenum. In many cases, responses decreased with increased doses of silver. The graph of the micronucleus data showed a highly variable positive control response. While indicating a positive response trend it was not particularly convincing and the frequency of micronuclei in lymphocytes derived from murine spleen cells cultured for 44 h ex vivo of male and female mice were not tabulated (it was however supplied for the Comet assay and appears reliable). Overall, the results are difficult to interpret since units and dose levels are unclear and there is no historical control data to compare results with. The reliability of the study is thus questionable.

RAC also considered the publication by Gromadzka-Ostrowska et al. (2012) not referred to in the genotoxicity section of the CLH report for silver but in the reproductive toxicity section (10.10). This study was considered to appear as "a reasonable study with some shortcomings" and is not GLP or

[16] Gromadzka-Ostrowska et al. Silver nanoparticles effects on epididymal sperm in rats. Toxicology Letters 214 (2012) 251– 258. http://dx.doi.org/10.1016/j.toxlet.2012.08.028.

OECD guideline compliant. Male animals were divided into 4 groups of 24 rats per group. Animals were injected (tail vein) with a single dose (5 mg/kg or 10 mg/kg) of 20 nm AgNPs (groups Ag I and Ag II, respectively) or with 5 mg/kg of 200 nm AgNPs (group Ag III). Sham-exposed rats were injected with 0.9% NaCl solution. Animals from the experimental and control groups were anesthetized by isoflurane inhalation and bleed by cardiac puncture 24 h, 7 days and 28 days after injection.

This test was effectively a positive in vivo Comet Assay. It was designed to investigate the acute effects of intravenously administered AgNPs on the sperm count, frequency of abnormal spermatozoa, germ cell DNA damage in sperm cells and testis morphometry in male Wistar rats.

There was an apparent decrease in epididymal sperm counts but the results are not particularly convincing and the frequency of abnormal spermatozoa in epididymal semen from experimental groups was not significantly different when compared with the control group.

AgNPs (20 nm) caused DNA damage in germ cells and the highest level of DNA damage was observed 24 h after injection, thereafter decreasing.

There were significant changes in the testicular morphology – increased lumen diameter, area and circumference of seminiferous tubules in the animals treated with 200 nm AgNPs (Ag III group) 28 days after AgNPs injection.

This study is taken as further evidence of a positive in vivo Comet Assay but there were some shortcomings that reduce the reliability weighting that may be placed on this study. There was no recording of the use of positive controls and no comparison with historical control data. A slice from whole testes was used which contains a mixture of somatic and germ cells and as indicated in OECD TG 489, it is not always clear if positive results under these conditions is reflective of germ cell damage. The morphometric results for the non-nanosized silver are questionable since it was not replicated for the 20nm nanoforms. In conclusion, the study while acceptable does present a number of uncertainties and the results were not considered to present the strength of evidence sufficient to fulfil criteria for Muta. 1B classification.

The RAC opinion also lists a positive result in a Comet Assay (OECD 489) performed in mice exposed to a single i.p. dose 44nm AgNP. The study was non-GLP but considered reliable (Al Gurabi et al., 2015). Another study listed with a positive result is a Comet assay in mice exposed to a single dose / single dose with 5nm AgNP once a week for 5 weeks (Awasthi et al., 2015). The study was also considered reliable. The opinion further lists a negative result from a Comet assay in rats administered a single i.v. dose of 20 / 200nm AgNP. The study was non-GLP but considered reliable and well conducted (Dobrzyńska et al., 2014). It was commented, however, in a minority opinion from a RAC member that the study lacks positive controls.

Genotoxic effects of AgNP exposure or exposure to silver acetate was investigated simultaneously by Boudreau et al., (2016)[17] in a micronucleus assay performed in male and female rats using flow cytometric analysis of peripheral blood. Bone marrow was exposed to Ag only in the acetate treated animals. Another study by Boudreau et al 2016 is relevant and well performed according to GLP and guidelines and should according to the RAC opinion rightly be given a greater weighting than the other studies. They investigated many different endpoints, including kinetics and micronuclei after repeated oral dosing with AgNPs (of different sizes) and silver acetate (AgOAc). Levels in blood were elevated after wk1 and wk12 with a weak dose response for AgNPs but a distinct one for AgOAc. The micronuclei assay was negative for each time point in the peripheral blood of rats treated with AgNP. Silver bioavailability was similar between 10nm AgNPs and AgOAc and higher doses were administered with the salt. The accumulation of silver in tissues and organs showed significant dose relationships, irrespective of the test substance or particle size, suggesting that the uptake and deposition of silver was proportional to the administered amount of silver content of the substance and the bioavailability of silver from each substance and form. There were no qualitative differences between the elemental composition of deposited particles or granules in any of the examined tissues of rats exposed to either AgNP or AgOAc. This study was considered to add to the negative in vivo mutagenicity for both silver nanoforms and a soluble salt, silver acetate. However, as also highlighted

[17] Differential Effects of Silver Nanoparticles and Silver Ions on Tissue Accumulation, Distribution, and Toxicity in the Sprague Dawley Rat Following Daily Oral Gavage Administration for 13 Weeks. Tox. Sciences, 150(1); 131-160

in the minority opinion for the RAC conclusion on silver, this type of study is not adequate to detect the concerns raised from the in vitro studies, i.e., gene mutations. As discussed in the minority opinion, the weight-of-evidence points toward positive results in Comet assays in vivo as four in vivo Comet assays, performed with different species exposed to AgNPs, showed statistically significant positive results (Patlolla et al., 2015; Al Gurabi et al., 2015; Awasthi et al., 2015; Gromadzka-Ostrowska et al., 2012), three of them being dose-dependent as well. In two studies, the animals were exposed orally, increasing the concern. Moreover, positive γ-H2AX immunofluorescence assay (Kovvuru et al., 2015) was also reported, and can be considered as supportive. Li et al. (2014) study includes two types of Comet assay (alkaline and enzyme modified) performed on mice exposed to AgNPs and shows equivocal results. On the other hand, from the 3 remaining in vivo Comet assays concluded negative, two of them showed concerning limitations that impact the reliability of the final results. The first one (IIIA 6.6.5-02) was performed with silver zinc zeolite, an ion exchanger which is not supported for read-across. The second comet assay (Dobrzyńska et al., 2014) was lacking positive controls, therefore questioning the relevance of the negative results.

An in vivo Comet assay demonstrated positive results in rat full testes after exposure to AgNPs (Gromadzka-Ostrowska et al., 2012). In addition, the majority of the data indicates an adverse effect on males germ cells in several species (see "fertility" part of this position). Finally, AgNPs clearance appears low in testes (Lee and al., 2013; Van der Zande et al., 2012) and a dose-dependent deposition in testes was detected in rat after oral and inhalation exposure to AgNPs (Kim and al., 2008; Kim and al., 2011)

10.8.2 Comparison with the CLP criteria

There is no robust data to assess if silver nitrate has a potential to induce germ cell mutagenicity. Results obtained among existing in vitro studies with silver nitrate and various ionic silver substances in mammalian cell gene mutation tests, micronucleus assays and chromosomal aberration tests provide a range of negative and equivocal results for mutagenic activity as well as positive findings. Most published studies were non-GLP and not performed according to any OECD test guideline but overall, the in vitro data indicates a concern for DNA damage / gene mutation and clastogenicity for silver nanoforms and other silver compounds including silver salts. Unfortunately, there is no appropriate in vivo data on silver nitrate. However, data on nanosilver and silver acetate available in the open literature is considered to provide information on the genotoxic potential of silver ions and thus the properties of silver nitrate at non-corrosive concentrations (see sections 6 and 10.8.1). Although data obtained at concentrations where corrosivity would determine the toxicity of silver nitrate and data would thus not be reliable for the assessment, it should be noted that since the threshold of corrosivity is not known, all data is currently considered in the weight of evidence approach used in this CLH report. Based on the results of the 28-day study presented in section 10.12, it can be concluded that at least systemic doses up to 100 mg/kg bw/d are well-tolerated by rats. Data on nanoparticles submitted by the applicant for the review under BPR includes two bone marrow micronucleus studies with negative results. Exposure of the target tissue was not demonstrated by the PCE/NCE ratio, but systemic availability is indicated by data showing distribution to blood, stomach, brain, liver, kidneys, lungs and testes and is considered sufficient evidence for target tissue exposure. In contrast to these negative bone marrow micronucleus studies, the results from another study found in the open literature indicate that nanoparticles of silver induce irreversible chromosomal damage in the bone marrow and cause large DNA deletions in developing embryos of mice. The study was not performed according to the principles of GLP or any OECD test guideline but is published in a peer-reviewed scientific journal and the results are thus considered reliable. This data gives no clear picture of the intrinsic properties of the substance. Whereas the two bone marrow micronucleus studies indicate a low concern for mutagenicity, the chromosomal damage in bone marrow and large DNA deletions in developing embryos of mice observed in a different study are alarming. Consequently, one study indicates that the second paragraph in CLP annex I: 3.5.2.2 is fulfilled whereas two others indicate the opposite.

An unclear picture remains also when taking into account all data available for different silver compounds and different types of in vivo tests. Results for SCAS such as silver zeolites and other ion exchangers are considered less relevant since they release only small amounts of silver ions that are

insufficient to be properly tested in these assays and will thus almost always give a negative response. The different results with nanoparticles among studies may be due to differences in how components used to stabilise and coat silver nanoparticles dissociate under physiological conditions and thus expose biological compartments to the pure silver cores allowing for dissolution to Ag0 and oxidation. Overall, the most relevant in vivo study to represent silver nitrate is the micronucleus study performed with silver acetate as it is both robust and performed with a soluble salt with no expected impact of confounding factors. Nevertheless, this type of study does not address the concerns for gene mutation indicated by in vitro tests and several in vivo comet assays. The CLP guidance states (page 363; ECHA, 2017) *"In vivo tests in somatic cells which provide information on genotoxicity include, for example, the Comet single cell gel electrophoresis assay for DNA strand breaks."*

Classification in Category 1A is based on positive evidence from human epidemiological studies. This type of information is not available for silver nitrate thus criteria are not fulfilled. According to annex I: 3.5.2.2, classification in Category 1B is based on:

– positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or

– positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or

– positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

Annex I: 3.5.2.3.3 states: *"Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13(3) of Regulation (EC) No 1907/2006 ('Test Method Regulation') such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification."*

Taking into account that the two criteria in the second paragraph (i.e. *"positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells"*) are fulfilled based on results from non-guideline studies with some limitations and that also negative results were obtained, data is not considered sufficiently robust to fulfil criteria for category 1B.

According to annex I: 3.5.2.2, classification in Category 2 is based on:

– Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

– Somatic cell mutagenicity tests in vivo, in mammals; or

– Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."

Based on the results obtained with nanosilver releasing silver ions, the criteria for classification are considered fulfilled. The data is difficult to interpret but the overall weight of evidence is considered to indicate that silver ions do not induce micronuclei but may induce gene mutations. Silver is detected in gonads and has been shown to cause adverse effects in germ cells (see section 10.10). However, considering the variations in data and the limitations of each individual study, data is not considered sufficiently robust to fulfil criteria for classification in category 1B and category 2 is thus considered appropriate. This is supported by the CLP guidance (page 366; ECHA, 2017) stating that: *"A complex data situation with positive and negative results might still lead to classification. This is because all tests detecting a certain type of mutation (e.g. point mutations) have been positive and all tests detecting chromosome mutations have been negative. Such circumstances clearly warrant classification although several tests have been negative which is plausible in this case.* Consequently, silver nitrate is considered to fulfil criteria for classification and labelling in category 2.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Silver nitrate is proposed to fulfil criteria for germ cell mutagenicity in category 2.

10.9 Carcinogenicity

Table 49: Summary table of animal studies on carcinogenicity

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Realibility	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
No substance-specific data available						

Table 50: Summary table of human data on carcinogenicity

Summary table of human carcinogenicity data				
Type of data/ report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
No evidence of cancer in humans has been reported. Reliability 3				IIIA 6.5(07) 6.7 (02) Anon. (1998): US EPA Integrated Risk Information System Reference dose for chronic oral exposure.

Table 51: Summary table of other studies relevant for carcinogenicity

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
Reliability 3	Rats	Colloidal silver, 14 months Intravenous subcutaneous		Fibrosarcomas Local sarcomas may arise due to solid state carcinogenesis. (according to the ATSDR in 6.2 (08), subcutaneous imbedding of silver foil however produced fibrosarcomas	The document summarises information on carcinogenicity found in the IRIS Background document	IIIA 6.5(07) 6.7 (02) Anon. (1998): US EPA Integrated Risk Information System Reference dose for chronic oral exposure.

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
				earlier and more frequently than several other metal foils). 8/26 (type not specified) 6/8 tumours claimed to be at the site of injection, The frequency of other tumours (2/26) appears to be above the spontaneous frequency of 1-3% at any site. No further analysis possible due to poor data (Schmahl and Steinhoff (1960)).		
Reliability 3	Fischer 344 rats 25/sex/ group	Metal powder suspended in trioctanoin 5 or 10 mg per dose (each animal was treated for five consecutive months at 5 mg/dose, ten for five months at 10 mg/dose, then at 5 mg/dose for the subsequent five months and lastly at 10 mg/dose for the last five months). Intramuscular injection		No fibrosarcomas developed at the injection sites for silver. A few cases of mild local inflammation were noted at injection sites but only in the latter stages of the study. At necropsy there were several incidences of encapsulation of the vehicle or injected metal powder but none of the injected legs showed muscular atrophy.		IIIA 6.7 (04) Furst, R. and Schlauder, M.C. (1977): Inactivity of two noble metals as carcinogens. J Environ Path Toxicol 1 Environ. Health Perspect 40.

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
Various	Rat	Colloidal silver dose and number of animals unknown		Inconclusive (no information about frequency in controls)	The document summarises effects of metals observed in different studies. Information relevant for silver is limited to a sentence stating that weekly injections of colloidal silver in rats have resulted in a few tumors (Schmahl and Steinhoff (1960).	
Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
	B6C3F1 mice (300/sex) Fischer 344 rats (350/sex)	Silver zinc zeolite, denoted in the article "Antibacterial Zeolite Zeomic" Silver content 2.6% average zinc content 14.5%. mice: 0.1%, 0.3% and 0.9% rats : 0.01, 0.03, 0.1 and 0.3% Oral (in diet)		See 6.5(05) and 6.5(06) The document seems to be a published report of the study presented in 6.5(05) and 6.5(06). The document does not add any further information than what is presented below.	Article in Japanese, only abstract available in English.	IIIA 6.5(02) 6.7(03) Japanese Journal of Food Chemistry Vol 2 (1) 1995
Combined chronic and	Mouse B6C3F175/sex*	Silver zinc zeolite, denoted AgION Zeomic AJ 10N	NOAEL not determined	**No statistically significant increase of tumours in**		IIIA 6.5-05 (1992a)

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
carcinogenicity Reliability 2-3		(2.3% Ag, 12.5% Zn) 0, 0.1, 0.3 and 0.9% "at least" 0, 67, 211 and 617 mg/kg bw/day 0, 0.67, 2.0 and 6.9 mg silver ion equivalents/kg bw Oral	LOAEL: 0.1% (~0.67 mg silver ion equivalents/kg bw)	**treated animals.** 0.9% ↓RBC, HCT, MCH, MCV, Hb ↑MCHC ↑ renal cysts* (M, F) ↑enlargement of Langerhan´s islands (M) ↓kidney (8%), liver (10%), brain, weight (10%) (F) ↑pancreas (19%, M) ↑pigmentation of liver and pancreas 0.3% ↓HCT, MCV, Hb ↑MCHC (F) ↑ ovarian cysts ↑pigmentation of liver and pancreas 0.1% ↑ ovarian cysts ↑pigmentation of liver and pancreas ***Other effects;*** *0.9%* *↓bodyweight gain <10% (M)* *↑severity of thrombi (M, F)* *↓spleen weight (37%, M)* *↓brain (10%, F)* *0.3%* *↓bodyweight gain <10% (M)* *↓spleen weight (31%, M)*		

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
				↓brain (6%, F) *0.1%* *↓spleen weight (31%, M)* *↓brain (6%, F)* *dose-response		
Combined chronic and carcinogenicity Reliability 2-3	Rat70/sex**	Silver zinc zeolite denoted AgION Zeomic AJ 10N (2.3% Ag, 12.5% Zn) 0.01, 0.03, 0.1 and 0.3% ("at least" 0, 3, 9, 30 and 87 mg /kg bw/day) 0.03, 0.09, 0.3, 0.9 mg silver ion equivalents/kg bw Oral 105 weeks	NOAEL: 0.01 % (~0.03 mg silver ion equivalents/kg bw/day)	**Statistically significant positive trends for:** **Leukemia (m,f)** **Pituitary adenomas (f)** ***Endometrial polyps*** 0.1 % ↑Pigmentation of liver, kidneys, pancreas, stomach, lymph nodes choroid plexus ↑ALT (M/F 175/58%), AST (F 96%), ALP (M/F 25/39%), LDL-C (M/F 28/19%) ↑endometrial polyps ↑WBC (F 134%) ↓ HCT (10%), MCH (3/3%), MCHC (F 3%), Hb (F 12%) 0.03% ↑endometrial polyps ***Other effects:*** *all dose levels* *↑Severity of hepatic bile duct proliferation* *↓AST*		IIIA 6.5-06 (1992b)

Summary table of carcinogenicity studies in animals						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance, Dose levels, Route of exposure, Duration of exposure**	**NOAEL, LOAEL**	**Results** *(Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects)*	**Remarks** *(e.g. major deviations)*	**Reference**
				(M ≤42%, at 12 months) *↑ALT* *(M ≤172%, at 24 months)* *↓LDH (F≤90%, at 24 months)* *0.3%* *↓thymus weight n.s.s(38%, F)* *0.1, 0.3%* *↓TP (M ≤10%, M ALB ≤10%*		
* Termination: five/sex at 3 months, ten/sex at six months, ten at 22 months and the remaining at 24 months. ** Termination: ten rats/sex at 6 and 12 months and the remaining at 24 months.						

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

There is no robust and relevant information to assess the carcinogenic potential of silver nitrate. The information is limited to studies available in the open literature and to a chronic/carcinogenicity study performed with silver zinc zeolite type AJ.

Little is thus known about the carcinogenic potential of silver substances and studies available from the open literature are generally poorly reported and data is thus insufficient to allow for a conclusion whether or not silver and/or silver ions have an intrinsic carcinogenic potential. According to review articles there is no known association between human exposure to silver and cancer but it may not be safe to rely on a historical "safe use" of silver as consumer uses of silver compounds are changing with emerging uses in treated articles such as textiles and dental mouth guards resulting in new types of exposure scenarios for silver ions.

The literature data submitted and summarised in 6.5(07)/6.7(02) and 6.7 (04-05)) is mainly based on a study by Schmahl and Steinhoff (1960) and a study by Furst, R. and Schlauder, M.C. (1977). In the study by Schmahl and Steinhoff, subcutaneous injections of colloidal silver resulted in tumours in rats surviving longer than 14 months. Six of the eight tumours found among the 26 rats (23%) were located at the injection site. There were no vehicle controls included in the study but the spontaneous tumour frequency at any site was stated to be 1-3%. Based on this scarce information, it seems as if the frequency of tumours located at other sites was 2/26 (7.7%) and thus above the spontaneous frequency. In contrast, no fibrosarcomas developed at the injection sites in Fischer 344 rats intramuscularly injected with silver metal powder (Furst and Schlauder). A few cases of mild local inflammation were noted at injection sites but only in the latter stages of the study. At necropsy there were several incidences of encapsulation of the vehicle or injected metal powder but none of the injected legs showed muscular atrophy. The summary document in 6.5(07)/ 6.7(02) states that local sarcomas have been observed after subcutaneous implantation of silver foil. The document refers to Furst (1979) who states that the relevance of such results for exposure via ingestion is difficult to interpret as they may arise due to a phenomenon called solid state carcinogenesis. The ATSDR report submitted in 6.2 (08) states that subcutaneous imbedding of silver foil seemed to produce fibrosarcomas earlier and more frequently than several other metal foils. However, the results were only preliminary since the analysis of some of the metals was not complete at the time of publication.

The quality of the original test data cannot be assessed from this second-hand information. Considering the poor quality of other studies in the dossier that were published around the same time (1956), the original publications are not expected to provide further information and they have thus not been requested from the applicant. Overall, no conclusion with respect to the carcinogenic potential of silver ions and silver nitrate can be made based on this data.

Other data: The carcinogenic potential of silver zinc zeolite, a silver substance containing approximately 2.4 % silver was investigated in a chronic toxicity/carcinogenicity study in mice.

Mice: at termination, the total number of tumours per animal was lower in high dose males (1.00) compared to controls (1.26) and comparable between high dose females and controls. A statistically significant increase in the incidence of ovarian cysts was evident although there was no clear dose-response. The frequency was increased already in the low dose group. Based on the results of this study, AgION type AJ is not considered carcinogenic in mice.

Rats: At termination, the total number of tumours per animal was lower in high dose males (1.86) compared to controls (1.96). In contrast, a higher number of total tumours was observed in high dose females (2.11) compared to controls (1.37) but the difference was not statistically significant. The statistical analysis did however reveal a dose-related increase in the frequency of leukemia and infiltration of leukemia cells into different tissues in both male and female rats. Since the tumorous/non-tumorous changes observed were combined for scheduled and intercurrent deaths, it is not clear when in time the leukemia developed. The increased frequency of leukemia was dismissed by the study author since the frequency was claimed to be within the range observed in historical control data (referred to as Tajima Y, Data of biological characteristics of experimental animals, Soft Science Inc., 1989). While historical control data may be useful when analysing deviations in isolated data points, it is not considered appropriate to disregard a positive trend based on historical data. The P values obtained in a Cochran-Armitage trend test are 0.026 and 0.019 (one sided) for females and males, respectively. The positive trend is thus clearly statistically significant, and it is considered unlikely that this would arise in both males and females in the absence of a true effect. According to the study report, tissues from the right femoral bone were collected but it is not clear if the bone marrow was analysed for histopathological changes. According to the study report, the dose related increase in pituitary adenomas and endometrial polyps observed in females were statistically significant but the findings were dismissed by the study authors since they were irregularly distributed and lower than the incidence in the historical control data referred to.

In similarity with the line of reasoning for leukemia, it is not considered accurate to dismiss a statistically significant trend by historical control data (especially since the historical control data referred to is not included in the report). The pituitary adenomas observed are therefore regarded as being related to treatment. However, the positive trend for endometrial polyps was dismissed by the Technical Meeting for Biocides in June 2013 on the basis that the dose-response for the endometrial polyps was not considered strong enough to justify it as a real effect. Therefore, it is not given further significance here.

No additional information is available in the REACH registration dossier however a recently published study investing RNA epression, DNA methylation and cell proliferation following 6-week treatment with nanoparticles of silver (10 and 75 nm in size) is available in the open literature. The results from this study indicate that treatment with nanosilver resulted in a different expression of a substantial number of genes whereas only marginal effects on DNA methylation were observed. Moreover, the results indicate that nanoparticles of silver are pro-fibrotic (increased collagen deposition), induces epithelial-mesenchymal transition (EMT) as evidenced by an increased invasion index, anchorage independent cell growth, as well as cadherin switching and induced cell transformation. While these results may indicate that nanosilver and perhaps the silver ion could have tumour-promoting properties, in the absence of robust animal data they are not considered as sufficient evidence to fulfil criteria for classification.

Table 52: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
No data								

10.9.2 Comparison with the CLP criteria

There is no data available considered appropriate to assess the intrinsic carcinogenic potential of silver nitrate or the silver ion. Results from published in vitro data indicate that nanosilver may have tumour-promoting properties however in the absence of robust animal data they are not considered as sufficient evidence to fulfil criteria for classification. According to section 3.6.1 of CLP, evidence for classification in category 2 *"may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."*

Based on the study in mice and rats, classification in category 2 was originally proposed for silver zinc zeolite taking into consideration the CLP guidance to criteria. Following public consultation and discussions during the 39th meeting, RAC concluded that the results of the study did not meet criteria for classification taking into consideration the following:

"i. the weak statistical significance of the reported incidences in pituitary adenomas without carcinomas

ii. the weak statistical significance of incidences in leukaemia in a very susceptible strain of rats and the absence of leukemia in mice;

iii. the similar cumulative survival rate and the mean survival time in rats and mice;

iv. the comparable ratio of tumours/animal among control and exposed rats and mice at the termination of the studies;

v. the doubts on the human relevance of the leukaemia reported in rats; and

vi. the apparent sex dependence of the reported tumours."

Nevertheless, since the highest concentrations of silver ion equivalents tested in the chronic/carcinogenicity study in rats and mice are 0.9 and 6.9 mg/kg bw/d respectively, the data on silver zinc zeolite is not considered representative of silver nitrate at doses up to the maximum tolerated dose below the limit for corrosivity. Since no other in vivo data of sufficient quality is available to assess the carcinogenic potential, the information is considered inconclusive for the assessment of carcinogenicity.

10.9.3 Conclusion on classification and labelling for carcinogenicity

The information available is considered inconclusive to assess if criteria for classification for carcinogencity are fulfilled. Therefore, no classification is proposed.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 53: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No substance-specific data available			

Table 54: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 55: Summary table of other studies relevant for toxicity on sexual function and fertility (effects in italics are discussed in section 10.10.5)

Method, Guideline, GLP status, Reliability	Species, Strain, Sex, No/ group	Test substance Dose levels, Duration of exposure	Results	Remarks *(e.g. major deviations)*	Reference
The study was performed according to the current protocols for testing foods and food additives (FDA CFSAN Redbook, 2000).	Sprague-Dawley [Crl:CD®(SD) IGS BR] 20/sex	Silver acetate KSCN %Ag: 63.7-65.5% 0, 0.4, 4.0 and 40.0 mg/kg bw/d approximately 0, 0.25, 2.5 and 25 Ag+ mg/kg bw/d	**Parental:** 40 mg/kg bw/d Organ weights (f): ↓ stomach (40%) ↓ liver (9%) ↓ Feed consumption (16%) until lactation day 18 (f) **Sexual function and fertility:** 40 mg/kg bw Female fertility index ↓ 10% (not stat analysed) Implantations ↓ 22% (11.3 compared to 14.4 in control)	The main deficiencies of this study include the lack of GLP compliance, lack of individual animal data and the lack of further investigations such as oestrus cycle, sperm parameters and histopathological analyses of reproductive tissues (i.e. histopathological examinations of vagina, uterus and ovaries)	IIIA 6.8.2-06

Silver Acetate: Preliminary Reproductive Performance Study in the Sprague Dawley Rat by Dietary Administration	Sprague-Dawley [Crl:CD(SD)] F0: 0, 4, 40, 80, 160, 320 mg/kg bw/d 12/sex F1: 0, 4, 40 mg/kg bw/d 10/sex	Silver acetate (AgAc) >99.5%	**Parental F0 females:** **320 mg/kg bw/d*** ↓ Bwg (pre-pairing): 62% ↓ Bwg (gestation, d 0-20): 58% 4/12 killed following total litter loss GD22-LD2; 8/12 killed for welfare reasons GD20-LD1 Pale inactive mammary tissue 4/12 **160 mg/kg bw/d females*** 2/12 killed following total litter loss GD22-LD1; 10/12 killed for welfare reasons GD21-LD4 Pale inactive mammary tissue 2/12 Abnormal colour and content of GI tract, abnormal colour of Liver, pancreas, spleen: and mesenteric lymph nodes **80 mg/kg bw/d females:** Abnormal colour of Pancreas: 11/12 Kidney: 10/12 Mesenteric lymph nodes: 8/12 **40 mg/kg bw/d females:** ↑ ALP (40%) ↑ P (80%) ↑ gGT (1 vs 0 in control) ↑ A/G (13%) Abnormal colour of Pancreas: 11/12 Mesenteric lymph nodes: 2/12	The purpose of this study was to assess the influence of Silver Acetate on reproductive performance to assist in dose level selection for an EOGRTS according to OECD443. As animals/offspring in 40 mg/kg bw/day group had not shown a significant response to treatment, a further dose level of 80 mg/kg bw/day was instigated to enable dose selection for the subsequent extended one generation study. An additional group (Group 7) comprising of 12 male and 12 female animals was allocated to study and treated for four weeks before pairing, during gestation and up to weaning on Day 21 of lactation when all adults and offspring were terminated. Bioanalysis of Cu, Se, ceruloplasmin and glutathione peroxidase activity: see text	Covance Study Number 8436495 Report Issue Date 28 June 2021 (Draft 3)

			Parental F0 males: 320 mg/kg bw/d ↓ Overall bwg (d1-64): 20% ↑ Platelet counts (35%) ↓ hematocrit (6%) ↓ hemoglobin (11%) ↓ MCH (12%) ↓ MCV (8%) ↓ MCHC (4%) ↑ ALP (27%) ↑ Cholesterol ↑ ALT (98%) Abnormal colour of Liver: 11/12 Pancreas: 12/12 Mesenteric lymph nodes: 5/12 Abnormal content of: Cecum: 11/12 Rectum: 4/12 **Parental F0 males: 160 mg/kg bw/d** ↑ Platelet counts (44%) ↓ hematocrit (9%) ↓ hemoglobin (14%) ↓ MCH (16%) ↓ MCV (11%) ↓ MCHC (5%) ↑ ALP (40%) ↑ Cholesterol (48%) Abnormal colour of Liver: 7/12 Pancreas: 10/12 Mesenteric lymph nodes: 5/12 Abnormal content of: Cecum: 7/12 Rectum: 4/12 **Parental F0 males: 40 mg/kg bw/d** ↑ Platelet counts (23%) ↑ ALP (40%)		

			↑ Cholesterol (47%) **Sexual function and fertility:** no adverse effects on pre-coital interval, mating performance and fertility **Offspring F1, 320 mg/kg bw/d:** Post-implantation survival index: 54.1% (control: 94.9%) Live birth index: 15.3% (control: 96%) ↓Bw (day 1): 24% **Offspring F1, 160mg/kg bw/d:** Post-implantation survival index: 78.7% (control: 94.9%) Live birth index: 37.8 % (control: 96%) ↓Bw (day 1): 25% ***terminated for welfare reasons between GD20 and LD4**		
Silver Acetate: Extended One Generation ReproductiveToxicity Study in the Sprague Dawley Rat by Dietary Administration	Sprague-Dawley [Crl:CD(SD)] F0: 0, 40, 80, 120 mg/kg bw/d 25/sex F1: 0, 40, 80, 120 mg/kg bw/d 10/sex Cohort 1A: 20/sex (23 m in 120 mg/kg bw/d group) Cohort 1B: 20/sex (17 m/18 f in 120 mg/kg bw/d group) Cohorts 2A, 2B, 3: 10/sex	Silver acetate (AgAc) >99.5%	**Parental F0 females:** **120 mg/kg bw/d*** Mortality: 1/25 ↑ HCT: w 10: 10%, st5% ↑ Hb, st* 4% ↑ RBC: w 10: 14%, st*: 10% ↓ MCH: w 10: 10%, st*: 5% ↓ MCHC: w 10: 7% ↓ MCV: w 10: 4%, st*: 4% ↑ RDW, st: 19%, Plt: ↓w 10: 37% st*: 16% ↓ PT: w 10: 8%, ↑ ALP w 10: 77% ↑ AST w 10: 17%	Abnormal colouration of tissues were observed in all treated animals	Labcorp Study Number 8437234 Report Issue Date 24 January 2022

			↑ gGT st*: 1 compared to 0 ↑ Chol w 10: 77%, st*45% ↓ K: w 10: 12, st*: 8% ↑ heart/rel bw: 10% ↑ epithelial degeneration of the glandular mucosa 5/23 (minimal), 5/23 (slight), 0 in cntrol **80 mg/kg bw/d females*** ↑ HCT: w 10: 9% st*: 6% ↑ Hb, st* 5% ↑ RBC: w 10: 13%, st* 10% ↓ MCH: w 10: 9%, st* 5% ↓ MCHC: w 10: 6% ↓ MCV: w 10: 3%, st* 4% ↑ RDW, st: 9% ↓ Plt: w 10: 42%, st: 21% ↑ ALP w 10: 39% ↑ Chol w 10: 63%, st*27% ↓ K: w 10: 11% ↑ heart/rel bw: 10% ↑ epithelial degeneration of the glandular mucosa 5/24 (minimal) **40 mg/kg bw/d females:** ↑ HCT: w 10: 7%, st* 6% ↑ Hb, st* 6% ↑ RBC: w 10: 10%, st* 8% ↓ MCH: w 10: 8% ↓ MCV: w 10: 3% ↓ Plt: w 10: 43%, st* 17% ↑ RDW, st: 7% ↑ ALP w 10: 39%		

			↑ Chol w 10: 46%, st*19% ↓ K: w 10: 17% ↑ heart/rel bw: 5% ↑ epithelial degeneration of the glandular mucosa 3/25 (minimal) **Parental F0 males:** **120 mg/kg bw/d** ↑ Mortality 2/25 ↓ Overall bwg (d0-19): 7% ↓ HCT, st: 5% ↓ Hb, st* 11% ↑ RBC, st*: 7% ↓ MCH, st*: 18% ↓ MCHC, st*: 6% ↓ MCV, st*: 12% ↑ RDW, st: 35%, ↑ Plt, st*: 27% ↑ WBC, st*: 25% ↑ L, st*: 33% ↑ E, st*: 64% ↑ Plt, st*: 27% ↑ ALP, st*: 27% ↓ Creat, st*: 7% ↑ Chol, st*: 61% ↓ K, st*: 5% ↓ P, st*: 13% ↑ spleen/rel bw: 15% ↓Abs weight left testis: 5% ↓Abs weight left epididymis: 4% ↓Abs/rel prostate: 13% ↑ extramedullary hematopoiesis in spleen: Minimal: 15/23 (6/25 in control) Slight: 1/23 (0/25 in control) Marked: 0/23 (6/25 in control) **Parental F0 males:** **80 mg/kg bw/d**		

			↓ Overall bwg (d0-19): 8% ↓ Hb, st* 7% ↑ RBC, st*: 8% ↓ MCH, st*: 14% ↓ MCHC, st*: 5% ↓ MCV, st*: 10% ↑ RDW, st: 28%, ↓ Creat, st*: 4% ↑ Chol, st*: 57% ↓ K, st*: 6% ↓Abs weight left testis: 7% ↓Abs weight left epididymis: 7% ↓Abs/rel prostate: 17% ↑ extramedullary hematopoiesis in spleen Minimal: 12/24 (6/25 in control) Slight: 0/24 (0/25 in control) Marked: 2/24 (6/25 in control) **Parental F0 males:** **40 mg/kg bw/d** ↓ MCH, st*: 5% ↓ MCV, st*: 3% ↑ RDW, st: 11%, ↓ Creat, st*: 14% ↑ Chol, st*: 66% Minimal: 6/25 (6/25 in control) ↓Abs/rel prostate: 10% **Sexual function and fertility:** **F0 120 mg/kg bw/d:** ↓ sperm weight: 6% ↓ total sperm: 15% ↓ percent slow sperm motion: 24% **F0 80 mg/kg bw/d:** ↓ sperm weight: 7%		

			F1, cohort 1A (all animals) **120 mg/kg bw/d:** ↓ Cauda epididymis weight 37% ↓ Cauda epididymis total sperm 45% ↓ testis weight 15% ↓ testis spermatid count (total) 18% **80 mg/kg bw/d:** ↓ Cauda epididymis weight 10% ↓ Cauda epididymis total sperm 19% ↓ testis weight 11% ↓ testis spermatid count 31% ↓ testis spermatid count (total) 37% **40 mg/kg bw/d:** ↓ Cauda epididymis total sperm 20% ↓ testis spermatid count 19% ↓ testis spermatid count (total) 24% **Offspring F1, 120 mg/kg bw/d:** ↓Live day 1 (before culling): 19% ↓Sex ratio (before culling): 9% ↓AGD m/f: 9/11% ↑ dark coloration of skin ↓thymus weight (m/f):40/38%, rel bw m/f: 30/27 ↓abs brain weight (m/f): 8/% rel: ↑5% (f) **Offspring F1, 80mg/kg bw/d:** ↓Sex ratio (before culling): 17%		

			↓thymus weight (m/f):25/32 %, rel bw m/f: 16/25 ↓abs brain weight (f): 4% **Offspring F1, 40mg/kg bw/d:** ↓Sex ratio (before culling): 13% ↓thymus weight rel bw: 13 (f:) **F1: 120 mg/kg bw**** Mortality (males): 9 (3 in 1A, 2 in 1B, 3 in 2A, 1 in 3) (females): 2 (both in 1A) ↑ Piloerection (m: 7/70, f 1/70) ↑ Hunched posture (m: 7/70, f 1/70) ↑ Abnormal gait (m: 4/70, f 1/70) ↓ Body weight at weaning m/f: 13/12% ↓ HCT f: 6% ↓ RBC f: 8% ↑ MCH m/f: 5/5% ↑ MCHC m/f: 3/3% ↓ RDW f: 5% ↑ WBC f: 128% ↑ N f: 109% ↑ L f: 142% ↑ E f: 180% ↑ B f: 300% ↑ M f: 150% ↑ LUC m/f: 133/150% ↓ Plt f: 15% ↓ Pt f: 2% ↓ APTT, f: 17% ↓ APTT, f: 17% ↑ ALP m/f: 215/218% ↑ ALT m/f: 71/115% ↑ AST f: 21% ↑ urea m: 26% ↓ creat m/f: 29/33% ↑ gluc m/f: 75/59%		

			↑ chol m/f: 70/58% ↑ K m/f: 28/13% ↑ Ca m: 3% ↓ Phos, m/f: 10/16% ↓ Total prot, f: 8% ↓ Total Alb, f: 10% ↓ A/G ratio, f: 9% **F1: 80 mg/kg bw** ↓Body weight at weaning m/f: 9/10% ↓Bodyweight gain (d 1-71) m: 14% ↑ HCT f: 5% ↑ RBC m/f: 6/7% ↓ MCH m: 8% ↑ MCHC m/f: 3/3% ↓ RDW f: 5% ↑ WBC f: 66% ↑ N f: 105% ↑ L f: 78% ↑ E f: 200% ↑ B f: 200% ↑ M f: 200% ↑ LUC f: 100% ↓ Plt f: 31% ↓ Pt f: 6% ↑ ALP m: 28% ↑ ALT m: 31% ↓ creat m/f: 7/9% ↑ chol m/f: 40/45% ↑ Ca m: 3% ↓ Phos, f: 16% **F1: 40 mg/kg bw** ↓Bodyweight gain (d 1-71) m: 6% ↑ RBC f: 4% ↑ WBC f: 51% ↑ B f: 100% ↑ M f: 100% ↑ LUC f: 150% ↑ ALP m: 21% ↓ creat m/f: 14/12% ↑ chol m/f: 60/29% **Cohort 1A:** **120 mg/kg bw/d:**		

			brain weight Abs/rel: M: ↓9/↑25% F: abs ↓7% heart weight Abs/rel: M: ↓12/↑18% F: rel ↑14% thymus weight Abs/rel: M: ↑31/75% F: ↑31/46% Adrenal weight M (abs): ↓24% F (abs/rel): ↓21/11% Kidney weight Abs/rel: M: ↓22/↑7% F (abs): ↓22 Pituitary weight Abs/rel: M(abs): ↓27 F ↑6/22% Thyroid and parathyroids: Abs/rel: M(abs): ↓21 F ↓13/10% Testis, left: Abs/rel: ↓19/↑13% Testis, right: Abs/rel: ↓12/↑22% Epididymis, left: Abs: ↓31% Epididymis, right: Abs/rel: ↓33/8% Prostate: Abs/rel: ↓39/18% Seminal vesicles: Abs/rel: ↓45/26% Uterus, cervix, oviducts: Abs/rel: ↓28/21% **80 mg/kg bw/d** ↑ brain weight Abs/rel: M: ↓4/↑7%		

			F: abs ↓7% heart weight rel m/f: ↑7/9% F: rel /↑14% Adrenal weight M (abs): ↓14% F (abs/rel): ↓15/13% Kidney weight Abs/rel: M(abs): ↓23 F ↓10/8% Pituitary weight Abs/rel: M: ↓20/10 Testis, left (abs): ↓19 Testis, right (abs): ↓10 Epididymis, left: Abs: ↓9% Epididymis, right: Abs: ↓11% Prostate: Abs: ↓15% **40 mg/kg bw/d** ↑ brain weight Abs (m):↓4 Adrenal weight M (abs): ↓10% F (abs/rel): ↓15/13% Kidney weight Abs/rel: M(abs): ↓14 F ↓9/8% Pituitary weight M (abs): ↓13% **Cohort 1B:** **120 mg/kg bw/d** heart weight Abs/rel: M: ↓15/↑24% Testes: Abs/rel: ↓15/↑27% Epididymis Abs: ↓37% Prostate: Abs/rel: ↓46/23%		

			Seminal vesicles: Abs/rel: ↓50/29% Uterus, cervix, oviducts: Abs/rel: ↓33/28% **80 mg/kg bw/d** heart weight M(rel): ↑12% Testes (abs): ↓10% Epididymis Abs: ↓9% Prostate (abs):↓19% Seminal vesicles (abs): ↓13% **40 mg/kg bw/d** heart weight M(rel): ↑8% Prostate (abs):↓13% **Cohort 2A:** **120 mg/kg bw/d:** brain weight Abs/rel: M: ↓4/↑14% **80 mg/kg bw/d:** brain weight Abs (m): ↓8% **40 mg/kg bw/d:** brain weight Abs (m): ↓4% **Cohort 3:** **120 mg/kg bw/d:** spleen weight rel (m/f): ↑34/29%		
*st = scheduled termination ** this group was terminated for welfare reasons at approximately 10 weeks of age Statistical analysis of organ weight data for high dose animals is not scientifically valid since data is not contemporary with controls.					
A review of reproductive and developmental toxicity of silver nanoparticles in laboratory animals	Various laboratory animals			Study summaries of publications referred to and performed in vivo via the oral route are	Makoto Emaa,*, Hirokazu Okudab, Masashi Gamoa,

				presented in an appendix.	Kazumasa Honda Reproductive Toxicology 67 (2017) 149–164
OECD TG 416 Oral in diet	Rat SpragueDawley Crl: CD® IGS BR 28/sex	Silver sodium zirconium hydrogenphosphate Exp.add 9823-37 (10% Ag) 1000, 5000 and 20000 ppm corresponding to 72.5/78.2, 363/400 and 1465/1612 mg a.s/kg bw in P males and females (premating). Approximately 1.9, 9.9 and 40 mg silver ion equivalents/kg bw/d in females). Maturation, mating, gestation and lactation for two successive generations.	**Parental:** P 20 000ppm: No mortalities ↑pigmentation (pancreas) ↓ thymus weight (20% m), adrenals (14%), kidneys (m, 16%) ↑spleen weight (m, 11%), rel brain weight (m, 9.7%) P 5000ppm: No mortalities ↑pigmentation (pancreas) ↑spleen weight (m, 20%) F1 20 000: ↑mortality (4m, 2f, none in control) ↓bodyweight: pairing (≤ 16%), gestation* (≤ 10%) lactation (≤ 10%) ↓food consumption: pairing (≤ 20), m), gestation, lactation (≤22%) ↑pigmentation (pancreas, lymph nodes, thymus) F1 5000 ppm: 1 mortality (f) ↑pigmentation (pancreas, lymph nodes, thymus) **Sexual function and fertility:** P: Statistically significantly decreased absolute weight of seminal vesicles/coagulating		IIIA 6.8.2-03 (2002)

			gland at 20000 ppm (14%), and 5000 ppm (14%) as compared to the ctrl. A dose-related decrease in the prostate weight (statistically non-significant): 0.847, 0.813, 0.799 and 0.744 g at 0, 1000, 5000 and 20000 ppm Semen parameters: statistically significantly lower lateral amplitude and higher straightness at the high dose as compared to the control. F1: Males at 20000 ppm: statistically significantly reduced absolute weight of seminal vesicles/coagulating gland and right testis, increased relative epididymides weight and reduced absolute and relative prostate weight. Males at 20000 ppm and 5000 ppm: Statistically significant differences in group mean sperm head area. Females at 20000 ppm: statistically significantly reduced absolute/relative uterus weight, 1 dystocia, increased pre-coital interval, lower total number of ovarian follicles, lower		

OECD TG 416	Rat SpragueDawley Crl: CD® (SD) IGS BR 30/sex	Silver zinc zeolite AgION Silver Antimicrobial Type AK Oral in diet m/f: 0, 1000, 6250, 12500 ppm ~72/87, 472/548, 984/1109 mg/kg bw (premating) This corresponds to approximately 1.5/1.8, 9.8/11.3; and 20.3/22.9 mg silver ion equivalents/kg bw/d in males and females Maturation, mating, gestation and lactation for two successive generations	**Parental effects:** **P 12500 ppm:** ↑ Mortality (m 10%) ↓Bodyweight (m≤10% (pre/post pairing, f 6% gestation day 20, ≤ 11%) ↓Bodyweight gain (m≤17% (pre pairing), f gestation 14-20:29% 0-20:16%) ↓Food consumption (premating m ≤8%, lactation 0-4:27%, 4-7: 12%, 7-14: 21%, 14-21: 27%) ↑RBC (m/f 13/15%), platelets (m/f 42/45) ↓Hb (m/f 16/12%), HCT (m 9%) MCH (m/f 25/23%) MCHC (m/f 7/6%), ↑Pigmentation of organs ↑Histopathological changes in kidneys (including hydronephrosis (8m/2f , 3m in controls) , urinary tract ↓ kidney weight (m abs/rel 14/3%, f rel brain 7%) rel brain weight (m, 9%) Spleen (m, 7%) **P 6250 ppm:** ↑ Mortality (m, 3.3%) ↑RBC (f 11%), ↓ MCV (m/f, 6/9%),	Read across	IIIA 6.8.2 (04) (2002)

			MCH (m/f 6/12%), MCHC (f, 3%) ↑Pigmentation of organs ↑Histopathological changes in kidneys (including hydronephrosis 7m/2f, 3m in controls)) ↓kidney weight (m, abs/rel bw 13/7%) spleen (m, abs/rel bw 14/21%) **P 1000 ppm:** ↑Pigmentation of organs **F1** **F1 12500 ppm:** ↑Mortality (m/f 93.3/76.7%) ↓Bodyweight (premating m/f ≤ 56/46%) ↓Bodyweight gain (premating m/f ≤ 47/40%) ↑Histopathological changes ↑Thymus atrophy **F1 6250 ppm:** ↑Mortality (m/f 23.3/3.3%) ↓Bodyweight (premating w1-10 m/f 25-13/19-2 (n.s.s)%, post-pairing m ≤12%, gestation n.s.s, lactation≤ 10%) ↑Histopathological changes (including hydronephrosis 10		

			m/4f , 0 in controls) ↑Kidney weight (m/f, abs 19/11%, rel bw 9/8%, rel brain 13/7%) ↓Brain (m/f, 7/5%) Adrenal (m, abs 18%, rel brain 12%) Spleen (m, rel bw 11%) Liver (f, 8%) ↑Thymus atrophy (thymus not weighed in F1 adults) **F1 1000 ppm:** ↑Mortality (m 3.3%) ↑Pigmentation of organs ↑ Hydronephrosis (3m, 1f, 0 in controls) **Sexual function and fertility:** **P:** P 12500 ppm: Slightly increased gestation length 22.3 vs 21.9 in ctrl (statistically significant) Increased relative weight of epididymis left/right (rel bw 11**/9%**) and testis (rel bw left/right 12**/10*%) P 6250 ppm: Slightly increased gestation length 22.3 vs 21.9 in ctrl ((statistically significant)		

			P 1000 ppm: Slightly increased gestation length 22.3 vs 21.9 in ctrl **F1:** F1 12500 ppm: Due to excessive toxicity the animals were killed prior to mating. ↑day of vaginal opening (day 59.9, control: 35.1) and preputial separation (day 56.7, control: day 44.5) Sexual organ weights determined only in 2 males and 7 females at terminal sacrifice, no statistical analyses performed. F1 6250 ppm: Statistically significant decrease in the absolute weight of epididymis, testis, prostate, seminal vesicle and uterus/oviducts/cervix as compared to ctrl. The weight of right testis and prostate relative to the brain weight were statistically significantly lower than those in the ctrl. Higher percentage of abnormal sperm (0.50 vs 0.18 in ctrl)		

			↑day of vaginal opening (day 39.8) and preputial separation (day 47.4) 1000 ppm: Higher percentage of abnormal sperm (1.41 in vs 0.18 in controls) * = Significantly different from control value ($p<0.05$) ** = Significantly different from control value ($p<0.01$) *** = Significantly different from control value ($p<0.001$)		

Abstracts (from the original publications) of additional studies on sexual function and fertility included in the REACH registration dossier on Silver EC number: 231-131-3; CAS number: 7440-22-4:

Gromadzka-Ostrowska et al., 2012. Silver nanoparticles effects on epididymal sperm in rats. Toxicology Letters 214, 251 - 258.

The motivation of our study was to examine the acute effects of intravenously administered a single bolus dose of silver nanoparticles (AgNPs) on rat spermatogenesis and seminiferous tubules morphology. In the treated rats compared to the vehicle treated control animals, the experiments revealed a size-dependent (20nm and 200nm), dose-dependent (5 and 10mg/kg body mass) and time-dependent (24h, 7 and 28days) decrease the epididymal sperm count measured by histological methods. In parallel AgNPs injection increased the level of DNA damage in germ cells, as measured by alkaline comet assay. Histological examination of the testes showed change in the testes seminiferous tubule morphometry in 200nm Ag NPs treated rats. No change of body weight, adipose tissue distribution and the frequency of abnormal spermatozoa was observed. Twenty nanometers AgNP appeared to be more toxic than 200nm ones.

Castellini et al., 2014. Long-term effects of silver nanoparticles on reproductive activity of rabbit buck. Syst. Biol. Resprod. Med. 60, 143-150

Using the rabbit as an animal model, this study evaluated the long-term effect of silver nanoparticles (NPs) administered intravenously (0.6 mg/kg bw) on reproductive activity and sperm quality. Semen analysis was performed by optical microscopy and sperm motility evaluation by computer assisted sperm analyzer (CASA). Mitochondria oxygen consumption, light and transmission electron microscopy of rabbit testis and ejaculated sperm were also carried out. Throughout the experiment NP-treated rabbits showed higher seminal reactive oxygen species (ROS), less motile sperm, and lower curvilinear velocity and oxygen consumption than control animals. In contrast, libido, serum testosterone, sperm concentration, and semen volume were hardly affected by NPs. Transmission electron microscopy analysis did not show any evident morphological damage in testes; however, Ag NPs are visible in spermatids and ejaculated sperm. These preliminary results show that Ag NPs can reach the testes, compromising sperm motility, sperm speed, and acrosome and mitochondria shape and function.

Rezaei-Zarchi et al., 2012. Effect of Silver nanoparticles on the LH, FSH and Testosterone Hormones in male rat. Med. Sci. 15, 25-29.

METHODS: In this study, silver nanoparticles with doses of 25, 50, 100 and 200 mg/kg body weight were orally administered for 28 days to 50 male rats in five groups of 10. Then blood samples were taken from rats. Hormonal tests of LH and FSH were performed by DIAPLUS Inc kit and hormonal test of testosterone was performed by DRG instruments kit and the groups were compared. FINDINGS: In this study any significant changes were not observed in the mentioned hormonal concentration up to 100 mg dose of silver nanoparticles. But significant changes in testosterone concentration (reduced concentration) in high doses, i.e. 200 mg (1.8±0.5 nmol/L vs. 2.5±0.6 nmol/L) were observed that could be due to inhibition effect of silver nanoparticles on the function of testosterone-producing cells ($p<0.05$).

Amraie et al., 2013. Investigation of the silver nanoparticle (Ag NPs) effects on the fertility potential of rats. Elixir Appl. Biology 59 (2013) 15587-15589.

In this study 75 male rats (body weight 150±20 gram, 4week year old) were used which were divided into 5 groups (1 control group and 4 experimental groups), with 15 rats in each group. Different dosages of Ag NPs (25, 50, 100, 200 mg/kg) were administered to the experimental rats in a period of spermatogenesis (35 days). After this time interval they were killed by spinal cord severing method, their epididymides were separated and in order to analyze the mobility of the sperms, a homogenous solution was prepared in the ham's medium. Moreover, in order to study the morphology of sperms we used the Giemsa-stained samples. Finally statistical data was analyzed by T-Test and SPSS software. Results showed by (p<0/05) administration of silver nanoparticle has a significant effect on the reduction of sperm mobility and its natural morphology (p<0/05).

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

There are no studies available specifically investigating the reproductive toxicity of silver nitrate. Information on the intrinsic properties of the silver ion may be obtained from studies performed with other silver substances as the silver ion is released from the substance used in the studies and is thereby indirectly tested. Based on this information it may be possible to estimate the reproductive toxicity of silver nitrate at non-corrosive concentrations. Although data obtained at concentrations where corrosivity would determine the toxicity of silver nitrate would not be reliable for the assessment, it should be noted that since the threshold of corrosivity is not known, all data is currently considered in the weight of evidence approach used in this CLH report. Based on the results of the 28-day study presented in section 10.12, it can be concluded that at least 28 systemic doses up to 100 mg/kg bw/d are well-tolerated by rats. However, since the substances differ with respect to silver content and silver release, factors determining the actual silver ion exposure, data for some substances takes precedence over other data. Some also

contain additional constituents that may influence the toxicity making them less appropriate to serve as source chemicals in a read across approach.

Recently (spring 2022), information on the reproductive toxicity of silver acetate became available in a targeted public consultation of new data relevant for classification and labelling of silver (May 2022). The information includes a preliminary reproductive toxicity study (dose-range finding study) and an extended one generation reproductive toxicity study performed in the Sprague Dawley rat. The results are presented and discussed below.

As shown in the table above there are also two fertility studies (two-generation studies) performed with silver zinc zeolite and silver sodium zirconium hydrogenphosphate, respectively, and a one-generation study performed with silver acetate. There are also many published studies performed with nanosilver available in the open literature and some of these are also included in the registration dossier submitted for silver under REACH. Due to the less complex chemical composition and the presumed exposure to silver ions (see section 10.10.3), data on silver acetate and nanosilver is considered more relevant to assess the toxicity of silver nitrate than data on silver zinc zeolite and silver sodium zirconium hydrogenphosphate. The two latter substances also contain other constituents that may influence the toxicity and are thus considered less appropriate to serve as source chemicals for read across although being performed according to recognised guidelines and the principles of GLP.

Results with silver acetate: Recently, the results of an EOGRTS as well as a preceding preliminary study performed with silver acetate became available and considered in the discussions on the classification and labelling of silver (RAC-61).

In the preliminary study performed to assist dose selection for the EOGRTS, groups of F0 rats (12/sex) received doses of 4, 40, 80, 160 and 320 mg AgAc/kg bw/day via the diet. Males were treated for 29 days before pairing and until necropsy after litters had weaned (after at least 65 days of treatment) whereas females were treated for 29 days before pairing, throughout pairing, gestation and until necropsy on Day 21 of lactation. In F1, three groups 10/sex received doses of 4 or 40 mg/kg bw/day from weaning, during late lactation and to their scheduled termination on Days 31-33 (i.e., Day 59-61 (+/- 2days) of age) following completion of sexual maturation. The F0 generation was checked for clinical condition, body weight, food consumption, estrous cycles, pre-coital interval, mating performance, fertility, gestation length, exposure assessment and biomarkers (ceruloplasmin activity, glutathione peroxidase (GPx) activity, copper, selenium and silver levels), organ weights and macroscopic and microscopic pathological investigations. In addition, haematology and blood chemistry investigations were made in all treated males and in females treated with 4 and 40 mg/kg bw/day. Litter size and survival, sex ratio, body weight, organ weights and macropathology were also assessed in all pups. The selected F1 pups administered 4 and 40 mg/kg bw/day were examined for clinical condition, body weight, food consumption, sexual maturation, exposure assessment and biomarkers, organ weights and subjected to macroscopic pathology investigations.
The following results were obtained (parameters considered adversely affected by treatment are marked in bold text):

Biomarkers:
<u>Ceruloplasmin:</u> activity decreased with increasing doses of Silver Acetate in F0 male and female animals on Day 12, in F0 males on Day 43 and female animals on Gestation Day (GD) 6. F1 males and females administered 4 mg/kg bw/day and 40 mg/kg bw/day followed the same trend on Day 21 whereas male and female animals administered 80 mg/kg bw/day showed results inconsistent with this trend.

<u>Glutathione Peroxidase:</u> (GPx) activity decreased in high dose female rats (37-42% lower than in Controls) at Day 12 and GD6. Both males and females administered 80 mg/kg bw/day showed low GPx activity.

<u>Silver:</u> levels generally increased with increasing dose levels with the highest concentrations detected in the milk pellets obtained from offspring on Day 4 of age.

<u>Copper</u> <u>and selenium:</u> serum levels tended to decrease with increasing dose and the observed effect was more marked for copper. There was evidence for a dose-related decrease in copper levels in the testes for F0 males and a non-dose related increase in selenium levels in the ovaries for F0 females. There was no

clear effect of treatment on the ovarian copper levels or the testis selenium levels. The significance of these changes in biomarkers are further discussed in section 10.10.5.

F0

Four females at 320 mg/kg bw/day and two at 160 mg/kg bw/day were killed between completion of parturition and Day 2 of lactation following **total litter loss** with offspring either found dead, missing (presumed cannibalized) or killed for welfare reasons. One of these females at 160 mg/kg bw/day had total litter loss on Day 1 of lactation, and the other dam was killed for welfare reasons on Day 1 of lactation, with 12 live offspring, three dead offspring and three missing. Three of these dams at 320 mg/kg bw/day had a total litter loss on Days 1/2 of lactation and one was killed for welfare reasons on Day 1 of lactation with three live and one dead offspring. The remaining females in these dose groups were terminated on welfare grounds between GD20 and LD4. At the macroscopic examination, the mammary tissue appeared pale and inactive in two females receiving 160 mg/kg/day and four females receiving 320 mg/kg bw/day.

Table: Summary of mortality and litter information of dams at 320 mg/kg bw/day and 160 mg/kg bw/day

Dose group	Day of death	Mode of death	Terminal signs	Litter information	Macropathology findings
320 mg/kg bw/day	GD 21	WE	Coat - Piloerection	14 implantations 14 early resorptions (implantation scars)	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal color, dark Kidneys - Granular, bilateral Liver - Abnormal color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Pancreas - Abnormal color, dark Rectum - Abnormal color, dark Rectum - Abnormal contents, dark Spleen - Abnormal color, dark
	GD 21	WE	Coat - Hair loss, Forelimbs Coat - Hair loss, Hindlimb	13 implantations	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal color, dark Duodenum - Abnormal contents, dark Ileum - Abnormal contents, dark Jejunum - Abnormal contents, dark Liver - Abnormal color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Pancreas - Abnormal color, dark Rectum - Abnormal color, dark Rectum - Abnormal contents, dark Spleen - Abnormal color, dark Stomach - Abnormal color, dark Stomach - Distended, 20-29mm
	LD2	TL	Within normal limits	14 implantations 12 offspring: 7-FD; 1-WE; 4-MI	Cecum - Abnormal contents, dark Duodenum - Abnormal contents, dark Ileum - Abnormal contents, dark Jejunum - Abnormal contents, dark Lymph Node Mesenteric - Abnormal color,

					dark, 2-5 (few) Mammary - Inactive, pale Pancreas - Abnormal color, dark Stomach - Distended, 20-29mm
	GD21	WE	Within normal limits	18 implantations Including 1 early resorption	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal color, dark Ileum - Abnormal color, dark Liver - Abnormal color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Pancreas - Abnormal color, dark Pancreas - Edematous, noted Rectum - Abnormal color, dark Rectum - Abnormal contents, dark Spleen -Abnormal color, dark
	GD21	WE	Coat - Hair loss, Dorsal surface Eyes - Dull, Bilateral	15 implantations	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal contents, dark Ileum - Abnormal contents, dark Jejunum - Abnormal contents, dark Liver - Abnormal color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Pancreas - Abnormal color, dark Rectum - Abnormal color, dark Rectum - Abnormal contents, dark Stomach - Abnormal color, glandular mucosa, dark
	LD1	TL	Coat - Hair loss, Dorsal surface Eyes - Dull, Bilateral Staining - Abnormal color, Brown, Upper dorsal surface	15 implantations 9 offspring: 9-FD	Cecum - Abnormal color, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Mammary - Inactive, pale Pancreas - Abnormal color, dark
	GD22	TL	Reason for dispatch - General poor clinical condition, Total litter loss.	15 implantations 12 offspring; 12-FD	Cecum - Abnormal contents, dark Colon - Abnormal contents, dark

			Skin color - Pallor, Whole body		
	GD21	WE	Coat - Hair loss, Dorsal surface	14 implantations	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal color, dark Duodenum - Abnormal contents, dark Ileum - Abnormal color, dark Ileum - Abnormal contents, dark Jejunum - Abnormal contents, dark Liver - Abnormal Color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Pancreas - Abnormal color, dark Rectum - Abnormal color, dark Rectum - Abnormal contents, dark Spleen - Abnormal color, dark Stomach - Abnormal color, dark Stomach - Abnormal contents, dark Stomach - Distended, 10-19mm
	LD1	TL	Within normal limits	14 implantations 9 offspring: 5-FD; 4-MI	Cecum - Abnormal color, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Mammary - Inactive, pale Stomach - Abnormal contents, fetal material
	GD20	WE	Within normal limits	10 implantations Including 1 early resorption	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal color, dark Duodenum - Abnormal contents, dark Ileum - Abnormal contents, dark Jejunum - Abnormal contents, dark Liver - Abnormal color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Pancreas - Abnormal color, dark Rectum - Abnormal color, dark Rectum - Abnormal contents, dark
	LD1	WE	Within normal limits	15 implantations 4 offspring: 1-FD; 3-WE	Mammary - Inactive, pale Pancreas - Abnormal color, dark
	GD22	WE	Within normal limits	13 implantations 1 offspring: 1-WE	Cecum - Abnormal contents, dark Duodenum - Abnormal contents, dark Ileum - Abnormal contents, dark

					Jejunum - Abnormal contents, dark Ovaries - Dark area(s), bilateral, </=1mm, 5+ (many) Pancreas - Abnormal color, dark Pancreas - Edematous, noted Rectum - Abnormal contents, dark Stomach - Abnormal contents, fetal material Stomach - Distended, 30-39mm
160 mg/kg bw/day	GD22	WE	Reason for dispatch - General poor clinical condition	14 implantations	Cecum - Abnormal contents, dark Duodenum - Abnormal color, dark Pancreas - Abnormal color, dark
	GD22	WE	Reason for dispatch - General poor clinical condition	13 implantations Including - 1 early resorption and 1 late resorption (dead fetus)	Cecum - Abnormal contents, dark Pancreas - Abnormal color, dark
	LD1	TL	Within normal limits	16 implantations 14 offspring: 13-FD; 1-WE	Pancreas - Abnormal color, dark
	LD1	TL	Eyes - Dark, Bilateral Eyes - Dull, Bilateral Skin color - Pallor, Whole body	16 implantations 7 offspring: 7-FD	Cecum - Abnormal color, dark Cecum - Abnormal contents, dark Colon - Abnormal color, dark Colon - Abnormal contents, dark Duodenum - Abnormal color, dark Duodenum - Abnormal contents, dark Ileum - Abnormal color, dark Ileum -Abnormal contents, dark Jejunum - Abnormal contents, dark Liver - Abnormal color, all lobes, dark Lymph Node Mesenteric - Abnormal color, dark, 5+ (many) Mammary - Inactive, pale Pancreas - Abnormal color, dark Rectum - Abnormal color, dark Rectum - Abnormal contents, dark
	LD1	WE	Reason for dispatch - General poor clinical condition	16 implantations 15 offspring: 6-FD; 1-MI; 8-WE	Cecum - Abnormal contents, dark Pancreas - Abnormal color, dark
	LD2	WE	Reason for dispatch - General poor clinical condition	2 implantations 1 offspring: 1-FD	Mammary - Abnormal color, pale Pancreas - Abnormal color, dark
	GD21	WE	Reason for dispatch - General poor	10 implantations	Cecum - Abnormal contents, dark Pancreas - Abnormal color, dark

			clinical condition		
	GD22	WE	Reason for dispatch - General poor clinical condition	18 implantations	Pancreas - Abnormal color, dark
	LD1	WE	Build (Deformity) - Kinked tail Reason for dispatch - General poor clinical condition	18 implantations 18 offspring: 12-FD; 6-WE	Pancreas - Abnormal color, dark
	LD3	WE	Reason for dispatch - General poor clinical condition	17 implantations 14 offspring: 5-FD; 6WE; 3-MI	Pancreas - Abnormal color, dark
	LD1	WE	Reason for dispatch - General poor clinical condition	18 implantations 18 offspring: 3-FD; 12-WE; 3MI	Mammary - Inactive, pale Pancreas - Abnormal color, dark
	LD4	WE	Reason for dispatch - General poor clinical condition	16 implantations 15 offspring: 5-FD; 5-WE; 5-MI	Pancreas - Abnormal color, dark

LD - lactation day, GD - gestation day, WE - Euthanized for welfare reasons, FD – Found dead, MI – Missing, presumed cannibalised

Routine physical examination of males from the start of treatment up to termination and for females before pairing, during gestation and lactation did not reveal any findings that could be attributed to administration of Silver Acetate at any dose levels; assessment of females during lactation was restricted to females at target dose levels of 4, 40 and 80 mg/kg bw/day.

Overall, the mean **body weight gain** for males administered 320 mg/kg bw/day was markedly low (approximately 20% of Controls), resulting in low mean body weight at the end of the treatment period. The overall body weight gain of high-dose F0 females during the four-week pre-pairing treatment period was low (approximately 38% of Controls) with an overall **gestational body weight gain** approximately 42% of Controls. Body weight gain during lactation was poor in females administered 80 mg/kg bw/day.

Table: Body weight - group mean values (g) for males (F0)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Day 1	4	8	11	15	18	22	25	29	32	36	39	43
Statistics test		Av	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	424	435	445	450	465	475	486	494	503	500	501	511	515
	SD	19.4	18.1	20.2	21.3	24.3	26.3	26.8	26.2	30.3	27.6	30.8	36.5	41.2
	N	12	12	12	12	12	12	12	12	12	12	12	12	12
2M	Mean	431	440	458	459	475	478	492	496	506	507	511	518	530
	SD	20.9	21.7	22.4	23.3	21.6	24.4	25.2	26.9	26.0	28.5	26.8	22.5	24.7
	N	12	12	12	12	12	12	12	12	12	12	12	12	12
3M	Mean	422	432	446	449	459	461	477	483	489	489	488	496	506
	SD	22.8	24.2	26.0	28.6	28.2	30.3	29.8	33.1	33.1	34.0	38.5	40.9	39.9
	N	12	12	12	12	12	12	12	12	12	12	12	12	12
4M	Mean	416	427	442	444	456	457	469	476	480	476	481	490	500
	SD	22.2	23.6	23.7	24.2	25.7	27.4	29.8	29.8	33.7	33.8	34.4	35.5	35.9
	N	12	12	12	12	12	12	12	12	11	11	11	11	11
5M	Mean	419	404**	407**	408**	422**	417**	424**	425**	430**	419**	429**	433**	440**
	SD	24.3	23.2	28.0	26.4	31.2	33.3	38.0	40.1	45.7	47.7	47.8	49.5	47.1
	N	12	12	12	12	12	12	12	12	12	12	12	12	12
7M	Mean	391	402	412	419	430	440	456	462	471	473	483	487	496
	SD	15.8	21.2	26.6	28.1	32.7	32.3	34.1	35.1	33.2	33.5	33.1	32.7	33.8
	N	12	12	12	12	12	12	11	11	11	11	11	11	11

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Day 46	50	53	57	60	64
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	524	533	540	547	548	557
	SD	37.6	39.0	41.1	38.6	43.7	47.6
	N	12	12	12	12	12	12
2M	Mean	531	544	545	552	555	562
	SD	25.7	26.9	29.9	29.5	31.4	30.8
	N	12	12	12	12	12	12
3M	Mean	509	522	524	532	534	541
	SD	42.4	44.5	43.6	42.9	43.0	43.1
	N	12	12	12	12	12	12
4M	Mean	503	512	514	523	526	531
	SD	37.1	39.2	39.2	40.4	42.7	44.7
	N	11	11	11	11	11	11
5M	Mean	439**	446**	442**	442**	442**	445**
	SD	46.7	48.2	47.3	50.6	51.9	52.0
	N	12	12	12	12	12	12
7M	Mean	501	513	513	522	518	526
	SD	32.8	33.2	34.0	31.5	33.8	33.6
	N	11	11	11	11	11	11

Table: Body weight - group mean values (g) for females before pairing (F0)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Day 1	4	8	11	15	18	22	25	29
Statistics test		Av	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	244	243	255	252	260	255	267	267	270
	SD	19.3	17.3	18.4	20.5	20.9	22.4	24.2	23.8	25.1
	N	12	12	12	12	12	12	12	12	12
2F	Mean	237	241	249	246	252	248	254	254	257
	SD	17.9	16.7	18.2	20.1	22.3	22.0	21.3	18.9	20.3
	N	12	12	12	12	12	12	12	12	12
3F	Mean	240	243	251	251	258	259	264	265	270
	SD	12.4	12.7	11.4	11.4	12.3	14.9	16.0	15.5	21.0
	N	12	12	12	12	12	12	12	12	12
4F	Mean	244	248	255	256	262	260	262	263	267
	SD	12.0	13.7	14.1	13.8	14.7	14.7	14.7	15.6	15.7
	N	12	12	12	12	12	12	12	12	12
5F	Mean	253	247	250	253	260	255	262	257	263
	SD	19.1	19.6	21.4	20.7	22.0	20.4	24.0	21.3	21.6
	N	12	12	12	12	12	12	12	12	12
7F	Mean	274	279	287	287	293	298	310	309	311
	SD	9.1	11.4	14.2	12.8	11.9	13.9	18.0	21.4	21.2
	N	12	12	12	12	12	12	12	12	12

Table: Table: Body weight - group mean values (g) for females during gestation (F0)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Day 0	3	6	10	14	17	20
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Du
1F	Mean	273	286	297	311	331	362	410
	SD	24.6	24.4	25.4	22.1	26.7	28.8	32.9
	N	12	12	12	12	12	12	12
2F	Mean	260	274	283	298	315	344	382
	SD	23.7	25.1	24.8	24.5	25.2	27.9	32.4
	N	11	11	11	11	11	11	11
3F	Mean	281	294	303	319	338	369	420
	SD	21.9	18.9	18.0	18.8	21.4	20.3	24.5
	N	11	11	11	11	11	11	11
4F	Mean	270	288	297	311	329	355	396
	SD	17.2	15.7	16.7	19.4	20.3	23.5	30.7
	N	12	12	12	12	12	12	12
5F	Mean	256	274	276*	278**	285**	291**	315**
	SD	20.3	25.1	26.5	29.0	31.9	30.0	29.5
	N	11	11	11	11	11	11	11
7F	Mean	321	331	341	354	373	408	456
	SD	25.2	20.7	21.2	21.4	20.7	19.9	22.6
	N	11	11	11	11	11	11	11

Table: Body weight - group mean values (g) for females during lactation (F0)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Day 1	4	7	11	14	18	21
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	306	318	331	344	348	342	330
	SD	26.1	31.3	31.6	24.5	28.6	20.8	22.8
	N	11	11	11	11	11	11	11
2F	Mean	288	297	310	329	329	334	318
	SD	26.1	28.0	29.0	33.4	28.8	28.3	29.4
	N	11	11	11	11	11	11	11
3F	Mean	312	325	335	350	354	355	341
	SD	17.0	19.0	18.4	16.0	21.1	19.1	16.3
	N	11	11	11	11	11	11	11
7F	Mean	348	361	362	373	363	360	346
	SD	18.7	20.3	21.2	16.3	15.0	22.5	20.2
	N	11	11	11	11	11	11	11

High dose F0 males showed statistically significantly low mean values during the majority of **food consumption** phases and high-dose females showed periods of low food consumption during prepairing and consistently throughout gestation.

At scheduled termination, mid and high-dose males showed statistically significant **changes in hematological parameters** (low hematocrit, hemoglobin level, mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume) but without dose response. **Platelet counts** for males receiving 40, 160 or 320 mg/kg bw/day were slightly but statistically significantly high.

Alkaline phosphatase activity and cholesterol levels were increased in males and females administered 40 mg/kg bw/day and statistically significantly high in males at 160 and 320 mg/kg bw/day (except female cholesterol); at 4 mg/kg bw/day the mean cholesterol levels for males was also slightly but statistically significantly high.

Test substance-related dark coloration of several organs such as the liver, cecum. rectum, pancreas, mesenteric lymph nodes, uterus, kidney, thymus, urinary, bladder and salivary gland was observed in males of females of several dose groups.

Sexual function and fertility:

P0 generation:

No adverse effects on estrous cycles, pre-coital interval, mating performance and fertility were observed. Gestation length at 160 mg/kg bw/day and 320 mg/kg bw/day in females that littered before the groups were prematurely terminated showed a shift towards a slightly longer gestation length. At 40 mg/kg/day there was a shift towards a slightly longer gestation length ($p<0.05$) but the gestation index was 100%.

Table: Mating performance and fertility - group values (P0)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	12	12	12	100	100	100
2M	12	12	11	100	92	92
3M	12	12	11	100	92	92
4M	12	12	12	100	100	100
5M	12	12	12	100	100	100
7M	11	11	10	100	91	91
1F	12	12	12	100	100	100
2F	12	12	11	100	92	92
3F	12	12	11	100	92	92
4F	12	12	12	100	100	100
5F	12	12	12	100	100	100
7F	12	12	11	100	92	92

Table: Mean number of implantation sites at 0 (group 1), 160 (group 4) and 320 (group 5) mg/kg bw/day

Group		Implantations
Statisticstest		Sh
1	Mean	15.8
	SD	2.68
	N	11
	N<100%	-
4	Mean	14.9
	SD	5.28
	N	8
	N<100%	
5	Mean	14.3
	SD	0.82
	N	6
	N<100%	-

Table: Gestation length and gestation index - group values (P0)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group	Number of pregnant animals		Gestation length (days) 21	21.5	22	22.5	23	23.5	24	Number of complete live litters born	Gestation index (%)
Statistics test						Lt					
1	12A	N	0	0	4	5	2	0	0	11	92
		(%)			(36)	(45)	(18)				
2	11	N	1	0	0	6	4	0	0	11	100
		(%)	(9)			(55)	(36)				
3*	11	N	0	0	0	3	8	0	0	11	100
		(%)				(27)	(73)				
4**	12B	N	0	0	0	1	6	0	1	α	α
		(%)				(13)	(75)		(13)		
5**	12C	N	0	0	0	3	1	2	0	α	α
		(%)				(50)	(17)	(33)			
7β	11	N	0	0	0	2	9	0	0	11	100
		(%)				(18)	(82)				

α Group 4 and 5 females euthanized for welfare reasons; data not included as females in different stages of gestation and parturition at termination
β Group 7 excluded from statistical analysis as not treated concurrently with controls
A Percentage distribution of gestation lengths calculated from 11 animals - one pregnant female failed to litter
B Percentage distribution of gestation lengths calculated from eight animals - four pregnant females euthanized for welfare reasons during gestation
C Percentage distribution of gestation lengths calculated from six animals - six pregnant females euthanized for welfare reasons during gestation

F1 generation:

Developmental toxicity: Females administered 160 mg/kg/day and 320 mg/kg/day were terminated prematurely after two and four total **litter losses**, respectively. **Post-implantation survival and the live birth index** were low at 160 and 320 mg/kg/day resulting in low litter sizes. The offspring **body weight** on Day 1 was low compared to controls and examinations predominately revealed an absence of milk in the stomach of the decedent offspring.

There was no effect on litter size, offspring survival, sex ratio and ano-genital distance up to and including 80 mg/kg bw/day.

Table: Litter size - group mean values (F1)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Post implantation survival index (%)	Live birth index (%)	Viability index (%) Day 4	Lactation index (%) Day 21
Statistics test		Wi	Ch	Ch	
1F	Mean	94.9	96.0	100.0	99.1
	SD	7.44	3.26	0.00	3.02
	N	11	11	11	11
	N<100%	5	7	0	1
2F	Mean	90.7	95.0	100.0	100.0
	SD	5.81	13.89	0.00	0.00
	N	11	11	11	11
	N<100%	9	2	0	0
3F	Mean	90.0	96.7	99.4	100.0
	SD	10.94	3.98	2.01	0.00
	N	11	11	11	11
	N<100%	7	5	1	0
7F	Mean	91.3	92.6	98.8	100.0
	SD	4.88	7.94	2.68	0.00
	N	11	11	11	11
	N<100%	10	7	2	0

The following data were used for the statistics tests, animal indices for post implantation survival index and animal indices dichotomized to 1 when 100% and 0 otherwise for live birth and viability indices.

Table: Offspring survival indices - group mean values (F1)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Implantations	Total @	Live on Day Before cull 1	4	After cull 4	7	11	14	18	21
Statistics test		Wi	Wi	Wi	Wi						
1F	Mean	15.8	15.3	14.6	14.6	10.0	10.0	10.0	10.0	9.9	9.9
	SD	2.68	3.23	3.01	3.01	0.00	0.00	0.00	0.00	0.30	0.30
	N	11	11	11	11	11	11	11	11	11	11
2F	Mean	14.9	13.5	12.9	12.9	9.5	9.5	9.5	9.5	9.5	9.5
	SD	2.55	2.62	3.33	3.33	1.04	1.04	1.04	1.04	1.04	1.04
	N	11	11	11	11	11	11	11	11	11	11
3F	Mean	16.6	15.0	14.5	14.4	10.0	10.0	10.0	10.0	10.0	10.0
	SD	2.50	2.97	2.62	2.62	0.00	0.00	0.00	0.00	0.00	0.00
	N	11	11	11	11	11	11	11	11	11	11
7F	Mean	17.6	16.3	15.0	14.8	10.0	10.0	10.0	10.0	10.0	10.0
	SD	2.80	2.76	2.45	2.40	0.00	0.00	0.00	0.00	0.00	0.00
	N	11	11	11	11	11	11	11	11	11	11

@ - Includes offspring that died prior to the designated Day 1 of age

Offspring **body weight gain** from Days 7-11 of age was slightly low when compared to controls. Offspring body weight at 80 mg/kg bw/day did not show any signs of overt toxicity but the assessment is limited by the absence of concurrent control.

Table: Body weight and body weight change - group mean values (g) for offspring (F1)

	Control	Silver Acetate				
Dose Group	1	2	3	4	5	7
Target Dose (mg/kg/day)	0	4	40	160	320	80

Group /Sex		Day of age (before cull) 1	1 @	4	Day of age (after cull) 4	7	11	14	18	21	Change 1-4	Change 4-7
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	6.8	6.9	9.5	9.6	15.6	25.0	31.8	40.3	51.4	2.7	6.0
	SD	0.62	0.62	1.09	1.03	1.22	1.78	1.52	1.97	3.45	0.80	0.59
	N	11	11	11	11	11	11	11	11	11	11	11
2M	Mean	6.9	7.0	9.8	9.8	15.3	23.9	30.4	38.8	49.9	2.9	5.5
	SD	0.68	0.72	1.32	1.32	1.92	2.90	3.56	4.50	6.74	0.77	0.99
	N	11	11	11	11	11	11	11	11	11	11	11
3M	Mean	7.0	7.1	9.9	9.9	15.6	23.8	30.2	37.9	49.4	2.8	5.7
	SD	0.71	0.71	1.09	1.13	1.15	1.97	2.39	2.95	4.80	0.49	0.72
	N	11	11	11	11	11	11	11	11	11	11	11
7M	Mean	6.8	6.9	9.9	10.0	15.9	24.7	30.6	38.5	47.7	3.2	5.9
	SD	0.63	0.63	1.18	1.21	1.60	2.18	2.35	2.48	3.86	0.63	0.71
	N	11	11	11	11	11	11	11	11	11	11	11

@ - Includes only those pups surviving after the cull

Group /Sex		Day of age (before cull) 1	1 @	4	Day of age (after cull) 4	7	11	14	18	21	Change 1-4	Change 4-7
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	6.3	6.4	9.1	9.1	14.9	24.0	30.8	38.8	49.5	2.7	5.8
	SD	0.59	0.60	1.09	1.09	1.46	2.04	2.03	2.50	4.04	0.74	0.53
	N	11	11	11	11	11	11	11	11	11	11	11
2F	Mean	6.5	6.6	9.3	9.4	14.7	22.8	29.2	37.5	48.0	2.8	5.3
	SD	0.51	0.55	1.02	1.04	1.47	2.47	2.94	3.80	5.60	0.69	0.85
	N	11	11	11	11	11	11	11	11	11	11	11
3F	Mean	6.7	6.8	9.4	9.4	14.9	22.8	29.0	36.5	47.7	2.6	5.5
	SD	0.59	0.55	1.10	1.05	1.25	2.06	2.55	2.92	4.06	0.58	0.64
	N	11	11	11	11	11	11	11	11	11	11	11
7F	Mean	6.3	6.3	9.3	9.3	15.0	23.3	29.1	36.2	45.1	3.0	5.7
	SD	0.77	0.74	1.55	1.51	2.21	2.69	2.98	3.63	5.16	0.79	0.90
	N	11	11	11	11	11	11	11	11	11	11	11

@ - Includes only those pups surviving after the cull

Macroscopic examination of offspring that died prior to scheduled termination did not reveal any findings at dose levels up to and including 80 mg/kg bw/day that could be attributed to administration of silver acetate. The offspring brain weight on Day 21 was unaffected by treatment at dose levels up to and including 80 mg/kg bw/day and the detailed brain histopathology of F1 offspring at Day 21 of age did not reveal any pathological changes or developmental abnormalities.

Females administered 160 mg/kg bw/day and 320 mg/kg bw/day were terminated prematurely during late gestation/late lactation and the assessment of F1 responses was therefore restricted to phase 1 animals receiving 4 or 40 mg/kg bw/day; litters administered 80 mg/kg bw/day were terminated on Day 21 of age.

There were no clinical signs observed and the mean body weight at weaning was similar across the groups for both males and females. However, the **overall gain for males** administered 4 and 40 mg/kg bw/day from Day 1 to Day 29 was low and the resultant mean body weight at these target dose levels were low compared to controls. The overall the body weight gain for females administered 40 mg/kg bw/day was slightly low at 90% of controls but this difference did not attain statistical significance.

The absolute mean brain weight for selected F1 females, but not males receiving 40 mg/kg bw/day was slightly but statistically significantly low but no abnormalities were discovered in the macroscopic examinations.

Sexual function and fertility: The age at completion of sexual maturation for both males and females was unaffected by administration of Silver Acetate at dose levels of 4 and 40 mg/kg bw/day. The mean body weight on the day of completion of preputial separation was slightly but statistically significantly low for males receiving 4 or 40 mg/kg bw/day.

Based on the results of this study it was concluded that dose levels of 160 and 320 mg/kg bw/day were not tolerated and thus unsuitable for the subsequent OECD 443 study.

Main study: In the extended one generation study claimed to be performed according to OECD TG 443 and the principles of GLP (unsigned in the version available to the DS), P0 animals were administered doses of 40, 80 or 120 mg silver acetate/kg bw/day orally in diet. Males were treated for ten weeks before pairing until necropsy made after litters were weaned. 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 (depending on cohort) at the same dose levels as the F0 generation, except for animals administered 120 mg/kg bw/day in Cohorts 1A and 1B which were terminated prematurely on welfare grounds at approximately 10 weeks of age rather than 13-14 weeks of age. The data for the F0 generation included clinical observations, body weight, food consumption, water consumption (by visual assessment), estrous cycles, mating performance and fertility, gestation length and parturition observations and reproductive performance. Clinical pathology (hematology and blood chemistry), analyses of thyroid hormones, blood silver, serum copper/selenium, sperm assessment, organ weight and macroscopic and microscopic pathological investigations were performed. For F1 offspring, clinical condition, litter size and survival, sex ratio, body weight, ano-genital distance, organ weights and macropathology were assessed. Nipple counts were performed on male offspring on Day 13 of age. Serum samples collected from litters on Day 4 were analyzed for serum copper/selenium levels and blood samples were analysed for silver levels. Serum samples from selected offspring on Day 22 of age were analyzed for thyroid-related hormones and serum copper/selenium levels, and blood samples were analysed for silver levels.

The F1 generation was split into five cohorts at weaning and the data recorded for each cohort included:

Cohort 1A: clinical condition, body weight, food consumption, sexual maturation and estrous cycles. Clinical pathology (hematology, blood chemistry, cardiac troponin, and urinalysis) and thyroid-related hormones, silver blood levels, serum copper/selenium levels sperm assessment, ovarian follicle and corpora lutea counts, organ weight, macroscopic pathology, full microscopic pathology and spleen immunophenotyping investigations.

Cohort 1B: clinical condition, body weight, food consumption, sexual maturation and estrous cycles. Silver blood levels and copper/selenium serum levels were assessed. Organ weight and macroscopic pathology investigations were performed.

Cohort 2A: clinical condition, body weight, food consumption and sexual maturation. Neurobehavioral screening, brain weight, brain morphometry, macroscopic pathology and microscopic pathology

Cohort 2B: no direct treatment and no specific in-life investigations. Animals were dispatched to necropsy at weaning for microscopic pathology investigations of the brain including brain morphometry.

Cohort 3: clinical signs, body weight, food consumption and sexual maturation. Spleen weight, macroscopic pathology and T-cell dependent antibody response investigations.

The following results were obtained (parameters considered adversely affected by treatment are marked in bold text):

P0:

General toxicity:

One male administered 80 mg/kg/day, two males administered 120 mg/kg/day died of unclear reason and one female administered 120 mg/kg/day was killed owing to a mammary lesion which was unrelated to treatment.

Body weight gain for males administered 80 and 120 was significantly reduced at termination whereas female body weight was unaffected by treatment. Intermittent, transient effects on food intake were observed but the efficiency of food utilization for animals before pairing and for females during gestation was unaffected by treatment.

At Week 10 (females) and at termination, differences in erythrocyte count, mean cell haemoglobin and mean cell volume, red cell distribution width, mean cell heamoglobin, mean haemocrit, haemoglobin concentration, platelet count were observed in males and females and white blood cell count with variable dose response and with males more affected than females. **Females administered 120 mg/kg/day showed high counts for eosinophils, monocytes and large unstained cells**.

At Week 10 (females) and at termination (both sexes) the biochemistry analyses showed **high alkaline phosphatase activity, high plasma cholesterol and low potassium levels**, in part but not fully according to a clear dose-response.

There were no statistically significant differences in thyroid stimulating hormone or thyroxine serum concentrations levels in any group or generation of males or females after dietary administration of silver acetate administered 40, 80 and 120 mg/kg/day when compared with controls.

In general **serum copper** and selenium levels decreased with increasing doses of silver acetate. The decreases in serum levels were not dose proportionate but tended to be greater for copper and for adult animals when compared with offspring on PND22. The consequences of silver-induced copper deficiency for development is further discussed in section 10.10.5.

Copper Serum levels (ng/mL)

Group/ sex	F0 Week 10		F1 PND22 offspring		F0 Males Term		F1 Cohort 1A Term		F1Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1M	1287	13.8	666	21.8	1515	18.8	1091	21.3	1211	13.1
2M	470	32.6	550	47.1	390	21.6	368	16.2	398	21.5
As % Control	*37*		*83*		*26*		*34*		*33*	
3M	316	23.4	337	30.1	255	19.3	245	43.2	254	26.5
As % Control	*25*		*51*		*17*		*22*		*21*	
4M	245	39.4	383	41.8	176	19.5	217	77.0	161	33.5
As % Control	*19*		*58*		*12*		*20*		*13*	

Group/ sex	F0 Week 10		F1 PND22 offspring		F0 Females PND28		F1 Cohort 1A Term		F1Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1F	1800	12.9	875	59.7	1840	9.73	1623	16.8	1578	19.2
2F	719	22.6	458	29.4	811	29.8	869	21.5	1020	14.3
As % Control	*40*		*52*		*44*		*54*		*65*	
3F	544	21.2	363	22.1	562	30.9	576	15.8	650	27.9
As % Control	*30*		*30*		*31*		*35*		*41*	
4F	374	33.2	449	78.4	448	28.5	286	29.1	378	36.1
As % Control	*21*		*51*		*54*		*18*		*24*	

Selenium Serum levels (ng/mL)

Group/ sex	PND22		F0 Males Term		F1 Cohort 1A Term		F1 Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1M	184	13.3	456	6.90	274	21.3	320	18.4
2M	127	9.73	253	15.0	166	15.6	233	17.6
As % Control	*69*		*55*		*61*		*73*	
3M	110	21.3	219	14.6	136	13.3	224	16.2
As % Control	*60*		*48*		*50*		*70*	
4M	111	15.2	219	8.61	108	24.6	169	11.7
As % Control	*60*		*48*		*39*		*53*	

Group/ sex	F1 PND22 offspring		F0 Females PND28		F1 Cohort 1A Term		F1 Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1F	192	6.53	381	5.04	216	19.3	252	10.0
2F	129	17.7	315	11.6	156	12.3	221	17.1
As % Control	*67*		*83*		*72*		*88*	
3F	115	20.9	268	7.33	132	13.2	210	32.0
As % Control	*60*		*70*		*61*		*95*	
4F	109	17.1	266	9.97	98.2	13.7	154	12.4
As % Control	*57*		*70*		*45*		*61*	

Body weight-relative heart weight was high for both males and females at all dose levels and all treated groups of males had low mean absolute pituitary weights. High mean body weight-relative spleen weight was observed in animals administered 120 mg/kg/day. Females receiving 120 mg/kg/day showed low absolute and body weight-relative mean **thymus weight**. The majority of macroscopic findings at scheduled termination included abnormal colouration of tissues, including the gastrointestinal tract, kidneys, lacrimal glands, liver, harderian glands, mesenteric lymph nodes, pancreas, salivary glands, thymus, thyroids and urinary bladder. At histopathology, extracellular pigment was observed in various organs/tissues and was considered to represent deposition of test item at these sites. In general, pigment was more prominent in females, and there was not always an apparent dose response. Pigment was not associated with any inflammatory or degenerative changes. Other histopathological findings included increased extramedullary hematopoiesis observed in the spleen of males administered 80 or 120 mg/kg/day and epithelial degeneration of the glandular mucosa of the stomach in females receiving 40, 80 or 120 mg/kg/day.

Sexual function and fertility in P0:

There was a treatment related effect on **testis weight and spermatid total millions, cauda epididymis sperm count and total millions** following treatment with silver acetate administered 120 mg/kg/day but motile, progressive, motion and morphology were unaffected. Low mean absolute testes weight was also observed in males administered 80 and 120 mg/kg/day. All treated groups of males had low absolute/body weight-relative mean prostate weights. At scheduled termination there was abnormal colouration of preputial/clitoral glands and uterus.

Table: Sperm analysis - group mean values (P0):

Dose Group	Control	Silver Acetate		
	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group		Motile sperm (%)	Progressively motile sperm (%)	---- Cauda epididymis ---- Weight (g)	Sperm count (millions/g)	Total (million)	----Testis ---- Weight (g)	Spermatid count (millions/g)	Total (million)
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1	Mean	90	36	0.299	455	136	1.96	85	166
	SD	13	13	0.047	113	36	0.20	19	40
	N	25	25	25	25	25	24	24	24
2	Mean	84	35	0.291	470	136	1.89	75	141
	SD	16	14	0.029	101	31	0.12	22	40
	N	25	25	25	25	25	25	25	25
3	Mean	89	38	0.285	532	152	1.83*	89	163
	SD	14	13	0.032	126	37	0.13	22	44
	N	23	23	24	24	24	24	24	24
4	Mean	90	41	0.293	419	123	1.85*	76	141*
	SD	19	13	0.038	98	34	0.14	16	33
	N	23	23	23	23	23	23	23	23

Table: Sperm motion data - group mean values (P0):

Group		VAP (µm/s)	VSL (µm/s)	VCL (µm/s)	ALH (µm)	BCF (Hz)	STR (%)	LIN (%)	Rapid (%)	Medium (%)	Slow (%)	Static (%)
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	IWi	Wi	Wi	Wi	Wi
1	Mean	153	89	349	25	36	60	28	54	3	34	10
	SD	17	12	52	2	3	4	2	16	2	12	13
	N	25	25	25	25	25	25	25	25	25	25	25
2	Mean	152	90	348	26	35	62	29	52	3	30	16
	SD	23	13	58	3	3	4	3	21	2	10	16
	N	25	25	25	25	25	25	25	25	25	25	25
3	Mean	154	90	348	25	35	60	28	59	2	28	11
	SD	16	9	34	2	3	3	1	18	1	10	14
	N	23	23	23	23	23	23	23	23	23	23	23
4	Mean	158	93	363	25	35	61	28	61	2	26*	10
	SD	20	14	45	2	3	3	2	18	2	11	19
	N	23	23	23	23	23	23	23	23	23	23	23

Table: Sperm morphology - group mean values (P0):

Dose Group	Control	Silver Acetate		
	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group	Number of animals	Total Number of sperm examined		Normal Number	Normal %	Total Abnormal Number	Total Abnormal %	Decapitate Number	Decapitate %
Statistics test					Wc		Wc		Wc
1.	25	5252	Mean	202	96.0	9	4.0	5	2.4
			SD	17	7.4	16	7.4	16	7.1
4.	23	4813	Mean	196	93.8	14	6.2	10	4.4
			SD	43	19.6	44	19.6	36	16.3

Group	Number of animals	Total Number of sperm examined		Head abnormal Number	Head abnormal %	Neck abnormal Number	Neck abnormal %	Midpiece abnormal Number	Midpiece abnormal %	Tail abnormal Number	Tail abnormal %
Statistics test					Tt		Fe		Fe		Tt
1.	25	5252	Mean	1	0.6	0.3	0.1	0.4	0.2	2	0.8
			SD	1	0.6	1	0.2	1	0.3	2	0.7
4.	23	4813	Mean	1	0.4	4	1.6	3	1.4	2	0.8
			SD	1	0.6	17	7.6	15	6.6	2	0.8

Group		Head flat Number	%	Pinhead Number	%	Head S-shaped Number	%	Eccentric insertion Number	%	Neck broken Number	%	Neck folded Number	%
Statistics test			Tt		Fe		Fe		Fe		Fe		Fe
1.	Mean	1	0.6	0.04	0.02	0.1	0.04	0.2	0.1	0.1	0.04	0.04	0.02
	SD	1	0.6	0.2	0.1	0.3	0.1	0.4	0.2	0.3	0.1	0.2	0.1
4.	Mean	1	0.4	0.04	0.02	0	0	0	0	1	0.6	0	0
	SD	1	0.5	0.2	0.1	0	0	0	0	7	3.0	0	0

Group		Neck frayed Number	%	Midpiece frayed Number	%	Midpiece broken Number	%	Midpiece thin (part) Number	%	MCD Number	%	Midpiece loop Number	%
Statistics test			Fe		Fe		Fe		Fe		Fe		Fe
1 cont	Mean	0	0	0.1	0.04	0.04	0.02	0.1	0.04	0.2	0.1	0	0
	SD	0	0	0.3	0.1	0.2	0.1	0.4	0.2	1	0.2	0	0
4 cont	Mean	3	1.3	2	0.7	2	0.7	1	0.4	0.04	0.02	0.04	0.02
	SD	14	6.4	7	3.3	7	3.3	4	1.7	0.2	0.1	0.2	0.1

Group		Tail bent/kink Number	%	Tail broken Number	%	Tail coiled Number	%	Tail folded Number	%	Tail detached Number	%	Tail looped Number	%
Statistics test			Tt		Fe		Fe		Fe		Tt		Fe
1 cont	Mean	1	0.4	0.1	0.1	0.04	0.02	0.1	0.04	0.5	0.2	0.04	0.02
	SD	1	0.6	0.4	0.2	0.2	0.1	0.3	0.1	1	0.3	0.2	0.1
4 cont	Mean	1	0.3	0.2	0.1	0	0	0.2	0.1	0.3	0.2	0.2	0.1
	SD	1	0.5	1	0.3	0	0	1	0.3	0.5	0.2	0.4	0.2

Group		Tail thin (part) Number	%	DCD Number	%
Statistics test			Fe		Fe
1 cont	Mean	0.04	0.02	0.04	0.02
	SD	0.2	0.1	0.2	0.1
4 cont	Mean	0.1	0.04	0	0
	SD	0.4	0.2	0	0

There was no effect of treatment on estrous cycles, pre-coital interval, mating performance or fertility, number of implantation sites and the gestation index (number of litters born/number of mated females) was 100% in all groups. A marginal shift toward a longer gestation length was observed in animals administered 120 mg/kg/day.

Table: Mating performance and fertility - group values (F0)

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	25	25	25	100	100	100
2M	25	25	25	100	100	100
3M	24	24	23	100	96	96
4M	24	23	23	96	100	96
1F	25	25	25	100	100	100
2F	25	25	25	100	100	100
3F	25	25	24	100	96	96
4F	25	24	24	96	100	96

Table: Gestation length and gestation index - group values (F0)

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group	Number of pregnant animals		Gestation length (days) 22	22.5	23	23.5	Number of complete live litters born	Gestation index (%)
Statistics test				Lt				
1	25	N	7	8	8	2	25	100
		(%)	(28)	(32)	(32)	(8)		
2	25	N	2	5	17	1	25	100
		(%)	(8)	(20)	(68)	(4)		
3	24	N	0	9	15	0	24	100
		(%)		(38)	(63)			
4*	24	N	0	6	18	0	24	100
		(%)		(25)	(75)			

F1 generation:

Developmental toxicity: There was an increased incidence of offspring mortality and dark coloration in animals administered 120 mg/kg/day whereas clinical condition of offspring administered 40 or 80 mg/kg/day was considered unaffected by treatment.

There was no effect of treatment on the number of implantation sites, mean litter size or sex ratio on postnatal Day 1. At 120 mg/kg/day the live birth and **viability indices** were low compared to controls, resulting in a **mean live litter size** on Day 1 of 13.0 compared to 14.5 in controls and 11.7 on Day 4 of compared to 14.4 in controls. There were no apparent differences in animals administered 40 or 80 mg/kg/day.

Following litter standardization on Day 4, the offspring survival was similar to controls at all dose levels.

Table: Litter size - group mean values (F1)

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group /Sex		Implantations	Total @	Live on Day Before cull 1	4	After cull 4	7	14	21
Statistics test		Wi	Wi	Wi	Wi				
1F	Mean	15.6	14.9	14.5	14.4	9.9	9.9	9.9	9.9
	SD	2.35	2.62	2.63	2.69	0.28	0.28	0.28	0.28
	N	25	25	25	25	25	25	25	25
2F	Mean	16.9	15.1	14.4	14.2	9.9	9.9	9.9	9.9
	SD	1.85	2.16	2.74	2.63	0.60	0.60	0.60	0.60
	N	25	25	25	25	25	25	25	25
3F	Mean	16.3	14.4	13.9	13.6	10.0	10.0	10.0	10.0
	SD	1.94	1.84	1.60	1.74	0.00	0.00	0.00	0.00
	N	24	24	24	24	24	24	24	24
4F	Mean	16.1	14.7	13.0	11.7**	9.3	9.3	9.3	9.3
	SD	1.95	2.18	2.84	3.20	1.55	1.55	1.55	1.55
	N	24	24	24	24	24	24	24	24

@ - Includes offspring that died prior to the designated Day 1 of age

Table: Offspring survival indices - group mean values (F1)

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group /Sex		Post implantation survival index (%)	Live birth index (%)	Viability index (%) Day 4	Lactation index (%) Day 21
Statistics test		Wi	CA	CA	
1F	Mean	94.5	97.3	99.1	100.0
	SD	6.50	4.73	3.09	0.00
	N	25	25	25	25
	N<100%	13	7	2	0
2F	Mean	89.3	94.7	99.0	100.0
	SD	9.84	9.01	2.88	0.00
	N	25	25	25	25
	N<100%	19	9	3	0
3F	Mean	88.8	96.6	97.9	100.0
	SD	8.06	5.56	4.97	0.00
	N	24	24	24	24
	N<100%	21	10	5	0
4F	Mean	91.1	89.1	90.2	99.6
	SD	9.21	15.32	16.09	2.04
	N	24	24	24	24
	N<100%	16	15*	12**	1

The following data were used for the statistics tests, animal indices for post implantation survival index and animal indices dichotomized to 1 when 100% and 0 otherwise for live birth and viability indices.

Mean **body weight** for male and female offspring administered 120 mg/kg/day was low compared to controls on postnatal day 1 and remained low until weaning. At 80 mg/kg/day, the mean offspring body weight on was similar to controls on postnatal day 1 but lower on postnatal days 14 and 21. There was no effect on offspring weight or weight gain in animals administered 40 mg/kg/day.

Table: Body weight - group mean values (g) for offspring (F1)

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group /Sex		Day of age (before cull) 1	1 @	4	Day of age (after cull) 4	7	14	21
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	6.9	6.9	9.6	9.6	15.6	32.6	53.9
	SD	0.65	0.67	1.11	1.12	1.51	2.26	4.36
	N	25	25	25	25	25	25	25
2M	Mean	6.9	7.0	9.9	9.9	16.1	32.7	53.0
	SD	0.64	0.57	1.26	1.20	1.51	3.20	4.84
	N	25	25	25	25	25	25	25
3M	Mean	6.6	6.6	9.5	9.5	15.1	30.9*	49.1**
	SD	0.64	0.62	0.98	0.99	1.53	2.45	4.20
	N	24	24	24	24	24	24	24
4M	Mean	6.3**	6.4**	8.9*	8.9*	14.2**	29.1**	46.6**
	SD	0.62	0.53	0.96	0.93	1.52	2.53	4.13
	N	24	24	24	24	24	24	24

@ - Includes only those surviving after the cull

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group /Sex		Day of age (before cull) 1	1 @	4	Day of age (after cull) 4	7	14	21
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	6.6	6.6	9.2	9.2	15.0	31.5	51.9
	SD	0.71	0.71	1.12	1.13	1.37	1.97	3.78
	N	25	25	25	25	25	25	25
2F	Mean	6.6	6.6	9.4	9.5	15.5	31.9	51.6
	SD	0.59	0.58	1.22	1.21	1.59	3.48	4.74
	N	25	25	25	25	25	25	25
3F	Mean	6.3	6.3	9.0	9.0	14.5	29.7*	47.3**
	SD	0.57	0.60	0.84	0.81	1.24	2.09	3.46
	N	24	24	24	24	24	24	24
4F	Mean	5.9**	6.0**	8.5*	8.6	13.8**	28.3**	45.2**
	SD	0.68	0.62	1.14	1.13	1.76	2.56	3.78
	N	24	24	24	24	24	24	24

@ - Includes only those surviving after the cull

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group /Sex		Day of age 21	25	Day 1	8	15	22	29	36	43	50	57	64	71
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	54	76	94	152	217	272	335	379	420	455	484	508	527
	SD	4.6	5.4	11.0	16.3	20.3	25.1	27.7	30.7	36.9	41.2	44.2	48.0	49.4
	N	70	60	60	60	60	60	60	60	50	40	40	40	20
2M	Mean	53	72**	86**	138**	196**	247**	307**	352**	391**	421**	448**	471**	492*
	SD	5.6	6.9	12.2	17.8	22.1	25.4	30.7	34.8	40.7	45.1	48.0	52.8	45.0
	N	70	60	60	60	60	60	59	59	49	39	39	39	20
3M	Mean	49**	67**	82**	131**	189**	239**	297**	340**	376**	405**	429**	451**	457**
	SD	4.9	6.8	9.5	14.2	18.4	21.6	24.5	26.1	30.2	28.9	30.5	32.7	38.4
	N	70	60	60	60	60	60	59	59	49	39	39	39	19
4M	Mean	47**	66**	82**	131**	187**	238**	294**	334**	361**				
	SD	5.2	6.0	10.1	15.0	19.5	22.2	26.7	33.5	47.7				
	N	68	60	60	60	60	58	56	53	44				

Group /Sex		Day of age 21	25	Day 1	8	15	22	29	36	43	50	57	64	71
Statistics test		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	52	70	86	129	163	183	206	220	233	244	253	263	263
	SD	4.2	5.2	9.0	11.0	11.7	14.6	16.0	17.3	18.5	20.5	20.6	22.0	23.8
	N	70	60	60	60	60	60	60	60	50	40	40	40	30
2F	Mean	51	69	80**	119**	151**	173**	198*	216	229	242	252	259	264
	SD	5.4	6.7	11.1	14.2	14.3	14.6	16.6	16.8	18.9	18.3	20.8	21.4	21.0
	N	70	60	60	60	60	60	60	60	50	50	40	40	30
3F	Mean	47**	64**	77**	114**	146**	169**	194**	211*	227	238	247	258	261
	SD	4.5	6.1	8.9	11.3	14.2	15.2	17.8	17.7	18.4	17.5	20.2	19.8	22.1
	N	70	60	60	60	60	60	60	60	50	50	40	40	30
4F	Mean	46**	62**	74**	111**	144**	167**	193**	211*	225	240			
	SD	5.0	6.0	9.2	12.3	14.8	16.9	21.0	21.6	25.0	23.7			
	N	70	60	60	60	60	60	60	58	48	18			

There was no effect of treatment on anogenital distance in male or female offspring and no effect on nipple count in males.

Macroscopic findings in offspring that died or were culled on postnatal day 4 were predominantly absence of milk in the stomach and/or autolysis. There were no significant macroscopic findings in offspring terminated on Day 22. At 80 or 120 mg/kg/day low mean absolute and body weight-relative thymus weights (p<0.01) were observed in both sexes, and females at 40 mg/kg/day showed low relative weights.

On PND22 **mean absolute brain weight** was low in females at 80 (4 % lower than controls) and 120 (12 % lower than controls) mg/kg/day. The absolute brain weight was 8% lower than controls in high dose males. When related to bodyweight, the brain weight was slightly increased in both sexes.

Silver Acetate-Related Effects in Organ Weight Parameters – Unselected F1 Generation Offspring on Day 22 of Age

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Brain								
Absolute Weight (g)	1.497	95	96	92**	1.457	98	96*	88*
Body Weight Relative (%)	2.619	102	107	107	2.697	100	106	105*
Thymus								
Absolute Weight (g)	0.236	89	75**	60**	0.255	87	68**	62**
Body Weight Relative (%)	0.4099	97	84**	70**	0.4715	87*	75**	73**

NA = Not applicable.
* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body weight) for dosed groups expressed as percentage of control mean value.

Nine male and two females died in the high dose group between Day 19 and Day 47. Seven of these decedents were subjected to full microscopic examination and in five the major factor contributing to death was **brain lesions**. Due to the high mortality, the high dose group was terminated prematurely at approximately 10 weeks of age.

Males administered 120 mg/kg/day showed a higher incidence of **piloerection, hunched posture and abnormal gait** and the same signs were also observed in a few females administered 120 mg/kg/day but at a low incidence.

At weaning (PND21), selected males and females receiving 80 or 120 mg/kg/day had low mean **bodyweight** and subsequent **bodyweight gain** up to PND25 was low at all target dose levels. Changes were statistically significant but without a clear dose response.

The mean body weight, mean body weight gain and food consumption was low from Day 1 of the F1 generation (PND28±2) up to termination in all treated males following a dose response. This was not seen in females. Overall, the efficiency of food utilization was unaffected by treatment for both males and females.

The hematological investigations made at termination (approximately 13 weeks of age) of males administered 80 mg/kg/day and females administered 40 or 80 mg/kg/day showed high mean erythrocyte counts. Males administered 80 mg/kg/day also showed low mean cell hemoglobin, low mean cell volume and high red cell distribution width and females administered 80 mg/kg/day showed high hemoglobin and high hematocrit. Conversely males and females administered 120 mg/kg/day that were terminated prematurely at approximately ten weeks of age showed low erythrocyte counts, high mean cell hemoglobin and high mean cell hemoglobin concentration. In addition, a low hematocrit, low red cell distribution width and high mean cell volume were noted in high dose females. Therefore, it can only be concluded that treatment causes a **disturbance of hematological parameters**.

White blood cell parameters at either scheduled or premature termination were high in treated females. Mean platelet counts were low in females administered 80 or 120 mg/kg/day, prothrombin times were shorter in all treated groups of females and activated partial thromboplastin time was shorter for females administered 120 mg/kg/day.

At premature termination, the **blood chemistry** analyses in high dose animals principally showed high alkaline phosphatase activity, high alanine amino-transferase activity, high aspartate amino-transferase activity (females only), high cholesterol, high glucose, high urea (males), low creatinine, low albumin/A:G ratio, high potassium, low phosphorous levels and low sodium(females).

At scheduled termination of animals at 40 or 80 mg/kg/day the blood chemistry investigations revealed high alkaline phosphatase activity (males), high alanine amino-transferase activity (males), high cholesterol and low creatinine. Troponin investigations made in the F1 generation revealed higher levels in treated males, with mean levels at 120 mg/kg/day that were slightly high but much lower than the mean

values at 40 or 80 mg/kg/day (no historical control data were available for comparison). Cardiac troponin levels were unaffected in females at all dose levels.

Urinalysis investigations of the F1 generation revealed a low total protein output and protein concentration in males at 80 mg/kg/day (urinalysis assessment was limited to control, low and mid dose animals).

Sexual function and fertility:

The mean age at completion of balano-preputial separation was essentially similar across the groups but the mean bodyweight at completion was statistically significantly lower than controls at all dose levels. The mean age at completion of **vaginal opening** in females receiving 120 mg/kg/day was 1.2 days later than controls and the mean bodyweight at completion was statistically significantly lower than controls at all dose levels. The interval between vaginal opening and the first estrus and estrous cycles were unaffected by administration.

Table: Sexual maturation and - group mean age and body weight at completion and vaginal opening to first estrus (F1)

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group		Balano preputial separation: Age at completion	Balano preputial separation: Body weight (g) at completion	Vaginal opening: Age at completion	Vaginal opening: Body weight (g) at completion
Statistics test		Wi	Wi	Wi	Wi
1	Mean	44.6	234.6	33.1	115.1
	SD	2.35	22.47	2.01	14.80
	N	60	60	60	60
2	Mean	44.9	219.9**	33.3	110.2*
	SD	3.24	23.55	2.27	13.05
	N	60	60	60	60
3	Mean	45.5	217.0**	33.2	104.3**
	SD	2.38	23.73	2.33	11.37
	N	60	60	60	60
4	Mean	45.4	212.5**	34.3**	108.0**
	SD	2.23	21.23	2.53	13.35
	N	59	59	60	60
Historical Control Data (8 studies 2012-2021)					
	n	362	362	362	362
	Mean	45.7	236.3	33.4	114.5
	Median	45.0	233.0	33.0	114.0
	SD	3.38	26.76	2.15	14.42
	MIN	38	173	29	62
	MAX	74	343	40	162

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group	Number of animals		Interval between vaginal opening and first estrus (days): 0-1	2-3	4-5
1	20	N	15	1	4
		(%)	(75)	(5)	(20)
2	20	N	13	4	3
		(%)	(65)	(20)	(15)
3	20	N	15	1	4
		(%)	(75)	(5)	(20)
4	20	N	13	3	4
		(%)	(65)	(15)	(20)

Mean ovarian follicle and corpora lutea counts at 120 mg/kg/day were similar to Controls and considered unaffected by administration. A number of small changes were observed among the 12 F1 males available for assessment of sperm parameters and were terminated approximately 3 weeks earlier than other groups. At 80 and 120 mg/kg/day there was a **statistically significant decrease in cauda epididymis and testicular weight** (p<0.01) and at all dose levels **testicular and cauda epididymal total spermatid and sperm counts (millions)** were low when compared with concurrent Control (p<0.01):

- reduced testis wt. (80, 120 mg/kg bw/d), both doses outside HCD, reduced by ≈ 11-15% of controls.
- reduced testis total spermatids (80, 120), -37% to -18% relative to control group, outside HCD for the 80 mg/kg bw/d group only.
- reduced cauda epididymis wt. (80, 120 mg/kg bw/d), both doses outside HCD, reduced by ≈ 10-37% of controls.
- reduced cauda epididymis total sperm count (40, 80, 120), -20%, -19% and -45% relative to control group, outside HCD for the 120 mg/kg bw/d group only. There was an effect on cauda epididymal spermatid counts and an increased incidence of abnormal morphology (particularly decapitates, mainly in one animal) at 120 mg/kg/day. The effects above were statistically significant unless single animals were excluded from the analysis.

Sperm analysis - group mean values (F1 Cohort 1A):

	Control	Silver Acetate		
Dose Group	1	2	3	4
Target Dose (mg/kg bw/day)	0	40	80	120

Group		Motile sperm (%)	Progressively motile sperm (%)	---- Cauda epididymis ---- Weight (g)	Sperm count (millions/g)	Total (million)	----Testis ---- Weight (g)	Spermatid count (millions/g)	Total (million)
Statistics test		Sh	Sh	Wi	Wi	Wi	Sh	Du	St
1	Mean	93	40	0.244	600	146	1.86	101	186
	SD	5	9	0.024	117	32	0.28	15	24
	N	20	20	20	20	20	20	20	20
2	Mean	90	38	0.236	499	117**	1.76	82**	142**
	SD	12	10	0.016	123	28	0.15	16	17
	N	18	18	18	18	18	18	18	18
3	Mean	88	39	0.220**	537	118**	1.66**	70**	117**
	SD	21	12	0.024	116	30	0.13	17	30
	N	20	20	20	20	20	20	20	20
3 0457 excluded$	Mean	93	41	0.220	556	123	1.66	73	122
	SD	6	7	0.025	81	24	0.13	12	22
	N	19	19	19	19	19	19	19	19
4	Mean	87	45*	0.153**	521	81**	1.59**	92	152**
	SD	24	19	0.031	140	26	0.50	25	45
	N	20	20	20	20	20	20	20	20
4. 0542 excluded$	Mean	92	48	0.157	546	86	1.66	97	160
	SD	13	16	0.025	87	19	0.40	13	29
	N	19	19	19	19	19	19	19	19

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Sperm motion data - group mean values (F1 Cohort 1A)

Group		VAP (µm/s)	VSL (µm/s)	VCL (µm/s)	ALH (µm)	BCF (Hz)	STR (%)	LIN (%)	Rapid (%)	Medium (%)	Slow (%)	Static (%)
Statistics test		Sh	Sh	Sh	Sh	Sh	Sh	Sh	Wi	Sh	Wi	Sh
1	Mean	167	94	393	28	37	58	26	63	2	29	7
	SD	11	7	31	2	2	3	1	12	1	10	5
	N	20	20	20	20	20	20	20	20	20	20	20
2	Mean	163	94	382	27	38	59	27	59	2	29	10
	SD	15	11	37	2	2	3	1	14	1	7	12
	N	18	18	18	18	18	18	18	18	18	18	18
3	Mean	158	90	372	26	35	56	25	62	1	25	12
	SD	39	22	92	6	9	14	6	17	1	10	21
	N	20	20	20	20	20	20	20	20	20	20	20
3 0457 excluded$	Mean	167	95	392	28	37	59	27	65	1	27	7
	SD	11	7	28	2	3	4	2	10	1	8	6
	N	19	19	19	19	19	19	19	19	19	19	19
4	Mean	148	94	330**	23**	32**	62**	30**	62	2	23	13
	SD	41	28	93	6	8	15	7	24	3	11	24
	N	20	20	20	20	20	20	20	20	20	20	20
4. 0542 excluded$	Mean	156	99	347	24	34	65	31	65	2	24	8
	SD	22	17	52	2	3	5	2	20	3	9	13
	N	19	19	19	19	19	19	19	19	19	19	19

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Sperm morphology - group mean values (F1 Cohort 1A)

Group	Number of animals	Total Number of sperm examined		Normal Number	Normal %	Total Abnormal Number	Total Abnormal %	Decapitate Number	Decapitate %
Statistics test					Sh		Sh		Sh
1.	20	4181	Mean	204	97.7	5	2.3	3	1.2
			SD	8	1.4	3	1.4	3	1.2
2.	18	3630	Mean	197	97.1	5	2.9	3	2.0
			SD	31	3.3	4	3.3	3	3.1
3.	20	4096	Mean	189	93.1	16	6.9	9	3.9
			SD	49	21.9	51	21.9	29	12.5
3 0457 excluded[$]	19	3863	Mean	199	98.0	4	2.0	2	1.1
			SD	22	1.1	2	1.1	2	0.9
4.	20	3979	Mean	183	91.3	16	8.7	13	6.8
			SD	42	11.9	25	11.9	23	11.2
4. 0542 excluded[$]	19	3942	Mean	191	92.0	17	8.0	13	6.3
			SD	23	11.8	25	11.8	24	11.2

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Group	Number of animals		Head abnormal Number	Head abnormal %	Neck abnormal Number	Neck abnormal %	Midpiece abnormal Number	Midpiece abnormal %	Tail abnormal Number	Tail abnormal %
Statistics test				Sh		Fe		Fe		Sh
1.	20	Mean	1	0.4	0.2	0.1	0.4	0.2	1	0.6
		SD	1	0.6	1	0.2	1	0.3	1	0.5
2.	18	Mean	1	0.5	0	0	0.4	0.2	1	0.5
		SD	1	0.5	0	0	1	0.4	1	0.9
3.	20	Mean	5	2.3	1	0.3	9	3.7	5	2.2
		SD	22	9.6	3	1.2	38	16.5	15	6.3
3 0457 excluded[$]	19	Mean	0.4	0.2	0.1	0.02	0.1	0.03	2	0.8
		SD	1	0.3	0.2	0.1	0.2	0.1	2	0.8
4.	20	Mean	1	0.7	0.1	0.05	0.3	0.1	2	1.3
		SD	1	0.8	0.3	0.1	0.5	0.2	3	1.5
4. 0542 excluded[$]	19	Mean	1	0.6	0.1	0.1	0.3	0.1	2	1.1
		SD	1	0.6	0.3	0.2	0.5	0.2	3	1.2

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Group		Head flat		Pinhead		Pronounced hook		Head misshapen		Eccentric insertion		Neck broken	
		Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Statistics test			Sh		Fe		Fe		Fe		Fe		Fe
1.	Mean	1	0.3	0.1	0.05	0	0	0.2	0.1	0	0	0.2	0.1
	SD	1	0.5	0.3	0.1	0	0	0.4	0.2	0	0	0.5	0.2
2.	Mean	1	0.4	0.1	0.1	0	0	0.2	0.1	0	0	0	0
	SD	1	0.4	0.3	0.2	0	0	0.4	0.2	0	0	0	0
3.	Mean	0.3	0.1	0	0	0	0	5	2.2	0	0	0.1	0.02
	SD	0.4	0.2	0	0	0	0	22	9.6	0	0	0.2	0.1
3 0457 excluded$	Mean	0.3	0.1	0	0	0	0	0.1	0.1	0	0	0.1	0.02
	SD	0.5	0.2	0	0	0	0	0.3	0.2	0	0	0.2	0.1
4.	Mean	1	0.5	0.1	0.2	0.1	0.05	0.1	0.05	0.1	0.02	0.1	0.02
	SD	1	0.5	0.3	0.6	0.3	0.1	0.3	0.2	0.2	0.1	0.2	0.1
4. 0542 excluded$	Mean	1	0.5	0.1	0.02	0.1	0.1	0.1	0.1	0.1	0.03	0.1	0.03
	SD	1	0.6	0.2	0.1	0.3	0.2	0.3	0.2	0.2	0.1	0.2	0.1

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Group		Neck folded		Neck frayed		Midpiece frayed		Midpiece folded		Midpiece broken		Midpiece thin (part)	
		Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Statistics test			Fe		Fe		Fe		Fe		Fe		Fe
1.	Mean	0.1	0.02	0	0	0	0	0	0	0.1	0.02	0	0
	SD	0.2	0.1	0	0	0	0	0	0	0.2	0.1	0	0
2.	Mean	0	0	0	0	0.1	0.03	0.1	0.03	0	0	0.1	0.03
	SD	0	0	0	0	0.2	0.1	0.2	0.1	0	0	0.2	0.1
3.	Mean	0	0	1	0.3	3	1.4	3	1.2	0	0	2	0.7
	SD	0	0	3	1.2	14	6.0	13	5.4	0	0	8	3.3
3 0457 excluded$	Mean	0	0	0	0	0.1	0.03	0	0	0	0	0	0
	SD	0	0	0	0	0.2	0.1	0	0	0	0	0	0
4.	Mean	0	0	0	0	0	0	0	0	0.1	0.05	0.1	0.02
	SD	0	0	0	0	0	0	0	0	0.3	0.1	0.2	0.1
4. 0542 excluded$	Mean	0	0	0	0	0	0	0	0	0.1	0.05	0.1	0.02
	SD	0	0	0	0	0	0	0	0	0.3	0.1	0.2	0.1

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Group		Midpiece irregular thin		MCD		Midpiece looped		Tail bent/kink		Tail broken		Tail folded	
		Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Statistics test			Fe		Fe		Fe		Sh		Fe		Fe
1.	Mean	0	0	0.2	0.1	0.2	0.1	1	0.3	0.1	0.02	0.1	0.02
	SD	0	0	0.4	0.2	0.5	0.2	1	0.4	0.2	0.1	0.2	0.1
2.	Mean	0	0	0.2	0.1	0.1	0.03	0.2	0.1	0.3	0.2	0	0
	SD	0	0	1	0.2	0.2	0.1	0.4	0.2	1	0.8	0	0
3.	Mean	1	0.4	0	0	0	0	1	0.6	0.1	0.05	2	0.7
	SD	4	1.8	0	0	0	0	1	0.6	0.3	0.1	6	2.6
3. 0457 excluded$	Mean	0	0	0	0	0	0	1	0.6	0.1	0.1	0.2	0.1
	SD	0	0	0	0	0	0	1	0.6	0.3	0.2	0.4	0.2
4.	Mean	0	0	0.1	0.05	0.1	0.02	1	0.6	0.3	0.1	0.2	0.1
	SD	0	0	0.3	0.1	0.2	0.1	2	0.7	1	0.3	0.5	0.2
4. 0542 excluded$	Mean	0	0	0.1	0.1	0.1	0.03	1	0.7	0.3	0.1	0.2	0.1
	SD	0	0	0.3	0.2	0.2	0.1	2	0.8	1	0.3	1	0.2

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Group		Tail frayed Number	%	Tail detached Number	%	Tail looped Number	%	Tail thin (part) Number	%	Tail Irregular thin Number	%
Statistics test			Fe		Fe		Fe		Fe		Fe
1.	Mean	0	0	0.3	0.1	0.2	0.1	0	0	0	0
	SD	0	0	1	0.3	0.4	0.2	0	0	0	0
2.	Mean	0	0	0.3	0.2	0.1	0.03	0	0	0	0
	SD	0	0	1	0.3	0.2	0.1	0	0	0	0
3.	Mean	0.1	0.02	1	0.4	0.1	0.05	0.3	0.1	1	0.4
	SD	0.2	0.1	3	1.4	0.4	0.2	1	0.6	4	1.8
3. 0457 excluded$	Mean	0.1	0.03	0.2	0.1	0.1	0.1	0	0	0	0
	SD	0.2	0.1	0.4	0.2	0.5	0.2	0	0	0	0
4.	Mean	0.1	0.02	0.3	0.2	0.2	0.2	0.2	0.1	0	0
	SD	0.2	0.1	1	0.6	0.4	0.6	0.4	0.2	0	0
4. 0542 excluded$	Mean	0.1	0.02	0.2	0.1	0.1	0.05	0	0	0.2	0.1
	SD	0.2	0.1	1	0.3	0.3	0.1	0	0	0.4	0.2

$- Animals excluded from group means due to atypical results and recorded here for comparison. Statistics test not performed on these groups.

Neurobehavioral testing (cohort 2A): results from the tests performed are presented in section 10.10.5.

Pathological findings among cohorts:

Cohort 1A: treatment related organ weight changes were noted in the heart in both sexes, in the brain of males administered 120 mg/kg/day, and in the thymus of both sexes administered 120 mg/kg/day.

Table: Incidence and Severity of Silver Acetate-Related Microscopic Findings – F1 Generation Cohort 1A at Scheduled Termination (Groups 1-3 at 13 weeks of Age and Group 4 at 10 weeks of Age)

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Brain								
Number Examined	20	19	20	20	20	20	20	20
Edema, Intramyelinic								
Minimal	0	0	2	6	0	0	0	3
Slight	0	0	1	2	0	0	0	0
Necrosis, Neuron, Hippocampus								
Minimal	0	0	8	11	0	0	3	9
Necrosis, Neuron/Glial Cell, Thalamus								
Minimal	0	0	3	3	0	0	2	3
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Pigment, Extracellular								
Minimal	0	11	8	5	0	2	9	6

Cohort 1B: males also showed treatment related organ weight changes in the heart.

Cohort 2A: males administered 120 mg/kg/day had high body weight relative brain weight; this was not evident in **Cohort 2B.**

Table: Incidence and Severity of Silver Acetate-Related Microscopic Findings – F1 Generation Cohort 2A at Scheduled Termination on Day 75 of Age

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Brain, Cerebrum								
Number Examined	10	10	10	7	10	10	10	10
Edema, Intramyelinic								
Minimal	0	0	0	1	0	0	0	3
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	1	0	0	0	1
Necrosis, Neuron, Hippocampus								
Minimal	0	0	5	5	0	0	3	7
Necrosis, Neuron/Glial Cell, Thalamus								
Minimal	0	0	0	2	0	0	0	4
Slight	0	0	0	1	0	0	0	1
Brain, Forebrain								
Number Examined	10	10	10	7	10	10	10	10
Edema, Intramyelinic								
Minimal	0	0	0	2	0	0	0	2
Brain, Medulla Oblongata								
Number Examined	10	10	10	7	10	10	10	10
Pigment, Extracellular								
Minimal	0	6	3	3	0	2	1	0

Table: Brain Morphometry – F1 Generation Cohort 2A at Scheduled Termination on Day 75 of Age

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Hippocampus								
Measurement (mm)	2.10	2.10	1.97*	1.91**	1.97	NA	NA	1.93

NA = Not applicable.
*;** = Statistically significant difference (absolute or relative) compared with respective control mean value

Cohort 3: higher than control mean absolute and body weight relative spleen weights were noted in both sexes at 120 mg/kg/day

Treatment-related macroscopic findings in F1 Cohorts 1A, 1B, 2A and 3 were generally observed in both sexes across all dose groups without a significant dose response and were generally limited to abnormal coloration (dark) of affected organs/tissues. This was generally observed at a lower incidence and in fewer tissues compared to F0 generation animals. This abnormal coloration was not apparent in F1 Cohort 2B animals that were necropsied at weaning. In the brain, dose **related neuronal necrosis in the hippocampus, and neuronal/glial cell necrosis in the thalamus** were observed in both sexes given 80 or 120 mg/kg/day. **Intramyelinic edema in the thalamus, caudate putamen and/or the corpus callosum** was also observed in males given 80 or 120 mg/kg/day and in females given 120 mg/k/day Brain morphometry measurements revealed statistically significantly **low hippocampus measurement** for males that received 80 or 120 mg/kg/day.

Cohort 3: the analysis of immunophenotyping parameters is presented in section 10.10.5.

In summary, treatment with silver acetate resulted in **reduced survival of F1 adults** administered 120 mg/kg/day. Survival was also decreased in F1 offspring administered 120 mg/kg/day with statistically significantly **reduced live birth index** (89% vs 97% in controls) and **viability index** at PND4 (90% vs 99% in controls) and thus a statistically significantly **reduced litter size** at PND4 (11.7 vs 14.4 in control group). Therefore, effects on survival in adult animals may reflect developmental toxicity rather than general systemic toxicity causing secondary effects. **F1 offspring body weights** were reduced in animals administered 80 or 120 mg/kg/day and **F1 neurobehaviour/sensory function and motor activity** for F1 males and females at 80 or 120 mg/kg/day further supporting developmental toxicity. **Sperm counts** were affected in high dose F0 males and at all dose levels for F1 males. Exposure to silver acetate resulted in significantly reduced Cu^{2+}serum levels. Weight changes were observed in several organs but with no other histopathological correlation than pigmentation. Therefore, these changes may be due to the deposition of a heavy metal and/or result from processes such as enzymatic induction (liver) or extramedullary haematopoesis (spleen). The effects on fertility in males (a statistically significant decrease in cauda epididymis and testicular weight, low testicular and cauda epididymal total spermatid and sperm counts (millions)) are considered a primary effect of the substance since the other effects observed are not expected to impact on these parameters.

Based on the above, the NOAELs set in this study were:

Parental NOAEL F0: could not be set since degeneration in stomach mucosa was observed in all treated females.

Parental NOAEL F1 adults: 40 mg/kg/day based on effects observed at 80 or 120 mg/kg/day including mortality, neurobehavioural changes (reduced activity and rearing in the arena, reduced reactivity, abnormal motor movement/gait, intramyelinic edema and neuronal and/or glial cell necrosis and F1 brain morphometry (low mean hippocampus). Changes in hematologocal and biochemical parameters and changes in organ weights (heart, thymus, spleen) at 120 mg/kg bw/d. It is noted that the study author did not include mortality and changes in hematological and biochemical parameters in the NOAEL set at 40 mg/kg bw/day.

Fertility NOAEL: the study author set the NOAEL for mating and performance at 120 mg/kg/day based on results obtained in the F0 generation. However, the DS do not consider it safe to exclude a toxicological significance of the effects on sperm counts observed in high dose F0 males and at all dose levels in F1 males. Although such effects may have little effect on the fertility of the rat, a substance causing a reduction of sperm counts could have different consequences for humans. Therefore, a NOAEL cannot be set.

Offspring NOAEL: 80 mg/kg/day (due to reduced offspring survival and reduced growth at 120 mg/kg/day).

The reproductive toxicity of silver acetate was also investigated in a rat one-generation study published in 2016. To mimic the most likely human exposure route, silver acetate was administered in the drinking water at dose levels of 0, 0.4, 4 and 40 mg/kg bw/d, equivalent to approximately 0, 0.25, 2.5 and 25 mg/kg bw/d silver. Groups of (P) rats (20/sex) were administered the test material throughout a 10-week pre-mating period and during mating. Females continued to be exposed during gestation and lactation; males were terminated following exposure for 90 days. The resulting (F) litters were culled (5/sex where possible) on PND4 and offspring were further selected following weaning on PND21 (1/sex/litter) and remained untreated until termination on PND26. Parental animals were observed for clinical signs; bodyweights, food and water consumption were measured periodically. Gross necropsy was performed on all parental animals; weights of selected organs were measured, and histopathological examinations were made for a limited selection of tissues and the testes of 10 males/group were additionally assessed using specific staining following perfusion fixation. The major deviations in the study include the lack of GLP compliance, lack of individual animal data and the lack of further investigations of important parameters such as oestrus cycle, sperm parameters and histopathological analyses of reproductive tissues (other than the testis). Nevertheless, the study is claimed to follow the current protocols for testing foods and food additives (FDA CFSAN Redbook, 2000) and overall, the study seems to be of good quality and results are considered reliable.

Only a few effects were noted in parental animals including a reduced fluid consumption that reached statistical significance on some occasions, reduced stomach weights and pigmentation of organs and tissues. As shown in the table below, reduced fertility indices and a reduced number of litters and implants were observed in the 40 mg/kg dose group.

	M				**F**			
	0	**0.4**	**4**	**40**	**0**	**0.4**	**4**	**40**
No. exposed to mating	19	20	20	20	20	20	20	20
No. (produced) plug or sperm-positive females	17	19	19	18	20	20	20	20
Mating index	89.5	95.0	95.0	90.0	100.0	100.0	100.0	100.0
(No. prod litter/no prod plugs/sperm-positive) ×100 (given	100.0	100.0	100.0	**88.9**	100.0	100.0	100.0	**80.0**

as "Fertility index 1" in the original article)								
(No. prod litter/no exposed to mating) ×100 (given as "Fertility index 2" in the original artcle)	89.5	95.0	95.0	**80.0**	100.0	100.0	100.0	**80.0**
Fertility index (number of females with implantations/number of sperm positive females) x 100					100	100	100	**90**
Producing litters (No.)	17	19	19	16	20	20	20	**16**
With implantations (No.)					20	20	20	**18**
Total resorption (No.)					-	-	-	**2**
Total litter loss (No.)					*1*	*1*	*1*	*2*
Non-viable pups only (No.)					-	-	*1*	-
Viable litters (No.)					*19*	*19*	*18*	***14***
Implantations (No.)					*14.4*	*14.0*	*14.3*	***11.3****
Litter size (No.)					*13.1*	*12.4*	*13.4*	***10.3****
Live pups (No.)					*13.0*	*12.3*	*12.8*	***10.5[a]***
*significantly different to controls (p≤0.05); a (p≤0)								

Results with silver sodium hydrogen zirconium phosphate: Silver sodium hydrogen zirconium phosphate in the form of Exp.add 9823-37 (also known as AlphaSan® RC2000) was tested in rats in a study performed in accordance with OECD TG 416. The test substance was administered in dietary doses of 1000, 5000 and 20000 ppm to two generations of rats throughout maturation, mating, gestation and lactation.

Parents P: There were no treatment-related deaths in the P generation and no effects on bodyweights or food consumption.

Increased relative weight of spleen was observed in high and mid dose males whereas a decreased absolute weight of thymus was observed in high dose males only. The pathological examinations showed pigmentation of pancreas in high and mid dose males and females.

Sexual function and fertility P: There were no effects on oestrous cycles, mating performance or number of pregnant females. The absolute weight of seminal vesicles and coagulating gland was statistically significantly lower at the high and mid dose as compared to the control (see the table below). For the semen parameters, the lateral amplitude was statistically significantly lower and the straightness was statistically significantly higher at the high dose as compared to the control. There were no effects on the semen morphology or on spermatid counts in testis or epididymes.

Absolute weight of seminal vesicles and coagulating gland in P males:

P organ weight: absolute (g)	0 (N=28)	1000 (N=28)	5000 (N=28)	20000 (N=28)

and relative to bw (%)				
Seminal vesicle and coagulating gland	2.881 0.451	2.627 0.407	2.480** 0.405	2.466** 0.399

Semen analysis of the P males:

Group	Dose Level (ppm)		Concen-tration (M/ml)	Motility (%)	Progres-sive motility (%)	Path velocity (µm/s)	Progres-sive velocity (µm/s)	Track speed (µm/s)	Lateral amplitude (µm)	Beat frequency (Hz)	Straight-ness (%)	Linearity (%)	Elonga-tion (%)	Area (µm sq)
1	0	Mean	293.9	33.3	3.2	172.6	90.3	399.3	24.7	41.2	53.1	24.1	40.9	348.7
		SD	247.08	15.07	1.83	13.24	6.43	31.57	4.28	2.83	3.05	2.04	4.44	170.28
		N	28	28	28	27	27	27	27	27	27	27	27	27
2	1000	Mean	312.1	41.1	3.7	169.6	90.0	388.1	24.2	40.9	53.5	24.2	40.5	350.0
		SD	263.59	15.04	1.93	11.85	7.03	31.19	3.65	3.16	2.86	1.55	4.48	118.22
		N	28	28	28	28	28	28	28	28	28	28	28	28
3	5000	Mean	272.8	39.5	3.9	174.0	92.0	401.4	23.3	40.4	53.6	24.4	41.7	410.5
		SD	244.11	18.41	2.49	13.33	7.13	32.61	4.19	2.98	2.61	1.68	6.14	171.24
		N	28	28	28	26	26	26	26	26	26	26	26	26
4	20000	Mean	301.6	33.9	4.0	164.1	92.4	373.7	21.6*	38.7	57.8*	26.9	39.5	346.2
		SD	263.32	18.58	2.24	18.77	10.40	52.91	4.59	4.84	7.97	4.93	6.23	129.59
		N	28	28	28	27	27	27	27	27	27	27	27	27

* = Significantly different from control value (p<0.05)

** = Significantly different from control value (p<0.01)

Parents F1: Four high dose males and two high dose females died in the F1 generation whereas all F1 control animals survived. One animal was killed due to suspected dystocia and pathological findings were observed in the stomach of two animals. For the remaining animals, the cause of death was unclear. The bodyweights of male rats were reduced the entire period before pairing and the bodyweights of female rats were reduced during the first three weeks before pairing and during the entire gestation and lactation periods. Food consumption was reduced in males during the last weeks of maturation and during the first days of gestation and lactation in females (≤ 10%). Pigmentation of pancreas, lymph nodes and thymus was observed in high and mid dose animals.

Sexual function and fertility F1: There were no effects on oestrous cycles, mating performance or number of pregnant females. There was a statistically significantly reduced absolute weight of seminal vesicles/coagulating gland (2.725, 2.717, 2.632 and 2. 368* g at 0, 1000, 5000 and 20000 ppm) and right testis (1.888, 1.925, 1.926, 1.747* g at 0, 1000, 5000 and 20000 ppm, respectively) at the top dose. At the top dose there was also an increased relative epididymides weight (left/right 9.6/19%) and a reduced absolute and relative prostate weight (abs/rel 33/25%). At the two highest doses there were statistically significant differences in group mean sperm head area: 7.7, 8.0, 8.3*** and 8.1*µm^2 at 0, 1000, 5000 and 20000 ppm. The only statistically significant change observed among organ weights in females was a reduced absolute/relative weight of uterus (28/13%) in the high dose group. The pre-coital interval was longer in high dose females compared to controls. Since this did not affect fertility (the group mean total implantation counts for females were 16, 16.3, 15.9 and 14.7 at 0, 1000, 5000 and 20000 ppm, respectively), it is not given further significance. According to the study author, there were no significant differences in the proportions of each of the follicle however the total number of follicles (small, medium and large) was lower in high dose animals (7.7/7.5/5.6 in (ovary 1/ ovary 2/ overall respectively) compared to controls (10.4/10.1/10.2 respectively). However, it is noted that a non-statistically significant reduction of the mean primordial follicle counts was noted in F1 animals in the study with silver zinc zeolite (group means of 83.1, 65.3 and 69 in control, 1000 ppm and 6250 ppm respectively. In the absence of statistical

significance and no effects on reproductive performance, the significance of these observations are unclear.

Results with silver zinc zeolite: In a two-generation reproduction and fertility study in rats, the silver zinc zeolite denoted AgION Silver Antimicrobial Type AK was administered through the maturation, mating, gestation and lactation periods for two successive generations.

Parents P: Three males administered the high dose and one male administered the mid dose died during the study. The cause of death could not be established but the deaths were considered related to treatment by the study author. Bodyweight and bodyweight gains were reduced in males during premating by ≤ 10 and 17% respectively. After mating, the male bodyweight gain was comparable for all groups. One female control animal died during the study but no deaths occurred among the treated P females. The bodyweights were reduced in high dose females at day 20 of gestation and at day 7, 14 and 21 of lactation but did not fall below 11% of the bodyweight in controls. In the high dose group females the bodyweight gain was reduced during gestation, during days 0-20 by 16% and days 14-20 by 29%. The adjusted mean maternal bodyweight change was not calculated but considering that the mean bodyweights of males and females pups were 15% lower compared to controls day 0 and that the number of pups born/litter was 15% lower than controls, the reduced bodyweight gain may have been an intrauterine rather than a maternal effect. This can also be illustrated by roughly adjusting the mean maternal body weight for foetal weights. The results indicate that the terminal bodyweights of high dose dams were actually higher compared to control dams when the total litter weight was subtracted. Therefore, the reduced bodyweight gain observed during gestation in 12500 ppm dams seems due to effects on foetal weight rather than maternal weight. The reduced body weight gain during gestation is thus not considered to indicate severe maternal toxicity in the P high dose females. The bodyweight gain during lactation was at some of the measurements significantly increased or decreased compared to controls, but the overall bodyweight gain during lactation (days 0-26) was not statistically significantly different from controls. Food consumption was reduced between 12 and 27% in the high dose group during lactation and the changes were statistically significant. The reduced bodyweight gain and food intake is further discussed in section 4.11.5. High dose males and females had increased levels of erythrocytes, platelets and decreased levels of haemoglobin (Hb), haematocrit (HCT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Some of these parameters were also slightly affected in mid dose males and females. The same effects were seen also in the repeated dose studies performed with silver zinc zeolite Type AK and were considered to be caused by zinc. According to the repeated dose study report, zinc prevents uptake of copper in the GI tract which suppresses production of ceruloplasmin. This in turn leads to decreased iron transport and decreased synthesis of haemoglobin. There were no clinical signs observed. Pigmentation was observed in several tissues of mid and high dose animals and mild pigmentation of pancreas and thymus was observed also in some females of the low dose group. Histopathological changes in the kidneys (including hydronephrosis) were noted in high and mid dose animals. Kidney weights were decreased in high dose male and females. The thymus was not weighed.

Sexual function and fertility P: There were no effects on sexual function and fertility apart from a slightly increased gestation length in treated animals (22.3 in all treated groups compared to 21.9 days in controls) that was statistically significant for the mid and high dose group. There were no effects on sperm parameters at any dose. At the top dose the weight of left and right epididymis and testis relative to body weight were statistically significantly increased (about 10 %) as compared to the controls, but there were no effects on the absolute weights of these organs. The top dose caused also excessive mortality (10%) in males, and therefore this dose level is not considered relevant for assessing effects on sexual function and fertility in males. Adverse effects on reproduction were manifested in high dose animals as reduced mean number of live and total pups at birth, reduced live birth index, increased number of stillborn pups and increased stillborn index (see tables in section 10.10.5). Complete pup mortality was observed in six females of the high dose group, all others retained litters to weaning. Considering that there was only a

slight decrease in the mean total implantation scars/litter (and even an increase in F1 dams) the increased mean stillborn index likely reflects an increase in post-implantation loss (see section 10.10.5).

Fertility parameters of P animals:

Endpoint	0 ppm	1000 ppm	6250 ppm	12500 ppm
No. Females on Study	30	30	30	30
No. Females Paired	29	30	30	30
No. Females Mated	29	28	29	30
No. Pregnant	28	26	28	28
Female Mating Index	100.0	93.3	96.7	100.0
Female Fertility Index	96.6	86.7	93.3	93.3
Female Fecundity Index	96.6	92.9	96.6	93.3
No. Males on Study	30	30	30	30
No. Males Paired	29	30	30	29
No. Males Mated	29	28	29	29
No. Males Impregnating a Female	28	26	28	27
Male Mating Index	100.0	93.3	96.7	100.0
Male Fertility Index	96.6	86.7	93.3	93.1
Male Fecundity Index	96.6	92.9	96.6	93.1

Total Implantation Scars/Litter		0 ppm	1000 ppm	6250 ppm	12500 ppm
	Mean	14.9	14.3	14.2	13.6
	SD	2.38	1.76	3.68	3.33
	N	28	26	28	28

Sperm parameters of P animals

Endpoint	0 ppm Mean	0 ppm SD	0 ppm N	1000 ppm Mean	1000 ppm SD	1000 ppm N	6250 ppm Mean	6250 ppm SD	6250 ppm N	12500 ppm Mean	12500 ppm SD	12500 ppm N
Sperm Motility												
Percent Motility	94.5	6.87	30	97.1	3.35	30	97.1	2.90	29	94.7	7.12	26
Percent Progressive Motility	77.8	8.40	30	80.2	4.80	30	78.4	6.63	29	74.7	8.26	26
Total Sperm Concentration per Cauda Epididymis x 10^{-8}	3.222	0.5782	30	3.384	0.6500	30	3.385	0.5503	29	3.332	0.7783	26
Sperm Concentration per gram Cauda Epididymis x 10^{-8}	9.707	1.3751	30	9.977	1.1818	30	10.493	1.4844	29	10.283	2.1481	26
Homogenization Resistant Sperm Head Count x 10^{-8}	2.106	0.4876	30	NA	NA	NA	NA	NA	NA	1.904	0.3646	26
Daily Sperm Production (DSP) No. Sperm Heads/Testis x 10^{-8}	0.345	0.0799	30	NA	NA	NA	NA	NA	NA	0.312	0.0598	26
Spermatogenic Efficiency DSP per gram Testis x 10^{-8}	0.191	0.0398	30	NA	NA	NA	NA	NA	NA	0.176	0.0273	26
Percent Abnormal	0.07	0.217	30	NA	NA	NA	NA	NA	NA	0.13	0.267	26

NA = not available

Parents F1: The mortality in the high dose (12500 ppm) animals was excessive as 28/30 males and 23/30 females died prior to the end of the premating period. The group was therefore terminated prior to mating and there were consequently no data on fertility parameters or pups from this group. The cause of death was not clearly established but discoloration of organs, histopathological changes in the kidneys, decreased size of thymus, enlarged heart and spleen, penile distention/extension and red discoloration were noted among the dead animals. The mortality rates as 6250 ppm were 23.3 and 3.3% for males and females, respectively. Body weights of F1 males administered 6250 or 12500 ppm were lower than controls at the start of and throughout the premating, pairing and post-pairing periods and until termination of the high dose group. The body weight gain in males administered 6250 ppm was however comparable to controls over the entire premating period. Bodyweights of mid dose F1 females were statistically lower during the first six weeks of premating and also at one time-point during lactation but there were no statistically significant effects on body weight gains during overall (week 1-12) premating, gestation or lactation. Food consumption was reduced in high dose animals and in mid dose males during the entire study.

The macroscopic examinations of F1 animals revealed changes in the urinary tract and in the kidneys. Effects on kidneys included mild caliculi, mild to moderate pelvic dilation and an increased incidence of mild to moderate cortical surface irregularity. Most often cortical surface irregularity corresponded to microscopical changes such as chronic interstitial nephritis and/or infarction. In addition, two males administered 6250 ppm had mild calculus formation in the urinary bladder. Low and mid dose animals had an increased frequency of hydronephrosis (increased frequency compared to P). Tan/brown discoloration of multiple organs were observed in animals (pancreas, thymus, glandular stomach, duodenum, jejunum, mandibular salivary glands, Harderian glands, exorbital lacrimal glands, pineal gland and urinary bladder. A low incidence of thymic athrophy was noted in animals administered 1000 (premating 71/87 mg/kg bw/d in males and females respectively)) or 6250 ppm (m/f: 477/582 mg/kg bw/d). Organ weight analysis of animals administered 6250 ppm showed an increased relative weight of spleen (only significant in males), reduced absolute brain weight in males and females. Reduced kidney weights were observed in males and females administered 1000 or 6250 ppm. Other statistically significant changes observed were not considered related to treatment. Splenomegaly correlated microscopically with increased extramedullary haematopoiesis and is assumed to be related to treatment since anaemia was observed in the P parents.

Sexual function and fertility F1: There were no statistically significant or clearly dose-related effects on the fertility parameters. It is noted however that the percentage of abnormal sperm was higher in treated

animals compared to controls (0.50 in the mid dose (6250) group, 1.41 in the low dose group and 0.18 in controls). In the absence of statistical significance and effects on fertility, the significance of this finding is unclear. A reduced absolute/relative weight of prostate, reduced absolute weight of seminal vesicle, reduced absolute/relative weight of both testes and reduced absolute weight of uterus/oviducts/cervix was observed but only changes in absolute weights achieved statistical significance. Effects are therefore considered secondary to the reduced bodyweight. Also the delay in sexual maturation (day of vaginal opening and preputial separation, respectively) are considered to be a consequence of the reduced bodyweight. The mean primordial follicle counts were reduced in treated animals (group means of 83.1, 65.3 and 69 in control, 1000 ppm and 6250 ppm respectively) but statistical significance was not achieved. In the absence of effects on reproductive performance the significance of this observation is unclear.

Sexual maturation of F1 animals:

Endpoint		0 ppm	1000 ppm	6250 ppm	12500 ppm
Vaginal Opening (Days)	Mean	35.1	35.6	39.8 [b]	59.9 [b]
	SD	2.25	2.16	3.59	10.82
	No. of Pups Passing	30	29	30	22
Body Weight on Day Passed Vaginal Opening, g	Mean	118.1	114.9	114.8	117.9
	SD	12.78	16.53	12.93	20.87
	No. of Pups	30	29	30	21
Preputial Separation (Days)	Mean	44.5	44.2	47.4 [b]	56.7 [b]
	SD	3.71	1.81	3.01	6.44
	No. of Pups Passing	30	30	30	23
Body Weight on Day Passed Preputial Separation, g	Mean	209.1	203.2	183.0 [b]	130.3 [b]
	SD	19.09	19.93	22.65	18.49
	No. of Pups	29	30	30	23

Sexual organ weights in F1 animals:

F1 (adults) organ weight: absolute (g) and relative to bw (% x 10^2)	0 ppm (N=30)	1000 ppm (N=29)	6250 ppm (N=23)	12500 ppm (N=2) No statistical analysis performed
Epididymes (left)	0.74 1.23	0.71 1.20	0.64** 1.21	0.48 1.83
Epididymes (right)	0.74 1.24	0.72 1.21	0.66** 1.23	0.47 1.79
Prostate	0.70 1.17	0.68 1.14	0.56** 1.06	0.42 1.58
Seminal vesicle	2.71 4.52	2.58 4.33	2.48* 4.67	1.19 4.53
Testis (left)	1.92	1.89	1.69**	1.51

	3.21	3.17	3.16	5.74
Testis (right)	1.91 3.19	1.88 3.15	1.68** 3.14	1.44 5.46
Ovary	0.115 3.38 (N=30)	0.116 3.54 (N=30)	0.128 3.93 (N=29)	0.061 3.83 (N=7)
Uterus/oviducts/cervix	0.679 0.2 (N=30)	0.646 0.2 (N=30)	0.543* 0.17 (N=29)	0.240 0.14 (N=7)

Fertility parameters in F1 animals:

Endpoint	0 ppm	1000 ppm	6250 ppm	12500 ppm[1]
No. Females on Study	30	30	30	
No. Females Paired	30	30	29	
No. Females Mated	27	25	27	
No. Pregnant	25	19	23	
Female Mating Index	90.0	83.3	93.1	
Female Fertility Index	83.3	63.3	79.3	
Female Fecundity Index	92.6	76.0	85.2	
No. Males on Study	30	30	30	
No. Males Paired	30	29	26	
No. Males Mated	27	24	25	
No. Males Impregnating a Female	25	18	22	
Male Mating Index	90.0	82.8	96.2	
Male Fertility Index	83.3	62.1	84.6	
Male Fecundity Index	92.6	75.0	88.0	

No. - Number
[1] Group 4 terminated prior to mating

*No statistical significance observed

Primordial follicle counts in F1 females*:

	0 ppm	1000 ppm[e]	6250 ppm
Number of Follicles per Group (10 Slides per Animal)	1246	1035	783
Number of Animals per Group (included in the statistical process)	15	12	15
Group Mean	83.1	65.3	69.0
Group Standard Deviation	33.71	42.01	23.47

*No statistical significance observed
[e]Not included in the statistical analysis

Sperm parameters in F1 males:

	0 ppm			1000 ppm			6250 ppm		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Sperm Motility									
Percent Motility	88.7	9.67	30	92.3	7.62	29	95.5[b]	4.14	22
Percent Progressive Motility	65.3	12.85	30	68.3	13.63	29	73.4[a]	9.33	22
Total Sperm Concentration per Cauda Epididymis x 10^{-8}	3.513	0.5746	30	3.480	0.6093	29	3.202	0.4202	23
Sperm Concentration per gram Cauda Epididymis x 10^{-8}	10.547	1.2809	30	10.392	0.9297	29	10.945	1.1363	23
Homogenization Resistant Sperm Head Count x 10^{-8}	1.983	0.2941	30	NA	NA	NA	1.842	0.2554	23
Daily Sperm Production (DSP) No. Sperm Heads/Testis x 10^{-8}	0.325	0.0483	30	NA	NA	NA	0.302	0.0418	23
Spermatogenic Efficiency DSP per gram Testis x 10^{-8}	0.170	0.0265	30	NA	NA	NA	0.181	0.0288	23
Percent Abnormal	0.18	0.404	30	1.41[b]	1.173	29	0.50	0.707	23

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number
NA - Not Available/Not Applicable

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Other effects of possible relevance to fertility were noted in the chronic/carcinogenicity study with silver zinc zeolite and include a statistically significant increased incidence of ovarian cysts in mice and a statistically significant trend for increased endometrial polyps in rats. However, the findings were dismissed by the study authors since they were irregularly distributed and the incidence of endometrial polyps was also claimed to be lower than the historical control data referred to. The increase in endometrial polyps was not considered to be a true effect by the Technical Meeting for Biocides in June 2013.

MICE	0	0.1	0.3	0.9	

Ovarian cysts	6/49	22/49***	19/50***	16/49***	
RATS	**0**	**0.01**	**0.03**	**0.1**	**0.3**
Endometrial polyps*	0/49	2/50	5/49	9/50**	7/49**
Pituitary adenomas*	M:1/50 **F:11/49**	M:0/49 **F: 16/50**	M:3/50 **F: 12/49**	M:0/48 **F: 19/50**	M:1/49 **F: 20/49**
* Statistically significant dose response relation ** Statistically significant p<0.05 *** Statistically significant p<0.01					

Published studies with nanosilver: There is an abundance of studies available in the open literature in which effects of nanosilver have been investigated. Due to the large amount of data available, all information cannot be presented here. However, published studies performed via oral route and referred to in a review of reproductive and developmental toxicity (Emaa et al[18]) describing adverse effects on sexual function and fertility are considered relevant to include in this assessment. These studies are presented in the table below. Further data such as tabulated numerical data and graphical illustrations are available in the study summaries attached in Annex I.

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
No guideline No GLP	Rats Sprague-Dawley 20/group 1 control group, 2 expermintal groups	Silver nanoparticles 10 and 25 nm, purity: 99.98% 28 days 1, 2, and 4 months Oral gavage 100 and 500 mg/kg/ once day	Not determined	The silver concentrations in the testes and brain did not decrease to the control levels, even after the 4-month recovery period, indicating that silver clearance is difficult across biological barriers, such as the blood–brain barrier or		IIIB 6.8.2-13 Lee, H., Kim Y.S., Song, K.S., Ryu, H.R., Sung, J.H., Park, J.D., Park, H.M, Song, N.W., Shin, B.S., Marshak, D., Ahn, K., Le, J.A., and Yu, I.J.

[18] Makoto Emaa, *et al*; A review of reproductive and developmental toxicity of silver nanoparticles in laboratory animals, Reproductive Toxicology 67 (2017) 149–164

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				blood-testis barrier. Silver concentration clearance order: blood > liver = kidneys > spleen > ovaries > testes = brain. Therefore, the silver clearance from tissues containing biological barriers would appear to be differently regulated. A certain level of liver toxicity was indicated by the increase of cholesterol, alkaline phosphatase, and aspartate aminotransferase (AST).		(2013): Biopersistence of silver nanoparticles in tissues from Sprague-Dawley rats. Part Fibre Toxicol.;10:36
No guideline No GLP	Rats Wistar (males) 8/group	Silver nanoparticles, 70 nm 25, 50, 100, and 200 “mg/kg concentration” Oral gavage 48 days	Not determined	Ag NPs have acute and significant effects on spermatogenesis and number of spermatogenic cells and also on acrosome reaction in sperm cells. Microscopic studies showed a significant reduction in number of primary spermatocytes	Numerical data is available in study summary	IIIB, 6.8.2-14 Miresmaeili, S.M., Halvaei, I., Fesahat, F., Fallah, A., Nikonahad, N., and Taherinejad, M. (2013): Evaluating the role of silver nanoparticles on

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				in all experimental groups except experimental group 1 (25 mg/kg) (p=0.012) as well as spermatids (p=0.03) and spermatozoa (p=0.03) compared to control group.		acrosomal reaction and spermatogenic cells in rat. Iran J. Reprod Med Vol 11 (5); 423-430
No guideline No GLP	Rats Wistar 15/group 1 control group, 4 experimental groups	Silver nanoparticles, 70 nm Oral gavage 25, 50, 100, and 200 mg/kg/day every 12 hours 45 days None Oral gavage 25, 50, 100, and 200 mg/kg every 12 hours	Not determined	Significant reduction in sperm progressive motility related to dose of nanoparticle uptake. Significant increase in spermatozoa with non-progressive motility and immotile spermatozoa. Significant dose-related reduction in percentage of normal spermatozoa related to the dose of nanoparticles. Significant reduction of the blood serum testosterone. Significant dose dependent increase of blood serum leuteinizing hormone (LH).	The decrease in sperm motility was stated to probably be due to the influence of silver nanoparticles on mitochondrial function, possibly via ROS/free radical formation. Numerical data is available in study summary	IIIB, 6.8.2-15 Baki, M.E., Miresmaili, S.M., Pourentezari, M., Amraii, E., Yousefi, V., Spenani, H.R., Talebi, A.R., Anvari, M., Fazilati, M., Falah, A.A., and Mangoli, E. (2014): Effects of silver nanoparticles on sperm parameters, number of Leydig cells and sex hormones in rats. Iran J. Reprod.

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				Significant reduction in the number of Leydig cells prominent in higher concentrations (100 mg/kg and 200 mg/kg).		Med Vol 12 (2); 139-144
No guideline No GLP	Rats Wistar 10 males/group (5 sacrificed at PND 53, 5 at PND 90).	60 nm AgNPs suspended in aqueous solution. 0 µg/kg bw, 15 µg/kg bw, 30 µg/kg bw Oral Gavage Daily for 35 days (Post Natal Day (PND) 23 to PND 58). Post-exposure period: 44 days (PND 58 to PND 102)	Not determined	Delay onset of puberty (weight at puberty not affected) AgNP exposure during the prepubertal period also decreased the sperm reserves in the caput, corpus, and cauda of the epididymis in both treatment groups at PND53 and PND90. Significant reduction of sperm transit time through the segments of the epididymis at PND53. No significant change of bw between treated groups and the control. Sexual partner preference score in the group that received 15 µg/kg AgNPs	Numerical data is available in study summary	IIIB, 6.8.2-17 Mathias, F. T.; Romano, R. M.; Kizys, M. M. L.; Kasamatsu, T.; Giannocco, G.; Chiamolera, M. I.; Dias-da-Silva, M. R.; Romano, M. A. (2015): Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				indicates a preference for the male sex.		
No guideline No GLP	Rats Wistar 8 males/group	Citrate capped nanosilver, 5 – 20 nm Oral Gavage 20 µg /kg bw Daily for 90 days	Not determined	Ultrastructural observations present evidence of severely impaired and apoptotic germ cells in the testis; severe cellular changes in the cytoplasm of spermatogeni, primary and secondary spermatocytes, round and elongating spermatids and Sertoli cells No deaths, clinical signs or bodyweight effects	Apoptosis occurring in testicular epithelium served as the major cause of reduced germ cell populations observed Results from transmission electron microscopy are shown in study summary	IIIB, 6.8.2-18 Thakur, M.; Gupta, H.; Singh, D.; Mohanty, R. I.; Maheswari, U.; Vanage, G. (2014): Histopathological and ultra-structural effects of nanoparticles on rat testis following 90 days (Chronic study) of repeated oral administration
OECD TG 408: Repeat dose 90-day oral toxicity study in rodents No GLP	Rats Sprague Dawley 6 males/group	PVP capped nanoparticles, 20 – 30 nm suspended in a 0.9% saline solution 99.95% Oral Gavage 0 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg	Not determined	No significant changes in sperm count were observed, while a non-significant decrease number of spermatozoa was noted at 200 mg/kg. Haematoxylin–nigrosin staining showed a decrease in sperm viability at 200 mg/kg	Numerical data is available in study summary	IIIB, 6.8.2-19 Lafuente, D.; Garcia, T.; Blanco, J.; Sánchez, D. J.; Sirvent, J. J.; Domingo, J. L.; Gómez, M. (2016): Effects of oral

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				after AgNPs treatment. No significant effects of PVP-AgNPs on sperm motility were found. An increased number of epididymal sperm morphological abnormalities at 100 mg/kg but not at 200 mg/kg. No significant changes in tissue morphology.		exposure to silver nanoparticles on the sperm of rats
No guideline No GLP	Rat Wistar albino 6 rats/group: 2 control groups 4 treatment groups	Administrations: Group 1 (control): phys saline 2 weeks Group 2 (control): phys saline 4 weeks Group 3 (30 mg/kg): AgNP 2 weeks Group 4 (300 mg/kg): AgNP 2 weeks Group 5 (30 mg/kg): AgNP 4 weeks Group 6 (30 mg/kg): AgNP 4 weeks	Not relevant	All treated groups: Statistically significant reduction of bodyweight gain (no further information available) Congested blood vessels in stroma with inflammatory mononuclear cell infiltration. Dose and duration dependant areas of congestion in the stroma with extravasations of blood Excess depositions of collagen fibres indicating fibrosis.	**Numerical data not available in the article** hence no study summary available	M. Amr El-Nouri et al. (2013) Life Science Journal 2013;10(2); Study of the Effects of Silver Nanoparticles Exposure on the Ovary of Rats

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				Dose and duration dependent positive reactions for Caspase3 indicating apoptosis. Relative increase in atretic and degenerated follicles.		
	Rats Sprague-Dawley Male Six week old Average weight 245g 5/group 2 control groups, 3 experimintal groups	Silver nanoparticles Non-coated AgNPs <20nm PVP-coated AgNPs <15nm Non-coated – matrix = 4% polyoxyethylene glycerol trioleate and 4% Tween 20 in H2O Coated - suspended in water Oral exposure 28 days exposure Post-exposure: wash out until days 36 and 84 Silver exposure: 90 mg/kg bw for the Ag < 20 and Ag < 15-PVP groups vs 9 mg/kg bw for the AgNO3 group),	Not determined	Main target organs for AgNPs and AgNO3: liver and spleen, followed by the testis, kidney, brain, and lungs, without differences in the distribution pattern between the two different AgNPs, or the AgNO3 exposed animals. Higher uptake of silver in blood and organs of AgNO3 exposed rats Elimination of silver occurred at an extremely slow rate in brain and testis, which still contained high concentrations of silver two months after	Fraction of soluble silver rather similar between the Ag < 20 nm and AgNO3 animals in blood and in organs with the exception of testis and spleen (see text). This indicates that silver is probably mainly bioavailable in the ionic form (see text) Nanoparticles are formed in vivo from silver ions and they are probably composed of silver salts.	IIIA, 6.8.2-12 Van der Zande, M., Vandebriel, R.J., van Doren, E., Kramer, E., Herrera Riviera, Z., Serrano-Rojero, C.S., Gremmer, E.R., Mast, J., Peters, R.J.B., Hollman, P.C.H., Hendricksen, P.J.M., Marvin, H.J.P., Peijenberg, A.A.C.M., and Bouwmeester, H. (2012):

Summary table of animal studies on adverse effects on fertility (further data available in Annex I						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				the final exposure.		Distribution, Elimination, and Toxicity of Silver Nanopartcles and Silver Ions in Rats after 28-day Oral Exposure. ACS Nano. 28;6(8):7427-42

The table above shows published in vivo studies performed via the oral route and referred to in a review of reproductive and developmental toxicity of silver nanoparticles. The review is based on a literature search performed in 2016 however since the studies presented are not performed in accordance with recognised guidelines or the principles of GLP it is difficult to assess their robustness and reliability. Nevertheless, consistency of effects (e.g., numerical reduction in sperm and sperm morphological abnormalities) among studies indicate a toxic effect of nanosilver on sexual function and fertility.

Nanosilver was detected in the testes of rats and in mice in several of the studies performed. The levels were comparable or higher than in other tissues unless the size of nanoparticles were larger than approximately 70 nm. The authors suggest that this is due to smaller sized particles being more easily absorbed. Following a four-month recovery period the levels of nanosilver gradually decreased in all investigated tissues but the testis thus clearance across the blood-testis barrier is apparently difficult. The review also includes a study describing histopathological alterations (e.g. inflammation, apoptosis, atretic and degenerated follicles) in ovaries of rats administered 30 or 300 mg/kg bw/d[19]. Unfortunately, numerical data is not available in the original publication.

Nanoparticles of silver were shown to have acute and significant effects on spermatogenesis and number of spermatogenic cells and also on acrosome reaction in sperm cells:

Miresmaeili, S.M et al (IIIB, 6.8.2-14): Significant reduction in number of primary spermatocytes in all experimental groups except one as well as of spermatids and spermatozoa compared to control group. General toxicity not reported.

Baki, M.E., (IIIB, 6.8.2-15): Significant reduction in sperm progressive motility related to dose of nanoparticle uptake. Significant increase in spermatozoa with non-progressive motility and immotile spermatozoa. Significant dose-related reduction in percentage of normal spermatozoa related to the dose of nanoparticles.

[19] M. Amr El-Nouri et al. (2013) Life Science Journal 2013;10(2); Study of the Effects of Silver Nanoparticles Exposure on the Ovary of Rats

Significant reduction of the blood serum testosterone and significant dose dependent increase of blood serum leuteinizing hormone (LH). Significant reduction in the number of Leydig cells prominent in higher concentrations (100 mg/kg and 200 mg/kg). General toxicity not reported.

Mathias, F. T et al (IIIB, 6.8.2-17): Delayed onset of puberty (weight at puberty not affected). AgNP exposure during the prepubertal period also decreased the sperm reserves in the caput, corpus, and cauda of the epididymis in both treatment groups at PND53 and PND90. Significant reduction of sperm transit time through the segments of the epididymis at PND53. There was no significant change of bw between treated and control animals. The sexual partner preference score in the group that received 15 µg/kg bw indicated a preference for the male sex. Although the effect was statistically significant ($p<0.01$) the toxicological significance of this observation is unclear since a preference for females was observed in the 30 µg/kg bw. The authors speculate that the effect could correlate with the non-significant increase in testosteron level also observed in the 15 µg/kg bw group.

Thakur, M. et al (IIIB, 6.8.2-18): Ultrastructural observations present evidence of severely impaired and apoptotic germ cells in the testis; severe cellular changes in the cytoplasm of spermatogenia, primary and secondary spermatocytes, round and elongating spermatids and Sertoli cells. No deaths occurred and there were no other signs of toxicity (bodyweight, behavioural effects).

Lafuente, D. et al (IIIB, 6.8.2-19): No significant changes in sperm count were observed, while a non-significant decrease in number of spermatozoa was noted at 200 mg/kg. Haematoxylin–nigrosin staining showed a decrease in sperm viability at 200 mg/kg after AgNPs treatment. No significant effects of PVP-AgNPs on sperm motility were found. An increased number of epididymal sperm morphological abnormalities at 100 mg/kg but not at 200 mg/kg. There were no significant changes in tissue morphology. A significant decrease in food intake was observed but there were no effects on bodyweight.

The REACH registration dossier also contains a study investigating sperm parameters in rats receiving a single dose (5 mg/kg or 10 mg/kg) of 20 nm silver nanoparticles or 5 mg/kg of 200 nm silver nanoparticles by intravenous administration (Gromadzka-Ostrowska, J. et al 2012). Some changes in the investigated parameters were noted:

- statistically significant decrease in gonadosomatic index (GSI) during the experiment ($p < 0.05$).

- apparent decrease in sperm count - statistically significant differences of the epididymal sperm count in respect to both, silver nanoparticles size and dose ($p < 0.002$). There was a statistically significant lower values in the 24-hour 5 mg/kg group (20 nm silver nanoparticles) in comparison to those observed in the 10 mg/kg (20 nm silver nanoparticles), 5 mg/kg (200 nm silver nanoparticles) and control groups ($p < 0.05$; $p < 0.001$ and $p <0.001$ respectively).

- statistically significant decrease in the germ cell counts following the 5 mg/kg group (20 nm silvernanoparticles) injection in the 28-day group, when compared with the control group ($p < 0.05$). However, no difference between the control animals and 10 mg/kg (20 nm silver nanoparticles) and 5 mg/kg (200 nm silver nanoparticles) groups was found in any time of measurements.

- in all treated groups the number of abnormal spermatozoa found 1 or 4 weeks after treatment was higher ($p < 0.05$), when compared with the results at 24 hours. Folded, amorphous spermatozoa, cells lacking or showing a small hook and cells with undulating or elongated head were the most frequent abnormal forms found. In addition, in all groups examined 1 and 4 weeks following the injection elongated heads and two or three headed forms appeared more often than in groups examined 24 hours after injection.

- the comet assay showed that DNA damage (% DNA in tail) in the germ cells was significantly increased at 24 hours in the 5 mg/kg group (20 nm silver nanoparticles) and 10 mg/kg (20 nm silver nanoparticles) groups ($p \leq 0.05$), then decreased at later time after injection, i.e. 7 and 28 days after treatment. At day 7 and 28 after injection, no significant difference in the extent of DNA damage between the silver nanoparticles treated groups and the control animals was found. No difference in the DNA damage level was found between the control group and the 5 mg/kg (200 nm silver nanoparticles) group at all time points.

- histomorphometry of seminiferous tubules: analysis showed that the values of all parameters differed significantly between silver nanoparticles treated groups ($p < 0.001$ for all parameters). Posthoc tests revealed the signficantly higher values only in the 5 mg/kg (200 nm silver nanoparticles) group. Histological

examination of the testes showed also the differences of seminiferous tubules morphology, namely wider intercellular space and higher vacuolization of the germinal epithelium in 5 mg/kg (200 nm silver nanoparticles) group rats. No differences were observed between the 5 mg/kg group (20 nm silver nanoparticles), 10 mg/kg (20 nm silver nanoparticles) and NaCl groups, nor time dependent changes of tested parameters within the particular groups.

10.10.3 Comparison with the CLP criteria

Silver nitrate has corrosive properties and the systemic toxicity of the substance is thus difficult to test. Consequently, the toxicological data on silver nitrate is very limited. There is no information specifically addressing the reproductive toxicity of silver nitrate but effects on fertility were noted in a one-generation rat study performed with silver acetate which is a soluble silver salt of simple chemical composition. Furthermore, effects on sperm parameters (sperm counts at 120 mg/kg/day for F0 males and all dose levels for F1 males; F1 sperm morphology at 120 mg/kg/day) were noted in an EOGRTS performed with silver acetate and similar effects were noted in several published studies performed with nanosilver. The effects can thus be ascribed the silver ion and data for this substance is thereby considered relevant to assess effects of silver nitrate at non-corrosive concentrations. Likewise, data obtained with other silver containing active substances releasing silver ions are considered relevant in a weight of evidence approach. Since the threshold for corrosivity (i.e., the dose where it would not be possible to distinguish between primary and secondary effects) is unknown, all effects of possible relevance and reliability are considered in this weight of evidence approach..

The most robust and relevant data in this section include an EOGRTS performed with silver acetate administered in diet at doses of 40, 80 and 120 mg/kg/day. In addition to effects on sperm parameters, treatment resulted in a number of other toxic effects including: F1 mortality at 120 mg/kg/day; F0/F1 red blood cell parameters at all dose levels; F1 offspring survival at 120 mg/kg/day; F1 offspring body weight at 80 or 120 mg/kg/day; F1 neurobehaviour/sensory function at 80 or 120 mg/kg/day; motor activity for F1 males and females at 80 or 120 mg/kg/day; Epithelial degeneration of the glandular mucosa of the stomach in F0 females at all dose levels; F1 histopathological findings of intramyelinic edema and neuronal and/or glial cell necrosis at 80 or 120 mg/kg/day and F1 brain morphometry (low mean hippocampus) at 80 or 120 mg/kg/day. The minor reductions recorded in F0 and F1 male body weight gain up to 8% and 14% lower than Controls respectively were judged non adverse. These effects are not considered to explain the effects on sperm parameters and thereby dismiss findings as being secondary to general toxicity.

The data available also includes robust two-generation studies performed with silver zinc zeolite and silver sodium zirconium hydrogenphosphate. There were no clear adverse effects on sexual function and fertility observed in these studies, but some changes of unknown significance were noted with silver sodium hydrogen zirconium phosphate (i.e., semen parameters (lateral amplitude and the straightness, mean sperm head area), pre-coital interval and number of ovarian follicles) and with silver zinc zeolite (slight increase of gestation length, percentage of abnormal sperm and the primordial follicle counts). It should be noted though that the silver ion exposure from these substances may differ from silver acetate and nanosilver. Although the estimated amount of released silver ions in gastrointestinal tract from highest tested dose was actually higher in the study with silver sodium hydrogen zirconium phosphate that did not show any clear adverse effects on sexual function and fertility compared to the one-generation study with silver acetate which showed reduced fertility indices (sperm parameters were not tested), the latter was administered in drinking water and thus in ionic form compared to silver sodium hydrogen zirconium phosphate which was administered mixed in diet. Silver ions easily bind to thiol groups of proteins and the formation of different silver complexes with biomolecules may at least theoretically limit the availability of silver ions for absorption in the gastrointestinal tract. Since the maximum dose used in the one-generation study with silver acetate administered in drinking water was lower than the doses used in the EOGRTS in which silver acetate was administered in diet, it is difficult to compare effect levels from different administration routes. The amount of silver ions released from the nanoparticles at the different doses used depend on dose, size, surface coating and test conditions. In the absence of such information in the publications (see annex 3), the silver ion exposure in the studies with nanosilver is unclear. Since

there is no robust data available for a soluble silver salt at concentrations up to a limit dose, the reproductive toxicity of the silver ion is assessed from indirect information from studies performed with different SCAS releasing silver ions. The data on silver zinc zeolite and silver sodium zirconium hydrogenphosphate are considered robust but the substances also contain additional elements of possible toxicological significance and the amount of silver ions tested are limited by silver content and release. Silver acetate and nanosilver are chemically less complex but the majority of the published studies on silver nanoparticles are not performed according to guidelines thus some parameters recommended in OECD guidelines are not analysed and the amount of silver ions released from nanoparticles is not known. Therefore, all data is considered in a weight of evidence approach and although findings in studies with low reliability (e.g., due to limited investigations or poor quality) may not in isolation be considered toxicologically significant, a similar pattern of positive findings noted for several SCAS raise concern for effects on sexual function and fertility even if studies are of limited reliability.

According to Annex I: 3.7.1.3 of the CLP regulation, adverse effects on sexual function and fertility includes *"Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."*

The reduction of the fertility index[20] (10%, not statistically analysed) and the statistically significant reduction of the number of implantations (22%, 11.3 compared to 14.4 in control) in dams observed in the published study with silver acetate at 40 mg/kg bw/day (IIIA 6.8.2-06) are considered to represent "clear" evidence of an adverse effect on sexual function and fertility and thus to fulfil criteria for classification in category 1B. The effect is assumed to be attributed to the silver ion only and based on the silver content of silver acetate the effect level is 26 mg silver ion/kg bw/d[21]. Oestrus cycle, sperm parameters and histopathological analyses of reproductive tissues (other than the testis) were not investigated in the one-generation study with silver acetate thus it is not known if the reduced fertility index results from a toxicological effect on germ cells. However, the results from a recent EOGRTS study showed statistically significant decreases in cauda epididymis and testicular weight in F1 males administered 80 and 120 mg/kg/day and low testicular and cauda epididymal total spermatid and sperm counts (millions) at all dose levels. Minor effects were also observed in high and mid dose males and in F0. The number of implantation sites (15.6, 16.9, 16.3 and 16.1 at 0, 40, 80 and 120 mg/kg bw/day, respectively) and fertility indices (100, 100, 96, 96% at 0, 40, 80 and 120 mg/kg bw/day, respectively) were not affected by treatment in the EOGRTS on silver acetate.

Furthermore, the results from several studies performed with nanosilver support an effect of silver ions on germ cells as they show **a reduced number of sperm and alterations in sperm morphology** (IIIB, 6.8.2-14, Miresmaeili et al., 2013; IIIB, 6.8.2-15, Baki, et al., 2014; IIIB, 6.8.2-17, Mathias et al., 2015; IIIB, 6.8.2-18, Thakur et al., 2014; IIIB, 6.8.2-19, Lafuente, et al., 2016; and Gromadzka-Ostrowska et al., 2012). The studies are not performed according to guidelines or the principles of GLP hence fewer animals and dose levels than required in guidelines were used in most of the studies. Therefore, it is difficult to assess the reliability and relevance of the results. However, since similar effects were observed in several studies and these are published in peer-reviewed scientific journals, the quality of studies is yet assumed to be acceptable. The results indicate that nanoparticles of silver have **acute and significant effects on spermatogenesis, the number of spermatogenic cells and on acrosome reaction in sperm cells. Effects on germ cells** were also noted in the two-generation studies performed with silver sodium hydrogen zirconium phosphate and silver zinc zeolite but **in the absence of statistical significance and effects on reproductive performance**, the significance is difficult to assess. Moreover, **a statistically significant delay in the onset of puberty was observed at both d**oses tested in one study with nanosilver although the weight at puberty was not

[20] According to the publication, the fertility index stated in the report reflects the no. produced litter/no. sperm-positive

[21] 108/167 g/mol = 0.64 × 40 = 26

affected (IIIB, 6.8.2-17, Mathias et al., 2015). A delay in sexual maturation was also observed in the two-generation study with silver zinc zeolite however in this case it was considered secondary to the pronounced effects on bodyweights.

Taken together, the effects observed with silver acetate and/or nanosilver **(reduced fertility index, number of implantations, effects on sperm parameters, spermatogenesis and number of spermatogenic cells and delay in onset of puberty)** are considered to clearly indicate an adverse effect on sexual function and fertility. However, the relevance of data on nanosilver for the assessment of the silver ion and silver nitrate need to be considered. At least some of the effects noted in the studies could result from oxidative stress leading to apoptosis. This may be caused by the nanoparticle itself or the silver ions released. The silver ion has been shown to induce oxidative stress in studies performed with silver nitrate available in the open literature[22] thus it is reasonable to assume that it is an intrinsic ability of the silver ion that is expressed also when dissolved from soluble salts like silver acetate and silver nitrate. Germ cells seem to be highly sensitive to this mechanism, but it is recognised that there may also be other modes of action for the effects observed.

It may also be discussed if nanoparticles of silver are distributed to and penetrate germ cells different from silver ions. The distribution of nanoparticles of silver as well as of silver nitrate was investigated in a 28-day study in rats (Van der Zande, M., et al, doc IIIB, 6.8.2-12). The results indicate a silver distribution pattern upon oral exposure to two different sizes of AgNPs and to AgNO3 with highest amounts in liver and spleen, followed by the testis, kidney, brain, and lungs, without differences in the distribution pattern between the two different AgNPs, or the AgNO3 exposed animals. The uptake of silver in blood and organs was higher in animals treated with AgNO3 compared to AgNP treated rats. However, when normalising the silver exposure dose in blood for soluble silver[23] the difference in blood was much smaller. This indicates that the major part of silver in plasma is ionic silver released from the nanoparticles. Normalising for soluble silver exposure dose in organs resulted in similar silver contents between Ag<20 and AgNO3 in all organs except for testis and spleen in which the silver dose was higher for Ag<20. The authors thus conclude that AgNP only contributes to the silver concentration in these two organs and to a much lower extent, a result stated to be in contrast with a different study indicating that not only soluble silver but a significant fraction of AgNPs contribute to silver in organs. Nevertheless, of significance for this assessment is the detection of silver from both nanoparticles of silver and AgNO3 in the testis meaning that it is reasonable to assume that effects discussed in this section are not specific to nanoparticles but also relevant for effects of ionic silver from salts administered via the oral route. This is also confirmed by the new EOGRTS data on silver acetate.

In conclusion, the effects noted are considered to fulfil the criteria for classification in agreement with (in bold) *"Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system,* ***adverse effects on onset of puberty, gamete production and transport,*** *reproductive cycle normality, sexual behaviour,* ***fertility,*** *parturition,* ***pregnancy outcomes, premature reproductive senescence****, or modifications in other functions that are dependent on the integrity of the reproductive systems."*

Substances are classified in category 1A based on evidence from humans. Such data is not available for silver nitrate thus criteria for this category are not fulfilled. Substances are classified in category 1B based on data from animal studies and according to CLP guidance: *"such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there*

[22] E.g. Cortese-Krott MM et al (2009), Free Radic Biol Med.; Shim I et al (2017) Appl Toxicol.

[23] "since the exposure dose of silver was not equal in all groups (90 mg/kg bw for the Ag < 20 and Ag < 15-PVP groups vs 9 mg/kg bw for the AgNO3 group), all results were normalized on the silver exposure dose and presented as the ratio between the measured silver concentration in blood or feces (μg silver/kg blood or feces) and the daily silver exposure dose (mg silver/kg body weight)."

is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Substances are classified in Category 2 based on *"some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."*

The data available on silver acetate and nanosilver indicate that the silver ion has the ability to cause adverse effects on sexual function and fertility possibly by a mechanism involving oxidative stress. According to the original report for the silver acetate study and the published studies with nanosilver there were no marked general toxicity indicating that effects were *"a non-specific consequence of the other toxic effects"*. There is no reason to expect a lower sensitivity of humans to these effects and therefore **criteria for classification in category 1B, H360F are considered fulfilled.**

10.10.4 Adverse effects on development

Table 56: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No substance-specific information available.			

Table 57: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
According to the summary prepared by the Agency for Toxic Substances and Disease Registry it is not known whether silver causes developmental toxicity in humans. There were no studies found regarding developmental effects in humans after exposure to silver, but the document refers to a study by Robkin et al. (1973) in which the possibility of a relationship between the concentration of silver in foetal tissues and the occurrence of developmental abnormalities was investigated. The authors reported that the concentration of silver in the foetal liver of 12 anencephalic human foetuses was higher (0.75±0.15 mg/kg) than the values from 12 foetuses obtained either through therapeutic abortions (0.23±0.05 mg/kg), or in 14 spontaneously aborted foetuses (0.21±0.05 mg/kg). The concentration in 9 premature infants was 0.68±0.22 mg/kg. **The authors could not determine if the higher concentration of silver in anencephalic foetuses were associated with the malformation, or with foetal age.**				Doc IIIA 6.2(08)

Table 58: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as	Observations	Reference

		applicable)		
Silver Acetate: Preliminary Reproductive Performance Study in the Sprague Dawley Rat by Dietary Administration	Silver acetate (AgAc) >99.5%	Sprague-Dawley [Crl:CD(SD)] F0: 0, 4, 40, 80, 160, 320 mg/kg bw/d 12/sex F1: 0, 4, 40 mg/kg bw/d 10/sex	**Parental F0 females:** **320 mg/kg bw/d*** ↓ Bwg (pre-pairing): 62% ↓ Bwg (gestation, d 0-20): 58% 4/12 killed following total litter loss GD22-LD2; ¨ 8/12 killed for welfare reasons GD20-LD1 Pale inactive mammary tissue 4/12 **160 mg/kg bw/d females*** 2/12 killed following total litter loss GD22-LD1; 10/12 killed for welfare reasons GD21-LD4 Pale inactive mammary tissue 2/12 Abnormal colour and content of GI tract, abnormal colour of liver, pancreas, spleen: and mesenteric lymph nodes **80 mg/kg bw/d females:** Abnormal colour of Pancreas: 11/12 Kidney: 10/12 Mesenteric lymph nodes: 8/12 **40 mg/kg bw/d females:** ↑ ALP (40%) ↑ P (80%) ↑ gGT (1 vs 0 in control) ↑ A/G (13%) Abnormal colour of Pancreas: 11/12 Mesenteric lymph nodes: 2/12 **Parental F0 males:** **320 mg/kg bw/d** ↓ Overall bwg (d1-64): 20% ↑ Platelet counts (35%) ↓ hematocrit (6%) ↓ hemoglobin (11%) ↓ MCH (12%) ↓ MCV (8%) ↓ MCHC (4%) ↑ ALP (27%) ↑ Cholesterol ↑ ALT (98%) Abnormal colour of	Covance Study Number 8436495 Report Issue Date 28 June 2021 (Draft 3)

			Liver: 11/12 Pancreas: 12/12 Mesenteric lymph nodes: 5/12 Abnormal content of: Cecum: 11/12 Rectum: 4/12 **Parental F0 males:** **160 mg/kg bw/d** ↑ Platelet counts (44%) ↓ hematocrit (9%) ↓ hemoglobin (14%) ↓ MCH (16%) ↓ MCV (11%) ↓ MCHC (5%) ↑ ALP (40%) ↑ Cholesterol (48%) Abnormal colour of Liver: 7/12 Pancreas: 10/12 Mesenteric lymph nodes: 5/12 Abnormal content of: Cecum: 7/12 Rectum: 4/12 **Parental F0 males:** **40 mg/kg bw/d** ↑ Platelet counts (23%) ↑ ALP (40%) ↑ Cholesterol (47%) **Offspring F1, 320 mg/kg bw/d:** Post-implantation survival index: 54.1% (control: 94.9%) Live birth index: 15.3% (control: 96%) ↓Bw (day 1): 24% **Offspring F1, 160mg/kg bw/d:** Post-implantation survival index: 78.7% (control: 94.9%) Live birth index: 37.8 % (control: 96%) ↓Bw (day 1): 25% * The purpose of this study was to assess the influence of Silver Acetate on reproductive performance to assist in dose level selection for an EOGRTS according to OECD TG 443. As animals/offspring in 40 mg/kg bw/day group had not shown a significant response to	

			treatment, a further dose level of 80 mg/kg bw/day was instigated to enable dose selection for the subsequent extended one generation study. An additional group (Group 7) comprising of 12 male and 12 female animals was allocated to study and treated for four weeks before pairing, during gestation and up to weaning on Day 21 of lactation when all adults and offspring were terminated. Bioanalysis of Cu, Se, ceruloplasmin **and glutathione peroxidase activity: see text**	
Silver Acetate: Extended One Generation Reproductive Toxicity Study in the Sprague Dawley Rat by Dietary Administration Sprague	Silver acetate (AgAc) >99.5%	Sprague-Dawley [Crl:CD(SD)] F0: 0, 40, 80, 120 mg/kg bw/d 25/sex F1: 0, 40, 80, 120 mg/kg bw/d 10/sex Cohort 1A: 20/sex (23 m in 120 mg/kg bw/d group) Cohort 1B: 20/sex (17 m/18 f in 120 mg/kg bw/d group) Cohorts 2A, 2B, 3: 10/sex	**Parental F0 females:** **120 mg/kg bw/d*** Mortality: 1/25 ↑ HCT: w 10: 10%, st 5% ↑ Hb, st* 4% ↑ RBC: w 10: 14%, st*: 10% ↓ MCH: w 10: 10%, st*: 5% ↓ MCHC: w 10: 7% ↓ MCV: w 10: 4%, st*: 4% ↑ RDW, st: 19%, Plt: ↓w 10: 37% st*: 16% ↓ PT: w 10: 8%, ↑ ALP w 10: 77% ↑ AST w 10: 17% ↑ gGT st*: 1 compared to 0 ↑ Chol w 10: 77%, st*45% ↓ K: w 10: 12, st*: 8% ↑ heart/rel bw: 10% ↑ epithelial degeneration of the glandular mucosa 5/23 (minimal), 5/23 (slight), 0 in cntrol **80 mg/kg bw/d females*** ↑ HCT: w 10: 9% st*: 6% ↑ Hb, st* 5% ↑ RBC: w 10: 13%, st* 10% ↓ MCH: w 10: 9%, st* 5% ↓ MCHC: w 10: 6% ↓ MCV: w 10: 3%, st* 4%	Labcorp Study Number 8437234 Report Issue Date 24 January 2022

			↑ RDW, st: 9% ↓ Plt: w 10: 42%, st: 21% ↑ ALP w 10: 39% ↑ Chol w 10: 63%, st*27% ↓ K: w 10: 11% ↑ heart/rel bw: 10% ↑ epithelial degeneration of the glandular mucosa 5/24 (minimal) 40 mg/kg bw/d females: ↑ HCT: w 10: 7%, st* 6% ↑ Hb, st* 6% ↑ RBC: w 10: 10%, st* 8% ↓ MCH: w 10: 8% ↓ MCV: w 10: 3% ↓ Plt: w 10: 43%, st* 17% ↑ RDW, st: 7% ↑ ALP w 10: 39% ↑ Chol w 10: 46%, st*19% ↓ K: w 10: 17% ↑ heart/rel bw: 5% ↑ epithelial degeneration of the glandular mucosa 3/25 (minimal) **Parental F0 males:** **120 mg/kg bw/d** ↑ Mortality 2/25 ↓ Overall bwg (d0-19): 7% ↓ HCT, st: 5% ↓ Hb, st* 11% ↑ RBC, st*: 7% ↓ MCH, st*: 18% ↓ MCHC, st*: 6% ↓ MCV, st*: 12% ↑ RDW, st: 35%, ↑ Plt, st*: 27% ↑ WBC, st*: 25% ↑ L, st*: 33% ↑ E, st*: 64% ↑ Plt, st*: 27% ↑ ALP, st*: 27%	

			↓ Creat, st*: 7% ↑ Chol, st*: 61% ↓ K, st*: 5% ↓ P, st*: 13% ↑ spleen/rel bw: 15% ↓Abs weight left testis: 5% ↓Abs weight left epididymis: 4% ↓Abs/rel prostate: 13% ↑ extramedullary hematopoiesis in spleen: Minimal: 15/23 (6/25 in control) Slight: 1/23 (0/25 in control) Marked: 0/23 (6/25 in control) Parental F0 males: **80 mg/kg bw/d** ↓ Overall bwg (d0-19): 8% ↓ Hb, st* 7% ↑ RBC, st*: 8% ↓ MCH, st*: 14% ↓ MCHC, st*: 5% ↓ MCV, st*: 10% ↑ RDW, st: 28%, ↓ Creat, st*: 4% ↑ Chol, st*: 57% ↓ K, st*: 6% ↓Abs weight left testis: 7% ↓Abs weight left epididymis: 7% ↓Abs/rel prostate: 17% ↑ extramedullary hematopoiesis in spleen Minimal: 12/24 (6/25 in control) Slight: 0/24 (0/25 in control) Marked: 2/24 (6/25 in control) **Parental F0 males:** **40 mg/kg bw/d** ↓ MCH, st*: 5% ↓ MCV, st*: 3% ↑ RDW, st: 11%, ↓ Creat, st*: 14% ↑ Chol, st*: 66% Minimal: 6/25 (6/25 in control) ↓Abs/rel prostate: 10%	

			Developmental toxicity: **Offspring F1, 120 mg/kg bw/d:** Live birth index: 89.1% vs 97.3% in ctrl Viability index (PND4): 90.2% vs 99.1% in ctrl Pup bw PND1: 8.7% and 10.6% lower than ctrl ↓Live day 1 (before culling): 19% ↓Sex ratio (before culling): 9% ↓AGD m/f: 9/11% ↑ dark coloration of skin ↓thymus weight (m/f):40/38%, rel bw m/f: 30/27 ↓abs brain weight (m/f): 8/% rel: ↑5% (f) **Offspring F1, 80mg/kg bw/d:** ↓Sex ratio (before culling): 17% ↓thymus weight (m/f):25/32 %, rel bw m/f: 16/25 ↓abs brain weight (f): 4% Offspring F1, 40mg/kg bw/d: ↓Sex ratio (before culling): 13% ↓thymus weight rel bw: 13 (f:) **F1: 120 mg/kg bw**** Mortality PND 19-47 (males): 9 (3 in 1A, 2 in 1B, 3 in 2A, 1 in 3) (females): 2 (both in 1A); of these seven decedents were subject to full microscopic examination which revealed five with brain lesions which were considered the major factor contributing to these deaths. Following the high incidence of mortality, the high dose group was terminated prematurely at approximately 10 weeks of age. ↑ Piloerection (m: 7/70, f 1/70) ↑ Hunched posture (m: 7/70, f 1/70) ↑ Abnormal gait (m: 4/70, f 1/70) ↓ Body weight at weaning m/f: 13/12% ↓ HCT f: 6% ↓ RBC f: 8% ↑ MCH m/f: 5/5% ↑ MCHC m/f: 3/3% ↓ RDW f: 5% ↑ WBC f: 128% ↑ N f: 109%	

			↑ L f: 142% ↑ E f: 180% ↑ B f: 300% ↑ M f: 150% ↑ LUC m/f: 133/150% ↓ Plt f: 15% ↓ Pt f: 2% ↓ APTT, f: 17% ↓ APTT, f: 17% ↑ ALP m/f: 215/218% ↑ ALT m/f: 71/115% ↑ AST f: 21% ↑ urea m: 26% ↓ creat m/f: 29/33% ↑ gluc m/f: 75/59% ↑ chol m/f: 70/58% ↑ K m/f: 28/13% ↑ Ca m: 3% ↓ Phos, m/f: 10/16% ↓ Total prot, f: 8% ↓ Total Alb, f: 10% ↓ A/G ratio, f: 9% ↓ **Absolute brain weight (m and f) PND 22** **F1: 80 mg/kg bw** ↓Body weight at weaning m/f: 9/10% ↓Bodyweight gain (d 1-71) m: 14% ↓ **Absolute brain weight (f) PND 22** ↑ HCT f: 5% ↑ RBC m/f: 6/7% ↓ MCH m: 8% ↑ MCHC m/f: 3/3% ↓ RDW f: 5% ↑ WBC f: 66% ↑ N f: 105% ↑ L f: 78% ↑ E f: 200% ↑ B f: 200% ↑ M f: 200% ↑ LUC f: 100% ↓ Plt f: 31% ↓ Pt f: 6% ↑ ALP m: 28% ↑ ALT m: 31% ↓ creat m/f: 7/9% ↑ chol m/f: 40/45% ↑ Ca m: 3%	

			↓ Phos, f: 16% **F1: 40 mg/kg bw** ↓Bodyweight gain (d 1-71) m: 6% ↑ RBC f: 4% ↑ WBC f: 51% ↑ B f: 100% ↑ M f: 100% ↑ LUC f: 150% ↑ ALP m: 21% ↓ creat m/f: 14/12% ↑ chol m/f: 60/29% **Cohort 1A:** **120 mg/kg bw/d:** **Cohort 1A:** Intramyelinic edema in brain Hippocampal neuronal necrosis Neuronal/glial cell necrosis in thalamus Extracellular pigment in brain brain weight Abs/rel: M: ↓9/↑25% F: abs ↓7% heart weight Abs/rel: M: ↓12/↑18% F: rel ↑14% thymus weight Abs/rel: M: ↑31/75% F: ↑31/46% Adrenal weight M (abs): ↓24% F (abs/rel): ↓21/11% Kidney weight Abs/rel: M: ↓22/↑7% F (abs): ↓22 Pituitary weight Abs/rel: M(abs): ↓27 F ↑6/22% Thyroid and parathyroids: Abs/rel: M(abs): ↓21 F ↓13/10%	

			Testis, left: Abs/rel: ↓19/↑13% Testis, right: Abs/rel: ↓12/↑22% Epididymis, left: Abs: ↓31% Epididymis, right: Abs/rel: ↓33/8% Prostate: Abs/rel: ↓39/18% Seminal vesicles: Abs/rel: ↓45/26% Uterus, cervix, oviducts: Abs/rel: ↓28/21% **80 mg/kg bw/d** Intramyelinic edema in brain Hippocampal neuronal necrosis Neuronal/glial cell necrosis in thalamus Extracellular pigment in brain ↑ brain weight Abs/rel: M: ↓4/↑7% F: abs ↓7% heart weight rel m/f: ↑7/9% F: rel /↑14% Adrenal weight M (abs): ↓14% F (abs/rel): ↓15/13% Kidney weight Abs/rel: M(abs): ↓23 F ↓10/8% Pituitary weight Abs/rel: M: ↓20/10 Testis, left (abs): ↓19 Testis, right (abs): ↓10 Epididymis, left: Abs: ↓9% Epididymis, right: Abs: ↓11% Prostate: Abs: ↓15% **40 mg/kg bw/d** ↑ brain weight	

			Abs (m):↓4 Adrenal weight M (abs): ↓10% F (abs/rel): ↓15/13% Kidney weight Abs/rel: M(abs): ↓14 F ↓9/8% Pituitary weight M (abs): ↓13% Extracellular pigment in brain **Cohort 1B:** **120 mg/kg bw/d** heart weight Abs/rel: M: ↓15/↑24% Testes: Abs/rel: ↓15/↑27% Epididymis Abs: ↓37% Prostate: Abs/rel: ↓46/23% Seminal vesicles: Abs/rel: ↓50/29% Uterus, cervix, oviducts: Abs/rel: ↓33/28% **80 mg/kg bw/d** heart weight M(rel): ↑12% Testes (abs): ↓10% Epididymis Abs: ↓9% Prostate (abs):↓19% Seminal vesicles (abs): ↓13% **40 mg/kg bw/d** heart weight M(rel): ↑8% Prostate (abs):↓13% **Cohort 2A:** **120 mg/kg bw/d:** brain weight Abs/rel: M: ↓4/↑14% Inramyelinic edema in brain (cerebrum and	

			forebrain) m: minimal: 6/20, slight: 2/20 (0 in controls) f: minimal: 3/20 (0 in controls) Hippocampal neuronal necrosis m: minimal: 11/20 (0 in controls) f: minimal: 9/20 (0 in controls) Neuronal/glial cell necrosis in thalamus m: minimal: 3/20, slight: 1/20, moderate: 1/20 (0 in controls) f: minimal: 3/20, slight: 1/20 (0 in controls) Extracellular pigment in brain (medulla oblongata) Brain morphometry: low mean hippocampus (mm). Auditory startle response: latency to peak values for females were low compared with controls and statistical significance was achieved during trials 31-40. Group mean auditory startle latency to peak values for males was high during trials 21-30. Group mean auditory startle peak amplitude values for males and females were low when compared with controls, with animals at 120 mg/kg/day showing the least habituation. The majority of group mean high (rearing) and low (ambulatory) beam activity scores were low for males and females Two males showed abnormal motor movements which included chewing mouth movements and licking around mouth. Three females failed the pupil closure reflex response with pupils that failed to dilate; In cage, in hand and arena observations included, but was not restricted to abnormal motor movements, gait and activity. Reactivity investigations showed a number of observations including reduced approach response, a weak startle response, failed pupil closure, high landing foot splay, reduced forelimb/hindlimb grip strength. Females showed slightly low body temperatures. **80 mg/kg bw/d:** brain weight Abs (m): ↓8% Inramyelinic edema in brain (cerebrum and forebrain). m: minimal: 2/20, slight: 1/20 (0 in controls)	

			Hippocampal neuronal necrosis m: minimal: 8/20 (0 in controls) f: minimal: 3/20 (0 in controls) Neuronal/glial cell necrosis in thalamus m: minimal: 3/20 (0 in controls) f: minimal: 2/20 (0 in controls) Extracellular pigment in brain (medulla oblongata) Brain morphometry: low mean hippocampus (mm) Total high and low beam scores for males, statistically significant. Two males showed abnormal motor movements which included chewing mouth movements and licking around mouth. Three females failed the pupil closure reflex response with pupils that failed to dilate **40 mg/kg bw/d:** brain weight Abs (m): ↓4% **Cohort 3:** **120 mg/kg bw/d:** spleen weight rel (m/f): ↑34/29% **Abnormal colouration of tissues was observed in all treated animals**	
*St = study termination				
OECD TG 416	Silver zinc zeolite denoted AgION Silver Antimicrobial Type AK	Rat SpragueDawley Crl: CD® (SD) IGS BR 30/sex Oral in diet m/f: 72/87, 472/548, 984/1109 mg/kg bw (premating) corresponding to approximately 1.5/1.8, 9.8/11.3; and 20.3/22.9 mg silver ion equivalents/kg bw/d in males	**Parental effects:** P 12500: ↑ Mortality (m 10%) ↓Bodyweight (m≤10% (pre/post pairing, f 6% gestation day 20, ≤ 11%) ↓Bodyweight gain (m≤17% (pre pairing), f gestation 14-20:29% 0-20:16%) ↓Food consumption (premating m ≤8%, lactation 0-4:27%, 4-7: 12%, 7-14: 21%, 14-21: 27%) ↑RBC (m/f 13/15%), platelets (m/f 42/45) ↓Hb (m/f 16/12%), HCT (m 9%) MCH (m/f 25/23%) MCHC (m/f 7/6%),	IIIA 6.8.2 (04) (2002)

		and females Administration during maturation, mating, gestation and lactation for two successive generations	↑Pigmentation of organs ↑Histopathological changes in kidneys (including hydronephrosis (8m/2f , 3m in controls) , urinary tract ↓ kidney weight (m abs/rel 14/3%, f rel brain 7%) rel brain weight (m, 9%) ↑ epididymis left/right (rel bw 11/9%) Spleen (m, 7%) Testis (rel left/right 12/10%) <u>P 6250:</u> ↑ Mortality (m, 3.3%) ↑RBC (f 11%), ↓ MCV (m/f, 6/9%), MCH (m/f 6/12%), MCHC (f, 3%) ↑Pigmentation of organs ↑Histopathological changes in kidneys (including hydronephrosis 7m/2f, 3m in controls)) ↓kidney weight (m, abs/rel bw 13/7%) spleen (m, abs/rel bw 14/21%) <u>P 1000:</u> ↑Pigmentation of organs <u>F1 12500:</u> ↑Mortality (m/f 93.3/76.7%) ↓Bodyweight (premating m/f ≤ 56/46%) ↓Bodyweight gain (premating m/f ≤ 47/40%) ↑Histopathological changes ↑Thymus atrophy <u>F1 6250:</u> ↑Mortality (m/f 23.3/3.3%) ↓Bodyweight (premating w1-10 m/f 25-13/19-2 (n.s.s)%, post-pairing m ≤12%, gestation n.s.s, lactation≤ 10%) ↑Histopathological changes (including hydronephrosis 10 m/4f , 0 in controls) ↑Kidney weight (m/f, abs 19/11%, rel bw 9/8%, rel brain 13/7%) ↓Brain (m/f, 7/5%) Adrenal (m, abs 18%, rel brain 12%) epididymis left/right (abs 14/11%, rel brain (left 9%)) Spleen (m, rel bw 11%) Testis (abs left/rel brain right 12/7%)	

			Prostate (rel brain 13%) Seminal vesicle (8%) Liver (f, 8%) ↑Thymus atrophy (thymus not weighed in F1 adults) F1 1000: ↑Mortality (m 3.3%) ↑Pigmentation of organs ↑ Hydronephrosis (3m, 1f, 0 in controls) Adverse effects on development: **F1 12500:** ↓total pups born/litter (15%) ↑stillborn index ↓ liveborn/litter (27%) ↓live birth index ↓ pup survival indices ↑ enlargement of heart ↑clinical signs ↓body weights M+f Day 0: 15% Day 4:pre/post culling: 19% Day 7: 23% Day 14: 26% Day 21: 36% Day 26: 47% ↓organ weights Brain 18% (rel bw ↑58%) Spleen 26% (rel bw ↑31%) Thymus (m/f abs 74/70%, rel bw 53/47%, rel brain 69/64%) ↑histopathological changes Effect on sex ratio ↑day of vaginal opening (day 59.9, control: 35.1) and preputial separation (day 56.7, control: day 44.5) Bodyweight on day passed vaginal opening/preputial separation: 117.9 (n.s.s)/130.3 (s.s) compared to 118.1/209.1 in controls **F1 6250:** ↑clinical signs ↓ body weights M+f Day 14: 13% Day 21: 25% Day 26: 47% ↓organ weights Brain 10%, rel bw ↑27%	

			Thymus (m/f abs 58/55%, rel bw 39/39%, rel brain 53/51%) ↑Spleen (m/f rel bw 31/32%) ↑histopathological changes ↑ enlargement of heart ↑day of vaginal opening (day 39.8) and preputial separation (day 47.4) **F1 1000:** ↓organ weights Thymus (m abs 13%, m/f rel bw 10/9%, m rel brain 11%) **F2 12500:** No pups due to high toxicity in parents **F2 6250:** ↑ stillborn index ↓live birth index ↓bodyweights Day 0: 5% Day 4: pre/post culling: 12% Day 7: 15% Day 14: 18% Day 21: 20% ↑histopathological changes ↓organ weights Brain (m/f 10/7%, rel bw ↑21/25%) Thymus (m/f abs 50/54%, rel bw 37/42%, rel brain 47/50%) Spleen (m abs 18%) ↑ enlargement of heart **F2 1000:** ↓Thymus weight (m rel bw 11%)	
OECD TG 416	Silver sodium zirconium hydrogenphosphate Exp.add 9823-37 (10% Ag) 1000, 5000 and 20000 ppm corresponding to 72.5/78.2, 363/400 and 1465/1612 mg a.s/kg bw in P	Rat SpragueDawley Crl: CD® IGS BR 28/sex Oral in diet Maturation, mating, gestation and lactation for two successive generations	**Parental:** P 20 000ppm: No mortality ↑pigmentation (pancreas) ↓ organ weights: thymus (20% m), seminal vesicle/coagulating gland (14%), adrenals (14%), kidneys (m, 16%) ↑spleen weight (m, 11%), rel brain weight (m, 9.7%) P 5000ppm: No mortality	IIIA 6.8.2-03 (2002)

	males and females (premating) approximately 1.9, 9.9 and 40 mg silver ion equivalents/kg bw/d in females)	NOAEL/LOAEL Parental P: 1000/5000 Parental F1: 1000/5000 Offspring F1:1000/5000 Offspring F2: 1000/5000 Reproduction: 5000/20 000	↑pigmentation (pancreas) ↑spleen weight (m, 20%) ↓seminal vesicle/coagulating gland weight (14%) P 1000ppm, 0 ppm: No mortality F1 20 000: ↑mortality (4m, 2f, none in control) ↓bodyweight pairing (≤ 16%), gestation* (≤ 10%) lactation (≤ 10%) ↓food consumption pairing (≤ 20), m), gestation, lactation (≤22%) ↑pigmentation (pancreas, lymph nodes, thymus) ↓organ weights: uterus (abs/rel 28/23%), prostate (abs/rel 33/25%) ↑relative epididymis weight (left/right 9.6/19%) F1 5000 ppm: Mortality: 1f (suspected hermafrodism, "although no female sex organs could be detected microscopically, the arrangement of sex organs at macroscopic examination did show some anomaly of sexual development." ↑pigmentation (pancreas, lymph nodes, thymus) **Adverse effects on development:** F1 20 000: ↓ group mean litter weights (8%, day 21), group mean individual weights (9%, day 21) thymus weight (m/f 38/32%) F1 5000 ppm: ↓ thymus weight (m 22%) F2 20 000: ↓ number born (11%) ↓live litter size (13%, day 1) ↓ group mean litter weights (13%, day 1), group mean individual weights (13%, day 21) ↓ thymus weight (m/f 38/37%) F2 5000: ↓ thymus weight (f 19%)	
OECD TG 414	Exp additive 9823-37 (10% Ag) 0, 100, 300, 1000	Sprague Dawley F/8 Further	maternal/ embryotox: >1000 mg/kg bw/day	IIIA 6.8.1(05) (1999)

	mg/kg bw/day (~2,5; 7.4 and 25 mg/kg bw respectively) gd 6-15	investigations to establish absorption and bioavailability of the test substance were not made.	Dams/foetuses: No effects observed	
OECD TG 414	Exp additive 9823-37 (10% Ag) 0, 100, 300, 1000 mg/kg bw/day (~2,5; 7.4 and 25 mg/kg bw)gd 6-15 Oral gavage	SpragueDawley F/25 Further investigations to establish absorption and bioavailability of the test substance were not made.	maternal/ embryotox: >1000 mg/kg bw/day >25 mg silver ion equivalents/kg bw) Dams/foetuses No effects observed	IIIA 6.8.1(06) (1999)
In accordance with the current protocols for testing foods and food additives (FDA CFSAN Redbook, 2000).	Silver acetate KSCN %Ag: 63.7-65.5% 0, 0.4, 4.0 and 40.0 mg/kg bw/d approximately 0, 0.25, 2.5 and 25 Ag+ mg/kg bw/d	Sprague-Dawley [Crl:CD®(SD) IGS BR] 20/sex The main deficiencies of this study include the lack of GLP compliance lack of individual animal data and the lack of further investigations such as oestrus cycle, sperm parameters and histopathological analyses of reproductive tissues (Histopathological, examinations of vagina, uterus and ovaries)	**Parental** <u>40 mg/kg bw/d</u> Organ weights (f): ↓ stomach (40%) ↓ liver (9%) ↓ Feed consumption (16%) until lactation day 18 (f) **Development** <u>40 mg/kg bw/d:</u> ↓ litter size at birth (21%) (10.3 compared to 13.1 in control) ↓ live pups at birth (19%) (10.5 compared to 13.0 in control)) F1 pups (40 mg/kg): reduced male pup survival ↓ foetal and pup survival (m):of 18 pregnant females 2 dams had a total resorption and 2 dams lost the full litter by PND4 (0/20 and 1/20, respectively in ctrl) ↓ pup weight PN day 26 (m): 8% <u>4.0 mg/kg</u> ↓ pup weight PN day 26 (f): 12% ↑ numbers of runts (Day 4 pre-cull: 35 tot/9 of 18 litters compared to 11 tot/7 of 19 in control) (Day 4 post-cull: 27 tot/8 of 18 litters compared to 7 tot in 4 of 19 in control) (Day 7: 25 tot in 10 of 18 litters compared to 7 tot in 4 of 19 in control) *Effects on fertility are discussed in section 10.10.2.*	IIIA 6.8.2-06
OECD TG 414*	Silver copper zeolite	Rat SpragueDawle	**2000 mg/kg bw:** ↑death (1/20)	Doc IIIA 6.8.1 (02)

Oral (gavage)	200, 700, 2000 mg/kg bw/day (~3, 10 and 29 mg Ag+/kg bw)	y F/30 Gd 6-15	↓body weight (13%) ↓bodyweight gain (25%) ↑clinical signs: sedation, void faeces, urogenital discharge, thinness **Foetuses:** No effects observed	
* The study followed OECD TG 414 (adopted 22nd january 2001) with a few deviations.				
Published research Oral gavage	Rats SpragueDawley (CD) F/25	Silver acetate (64.6% Ag) 0, 10, 30, 100 mg/kg bw/day No information on Ag+ solubility. Assuming 100% release in GI tract: 6.5, 19, and 65 mg Ag+/kg bw/d) gd 6-19	100 mg/kg bw/ **Dams** ↑piloerection, alopecia **Foetuses** ↑percentage litters with late foetal deaths (10%) (2/20 compared to 0/24, 0/23 and 0/25 in control, low and mid dose respectively). ↓male bodyweight/litter(5%) ↓foetal bodyweight/litter (5%) Other effects observed: 100 mg/kg bw: ↑ skeletal variations	IIIA 6.8.1 (07)
Published research	Rat Albino F/5-36**	Silver chloride 50 mg/day (250 mg/kg bw/d) no information on Ag+ solubility gd 1-20 gd 7-15	**Dams** ↓ceruloplasmin **Foetuses** (treated during gd 1-20) ↑postimplantational deaths (26%) ↑chryptorchidism (33%) ↑hydronephrosis (25%) ↓ceruloplasmin ↓bodyweight (22%) ↓viability index (100% deaths)	IIIA 6.8.1(03)
** Five females were treated during gestation days 7-15 and 20 females during gestation days 1-20. The control group included 36 females				

Summary table of published studies with nanosilver relevant for developmental toxicity (more details available in Annex I)

Method, Guideline, GLP status, Reliability	Species, Strain, Sex, No/ group	Test substance Dose levels, Duration of exposure	NOAELs, LOAELs	Results	Remarks *(e.g. major deviations)*	Reference
Dams euthanised on GD 19, uterus examined for resorptions and foetuses were removed.	Mice CD-1 3 females and 1 male/group, 10-18 litters per group	Oral, gavage A single dose of 10, 100, or 1000 mg/kg at gestation day 9 20 nm in size (passivated) 24-47 nm, average size of	Not determined	Increase in the number of non-viable foetuses (only stat sign at 10 mg/kg bw/d) 1000 mg/kg: 6.1% (n.s.s) 100 mg/kg: 5.5% (n.s.s) 10 mg/kg: 9.6% (stat sign) Control: 3.3%	The lack of effects at higher doses was postulated to be due to agglomeration of nanoparticles after	IIIB 6.8.2-07 Philbrook, N.A., Winn, L.M., Nabiul Afrooz, A.R.M., Saleh,

<table>
<tr><td colspan="7">Summary table of published studies with nanosilver relevant for developmental toxicity (more details available in Annex I)</td></tr>
<tr><td>Method, Guideline, GLP status, Reliability</td><td>Species, Strain, Sex, No/ group</td><td>Test substance Dose levels, Duration of exposure</td><td>NOAELs, LOAELs</td><td>Results</td><td>Remarks (e.g. major deviations)</td><td>Reference</td></tr>
<tr><td>Examination of dams and some developmental parameters (resorptions, pup viability, bw, skeletal and soft tissue abnormalities
No guideline
No GLP</td><td></td><td>35.3+/−5.8 nm (in DI water)
144-260 nm, average
220+/−21.1 nm (tragacanth Gum)
No further information regarding coating or amount of release of silver ions</td><td></td><td>No effects on maternal toxicity.

No other effects of developmental parameters (litter size, mean foetal length or weight, skeletal defects cell death or inflammation in foetal liver or kidneys</td><td>administration resulting in reduced toxicity and clearance by the animals.</td><td>N.B., and Walker, V.K. (2011): The effect of TiO2 and Ag nanoparticles on reproduction and development of Drosophila melanogaster and CD-1 mice. Tox. and Appl. Pharm. 257; 429-436</td></tr>
<tr><td>Necropsy day 4 postpartum: concentration of silver was analysed in pup liver, kidney, lung and brain
OECD TG 422
No GLP</td><td>Rat (Sprague-Dawley)</td><td>Citrate-capped AgNPs (ABC Nanotech, Korea).
7.9 ± 0.95 nm
Oral gavage,
62.5, 125 or 250 mg/kg bw/d
Exposure:
Males: 14 days before and during mating
Females: 14 days before and during mating, during gestation, and 4 days after parturition</td><td>Not determined</td><td>AgNPs observed in livers, kidneys, brain and lungs of the offspring:
<table><tr><td></td><td>Liver</td><td>Kidney</td><td>Lung</td><td>Brain</td></tr><tr><td>Control</td><td>4.7 ± 0.59</td><td>10.8 ± 1.4</td><td>7.1 ± 2.1</td><td>5.8 ± 2.5</td></tr><tr><td>AgNPs</td><td>37.3 ± 11.3*</td><td>132.4 ± 43.9*</td><td>42.0 ± 8.6*</td><td>31.1 ± 4.3*</td></tr></table>
*Statistically significant compared to control value (P < 0.01); unit: ng/g.</td><td>As AgNPs were also identified in the brain of offspring, AgNPs may reach the brain before the blood-brain barrier is formed in the foetus or they may directly pass the barrier.</td><td>IIIB 6.8.2-08
Lee, Y., Choi, J., Kim, P., Choi, K., Kim, S., Shon, W., and Park, K. (2012): A Transfer of Silver Nanoparticles from Pregnant Rat to Offspring. Toxicol. Res. Vol. 28 (3) 139-141</td></tr>
<tr><td>Pregnant rats were sacrificed 24</td><td>Rat (Wistar), 3 females</td><td>110mAg radio-labelled silver</td><td>Not determined</td><td>Average NP level in foetuses: 0.085–0.147% of the administered dose.</td><td>This study provides some</td><td>IIIA, 6.8.2-09</td></tr>
</table>

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Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
hours after treatment Infant rats were sacrificed 48 hours after treatment of the nursing rats. Examinations of foetuses: nanoparticle content of liver and brain Infant rats: gastrointestinal tract, liver, kidneys, spleen No guideline No GLP		nanoparticles, 34.9 ± 14.8 nm Pregnant rats received a single dose of 1.69 mg/kg bw (3 females) or 2.21 mg/kg bw (4 females); lactating 9 female rats were dosed once at 2.11 mg/kg bw Oral gavage		Accumulation of NP in female pregnant rats: Liver: 0.3–0.5% of the administered dose. Brain: 0.0035% of the administered dose Thus the NP penetration in liver exceeded the penetration of NPs through the hematoencephalic barrier into the brain of female rats by at least 10-100 times (3.5.× 10-3 %). Total inflow of [110mAg]-NPs into the milk: 1.94 ± 0.29% of the administered dose over a 48-hour period 25% of the amount was absorbed in the digestive tract of infant rats.	evidence for the transfer of silver NPs from a mother to offspring through the placenta and breast milk; although the presence of silver NPs in milk was not directly investigate.	Melnik, E.A., Buzulukov, Y.P., Demin, V.F., Demin, V.A., Gmoshinski, I.V., Tyshko, N.V., and Tutelyan, V.A. (2013): Transfer of Silver Nanoparticles through the Placenta and Breast Milk during in vivo Experiments on Rats. Acta Naturae Vol. 5 (3) 18; 107-115
Dams and offspring were, weighed, sexed and sacrificed on postnatal day 2. Determination of Ag in tissue (all dams and 2-3 pups per litter) and milk Biochemical and	Rat Sprague-Dawley 10/group	Citrate-capped silver nanoparticles, 55nm Pregnant female rats dosed orally once daily from Day 7 to Day 20 of gestation with 0, 0.2, 2, 20 mg/kg bw/day AgNPs or 20 mg Ag/kg bw/day as AgNO3	Not determined	Reduced bw gain in AgNO3 treated dams. The relative weights of the heart, uterus and brain were significantly higher at the highest dose of Ag-NPs compared to the controls ($p < 0.05$). Administration of AgNO3 lead to higher tissue contents of Ag in dams than administration of Ag-NPs especially for heart and plasma. Offspring tissue levels of Ag were generally similar or lower if their dams had	The ionic Ag was associated with a higher degree of toxicity. The Ag in both nanoparticle and ionic forms induced oxidative stress in dams and pups, with the ionic form being more potent.	IIIA, 6.8.2-10 Charehsaz, M., Hougaard, K.S., Sipahi, H., Ekici, A.I.D., Kaspar, C., Culha, M., Bucurgat, U.U., and Aydin, A. (2016): Effects of developm

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inflammatory analysis Measurement of oxidative stress Histopathology: brain (coronal sections from temporal and hippocampal areas), lung, spleen, heart, kidney, uterus, ovaries and liver from dams, and brain, heart, liver, lung and kidney from pups No guideline No GLP		oral gavage		been exposed to AgNO3 rather than the Ag-NPs. Only in plasma AgNO3 offspring had higher Ag levels than Ag-NP offspring. Ag content higher in all treated groups including milk from suckling pups. Accumulation of Ag in offspring confirms that Ag is able to cross the placenta. Kidney seems to be the main organ of fetal accumulation, followed by lung, liver and brain.	Observation of hippocampal sclerosis in dams even at the lowest dose level of 0.2 mg/kg bw/day. Oxidative stress in offspring brain tissue: increase of glutathione peroxidase Stat sign for AgNO3 Non-sign but dose-dependent by AgNP.	ental exposure to silver in ionic and nanoparticle form: A study in rats. Journal of Pharmaceutical Sciences Vol 24:24
Guideline/GLP not reported	Rats Sprague-Dawley Male Six week old Average weight 245g 5/group 2 control groups, 3 experimintal groups	Silver nanoparticles Non-coated AgNPs <20nm PVP-coated AgNPs <15nm Non-coated – matrix = 4% polyoxyethylene glycerol trioleate and 4% Tween 20 in H2O Coated - suspended in water Oral exposure	Not determined	Main target organs for AgNPs and AgNO3: liver and spleen, followed by the testis, kidney, brain, and lungs, without differences in the distribution pattern between the two different AgNPs, or the AgNO3 exposed animals. Higher uptake of silver in blood and organs of AgNO3 exposed rats Elimination of silver occurred at an extremely slow rate in brain and testis, which still contained high concentrations of silver	Fraction of soluble silver rather similar between the Ag < 20 nm and AgNO3 animals in blood and in organs with the exception of testis and spleen (see text). This indicates that silver is probably mainly	IIIA, 6.8.2-12 Van der Zande, M., Vandebriel, R.J., van Doren, E., Kramer, E., Herrera Riviera, Z., Serrano-Rojero, C.S., Gremmer, E.R., Mast, J.,

Summary table of published studies with nanosilver relevant for developmental toxicity (more details available in Annex I)						
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		28 days exposure Post-exposure: wash out until days 36 and 84 Silver exposure: 90 mg/kg bw for the Ag < 20 and Ag < 15-PVP groups vs 9 mg/kg bw for the $AgNO_3$ group),		two months after the final exposure.	bioavailable in the ionic form (see text) Nanoparticles are formed in vivo from silver ions and they are probably composed of silver salts. **The study thus demonstrates the relevance of data on nanosilver for ionic silver**	Peters, R.J.B., Hollman, P.C.H., Hendricksen, P.J.M., Marvin, H.J.P., Peijenberg, A.A.C.M., and Bouwmeester, H. (2012): Distribution, Elimination, and Toxicity of Silver Nanoparticles and Silver Ions in Rats after 28-day Oral Exposure. ACS Nano. 28;6(8):7427-42
Guideline/GLP not reported	Rats Wistar 10 males/group (5 sacrificed at PND 53, 5 at PND 90).	60 nm AgNPs suspended in aqueous solution. 0 µg/kg bw, 15 µg/kg bw, 30 µg/kg bw Oral Gavage Daily for 35 days (Post Natal Day (PND) 23 to PND 58).	Not determined	Delay onset of puberty (weight at puberty not affected) No significant change of bw between treated groups and the control. Sexual partner preference score in the group that received 15 µg/kg AgNPs indicates a preference for the male sex.	Numerical data is available in study summary	IIIB, 6.8.2-17 Mathias, F. T.; Romano, R. M.; Kizys, M. M. L.; Kasamatsu, T.; Giannocco, G.; Chiamolera, M. I.; Dias-da-Silva, M.

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		Post-exposure period: 44 days (PND 58 to PND 102)				R.; Romano, M. A. (2015): Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters
Guideline/GLP not reported	Mice Mice C57BL/6J pun/pun Wildtype: 6-7 Myh-/-: 9-10	AgNPs coated with 0.2% PVP Oral Gavage 500 mg/kg bw/day Pregnant dams were treated from 9.5 to 13.5 days post coitum	Not determined	Genotoxic and mutagenic in developing embryos: large DNA deletions, micronucleus formation in peripheral blood and in bone marrow and markedly increased DNA double strand breaks (DSB) in mononuclear cells in the bone marrow and leukocytes in peripheral blood.	The permanent genome alterationes were associated with DNA damage, increased double strand breaks and downregulation of DNA repair genes	IIIB 6.8.2-20 Kovvuru, P.; Mancilla, P. E.; Shirode, A. B.; Murray, T. M.; Begley T. J.; Reliene, R. (2015): Oral ingestion of silver nanoparticles induces genomic instability and DNA damage in multiple tissues

Summary table of published studies with nanosilver relevant for developmental toxicity (more details available in Annex I)						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
Modified OECD TG 414 No GLP	Rat Sprague-Dawley 11 females/group	Silver NPs suspended in 0.5% carboxymethyl cellulose aqueous solution Particle size 7.5 ± 2.5 nm Oral Gavage GD 6-20	NOAEL maternal: 100 mg/kg bw/day NOAEL developmental: 1000 mg/kg bw/day	Dams, 100 mg/kg bw/day oxidative stress observed in hepatic tissues Reduced glutathione reductase Reduced catalase Pre-implantation loss: 0: 2.4% 100: 14.5% 300: 3.8% 1000: 25.5%	Fewer litters (8-11/group) examined than required in guideline	IIIB 6.8.2-21 Yu, W.-J.; Son, J.M.; Lee1, J.; Kim, S.-H.; Lee, I.-C.; Baek, H.-S.; Shin, I.-S.; Moon, C.; Kim, S.-H.; Kim, J.-C. (2013): Effects of silver nanoparticles on pregnant dams and embryo-fetal development in rats
No guideline No GLP	Rat Wistar 45 females/group	Nanoparticles with sodium citrate buffer 20 ± 4 nm 25 mg/kg bw/d GD 9 to parturition Intragastric administration Offspring were sacrificed day after birth.	Not determined	Maternal tox: No significant differences in weight gain. Offspring (effects in the treated group as compared to control): Increase of silver and the number of microvacuolar structures in brain (612 vs 159 in ctrl). Reduced antioxidant activity and increased peroxidation Statistically significant decrease in bw on PND 0 in the treated groups as compared to control. Statistically significant decrease in the ratio of brain/body weight.		IIIB 6.8.2-22 Fatemi, M.; Hayatiroodbari, N.; Ghaedi, K; Naderi, G; (2013): The effects of prenatal exposure to silver nanoparticles on the developing brain in neonatal rats.

Abstracts (from the original publications) of additional studies on development included in the REACH registration dossier on Silver EC number: 231-131-3; CAS number: 7440-22-4:

Austin et al., 2012. Distribution of silver nanoparticles in pregnant mice and developing embryos. Nanotoxicology, December 2012; 6(8):912–922

Silver NPs (average diameter 50 nm) were intravenously injected into pregnant CD-1 mice on gestation days (GDs) 7, 8, and 9 at dose levels of 0, 35, or 66 µg Ag/mouse. Mice were euthanised on GD10, and tissue samples were collected and analysed for silver content. Compared with control animals injected with citrate buffer vehicle, silver content was significantly increased ($p < 0.05$) in nearly all tissues from silver NP-treated mice. Silver accumulation was significantly higher in liver, spleen, lung, tail (injection site), visceral yolk sac, and endometrium compared with other organs from silver NP-treated mice. Furthermore, silver NPs were identified in vesicles in endodermal cells of the visceral yolk sac. In summary, the results demonstrated that silver NPs distributed to most maternal organs, extra-embryonic tissues, and embryos, but did not accumulate significantly in embryos.

Mahabady et al., 2012. The evaluation of teratogenicity of nanosilver on skeletal system and placenta of rat fetuses in prenatal period. African Journal of Pharmacy and Pharmacology Vol. 6(6), pp. 419-424

This study was performed on 30 pregnant rats that were divided into five groups. Control group received normal saline and test groups received nanosilver (0.4 and 0.8 mg/kg) intraperitoneally at 8 and 9th day of gestation, respectively. Fetuses were collected at 20th day of gestation. After determination of weight and length; the fetuses were stained by Alizarin red-Alcian blue method. Also, placenta were weighed, and width and volume were measured and examined macroscopically. The mean of weight of animals' fetuses that received nanosilver (0.4 and 0.8 mg/kg) in 8th day and weight and length (0.8 mg/kg) in 9th day was significantly decreased in comparison with normal saline group. The weight, volume and width of placentas in treated animals were lesser than in control group. No macroscopic anomalies were seen in all the groups. Thus, nanosilver had no effect on skeletal system of rat fetuses and it is necessary to determine the association between the period of exposure and histopathologic changes with different doses over different time periods.

Babu et al., 2016. Effects of maternal silver acetate exposure on immune biomarkers in a rodent model. Food and Chemical Toxicology 98, 195-200.

Male and female rats (26-day old) were exposed to 0.0, 0.4, 4 or 40 mg/kg body weight silver acetate (AgAc) in drinking water for 10 weeks prior to and during mating. Sperm positive females remained within their dose groups and were exposed to AgAc during gestation and lactation. Splenic and thymic lymphocyte subsets from F1 generation PD (postnatal day) 4 and 26 pups were assessed by flow cytometry for changes in phenotypic markers. Spleens from PD4 pups had lower percentages of CD8+ lymphocytes in 4 and 40 mg/kg AgAc exposed groups and reduced Concanavalin A (Con A) response at all AgAc exposure groups. Splenic maturation increased in PD26 pups compared to PD4 pups. Con A and lipopolysaccharide (LPS) mediated splenic responses were lower in PD26 pups exposed to 40 mg/kg AgAc. Changes in PD 26 pup splenocyte phenotypic markers included lower TCR + cells at 4 and 40 mg/kg AgAc exposure and higher B cell population in the 40 mg/kg AgAc. PD26 pup splenic natural killer cell (NK) activity was higher in the 0.4 AgAc group and unchanged in 4 and 40 mg/kg AgAc groups. In conclusion, maternal exposure to AgAc had a significant impact on rat splenic development during the early lactation period.

Hang et al., 2013. Effect on rabbit reproduction of adding silvernano suspension to the drinking water. Rural Develop. 25 (9).

This study evaluated the effect on rabbit reproduction of a silver-nano suspension added to the drinking water. Sixteen rabbit does, 10 months of age were located in individual cages and allocated to two treatments: with or wothout 1% AgNano suspension added to the drinking water. The does were fed a pelleted concentrate at

3% of live weight and natural grass ad-libitum. There was no effect of AgNano treatment on changes in live weight of the does before and after parturition, nor on time to re-mating. Litter size and live weights of the kits at birth, at 24h and at weaning was not affected by incorporation of AgNano in the drinking water. The AgNano treatment reduced the incidence of diarrhea in the kits, increased their survival rate to weaning aand appeared to improve feed conversion during lactation as measured by feed consumed by the doe per unit gain in weight of the litter to weaning.

Taylor et al., 2014. Injection of ligand-free gold and silver nanoparticles into murine embryos does not impact pre-implantation development. Nanotechnol. 5, 677-688.

In this study the toxicity of gold and silver nanoparticles on mammalian preimplantation development was assessed by injecting nanoparticles into one blastomere of murine 2 cell-embryos, while the sister blastomere served as an internal control. After treatment, embryos were cultured and embryo development up to the blastocyst stage was assessed. Development rates did not differ between microinjected and control groups (gold nanoparticles: 67.3%, silver nanoparticles: 61.5%, sham: 66.2%, handling control: 79.4%). Real-time PCR analysis of six developmentally important genes (BAX, BCL2L2, TP53, OCT4, NANOG, DNMT3A) did not reveal an influence on gene expression in blastocysts. Contrary to silver nanoparticles, exposure to comparable Ag+-ion concentrations resulted in an immediate arrest of embryo development. In conclusion, the results do not indicate any detrimental effect of colloidal gold or silver nanoparticles on the development of murine embryos.

Gao et al., 2015. Toxicogenomic study in rat thymus of F1 generation offspringfollowing maternal exposure to silver ion. Toxicol. Rep. 2, 341-350.

Male and female rats (26-day-old) were exposed to 0.0, 0.4, 4 or 40 mg/kg body weight silver acetate (AgAc) in drinking water for 10 weeks prior to and during mating. Sperm-positive females remained within their dose groups and were exposed to silver acetate during gestation and lactation. At postnatal day 26, the effect of silver ions on the developing F1 generation rat thymus was evaluated at the transcriptional level using whole-genome microarrays. Gene expression profiling analyses identified a dozen differentially expressed genes (DEGs) in each dose group using a loose criterion of fold change (FC) >1.5 and unadjusted p < 0.05, regardless of whether the analysis was conducted within each gender group or with both gender groups combined. No dose-dependent effect was observed on the number of DEGs. In addition, none of these genes had a false discovery rate (FDR) <0.05 after correction for multiple testing. These results in combination with the observation that thymus-to-body-weight ratios were not affected and no histopathological abnormalities were identified indicate that in utero exposure to silver ions up to 26.0 mg/kg (equivalent to 40.0 mg/kg silver acetate) did not have an adverse effect on the developing thymus.

Ghaderi et al., 2015. Induced adverse effects of prenatal exposure to silver nanoparticles on neurobehavioral developement of offspring of mice. Toxicol. Sci. 40, 263-275.

Thirty virgin female NMRI mice were mated and treated subcutaneously once every three days from gestation day 3 until delivery, by 0, 0.2 and 2 mg/kg of bodyweight (BW) of Ag-NPs. Behavioral functions of adult offspring including spatial memory, passive avoidance learning, stress, anxiety-like behaviors and locomotor activities were assessed by commonly used neurobehavioral paradigms and the results were compared according to treatment and sex. Prenatal exposure to Ag-NPs significantly impaired their cognitive behavior in the Morris water maze. Although no evidence was observed indicating more anxiety-like behaviors in the treated offspring in the elevated plus maze, the number of defecations and leanings in the open field assay and number of passages in the light-dark box were greater in groups prenatally treated by Ag-NPs. Most of the impairments were more apparent in the offspring which had been prenatally exposed to high doses of Ag-NPs, particularly female ones. The present study indicated that the exposure of pregnant animals to Ag-NPs may lead to various neurobehavioral disorders in their offspring.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

There are no robust studies investigating the developmental toxicity of silver nitrate. However, the silver ion was indirectly investigated in several studies performed with silver containing active substances or with silver nanoparticles. In two OECD TG 414 studies performed with silver sodium zirconium hydrogenphosphate (6.8.1-06 (and preliminary study in 6.8.1(05)) and silver copper zeolite (6.8.1-02) no adverse effects on development were noted except for a statistically significant dose-related increase of the percentage males per litter in high dose animals treated with silver sodium zirconium hydrogen phosphate (56.8% compared to 43% in controls). The toxicological significance of this finding is unclear since the opposite pattern was observed in a preliminary study (40.3% in high dose and 50.6% in controls). Nevertheless, it is clear from a published developmental toxicity study performed with silver chloride (6.8.1-03 and discussed below) and from other data in this section that embryotoxic effects of silver are rarely observed if exposure is limited to the period of organogenesis only, (i.e., days 7-15). Moreover, the severity of effects seems to depend on the silver ion exposure from the silver substance which in turn depends on silver content and release.

Recently, the results of an EOGRTS as well as a preceding preliminary study performed with silver acetate became available. The results were discussed and considered in the classification and labelling of silver (RAC-61).

The preliminary study was performed to assist in the dose selection for the EOGRTS. In the study, four groups of 12 male and 12 female F0 rats received doses of 4, 40, 80, 160 and 320 mg AgOAc/kg bw/day via the diet. Males were treated for 29 days before pairing and until necropsy after litters had weaned (after at least 65 days of treatment) whereas females were treated for 29 days before pairing, throughout pairing, gestation and until necropsy on Day 21 of lactation. In F1, three groups of 10 males and 10 females received doses of 4 or 40 mg/kg bw/day from weaning, during late lactation and to their scheduled termination on Days 31-33 (i.e. Day 59-61 (+/- 2days) of age) following completion of sexual maturation. The F0 generation was checked for clinical condition, body weight, food consumption, estrous cycles, pre-coital interval, mating performance, fertility, gestation length, exposure assessment and biomarkers (ceruloplasmin activity, glutathione Peroxidase (GPx) activity), copper, selenium and silver levels), organ weights and macroscopic and microscopy pathology investigations. In addition, haematology and blood chemistry investigations were made in all treated males and in females treated with 4 and 40 mg/kg bw/day. Litter size and survival, sex ratio, body weight, organ weights and macropathology were also assessed in all pups. The selected F1 pups administered 4 and 40 mg/kg bw/day were examined for clinical condition, body weight, food consumption, sexual maturation, exposure assessment and biomarkers, organ weights and subjected to macroscopic pathology investigations. The following results were obtained (parameters considered adversely affected by treatment are marked in bold text):

Biomarkers

Ceruloplasmin activity decreased with increasing doses of Silver Acetate in F0 male and female animals on Day 12, F0 males on Day 43 and female animals on Gestation Day (GD) 6. F1 Male and female animals followed the same trend on Day 21 at 4 mg/kg bw/day and 40 mg/kg bw/day whereas male and female animals dosed at 80 mg/kg bw/day showed results inconsistent with this trend.

Glutathione Peroxidase (GPx) activity decreased in high dose female rats (37-42% lower than in Controls) at Day 12 and GD6. Both male and female animals at 80 mg/kg bw/day showed low GPx activity.

Silver generally increased with increasing target dose levels; silver levels in the rat tissue samples increased with increasing target dose levels, with the highest concentrations **detected in the milk pellets obtained from offspring on Day 4 of age.**

Copper and selenium serum levels tended to decrease as the target dose level increased; the observed effect was more marked for copper. There was evidence for a dose-related decrease in copper levels in the testes for F0 males and a non-dose related increase in selenium levels in the ovaries for F0 females. There was no clear effect of treatment on the ovarian copper levels or the testis selenium levels.

F0

Four females at 320 mg/kg bw/day and two at 160 mg/kg bw/day were killed between completion of parturition and Day 2 of lactation following **total litter loss** with offspring either found dead, missing (presumed cannibalized) or killed for welfare reasons. The remaining females in these dose groups were terminated on welfare grounds between GD20 and LD4. At the macroscopic examination, the mammary tissue appeared pale and inactive in two females that received 160 mg/kg/day and four females that received 320 mg/kg bw/day.

Overall, the **mean body weight gain** for males at 320 mg/kg bw/day was markedly low (approximately 20% of Controls), resulting in low mean body weight at the end of the treatment period. The overall body weight gain of high-dose F0 females during the four-week pre-pairing treatment period was low (approximately 38% of Controls) with an overall gestational body weight gain approximately 42% of Controls. Body weight gain during lactation was poor in females administered 80 mg/kg bw/day.

High dose F0 males showed statistically significantly low mean values during most **food consumption** phases and high-dose females showed periods of low food consumption during prepairing and consistently throughout gestation.

At scheduled termination mid and high-dose males showed statistically significant **changes in hematological parameters**; low hematocrit, hemoglobin level, mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume but without dose response. **Platelet counts** for males that received 40, 160 or 320 mg/kg bw/day were slightly but statistically significantly high.

Alkaline phosphatase activity and cholesterol levels were increased in males and females administered 40 mg/kg bw/day and statistically significantly high in males at 160 and 320 mg/kg bw/day (except female cholesterol); at 4 mg/kg bw/day the mean cholesterol levels for males was also slightly but statistically significantly high.

Test substance-related dark coloration of several organs such as the liver, cecum. rectum, pancreas, mesenteric lymph nodes, uterus, kidney, thymus, urinary, bladder and salivary gland was observed in males of females of several dose groups.

F1 generation

Females administered 160 mg/kg/day and 320 mg/kg/day had two and four total **litter losses**, respectively. **Post-implantation survival and the live birth index** were low at 160 and 320 mg/kg/day resulting in low litter sizes. The **offspring body weight** on Day 1 was low compared to controls and examinations predominately revealed an absence of milk in the stomach of the decedent offspring. All dams (and litters) in these dose groups were terminated by LD4, and the assessment of F1 responses during later time points was therefore restricted to phase 1 animals receiving 4 or 40 mg/kg bw/day and litters administered 80 mg/kg bw/day that were terminated on Day 21 of age.

There was no effect on litter size, offspring survival, sex ratio and ano-genital distance up to and including 80 mg/kg bw/day.

Offspring bodyweight gain from Days 7-11 of age was slightly low when compared with Controls.

Macroscopic examination of offspring that died prior to scheduled termination did not reveal any findings at dose levels up to and including 80 mg/kg bw/day that could be attributed to administration of Silver Acetate. Offspring Brain Weight on Day 21 was unaffected by treatment at dose levels up to and including 80 mg/kg bw/day and detailed brain histopathology of F1 offspring at Day 21 of age did not reveal any pathological changes or developmental abnormalities.

There were no clinical signs observed and the mean body weight at weaning was similar across the remaining groups for both males and females. However, the overall gain for **males** from Day 1 to Day 29 was low at 4 and 40 mg/kg bw/day and the resultant mean body weight at these target dose levels were low compared to controls. The overall body weight gain for females at 40 mg/kg bw/day was slightly low at 90% of controls but this difference did not attain statistical significance.

The absolute mean brain weight for selected F1 females, but not males that received 40 mg/kg bw/day was slightly but statistically significantly low, but no abnormalities were discovered in the macroscopic examinations.

Based on the results of this study it was concluded that dose levels of 160 and 320 mg/kg bw/day were not tolerated and thus unsuitable for the subsequent OECD 443 study.

Main study (EOGRTS on silver acetate): In the extended one generation study claimed to be performed according to OECD TG 443 and the principles of GLP (unsigned in the version available to the DS), F0 animals were administered doses of 40, 80 or 120 mg/kg bw/day orally in diet. Males were treated for ten weeks before pairing, up to necropsy after litters were weaned. 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 (depending on cohort) at the same dose levels and volume-dose as the F0 generation, with exception of animals at 120 mg/kg bw/day in Cohorts 1A and 1B which were terminated prematurely on welfare grounds at approximately 10 weeks of age rather than 13-14 weeks of age. The data for the F0 generation included clinical observations, body weight, food consumption, water consumption (by visual assessment), estrous cycles, mating performance and fertility, gestation length and parturition observations and reproductive performance. Clinical pathology (hematology and blood chemistry) and thyroid hormone analysis, blood silver analysis, serum copper/selenium analysis, sperm assessment, organ weight and macroscopic pathology and microscopic pathology investigations were performed. For F1 offspring, clinical condition, litter size and survival, sex ratio, body weight, ano-genital distance, organ weights and macropathology were assessed. Nipple counts were performed on male offspring on Day 13 of age. Serum samples that were collected from litters on Day 4 of age were analyzed for serum copper/selenium levels and blood samples were analysed for silver levels. Serum samples from selected offspring on Day 22 of age were analyzed for thyroid-related hormones and serum copper/selenium levels, and blood samples were analysed for silver levels.

The F1 generation was split into five cohorts at weaning and the data recorded for each cohort included:

Cohort 1A: clinical condition, body weight, food consumption, sexual maturation and estrous cycles. Clinical pathology (hematology, blood chemistry, cardiac troponin, and urinalysis) and thyroid-related hormones, silver blood levels, serum copper/selenium levels sperm assessment, ovarian follicle and corpora lutea counts, organ weight, macroscopic pathology, full microscopic pathology and spleen immunophenotyping investigations.

Cohort 1B: clinical condition, body weight, food consumption, sexual maturation and estrous cycles. Silver blood levels and copper/selenium serum levels were assessed. Organ weight and macroscopic pathology investigations were performed.

Cohort 2A: clinical condition, body weight, food consumption and sexual maturation. Neurobehavioral screening, brain weight, brain morphometry, macroscopic pathology and microscopic pathology

Cohort 2B: no direct treatment and no specific in-life investigations. Animals were dispatched to necropsy at weaning for microscopic pathology investigations of the brain including brain morphometry.

Cohort 3: clinical signs, body weight, food consumption and sexual maturation. Spleen weight, macroscopic pathology and T-cell dependent antibody response investigations.

F0:

One male at 80 mg/kg/day, two males at 120 mg/kg/day died of unclear reason and one female at 120 mg/kg/day was killed owing to a mammary lesion which was unrelated to treatment.

Body weight gain for males at 80 and 120 was significantly reduced at termination whereas female body weight was unaffected by treatment. Intermittent, transient effects on food intake were observed but the efficiency of food utilization for animals before pairing and for females during gestation was unaffected by treatment.

At Week 10 (females) and at termination (males and females) differences in erythrocyte count, mean cell haemoglobin and mean cell volume, red cell distribution width, mean cell heamoglobin, mean haemocrit, haemoglobin concentration, platelet count were observed and white blood cell count with variable dose

response and with males more affected than females. **Females at 120 mg/kg/day showed high counts for eosinophils, monocytes and large unstained cells**. None of the other parameters measured showed any effect of treatment.

At Week 10 (females) and at termination (both sexes) biochemistry analyses showed **high alkaline phosphatase activity, high plasma cholesterol** and low potassium levels but differences were not always dose proportionate.

There were no statistically significant differences in thyroid stimulating hormone or thyroxine serum concentrations levels in any group or generation of males or females after dietary administration of Silver Acetate at 40, 80 and 120 mg/kg/day when compared with controls.

In general s**erum copper** and selenium levels decreased as the target dose increased, the decreases in serum levels were not dose proportionate but tended to be greater for copper and for adult animals when compared with offspring on PND22. The study report states that copper is an essential trace element since it is a cofactor of several enzymes and proteins and plays a pivotal role in several biological functions (for example - respiration, protection from oxidative damage, iron metabolism), also including the central nervous system development and functioning (for example synthesis of neurotransmitters, myelination, activation of neuro peptides). Therefore, copper dysmetabolism is associated with different toxic effects including neurogenerative disorders (Giampietro et al, 2018) with low bioavailability of copper associated with demyelination and neurodegeneration (Spisni et al, 2009)

Copper Serum levels (ng/mL)

Group/ sex	F0 Week 10		F1 PND22 offspring		F0 Males Term		F1 Cohort 1A Term		F1Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1M	1287	13.8	666	21.8	1515	18.8	1091	21.3	1211	13.1
2M	470	32.6	550	47.1	390	21.6	368	16.2	398	21.5
As % Control	*37*		*83*		*26*		*34*		*33*	
3M	316	23.4	337	30.1	255	19.3	245	43.2	254	26.5
As % Control	*25*		*51*		*17*		*22*		*21*	
4M	245	39.4	383	41.8	176	19.5	217	77.0	161	33.5
As % Control	*19*		*58*		*12*		*20*		*13*	

Group/ sex	F0 Week 10		F1 PND22 offspring		F0 Females PND28		F1 Cohort 1A Term		F1Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1F	1800	12.9	875	59.7	1840	9.73	1623	16.8	1578	19.2
2F	719	22.6	458	29.4	811	29.8	869	21.5	1020	14.3
As % Control	*40*		*52*		*44*		*54*		*65*	
3F	544	21.2	363	22.1	562	30.9	576	15.8	650	27.9
As % Control	*30*		*30*		*31*		*35*		*41*	
4F	374	33.2	449	78.4	448	28.5	286	29.1	378	36.1
As % Control	*21*		*51*		*54*		*18*		*24*	

Selenium Serum levels (ng/mL)

Group/ sex	PND22		F0 Males Term		F1 Cohort 1A Term		F1 Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1M	184	13.3	456	6.90	274	21.3	320	18.4
2M	127	9.73	253	15.0	166	15.6	233	17.6
As % Control	*69*		*55*		*61*		*73*	
3M	110	21.3	219	14.6	136	13.3	224	16.2
As % Control	*60*		*48*		*50*		*70*	
4M	111	15.2	219	8.61	108	24.6	169	11.7
As % Control	*60*		*48*		*39*		*53*	

Group/ sex	F1 PND22 offspring		F0 Females PND28		F1 Cohort 1A Term		F1 Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1F	192	6.53	381	5.04	216	19.3	252	10.0
2F	129	17.7	315	11.6	156	12.3	221	17.1
As % Control	*67*		*83*		*72*		*88*	
3F	115	20.9	268	7.33	132	13.2	210	32.0
As % Control	*60*		*70*		*61*		*95*	
4F	109	17.1	266	9.97	98.2	13.7	154	12.4
As % Control	*57*		*70*		*45*		*61*	

Body weight-relative heart weight was high for both males and females at all dose levels and all treated groups of males had low mean absolute pituitary weights and absolute/body weight-relative

mean prostate weights. Low mean absolute testes weight was also observed at 80 and 120 mg/kg/day and high mean body weight-relative spleen weight was observed at 120 mg/kg/day. Females that received 120 mg/kg/day showed low absolute and body weight-relative mean **thymus weight**.

The majority of macroscopic findings at scheduled termination comprised abnormal colouration of tissues, including the gastrointestinal tract, kidneys, lacrimal glands, liver, harderian glands, mesenteric lymph nodes, pancreas, preputial/clitoral glands, salivary glands, thymus, thyroids, urinary bladder and uterus. At histopathology, extracellular pigment was observed in various organs/tissues and was considered to represent deposition of test item at these sites. In general, pigment was more prominent in females, and there was not always an apparent dose response. Pigment was not associated with any inflammatory or degenerative changes. Other histopathological findings included increased extramedullary hematopoiesis observed in the spleen of males at 80 or 120 mg/kg/day and epithelial degeneration of the glandular mucosa of the stomach in females that received 40, 80 or 120 mg/kg/day.

Developmental toxicity:

The gestation index (number of litters born/number of mated females) was 100% in all groups.

There was an increased incidence of offspring mortality at 120 mg/kg bw/day (3 males on days 27 and 36 and 2 females on days 33 and 36 in cohort 1A, 2 males in cohort 1B on days 19 and 32, 3 males in cohort 2A on days 19, 23, 47 and one in cohort 3 on day 19) and dark coloration at 120 mg/kg/day whereas there was one mortality each in mid and low dose groups (cohorts 1B on day 28 and 1A on day 26, respectively) and the clinical condition of offspring at 40 or 80 mg/kg/day was considered unaffected by treatment.

There was no effect of treatment on the number of implantation sites, mean litter size or sex ratio on postnatal Day 1. At 120 mg/kg/day the live birth and **viability indices** were low when compared with Controls, resulting in a mean **live litter size** on Day 1 of 13.0 compared to 14.5 in Controls and 11.7 on Day 4 of compared to 14.4 in Controls; no differences were apparent at 40 or 80 mg/kg/day.

Following litter standardization on Day 4 of age offspring survival at all dose levels was similar to Controls.

Mean **body weight** for male and female offspring at 120 mg/kg/day was low when compared with Controls on Day 1 of age and remained low until weaning. At 80 mg/kg/day mean offspring body weight on Day 1 was similar to Controls but lower than Controls on Days 14 and 21. There was no effect on offspring weight or weight gain at 40 mg/kg/day.

There was no effect of treatment on anogenital distance in male or female offspring and no effect on nipple count in males.

Macroscopic findings for offspring that died or were culled on Day 4 of age were predominantly absence of milk in the stomach and/or autolysis.

There were no significant macroscopic findings in offspring terminated on Day 22 of age. At 80 or 120 mg/kg/day low mean absolute and body weight-relative thymus weights ($p<0.01$) were observed in both sexes, and females at 40 mg/kg/day showed low relative weights.

On PND22 **mean absolute brain weight** was low in females at 80 and 120 mg/kg/day.

Nine male and two females died in the high dose group between Day 19 and Day 47. Seven of these decedents were subject to full microscopic examination and in five the major factor contributing to death was **brain lesions**. Due to the high mortality, the high dose group was terminated prematurely at approximately 10 weeks of age.

Males at the target dose of 120 mg/kg/day showed a higher incidence of **piloerection, hunched posture and abnormal gait**; these signs were also observed in a few females at 120 mg/kg/day but at a low incidence.

At weaning on PND21 selected males and females receiving 80 or 120 mg/kg/day had low mean **bodyweight** and subsequent **bodyweight gain** up to PND25 was low at all target dose levels. Changes were statistically significant but with no clear dose response.

From Day 1 of the F1 generation (PND28±2) up to termination mean body weight, mean body weight gain and food consumption was low for males at all dose levels showing a dose response. This was not seen in females. Overall, the efficiency of food utilization was unaffected by treatment for both males and females.

The hematological investigations made at termination (approximately 13 weeks of age) of males administered 80 mg/kg/day and females at 40 or 80 mg/kg/day showed high mean erythrocyte counts. Males at 80 mg/kg/day also showed low mean cell hemoglobin, low mean cell volume and high red cell distribution width and females at 80 mg/kg/day showed high hemoglobin and high hematocrit. Conversely males and females at 120 mg/kg/day that were terminated prematurely at approximately ten weeks of age showed low erythrocyte counts, high mean cell hemoglobin and high mean cell hemoglobin concentration. In addition, a low hematocrit, low red cell distribution width and high mean cell volume were noted in high dose females. Therefore it can only be concluded that **treatment caused a disturbance of hematological parameters.**

White blood cell parameters at either scheduled or premature termination were high in treated females. Mean platelet counts were low for females at 80 or 120 mg/kg/day, prothrombin times were shorter in all treated groups of females and activated partial thromboplastin time was shorter for females at 120 mg/kg/day.

At premature termination the **blood chemistry** analyses in high dose animals principally showed high alkaline phosphatase activity, high alanine amino-transferase activity, high aspartate amino-transferase activity (females only), high cholesterol, high glucose, high urea (males), low creatinine, low albumin/A:G ratio, high potassium, low phosphorous levels and low sodium(females).

At scheduled termination of animals at 40 or 80 mg/kg/day the blood chemistry investigations revealed high alkaline phosphatase activity (males), high alanine amino-transferase activity (males), high cholesterol and low creatinine. Troponin investigations made in the F1 generation revealed higher levels in treated males, with mean levels at 120 mg/kg/day that were slightly high but much lower than the mean values at 40 or 80 mg/kg/day (no historical control data were available for comparison). Cardiac troponin levels were unaffected in females at all dose levels.

Urinalysis investigations of the F1 generation revealed a low total protein output and protein concentration in males at 80 mg/kg/day (urinalysis assessment was limited to control, low and mid dose animals).

Neurobehavioral testing (cohort 2A): Observations possibly indicative of developmental neurotoxicity included abnormal motor movements (chewing mouth movements and licking around mouth) in two males administered 80 mg/kg/day and two males administered 120 mg/kg/day. Two females administered 120 mg/kg/day were observed with their eyelids completely closed; one recorded as being asleep, the other as being awake which is not normally an observation when the eyelids are completely closed. Three males administered 120 mg/kg/day were considered “slightly awkward to handle” during the in-hand assessment. During the arena assessment there were several findings predominately in males and females at 120 mg/kg/day and a few animals at 80 mg/kg/day.

• Palpebral closure (varying degrees): 3/9 males, 1 female administered 120 mg/kg/day.

• Gait observations (e.g. flattened, staggering or unsteady gait) in treated groups different from controls.

• Tremor in two males (short/long period, respectively) and in one female (short period) administered 120 mg/kg/day, one male in the Control group.

• Abnormal motor movements in males and females at 80 or 120 mg/kg/day such as licking around mouth and chewing mouth movements although there was also one male in the control group also showed licking around mouth.

• Both activity and rearing counts were low in all treated males and females in all treated groups with statistical significance achieved for males at 80 or 120 mg/kg/day and females at all dose levels. All activity and rearing count values for males in all treated groups were below the HCD minimum as were the activity count values for females in all treated groups; however, activity counts in control females were above the HCD range. The only rearing count value that was below the HCD minimum was in the females at 120 mg/kg/day, but in general, these low levels of activity mimic what was seen in the

automated activity system although the activity and rearing counts in the arena suggest effects at 80 or 120 mg/kg/day.

Many of the results in auditory startle response differed from control values and were statistically significant but fell within the historical control range or were slightly outside of the range. Neither male or female habituation values attained statistical significance, however, the values for females at 40 or 120 mg/kg/day were below the HCD range values at 80 mg/kg/day were within the HCD range. In conclusion, there was a suggestion of an effect on startle latency to peak response and habituation at 120 mg/kg/day.

Motor Activity measurements in cohort 2A showed low group mean high (rearing) and low (ambulatory) beam activity scores for all groups of treated males that were statistically significant at many of the 6-minute intervals, and total high and low beam scores for males in at 80 or 120 mg/kg/day also achieved statistical significance. Most high and low beam scores for males at 120 mg/kg/day and some at 80 mg/kg/day, for the first 24-minutes were below the HCD minimum. The first half of the 1-hour recording period is when the majority of exploratory behaviour occurs indicating that activity levels were low from the start of assessment. A dose-relationship was also evident although only the high beam total score fell below the HCD minimum. The low beam total score was within the HCD range. The majority of high and low beam scores for females at 120 mg/kg/day and to a lesser extent, at 80 mg/kg/day were also low when compared with controls, again with statistical significances attained at many of the 6-minute intervals and the total score (high beam at 120 mg/kg/day only). As above, many of the 6-minute interval scores and the total scores for females at 80 or 120 mg/kg/day were below the HCD minimum and these continued throughout the duration of the 1-hour recording period. All total high and low beam scores for males and females at 40 mg/kg/day were within the HCD range so no effect of treatment was inferred.

During the reactivity investigations, four males at 120 mg/kg/day showed a reduced approach response, showing no reaction to the probe, compared to one in the control group and two females showed a weak response to the startle reflex, compared to one in the control group. Three females in each of the groups receiving 80 or 120 mg/kg/day failed the pupil closure reflex response with pupils that failed to dilate; two males at 120 mg/kg/day also failed pupil closure however two control males also failed. One male and one female at 120 mg/kg/day showed a slow or partial response when assessed for proprioception.

Group mean bodyweights for males at all dose levels and females at 120 mg/kg/day were low when compared with controls: with values for males at 80 or 120 mg/kg/day attaining statistical significance. These values were also below the HCD range.

Group mean landing foot splay values for males at 80 or 120 mg/kg/day were increased, a typical sign of neurotoxicity, and attained statistical significance in high dose males although both values were above the HCD range. However, the same pattern in landing foot splay was not seen in the females.

Group mean forelimb grip strength values for males and females at 120 mg/kg/day were low with the value for females attaining statistical significance. In addition, hindlimb grip strength values for all treated males and females were low with the values for males at 80 or 120 mg/kg/day and for females at females at 120 mg/kg/day attaining statistical significance. All values were within the HCD range or slightly above, however the low values at 120 mg/kg/day are considered related to treatment.

In summary, F1adult animals at 120 mg/kg/day showed an increased **incidence of piloerection, hunched posture and abnormal gait** at routine physical examination and during behavioural assessment of Cohort 2A animals (in cage, in hand and arena observations) abnormal motor movements/gait and activity were also observed at 120 mg/kg/day but also in a few animals at 80 mg/kg/day. In addition, animals at 120 mg/kg/day showed possible treatment related effects on the **auditory startle response and reduced habituation**, animals at 80 or 120 mg/kg/day had treatment related difference following **reactivity investigations** (weak startle response, reduced approach response, failed pupil closure, high landing foot splay, reduced limb strength) and males and females at 80 or 120 mg/kg/day had low activity scores (rearing and ambulatory). According to the study report, the clinical signs observed may be associated with neuronal/glial cell necrosis in the thalamus since the thalamus is heavily involved in relaying information between the cortex and the brain stem, as well as within the different cortical structures, it contributes to many brain processes, including sensory relay (visual, auditory, somatosensory and gustatory systems), contributions to perception, relaying motor/sensory information, memory, alertness/attention, consciousness/awareness and contributes to cognition. Damage to this area can

therefore impact on many brain functions and can manifest as impaired movement, impaired posture, impaired processing of sensory information and has been associated with fatal coma due to its involvement in sleep regulation (Guy-Evans, 2021).

Pathological findings among cohorts:

Cohort 1A: treatment related organ weight changes were noted in the heart in both sexes, the brain in males given 120 mg/kg/day, and the thymus in both sexes at 120 mg/kg/day. F1 Cohort 1B males also showed treatment related organ weight changes in the heart.

Table: Incidence and Severity of Silver Acetate-Related Microscopic Findings – F1 Generation Cohort 1A at Scheduled Termination (Groups 1-3 at 13 weeks of Age and Group 4 at 10 weeks of Age)

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Brain								
Number Examined	20	19	20	20	20	20	20	20
Edema, Intramyelinic								
Minimal	0	0	2	6	0	0	0	3
Slight	0	0	1	2	0	0	0	0
Necrosis, Neuron, Hippocampus								
Minimal	0	0	8	11	0	0	3	9
Necrosis, Neuron/Glial Cell, Thalamus								
Minimal	0	0	3	3	0	0	2	3
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Pigment, Extracellular								
Minimal	0	11	8	5	0	2	9	6

Cohort 2A: males at 120 mg/kg/day had high body weight relative brain weight; this was not evident in **Cohort 2B.**

Table: Incidence and Severity of Silver Acetate-Related Microscopic Findings – F1 Generation Cohort 2A at Scheduled Termination on Day 75 of Age

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Brain, Cerebrum								
Number Examined	10	10	10	7	10	10	10	10
Edema, Intramyelinic								
Minimal	0	0	0	1	0	0	0	3
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	1	0	0	0	1
Necrosis, Neuron, Hippocampus								
Minimal	0	0	5	5	0	0	3	7
Necrosis, Neuron/Glial Cell, Thalamus								
Minimal	0	0	0	2	0	0	0	4
Slight	0	0	0	1	0	0	0	1
Brain, Forebrain								
Number Examined	10	10	10	7	10	10	10	10
Edema, Intramyelinic								
Minimal	0	0	0	2	0	0	0	2
Brain, Medulla Oblongata								
Number Examined	10	10	10	7	10	10	10	10
Pigment, Extracellular								
Minimal	0	6	3	3	0	2	1	0

Table: Brain Morphometry – F1 Generation Cohort 2A at Scheduled Termination on Day 75 of Age

	Silver Acetate							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	40	80	120	0	40	80	120
Hippocampus								
Measurement (mm)	2.10	2.10	1.97*	1.91**	1.97	NA	NA	1.93

NA = Not applicable.
*;** = Statistically significant difference (absolute or relative) compared with respective control mean value

Cohort 3: higher than control mean absolute and body weight relative spleen weights were noted in both sexes at 120 mg/kg/day

Treatment-related macroscopic findings in F1 Cohorts 1A, 1B, 2A and 3 were generally observed in both sexes across all dose groups without a significant dose response and were generally limited to abnormal coloration (dark) of affected organs/tissues. This was generally observed at a lower incidence and in fewer tissues compared to F0 generation animals. This abnormal coloration was no apparent in F1 Cohort 2B animals that were necropsied at weaning. In the brain, dose related **neuronal necrosis in the hippocampus, and neuronal/glial cell necrosis in the thalamus** were observed in both sexes given 80 or 120 mg/kg/day. **Intramyelinic edema in the thalamus, caudate putamen and/or the corpus callosum** was also observed in males given 80 or 120 mg/kg/day and in females given 120 mg/k/day Brain morphometry measurements revealed statistically significantly **low hippocampus measurement** for males that received 80 or 120 mg/kg/day.

Cohort 3: the analysis of immunophenotyping parameters showed minor changes in the spleen leukocytes of treated rats as well as a statistically significant increase ($p<0.05$) in the percentage of B cells in all treated females. According to the lab, this may be related to treatment but the increase in the percentage of B cells did not translate into an increase in IgM production as no significant differences were found in the anti-keyhole limpet hemocyanin (KLH) IgM analyses (TDAR). DS agrees that no statistically significant changes in IgM production were observed but it is noted that the IgM production was in fact consistently lower in all treated animals, although statistical significance was not achieved.

In summary, treatment with silver acetate resulted in **reduced survival of F1** offspring (nine males and two females) between postnatal day 19 and 47 administered 120 mg/kg/day. Seven of these decedents were subjected to full microscopic examination and in five the major factor contributing to death was **brain lesions**. Survival was also decreased in F1 offspring administered 120 mg/kg/day with statistically significantly **reduced live birth index** (89% vs 97% in controls) and **viability index** at PND4 (90% vs 99% in controls) and thus a statistically significantly **reduced litter size** at PND4 (11.7 vs 14.4 in control group). **F1 offspring body weights** were reduced in animals administered 80 or 120 mg/kg/day and **F1 showed developmental neurotoxicity including effects on neurobehaviour (e.g. in sensory and motor functions in** F1 males and females at 80 or 120 mg/kg/day). There were also effects in brain morphometry and histopathology at 80 or 120 mg/kg/day. Exposure to silver acetate resulted in significantly reduced Cu^{2+}serum levels. Weight changes were observed in several organs but with no other histopathological correlation than pigmentation. Therefore, these changes may be due to the deposition of a heavy metal and/or result from processes such as enzymatic induction (liver) or extramedullary haematopoesis. It is noted, though, that low mean absolute and body weight-relative thymus weights observed in both sexes at 80 or 120 mg/kg/day and low relative weights in females at 40 mg/kg/day was also observed in the fertility study, a 90-day repeated dose and the combined chronic-carcinogenicity studies with silver zinc zeolite.

Based on the above, the NOAELs set in this study were:

Parental NOAEL F0: could not be set since degeneration in stomach mucosa was observed in all treated females.

Parental NOAEL F1 adults: 40 mg/kg/day based on effects observed at 80 or 120 mg/kg/day including neurobehavioural changes (reduced activity and rearing in the arena, reduced reactivity, abnormal motor movement/gait), intramyelinic edema and neuronal and glial cell necrosis and F1 brain morphometry (low mean hippocampus). Effects on bodyweight and bodyweight gain, changes in hematologocal and biochemical parameters and changes in organweights (heart, thymus, spleen) were observed in animals administered 120 mg/kg bw/d.

Offspring NOAEL: 80 mg/kg/day (due to reduced offspring survival and reduced growth at 120 mg/kg/day).

Developmental neurotoxicity in selected F1 animals: 40 mg/kg/day (due to the following effects of treatment at 80 or 120 mg/kg/day: reduced activity and rearing of males and females in the arena, reduced reactivity, abnormal motor movement/gait, intramyelinic edema and neuronal and/or glial cell necrosis and F1 brain morphometry (low mean hippocampus) – mean achieved doses of 36 mg/kg/day in males and 40 mg/kg/day in females.

Developmental immunotoxicity in selected F1 animals: 120 mg/kg/day

Developmental effects were also noted in a one-generation and a two-generation studies performed with silver acetate (6.8.2-06) and silver zinc zeolite (6.8.1-04), respectively (also presented in section 10.10.2), studies in which exposure was continuous during the entire period of gestation. Furthermore, indications of developmental toxicity were observed in a published study performed with nanosilver (IIIB 6.8.2-07) and in a study with silver chloride (6.8.1-03). There were also to some extent indications of developmental toxicity in a two-generation study performed with silver sodium zirconium hydrogen phosphate resembling those observed in the two-generation study with silver zinc zeolite and the one-generation study with silver acetate. The effects were however less pronounced than those observed with silver zinc zeolite and silver acetate. No developmental effects were observed in a developmental toxicity study performed with silver sodium zirconium hydrogen phosphate (6.8.2-06)) despite that the silver ion exposure at the doses used was actually higher compared to the doses of silver zinc zeolite and silver acetate. Silver zinc zeolite contains both silver and zinc ions that may share the ability to compete with copper for binding to ceruloplasmin (see below). This would explain why clear adverse effects are observed with this substance at lower levels of silver ion exposure. The difference between results with silver sodium zirconium hydrogen phosphate and silver acetate could be a consequence of differences in administration routes. Silver acetate was administered in drinking water and thus in soluble form whereas silver sodium hydrogen zirconium phosphate was administered mixed in diet. Silver ions easily bind thiol groups of proteins and formation of different complexes may at least theoretically limit the amount available for absorption in the gastrointestinal tract.

Published data on silver chloride (Doc IIIA, 6.8.1(03)): In a published study by Shavlovski et al., a dose of 50 mg silver chloride/animal (less than approximately 250 mg/kg bw/day) was administered in diet to 20 inbred albino female rats from the first day of the study to termination (gestation day 20). A group of five rats was also used to study the effect of silver during the period of organogenesis (days 7-15 only). The study also investigated effects in untreated control rats, in rats administered silver chloride in diet and also injections of human ceruloplasmin[24], and in rats administered silver chloride in diet and also bipyridyl or penicillin (Cu/Fe chelators) throughout gestation. The results show that if dams were exposed to silver chloride between gestation days 1-20, the incidence of post-implantation deaths (36%) increased compared to control (9.6%) and historical controls (8.7%) and all newborn animals died within 24 hours. Moreover, the incidences of hydronephrosis (31%) and cryptorchidism (35%) increased substantially compared to controls (5.3 and 1.3% for hydronephrosis and cryptorchidism respectively) and historical controls (1.2 and 0.8% respectively)

[24] Administered during days 2 and 14 or 8 and 21 of gestation, respectively; it is not fully clear to the DS if silver was administered only on days 2-14 or 8-21 when co-applied with cerulolasmin but it is assumed that these rats also received silver on days 1-20.

	Periods of intoxication		Control group	Historical control
	day 7–15	day 1–20		
No. of pregnant rats in the experiment	5	20	36	237
No. of *corpora lutei*	63	231	430	2758
No. of:				
implanted embryos	59	222	384	2537
pre-implantational deaths (also %)	4 (6.3)	9 (3.9)	46 (10.7)	221 (8.0)
post-implantational deaths (also %)	3 (5.0)	80 (36.0)	37 (9.6)	220 (8.7)
live fetuses	56	42	347	2317
visible abnormalities	0	5	0	0
Average mass of a fetus (g)	2.2	1.75	2.24	2.26
Visceral pathology				
No. of fetuses examined	51	98	150	659
of those having (also %):				
atrial dilatation	2 (3.9)	1 (1.0)	0	0
thoracal hemorrhage	0	8 (8.2)	1 (0.7)	78 (11.8)
abdominal hemorrhage	0	19 (19.4)	7 (4.7)	48 (7.3)
hepatic hemorrhage	0	8 (8.2)	0	6 (0.9)
hydronephrosis	2 (3.9)	30 (30.6)	8 (5.3)	8 (1.2)
cryptorchism	0	34 (34.7)	2 (1.3)	5 (0.8)

The survival of newborns was improved if intraperitoneal injections of human ceruloplasmin were received during gestation days 2-14 and survival was almost comparable to controls if CP injections were received during gestation days 8-21:

	Effect of			Controls
	AgCl (day 1–20)	AgCl + CP (day 2–14)	AgCl + CP (day 8–21)	
No. of fertile rats	5[a]	6	6	4
No. of newborn animals	33	61	51	32
Of those, no. that died (also %)	33 (100)	21 (34.5)	3 (5.9)	0
Index of viability[b]	0	0.26	0.53	0.84
Index of lactation[c]	0	0.58	0.89	0.85
Average body mass on day 18 of life (g)	0	24.0	28.8	27.0
Index of the body mass increase[d]	0	3.9	3.6	3.2

Embryotoxic effects of silver chloride and the influence by co-administered CP:

	Introduction throughout the whole term of	
	AgCl	AgCl + CP
No. of pregnant rats in the experiment	20	22
No. of *corpora lutei*	231	267
No. of:		
implanted embryos	222	262
pre-implantation deaths (also %)	9 (3.9)	5 (1.9)
post-implantation deaths (also %)	80 (36)	25 (9.5)
live fetuses	142	237
visible abnormalities	5	0
Average mass of a fetus (g)	1.75	2.04
Visceral pathology		
No. of fetuses examined	98	220
Of those having (also %):		
atrial dilatation	1 (1)	22 (10)
thoracal hemorrhage	8 (8.2)	24 (10.9)
abdominal hemorrhage	19 (19.4)	38 (17.2)
hepatic hemorrhage	8 (8.2)	23 (10.5)
hydronephrosis	30 (30.6)	8 (3.6)
cryptorchism	34 (34.7)	0

The deaths of embryos and newborns were explained as a consequence of copper deficiency caused by silver inhibiting copper from binding to the transport protein ceruloplasmin. This theory was supported by the increased survival (and reduced frequency of teratogenic effects) in AgCl-treated rats who received injections of human ceruloplasmin as well as by the lack of copper in placenta, embryos and blood serum of adult rats treated only with AgCl.

Copper content in organs:

	Introduction throughout the whole term of		Controls
	AgCl	AgCl + CP	
Liver of adult female rats	11.5 ± 2.7	16.4 ± 1.7	17.5 ± 2.7
Heart of adult female rats	11.2 ± 1.3	16.3 ± 0.7	17.6 ± 2.6
Kidneys of adult female rats	8.6 ± 0.9	10.6 ± 0.7	13.9 ± 0.7
Placenta	0	4.3 ± 0.6	7.3 ± 0.6
Embryos	0	4.6 ± 0.6	5.8 ± 1.1
Blood serum of adult female rats ($mg\ ml^{-1}$)	0	0.7 ± 0.05	1.3 ± 0.05

In addition, malformations were exacerbated when sub embryotoxic doses of bipyridyl and penicillamin that chelate iron and copper, respectively, were injected throughout the whole term in rats concomitantly fed AgCl diet. Since CP also oxidases Fe2+ to Fe3+ and mobilises iron from tissues, inactivation of the enzyme by silver and chelation of Fe is expected to change the iron metabolism and potentiate the toxicity of silver. Penicillamin chelates copper and is thus also expected to increase effects of silver. The potentiating effect of the two chelators on silver toxicity was considered to further indicate that ceruloplasmin and copper deficiency are involved in the mechanism of silver toxicity. There were no effects in rats treated with AgCl during organogenesis only and this was considered to be due to active ceruloplasmin gradually decreasing from blood. Although the study was not performed according to GLP or a recognised guideline, the result is considered reliable since the publication has been peer-reviewed and the experiment seems to be well conducted. Several parameters requested in OECD TG 414 were not investigated but the study yet raises serious concern for developmental toxicity of silver, especially since the author states that treatment did not alter the physiological functions of the dams. Since effects were noted at the only dose level tested, a LOAEL for teratogenic effects cannot be be set for this study.

Assessment of the potential influence of co-occurring maternal toxicity on developmental effects by silver chloride: according to the article, the treated maternal animals did not differ from the controls with respect to their body mass, behaviour, food intake etc. The copper levels in blood serum of adult rats, placenta and embryos were zero in silver chloride-treated animals which may be the specific mechanism causing the deaths of embryos and newborns. The assessment of maternal effects is considered to demonstrate that the developmental effects (i.e. increased postimplantational deaths, chryptorchidism, hydronephrosis, reduced bodyweight and reduced viability index) are no secondary non-specific consequences of maternal toxicity. When exposure was continuous during the entire gestation period severe effects on foetal and pup viability were observed also in one- or two-generation studies performed with silver acetate, silver zinc zeolite and to some extent also silver sodium zirconium hydrogenphosphate.

Published data on silver acetate:

Developmental toxicity of silver acetate in CD albino rats during days 6-19 of gestation was investigated at doses of 10, 30, or 100 mg/kg/day (6.8.1-07). All animals survived treatment except a high dose dam exhibiting signs of morbidity and another high dose dam which was excluded due to a misdirected dose. Clinical signs such as piloerection and minor bodyweight changes were noted in all animals and other signs indicative of toxicity such as alopecia and rooting after dosing were observed in high dose animals. There were no significant effects on maternal body weight gain, food or water consumption during pre-treatment, treatment and gestation period. The number of pregnant dams was reduced in high dose dams (87.5% compared to 96% in control) but the difference was not statistically significant and did not show a dose-response. Other fertility parameters did not differ from controls. The incidence of litters with late foetal deaths was increased in the high dose group (incidences: 0/24, 0/23, 0/25 and 2/20) resulting in a statistically significant positive trend in the Cochran-Armitage test. The incidence was above historical control data (0-4.35% and 0-1 litters) but the study authors did not regard the result of this study as clear evidence of prenatal mortality since no significant treatment effect was observed for the percent litters with late fetal deaths in a Chi-Square Test. However, although not statistically significant, the percentage late fetal deaths /litter was 1.22 in high dose group compared to none in control and the lower dose groups. Moreover, a negative statistically significant trend was observed with respect to average fetal bodyweight per litter (sexes combined) and average male foetal bodyweight/litter but there were no significant pairwise differences. The incidence of malformations (external, visceral, skeletal) was lower in the high dose group compared to the control. The percentage of litters with skeletal variations (58.3, 78.3, 64 and 85% at 0, 10, 30, or 100 mg/kg/day, respectively) and the percentage of litters with any variation (70.8%, 91.3%, 80% and 95% at 0, 10, 30, or 100 mg/kg/day, respectively) was increased in high dose animals compared to controls. The skeletal variations included unossified sternebrae, rudimentary rib, short rib, bipartite ossification center. Considering that there was no dose-response and that the difference was not statistically significant, the observation is not given further toxicological significance. The LOAEL set

for maternal toxicity was 100 mg/kg bw based on clinical signs of toxicity and the LOAEL for pups was 100 mg/kg bw based on the decreased average male foetal bodyweight/litter and average total foetal bodyweight/litter. The LOAEL for embryotoxicity/teratogenicity is 100 mg/kg bw based on the increased incidence of the percent litters with late foetal deaths in the high dose group. Based on a silver content of 64.6% and the assumption that silver acetate is completely dissolved in the stomach, this LOAEL would correspond to 65 mg silver ion equivalents/kg bw.

Assessment of the potential influence of co-occurring maternal toxicity on developmental effects: one pregnant high-dose dam was euthanized on day 12 due to morbidity and one animal was removed from the high dose group due to a misdirected dose. There were no significant effects on maternal body weight gain, food or water consumption during pre-treatment, treatment and gestation period. Piloerection, alopecia and rooting after dosing were observed mainly in high dose animals but some clinical signs were also noted in single animals of all groups, including controls. Single incidences of alopecia, red amniotic fluid in uterine horns, or small intestine/stomach full of gas were reported in high dose animals. The assessment of maternal effects is considered not to demonstrate that the indications of adverse developmental effects (i.e. a 10% incidence of litters with late foetal deaths at the top dose (0% in controls), and a negative trend for average male foetal bodyweight/litter) are secondary non-specific consequences of maternal toxicity.

The reproductive toxicity of silver acetate was also investigated in a rat one-generation study published in 2016 (6.8.2-06). To mimic the most likely human exposure route, silver acetate was administered in the drinking water at dose levels of 0, 0.4, 4 and 40 mg/kg bw/d, equivalent to approximately 0, 0.25, 2.5 and 25 mg/kg bw/d silver. Groups of (P) rats (20/sex) were administered the test material throughout a 10-week pre-mating period and during mating. Females continued to be exposed during gestation and lactation; males were terminated following exposure for 90 days. The resulting (F) litters were culled (5/sex where possible) on PND4 and offspring were further selected following weaning on PND21 (1/sex/litter) and remained untreated until termination on PND26. Parental animals were observed for clinical signs; bodyweights, food and water consumption were measured periodically. Gross necropsy was performed on all parental animals; weights of selected organs were measured, and histopathological examinations were made for a limited selection of tissues. The testes of 10 males/group were additionally assessed using specific staining following perfusion fixation.

The major deviations in the study include the lack of GLP compliance, lack of individual animal data and the lack of further investigations of important parameters such as oestrus cycle, sperm parameters and histopathological analyses of reproductive tissues. Nevertheless, the study is claimed to follow the current protocols for testing foods and food additives (FDA CFSAN Redbook, 2000) and overall, the study seems to be of good quality and results are considered reliable. Only a few effects were noted in parental animals including a reduced fluid consumption that reached statistical significance on some occasions, reduced stomach weights and pigmentation of organs and tissues. The severity of pigmentation was dose-related and occurred in all treated animals thus a parental NOAEL cannot be set. All high-dose dams were sperm positive however of four animals not producing a litter, two dams did not have implantation sites whereas in the other two dams the litters were reabsorbed. This was reflected in the mean number of pups born per litter and the mean number of live pups born per litter (see the table below). Altogether, the following severe developmental effects were noted (see the table below):

- Reduced number of dams with viable litter on PND 21 at the top dose (a slight reduction also at the middle dose) (77.7, 90, 95 and 95 % of pregnant dams at 40, 4, 0.4 and 0 mg/kg bw/day):
    - Two dams having a total resorption (11.1% of pregnant dams) at the top dose (0% of dams in the controls and other dose groups). A reduction in survival at birth/post-natal survival due to a total litter loss in two dams (12.5% of dams with litter) at 40 mg/kg bw/d, 2 dams (10% of dams with litter) at 4 mg/kg bw/day compared to one dam (5% of dams with litter) in control and low dose group.
- A reduction in pup body weight and an increase in the numbers of runts (see table below) in the 4.0 mg/kg dose group.

- A reduction in female pup weight and male pup weight at PN day 26 in the 4.0 mg/kg and 40 mg/kg dose groups, respectively

	M				F			
	0	**0.4**	**4**	**40**	**0**	**0.4**	**4**	**40**
No. exposed to mating	19	20	20	20	20	20	20	20
No. (produced) plug or sperm-positive females	17	19	19	18	20	20	20	20
Mating index	89.5	95.0	95.0	90.0	100.0	100.0	100.0	100.0
***(No. prod litter/ No. prod plugs/sperm-positive) ×100* (given as "Fertility index 1" in the original article)**	100.0	100.0	100.0	**88.9**	100.0	100.0	100.0	**80.0**
***(No. prod litter/ No. exposed to mating) ×100* (given as "Fertility index 2" in the original article)**	89.5	95.0	95.0	**80.0**	100.0	100.0	100.0	**80.0**
Producing litters (*No.*)	17	17	19	16	20	20	20	**16**
With implantations (*No.*)					20	20	20	**18**
Total resorption (*No.*)					-	-	-	**2**
Total litter loss during lactation **(*No.*)**					1	1	1	2
Non-viable pups only at birth (*No.*)					-	-	1	-
Viable litters (remaining at LD 21)					19	19	18	**14**
Implantations (*No.*)					14.4	14.0	14.3	**11.3***
Litter size on PND0 (Mean No. pups					13.1 (20)	12.4 (20)	13.4 (19)	**10.3 (16)[d]***

born per litter)								
Live pups on PND0 (Mean No. live pups born per litter)					13.0 (20)	12.3 (20)	12.8 (19)	**10.5** (16)[a]
Live pups on PND4 (pre-cull)					11.9	11.4	12.4	9.2
Live pups on PND4 (post cull)					9.8	8.9	9.3	8.9
Live pups on PND7					9.7	8.9	8.9	8.9
Live pups on PND14					9.2	7.9	8.1	8.7
Live pups on PND21					9.0	8.2	7.9	8.5
Post-natal day 26 bodyweight (g)	**83.4±1.85**	**80.1±1.71**	**78.8±1.43**	**77.0±1.46***	**77.5±1.94**	**78.4±1.71**	**68.5±3.08***	**76.4±2.41**

*significantly different to controls (p≤0.05); a (p≤0.1)

d Two animals were found to have total resorptions and two dams did not have any implantations.

Total number of runts (number of litters with runts, number of litters)

	0 mg/kg		0.4 mg/kg		4.0 mg/kg		40.0 mg/kg	
	m	f	m	f	m	f	m	f
Day 0	3 (3, 20)	1 (1,20)	0 (0, 20)	0 (0,20)	7 (3, 18c)	4 (2,18)	1 (1, 15d)	2 (2, 15d)
Day 4, pre-culling	5 (5, 19a)	6 (6, 19)	3 (3, 20)	4 (3, 19b)	17 (5*, 18)	18 (8*, 18)	3 (2, 13)	7 (2, 13)
Day 4, post-culling	3 (3, 19)	4 (4, 19)	3 (3, 20)	2 (2, 19)	14 (5*, 18)	13 (6**, 18)	3 (2, 13)	5 (1, 13)
Day 7	4 (3, 19)	3 (3, 19)	12 (7, 20)	6 (4, 19)	9 (6, 18)	16 (8*, 18)	2 (2, 13)	4 (1, 13)
Day 14	3 (3, 19)	2 (2, 19)	0 (0, 19b)	5 (3, 19)	4 (3, 17c)	6 (5, 18)	1 (1, 13)	1 (1, 13)
Day 21	1 (1, 19)	0 (0, 19)	0 (0, 19)	2 (1, 19)	2 (2, 17)	6 (5, 18)	0 (0, 13)	0 (0, 13)

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a. One control female lost all pups day 4;
b. One female at 0.4 mg/kg bw/d lost all pups by PND14 (male pups by day 14 and female pups by day 4);
c. Day 0: one litter lost; day 14: all males lost in second litter;
d. Two litters lost by day 4. One litter had only males and one litter had only females;
* = p < 0.05; ** = p<0.1

Offspring bodyweight (g):

	M				F			
	0	0.4	4	40	0	0.4	4	40
PND0	6.9	7.1	6.3[a]	6.4	6.6	6.7	6.1	6.2
PND4 (pre-cull)	11.3	11.3	9.7*	10.4	10.9	10.9	9.3*	10.2
PND4 (post cull)	11.3	11.3	9.7*	10.4	10.9	11.0	9.6*	10.2
PND7	18.0	17.2	15.7a	16.9	17.6	17.4	15.0*	16.5
PND14	35.8	36.2	35.0	33.9	35.2	34.6	32.7	33.3
PND21	62.5	60.5	57.9	56.9[a]	59.2	58.9	53.2[a]	55.9

**significantly different to controls ($p \leq 0.05$); [a]$p \leq 0.1$*

The reason why the higher and statistically significant number of runts in the 4.0 mg/kg group was not as clearly observed in the 40 mg/kg dose group may be due to the foetal/pup mortality in this group masking such effects. According to the study authors, these effects are most likely a consequence of compound exposure and not compound-induced maternal toxicity.

Assessment of the potential influence of co-occurring maternal toxicity on developmental effects: Mortality was limited to the death of a control male during premating and is not considered to influence the interpreatation of developmental effects observed. In females at 40 mg/kg bw/d the bodyweight gain during pre-mating was marginally (~4%) reduced in females and slightly (but not significantly) lower (~9.5%) during gestation. The publication does not state whether or not the body weight gain reported is corrected for gravid uterine weight. A statistically significant increase in glucose (18%) was observed in high dose females but no other significant changes of clinical parameters were reported at this dose level. Deposition of silver in a number of tissues was observed in all treated groups.

Assessment of maternal effects is considered not to demonstrate that the developmental effects (i.e. reduced litter size which is partly a consequence of a decreased number of dams with implantations and thus a fertility effect at the top dose, but also due to an adverse effect on development as at the top dose 2/18 pregnant dams had a total resorption and two additional dams lost their full litter by PND4 and at the mid dose one female gave birth to only dead pups and another dam lost all male pups by day 14); decreased pup weight (statistically significant at different time points in males at 4 or 40 mg/kg bw/day and in females at 4 mg/kg bw day); increased number of runts at the mid dose) are secondary non-specific consequences of maternal toxicity.

Results obtained in a fertility study with silver zinc zeolite: In a two-generation study in rats, the silver zinc zeolite denoted AgION Silver Antimicrobial Type AK was administered through the maturation, mating, gestation and lactation periods for two successive generations.

Parents P: Three males administered the high dose and one male administered the mid dose died during the study. The cause of death could not be established but the deaths were considered related to treatment by the study author. Bodyweight and bodyweight gains were reduced in males during premating by ≤ 10 and 17% respectively as compared to controls. After mating, the male bodyweight gain was comparable for all groups. One female control animal died during the study but no deaths occurred among the treated P females. The bodyweights were reduced in high dose females at day 20 of gestation and at day 7, 14 and 21 of lactation but did not fall below 11% of the bodyweight in controls. The bodyweight gain was reduced during gestation, during days 0-20 by 16% and days 14-20 by 29%. The bodyweight gain during lactation was at some of the measurements significantly increased or decreased compared to controls, but the overall bodyweight gain during lactation (days 0-26) was not statistically significantly different from controls. Food consumption was reduced between 12 and 27% in the high dose group during lactation and the changes were statistically significant. High dose males and females had increased levels of

erythrocytes, platelets and decreased levels of haemoglobin (Hb), haematocrit (HCT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Some of these parameters were also slightly affected in mid dose males and females. The same effects were seen also in the repeated dose studies performed with silver zinc zeolite Type AK and were considered to be caused by zinc. According to the repeated dose study report, zinc prevents uptake of copper in the GI tract which suppresses production of ceruloplasmin. This in turn leads to decreased iron transport and decreased synthesis of haemoglobin. There were no clinical signs observed and no effects on functional fertility parameters that were statistically significant. Pigmentation was observed in several tissues of mid and high dose animals and mild pigmentation of pancreas and thymus was observed also in some females of the low dose group. Histopathological changes in the kidneys (including hydronephrosis) were noted in high and mid dose animals. Kidney weights were decreased in high dose male and females. The thymus was not weighed. The gestation length was slightly increased (22.3 compared to 21.9 days in controls) in treated animals and the change was statistically significant for the mid and high dose group.

Parents F1: The mortality in the high dose (12500 ppm) animals was excessive as 28/30 males and 23/30 females died prior to the end of the premating period. The group was therefore terminated after this phase and there were consequently no pups from this group. The cause of death was not clearly established but discoloration of organs, histopathological changes in the kidneys, decreased size of thymus, enlarged heart and spleen, penile distention/extension and red discoloration were noted among the dead animals. Body weights of F1 males administered 6250 or 12500 ppm were lower than controls at the start of and throughout the premating, pairing and post-pairing periods and until termination in the high dose group. The body weight gain in males administered 6250 ppm was however comparable to controls over the entire premating period. Bodyweights of mid dose F1 females were statistically significantly lower during the first six weeks of premating and also at one time-point during lactation but there were no statistically significant effects on body weight gains during the overall premating period (week 1-12), gestation or lactation. Food consumption was reduced in high dose animals and in mid dose males during the entire study. The macroscopic examinations at termination of F1 animals revealed changes in the urinary tract and in the kidneys. Effects on kidneys observed in animals treated with 6250 and 12500 ppm included mild caliculi, mild to moderate pelvic dilation and an increased incidence of mild to moderate cortical surface irregularity. Most often cortical surface irregularity corresponded to microscopical changes such as chronic interstitial nephritis and/or infarction. In addition, two males administered 6250 ppm had mild calculus formation in the urinary bladder. Low and mid dose animals had an increased frequency of hydronephrosis (increased frequency compared to P generation). Tan/brown discoloration of multiple organs were observed in animals (pancreas, thymus, glandular stomach, duodenum, jejunum, mandibular salivary glands, Harderian glands, exorbital lacrimal glands, pineal gland and urinary bladder) predominantely in 12500 ppm animals but also in mid and low dose. A low incidence of thymic athrophy was noted in animals administered 1000 (premating 71/87 mg/kg bw/d in males and females respectively)) or 6250 ppm (m/f: 477/582 mg/kg bw/d). Organ weight analysis of animals administered 6250 ppm showed an increased relative weight of spleen (only significant in males), reduced absolute brain weight in males and females, reduced absolute/relative weight of prostate, reduced absolute weight of seminal vesicle, reduced absolute/relative weight of both testes and reduced absolute weight of uterus/oviducts/cervix. Reduced kidney weights were observed in males and females administered 1000 or 6250 ppm. Other statistically significant changes observed were not considered related to treatment. Splenomegaly correlated microscopically with increased extramedullary haematopoiesis and is assumed to be related to treatment since anaemia was observed in the P parents. There were no statistically significant or clearly dose-related effects on the functional fertility.

Developmental toxicity, F1 pups: Adverse effects on development were manifested in high dose animals as reduced mean number of live and total pups at birth, reduced live birth index, increased number of stillborn pups and increased stillborn index (see the tables below). Complete pup mortality was observed in six females of the high dose group on PND4. Considering that there was only a slight decrease in the mean total implantation scars/litter (and even an increase in F1 dams) the increased mean stillborn index likely reflects an increase in post-implantation loss. Day 0-4 pup survival was low in the high dose group

(53.1% compared to 98.9% in controls) and 5/27 females that delivered litters with live pups failed to retain live pups to Day 4. The male/female sex ratio was reduced at day 0, 4 (pre/post culling), day 21 and 26 but the effect was only statistically significant on day 4 (preculling). Clinical signs in pups pre-weaning included decreased activity in mid and high dose animals and discoloured skin (blue/pale) and difficult breathing in high dose animals. The discoloration was mainly observed at day 26 whereas decreased activity and breathing difficulties were observed at day 0 or 4. There were no abnormalities detected in the clinical observations of dams made during lactation. Statistically significantly reduced bodyweights were observed at all measurements of male and female pups administered 12500 ppm and at day 14, 21 and 26 in male and female pups administered 6250 ppm. The absolute weights of brain, spleen and thymus were reduced in pups administered 6250 and 12500 ppm. These changes were statistically significant (except for spleen in 6250 pups). The changes remained statistically significant also when these organ weights (except for the spleen) were related to bodyweights. A dose-related delay in the day of vaginal opening and preputial separation was observed in all treated animals and the delay was significant in the mid and high dose group. Since the bodyweights were comparable between treated females and controls on the day of vaginal opening, the delay seems related to the reduced bodyweights. The bodyweights of 6250 and 12500 ppm males were yet reduced by 12,5 and 38% respectively at the time of preputial separation. There were no treatment related histopathological findings in the stillborn pups or in day 4 culled pups. Changes in the kidney (pale, dilation, cyst) liver (pale) were observed at day 26 in males and females administered 6250 or 12500 ppm. Moreover, cardiac changes were observed in both sexes of high and mid dose animals; mildly enlarged heart in 6/14 males and 6/18 females in 12500 group and 5/27 males and 4/26 females in 6250 group compared to 0 in controls). Small thymus was observed in 2/14 high dose males and 2/18 females.

Developmental toxicity;

F2 pups: The percentage of females delivering litters with stillborn pups (5.4 * vs 1.1% in ctrl) was increased in the 6250 ppm group and this was also reflected as an increased stillborn index and decreased live birth index (93.1* vs 98.3% in ctrl). The number of live pups/litter was decreased in the low dose group at day 4, 14 and 21 due to the complete loss of pups in two litters but there was no effect in the 6250 ppm animals. Pup body weights were lower in 6250 ppm pups than in controls at birth and were further reduced throughout the pre weaning period. Organ weight analysis showed reduced absolute/relative thymus and brain weights in males and females administered 6250 ppm. The macroscopic examinations of F2 pups at day 21 (weaning) revealed mild to moderate decreased size of thymus, mild cardiac enlargement (27/81 in males and 16/90 in females), mild renal pallor, mild hepatic pallor and mild pulmonary pallor in animals of the 6250 ppm group. Analysis of copper, silver and zinc in homogenates of three whole pups from control, 1000 and 6250 pups showed a general decrease of copper in the treated groups whereas the levels of silver and zinc were generally increased (table below). This analysis does not confirm but supports the mechanism proposed by Shavlovski.

Zinc, silver and copper levels (mg/kg bw) of F2 Day 4 culled pups

	control		1000		6250	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Silver	<1	<1	1.04	1.06	1.68	2.2
	<1	<1	1.06	<1	1.1	<1
	<1	<1	<1	<1	1.07	1.84
Zinc	7.77	10	8.87	8.05	8.65	10.4
	6.44	6.31	11.8	6.88	7.32	7.56
	8.01	7.62	5.57	5.63	8.85	11.9
Copper	2.24	2.18	1.97	1.67	<1.5	1.86
	2.07	2.49	2.19	1.61	<1.5	<1.5
	2.15	2.72	1.61	1.76	1.96	1.52

Overview of findings in the two-generation study with silver zinc zeolite

	Mortality	**Bodyweight (% change as compared to ctrl)**	**Bodyweight gain (% change as compared to ctrl)**	**Sexual maturation**	**Haematology (% change as compared to ctrl)**
12500					
P m P f	10% (3/30) 0%	**Premating (end):** ↓11% n.s.s in females **Gest:** ↓6% (only sign day 20) **Lact**:≤11%	**Premating** (d1-11): ↓17% n.s.s in females* **Gest**: Day 14-20:↓29% Day 0-20: ↓16%** **Lact**: No consistent pattern***	Not determined	**m/f** Hb: ↓16/12 RBC: ↑13/15 MCV: ↓20/19 MCH:↓25/23 MCHC:↓7/6 Plat:↑42/45
*stat sign increase certain weeks **see text for a discussion on adjusted maternal weight ***Increases/decreases compared to controls certain weeks but no consistent pattern.					
F1 (p)m F1 (p) f	93.3% (28/30) 76.7% (23/30) **Found dead**	**Premating (start):** ↓55% (m) ↓45% (f)	**Premating (Day 1-12):** ↓47% (m)	Day 59.9 Day 56.7	No data

	Mortality	Bodyweight (% change as compared to ctrl)	Bodyweight gain (% change as compared to ctrl)	Sexual maturation	Haematology (% change as compared to ctrl)
	Days (premating) 0-10 3 m 3 f 11-20 6 m 3 f 21-30 9 m 6 f 31-40 3 m 5 f 41-50 3 m 3 f 51-60 3 m 2 f 61-70 0 m 1 f 71-80 1 m -	**Premating (end):** ↓56% (m) ↓44% (f) **Gest:** n.s.s **Lact:** see text	↓40% (f) **Gest:**n.s.s **Lact:** see text	(F1 Control: 35.1/44.5)	
F1 pups (P dams)	**Total pups born/litter:** 12.1 (↓15%) **Liveborn/litter:** 10.3 (↓27%) **Stillborn/litter:** 1.5 (↑750%) **Live birth index:**85.5% **Stillborn index:** 12.2% **Pup survival indices:** PND 0-4: 53.1% PND 4*-21: 90. 3% (n.s.s) PND 4*-26: n.s.s	**M+f** Day 0: ↓15 Day 4: pre/post culling: ↓19 Day 7: ↓23 Day 14: ↓26 Day 21: ↓36 Day 26: ↓47	Not determined	Not relevant	No data
F2 pups (F1 dams)	No data F1 terminated prior to mating	No data F1 terminated prior to mating	No data F1 terminated prior to mating	Not relevant	No data F1 terminated prior to mating
6250					
P (m) P (f)	3.3% (1/30) 0%	**Premating (end):** ↓7% ↓19-9% on single occasions week 1-6 in females **Gest:** not stat sign **Lact:** ↓7% (day 14 only)	**Premating (Day 1-11):** ↓12% n.s.s in females **Gest:** Day 14-20:n.s.s Day 0-20: n.s.s **Lact:** No consistent pattern	Not determined	Hb: n.s.s RBC: n.s.s/↑11 MCV: ↓6/9 MCH:↓6/12 MCHC:↓7/3 Plat:n.s.s
F1 (m) p F1 (f) p	23.3% (7/30) 3.3% (1/30)	**Premating (start):** ↓25% (m)	**Premating (Day 1-12):** n.s.s	Day 39.8 Day 47.4	No data

	Mortality	Bodyweight (% change as compared to ctrl)	Bodyweight gain (% change as compared to ctrl)	Sexual maturation	Haematology (% change as compared to ctrl)
	Found dead **Days (premating)** m f 10-20 - 1 20-30 2 - 30-40 1 - 50-60 1 - 110-120 1 - 120-130 2 -	↓19% (f) **Premating (end, week 12):** ↓13% (m) n.s.s in females* **Gest:** not stat sign **Lact:** ≤10%	**Gest:** n.s.s **Lact:** (↓65% day 4, see report)	(F1 Control: 35.1/44.5)	
*bw stat sign reduced weeks 1-6 only.					
F1 pups	**Total pups born/litter:** n.s.s (13.1, ↓8%)) **Liveborn/litter:** n.s.s (12.8, ↓9%)) **Stillborn/litter:** n.s.s (0.4, ↑400%) **Live birth index:** n.s.s (97.4%) **Stillborn index:** n.s.s (2.6%) **Pup survival indices:** n.s.s (day 0-4: 96%)	**M+f** Day 0: n.s.s Day 4: pre/post culling: n.s.s Day 7: n.s.s Day 14: ↓13 Day 21: ↓25 Day 26: ↓29	Not determined	Not relevant	No data
F2 pups	**Total pups born/litter:** n.s.s (13, ↓1%) **Liveborn/litter:** **n.s.s** (12.2, ↓ 5%) **Stillborn/litter:** n.s.s (0.7, ↑350%) **Live birth index:** 93.1 % **Stillborn index:** 5.4 % **Pup survival indices:** n.s.s (day 0-4: 93.2%)	**M+f** Day 0: ↓5 Day 4: pre/post culling: ↓12 Day 7: ↓15 Day 14: ↓18 Day 21: ↓20 Day 26: n.d	Not determined	Not relevant	No data
1000					
P males P females	0% 0%	**Premating (end):** n.s.s M/F **Gest:** not stat sign **Lact:** not stat sign	**Premating (Day 1-11):** ↓6% n.s.s in females **Gest:** Day 14-20: n.s.s Day 0-20: n.s.s **Lact:** n.s.s	Not determined	

	Mortality	Bodyweight (% change as compared to ctrl)	Bodyweight gain (% change as compared to ctrl)	Sexual maturation	Haematology (% change as compared to ctrl)
F1 (m) p F1 (f) p	3.3% (1/30) 0%	**Premating (start/end):** n.s.s in m/f **Gest:** not stat sign **Lact:** ↓7% (day 4 only)	**Premating (Day 1-12):** **n.s.s** **Gest:** Day 14-20: n.s.s Day 0-20: n.s.s **Lact: n.s.s** (see text)	n.s.s	No data
F1 pups	**Total pups born/litter:** n.s.s (13.2, ↓7%) **Liveborn/litter:** n.s.s (↓9%) **Stillborn/litter:** n.s.s (0.3, ↑300%) **Live birth index:** n.s.s (97.6%) **Stillborn index:** n.s.s (2.0%) **Pup survival indices:** n.s.s (day 0-4: 98.8%)	**m+f** Day 0, 4 (pre/post culling), day 7, 14, 21, 26: n.s.s	Not determined	Not relevant	No data
F2 pups	**Total pups born/litter:** n.s.s (11.3, ↓14%) **Liveborn/litter:** n.s.s (10.9, ↓16%) **Stillborn/litter:** n.s.s (0.3, ↑150%) **Live birth index:** n.s.s (96%) **Stillborn index:** n.s.s (2.6%) **Pup survival indices**: n.s.s (day 0-4. 83.4%)	**m+f** Day 0, 4 (pre/post culling), day 7, 14, 21, 26: n.s.s	Not determined	Not relevant	
0					
P males P females	0% 3.3% (1/30)	- -	- -	Not determined	
F1 (m) p F1 (f) p	0% 0%	- -	- -	35.1 44.5	
F1 pups	**Total pups born/litter:** 14.2 **Liveborn/litter:** 14.1 **Stillborn/litter:** 0.1 **Live birth index:** 99.2%	- -	n.d	Not relevant	

	Mortality	Bodyweight (% change as compared to ctrl)	Bodyweight gain (% change as compared to ctrl)	Sexual maturation	Haematology (% change as compared to ctrl)
	Stillborn index: 0.8% **Pup survival indices:** PND 0-4: 98.9% PND 4*-21: 100% PND 4*-26:100%				
F2 pups	**Total pups born/litter:** 13.1 **Liveborn/litter:** 12.9 **Stillborn/litter:** 0.2 **Live birth index:** 98.3% **Stillborn index:** 1.1% **Pup survival indices:** PND 0-4: 95% PND 4*-21: 99.5%	- -	n.d	Not relevant	
*post culling					

Pathological findings (terminal sacrifice) in several generations in the two-generation study with silver zinc zeolite

	12500	6250	1000	Control
Incidences of hydronephrosis				
P	8m, 2f	7m, 2f	2m,1f	3m
F1	terminated	10m, 4f	3m, 1f	-
Reduced thymus weight (% lower than controls)				
P	not weighed; no histopathological findings			
F1 pups[25]	(m/f) abs 74/70%, rel bw 53/47% rel brain 69/64%	(m/f) abs 58/55% rel bw 39/39% rel brain 53/51%	m, abs 13%, m/f rel bw 10/9% m, rel brain 11%	-
F1 adults	not weighed thymus atrophy noted in males/females	not weighed	not weighed	not weighed
F2 pups	Not available due to termination of F1.	(m/f) abs 50/54%,	m, rel bw 11%	-

[25] According to the study report, complete necropsy examinations were performed on F1 and F2 pups culled on day 4 of lactation, on F1 pups not chosen to cobntinue on study from each litter at weaning and all F2 pups at weaning.

		rel bw 37/42%, rel brain 47/50%		

Assessment of the potential influence of co-occurring maternal toxicity on developmental effects: The high parental mortality rate in the study was more or less restricted to the P males of the high dose group (10%) and F1 males of the 6250 ppm group (23%). According to the CLP, maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation. The mortality rate in high dose P females was 0% and the rate in F1 females of the 6250 ppm group was not higher than observed in P controls (3.3% or 1/30). Thus the data on developmental toxicity cannot be dismissed based on maternal mortality at any dose. Considering the higher frequency of histopathological changes in kidneys and the urinary tract of males compared to females, it may be speculated that anatomical and/or biochemical differences make the males more sensitive to the substance and ultimately results in organ failure and death. However, at the top dose of the F1 generation, the mortality rates were considerable in young animals of both sexes during the pre-mating period starting after weaning (28/30 males and 23/30 females). This indicates a higher sensitivity of the developing animals (F1 generation) compared to adults (mortality rate of the P males 3/30 and P females 0/30 in at the top dose) possibly as a consequence of a copper deficiency as postulated by Shavlovski (see section 10.10.5) and supported by the results from the analysis of copper, silver and zinc made in homogenates of pups. According to CLP, developmental effects can manifest at any time point in the life span of the organism. The fact that also the pre- and post-natal pup mortality rates were high in the top dose of the F1 generation (stillborn index being 12.2% vs 0.8% in control and pup survival index between PND 0-4 being 53.1%) support the assumption that the high mortality rates of both sexes at the top dose of the F1 generation also after weaning results from developmental toxicity. A similar difference in sensitivity was also observed in young adults at 6250 ppm between P (3.3.% mortality in males, 0% in females) and F1 generation (23.3% mortality in males and 3.3.% in females) further suggesting that the consequences of developmental exposure are more severe than the consequences of adulthood exposure. There was also a statistically significant increase and decrease in stillborn and livebirth indeces at 6250 ppm in the F2 pups (5.4/93.1% compared to 1.1/98.3% in F2 control pups) providing further evidence for severe developmental effects in the absence of severe maternal toxicity.

The bodyweight of P dams in the 12500 group was reduced by 6% on day 20 of gestation and the bodyweight gain was reduced by 16% and 29% during days 0-20 and 14-20 of gestation respectively compared to controls. The adjusted mean maternal bodyweight change was not calculated but considering that the mean bodyweights of males and females pups were 15% lower compared to controls day 0 and that the number of pups born/litter was 15% lower than controls, the reduced bodyweight gain may have been an intrauterine rather than a maternal effect. This can also be illustrated by roughly adjusting the mean maternal body weight for foetal weights. The results indicate that the terminal bodyweights of high dose dams were actually higher compared to control dams when the total litter weight was subtracted. Therefore, the reduced bodyweight gain observed during gestation in 12500 ppm dams seems due to effects on foetal weight rather than maternal weight. The reduced body weight gain during gestation is thus not considered to indicate severe maternal toxicity in the P high dose females. There were no statistically significant changes in bodyweights/bodyweight gains in F1 6250 dams (the highest dose in the F1 generation) during gestation yet a statistically significant increase/decrease in stillborn and livebirth indeces were observed also in the offspring of this generation (F2 pups) (5.4/93.1% compared to 1.1/98.3% in F2 control pups). This is a further indication that the adverse effects in pups were not due to bodyweight changes of their mothers during gestation. In any case, according to OECD guidance document on mammalian reproductive toxicity testing and assessment (number 43), a feed restriction study clearly showed that severe weight loss or decrease in body weight gain in dams per see induced minor changes in skeleton development but no effects on viability or malformations in the rat (Fleeman, 2005).

None of the clinical signs of maternal intoxication listed in the CLP Regulation (i.e. excessive mortality, coma, prostration, hyperactivity, loss of righting reflex, ataxia or laboured breathing) were observed among P or F1 dams during the gestation or lactation periods (the high dose F1 animals were sacrificed prior to mating due to excessive mortality rates in both sexes and no offspring was generated from this generation). Haematological parameters were only analysed in the P females and showed some effects in 12500 and 6250 ppm dams but

effects noted are not considered severe (see table). Mild extramedullary haematopoiesis was observed in a single high dose P dam but there were no such observation made among the F1 6250 dams. A reduced food intake was observed in high dose P females compared to controls during lactation. However, according to the clinical observations made there were no abnormalities detected in any of these high dose dams during lactation. Considering that many of the dams lost some of their pups during the first postnatal days, the reduced food intake could solely illustrate the food demand being lower due to less lactating pups. Histopathological changes of kidneys and urinary tract were observed in all treated animals. The effects appear to be more severe in males based on higher incidences/severity of chronic interstitial nephritis, calculi and hydronephrosis. The frequencies were higher in F1 animals compared to P animals thus effects appear to increase over generations, probably a consequence of longer exposure duration and/or due to increased sensitivity of the developing foetus/pup. A reduced weight of thymus or thymus atrophy was observed in F1 pups (reduced thymus weight at all doses) and adults (atrophy, thymus not weighed) and F2 pups (reduced thymus weight at both doses). The thymus of P parents was not weighed, but no histopathological findings were observed. Effects on thymus were also observed in the two-generation study with silver sodium hydrogen zirconium phosphate (a decreased absolute weight of thymus in high dose P males and in F1 and F2 female and male pups at two highest doses). Thus, also the effects on thymus appeared to be more severe and occur at lower doses if exposure period included the developmental phase.

The effects seen in pups (i.e. reduced number of pups, reduced livebirth/increased stillborn index, reduced bodyweight gain, reduced pup and young animal survival indices, clinical signs (pale), histopathological changes in kidneys, heart, liver and reduced thymus weight) can thus not be considered being due to maternal neglect. Moreover, reduced bodyweights and subsequent delayed day of vaginal opening and preputial separation was observed in F1 offspring of 6250 ppm P females who did not differ significantly from controls with respect to mortality, bodyweight and bodyweight gain during gestation.

Results with silver sodium hydrogen zirconium phosphate: Silver sodium hydrogen zirconium phosphate in the form of Exp.add 9823-37 (also known as AlphaSan® RC2000) was tested in rats in a study performed in accordance with OECD TG 416. The test substance was administered in dietary doses of 1000, 5000 and 20000 ppm to two generations of rats throughout maturation, mating, gestation and lactation.

Parents P: There were no treatment related deaths and no effects on bodyweights or food consumption. Increased relative weight of spleen and decreased absolute weight of seminal vesicles/coagulating gland was observed in high and mid dose males whereas a decreased absolute weight of thymus was observed in high dose males only. The pathological examinations showed pigmentation of pancreas in high and mid dose males and females.

Development, F1 pups: There were no effects on litter parameters (litter size and viability). The litter weights and the mean individual weights were reduced by 8 and 9% at the end of lactation period (PND 21). There were no effects on landmarks of development (pinna unfolding, tooth eruption and eye opening) or on reflexological responses (surface righting reflex, mid-air righting reflex, startle reflex, pupillary reflex). The weight of thymus was reduced in both male and female mid and high dose pups. The pathological examination showed pigmentation of pancreas and the mesenteric lymph nodes in high and mid dose males and females.

Parents F1: Four high dose males and two high dose females died whereas all control animals survived. One animal was killed due to suspected dystocia and pathological findings were observed in the stomach of two animals. For the remaining animals, the cause of death was unclear. The bodyweights of male rats were reduced the entire period before pairing and the bodyweights of female rats were reduced during the first three weeks before pairing and during the entire gestation and lactation periods. Food consumption was reduced in males during the last weeks of maturation and during the first days of gestation and lactation in females (≤ 10%). The absolute weights of adrenals, kidneys, seminal vesicles/coagulating gland and right testis were reduced in high dose males and the relative brain weight, epididymides was increased in this group. The absolute and relative prostate weight was reduced more than 25% in high dose males. A dose-related decrease in prostate weight

was also observed in P males but statistical significance was not achieved. The only statistically significant change observed among organ weights in females was a reduced absolute/relative weight of uterus (28/13%) in the high dose group. Pigmentation of pancreas, lymph nodes and thymus was observed in high and mid dose animals.

Development, F2 pups: There were no effects on live birth index or the viability index but the number born and the litter size at day 1 were statistically significantly reduced in high dose females compared to controls.

Group mean litter sizes of F1 parental animals:

Group	Dose level (ppm)		Total Implantation Count	Number born	Litter Size				
					Day 1	Day 4	Day 7	Day 14	Day 21
1	0	Mean	16.0	15.1	14.8	14.2	7.8	7.6	7.6
		SD	3.5	1.7	2.1	2.7	0.7	1.0	1.0
		N	22	21	21	21	21	21	21
2	1000	Mean	16.3	15.5	14.7	14.0	8.0	8.0	8.0
		SD	2.4	2.4	3.1	3.3	0.0	0.0	0.2
		N	24	24	24	23	21	21	21
3	5000	Mean	15.9	15.2	15.0	14.8	8.0	8.0	8.0
		SD	2.4	2.6	2.5	2.4	0.0	0.0	0.2
		N	22	22	22	22	22	22	22
4	20000	Mean	14.7	13.4*	12.9*	12.4	8.0	8.0	8.0
		SD	1.8	1.5	1.4	1.9	0.0	0.0	0.0
		N	20	21	21	21	21	21	21

The litter weights were reduced by 13% at day 1 of lactation and the mean individual weights were reduced by 13% at the end of lactation (day 21). There were no effects on landmarks of development (pinna unfolding, tooth eruption and eye opening) or on reflexological responses (surface righting reflex, mid-air righting reflex, startle reflex, pupillary reflex). The weight of thymus was reduced in both male and female mid and high dose pups. Pigmentation of pancreas and the mesenteric lymph nodes was observed in high and mid dose males and females. The frequency of increased renal pelvic cavitation seemed to be slightly higher in high dose males (6) than in controls (1).

Assessment of the potential influence of co-occurring maternal toxicity on developmental effects:

There are some indications of developmental toxicity in the study pointing to similar direction as e.g. the study on silver zinc zeolite. The increased mortality rates at the top dose of the F1 generation (4/28 males, 2/28 females) as compared to the P generation (0% mortality in both sexes) may be a consequence of developmental exposure. Also the effects on thymus weight were more severe and occurred at lower doses if the exposure period included the developmental phase. In addition, there were no effect on bw of parental P animals, whereas the bw of F1 and F2 pups were decreased at the top doses. The number born and litter size were reduced in F2 generation. These indications of developmental toxicity are not considered to be secondary non-specific consequences of maternal toxicity as there was no excessive mortality, coma, prostration, hyperactivity, loss of righting reflex, ataxia or laboured breathing in dams.

Published studies with nanosilver:

The summary table of published studies performed with nanosilver presented includes in vivo studies performed via the oral route (gavage). The studies are referred to in a review compiling information on reproductive and developmental toxicity of silver nanoparticles identified in a literature search performed in 2016. The studies presented are not performed according to recognised guidelines or the principles of GLP complicating the assessment of robustness and reliability. However, effects observed among studies resemble

those in studies with other SCAS (e.g. silver in fetal tissues, foetal mortality (Philbrook, N.A. et al., 2011), delay of onset of puberty (Mathias et al., 2015) and decrease in pup weight as compared to controls (Fatemi et a., 2013) and indicate a toxic effect of nanosilver on the developing foetus. In addition to these effects, nanoparticles of silver were shown to induce large DNA deletions, micronucleus formation and DNA double strand breaks (DSB) in bone marrow and peripheral blood in developing mice foetuses (Kovvuru et al., 2015) (further discussed in section 10.8.1). In pups on the day after birth silver nanoparticles caused also a significant increase in microvacuolar structures in brains (612 vs 159 in ctrl), statistically significant decrease in the ratio of pup brain/body weight, silver accumulation in the brain and reduced antioxidant activity and increased peroxidation (Fatemi et al., 2013). The available data on maternal effects does not indicate severe toxicity and therefore the observed effects on silver nanoparticles are considered not to be secondary non-specific consequences of maternal toxicity. Since studies with nanosilver rarely are performed with ionic silver as a concurrent control, the data available in the open literature do not give a consistent view whether or not effects observed with nanoparticles result from silver ions released or if they are caused by the nanoparticles *per se*. Induction of oxidative stress is often reported as a nano-specific effect but is also observed with ionic silver[26] and as stated in Charehsaz et al (IIIA, 6.8.2-10) *"It can be concluded that the oxidative response/damage of Ag-NPs reported in previous studies depends not only on the NPs, but also the amount of Ag ions released from the surface of the NPs."* Consequently, even if some effects discussed in this section may result from a mode of action involving oxidative stress it is not scientifically justified to consider these as nano-specific since it is clearly an intrinsic property of the silver ion. Nevertheless, it is possible that nanoparticles cause a higher ROS production due to its oxidative potential. As discussed in section 6, the distribution of silver nanoparticles may differ from ionic silver dissolved from different silver salts and nanoparticles may thus reach different tissues where silver ions are released. This has been reported following inhalation of nanoparticles that are distributed to the brain via the olfactory nerve circumventing the blood-brain barrier. The distribution of nanoparticles of silver as well as of silver nitrate following oral administration was investigated in a 28-day study in rats (Van der Zande, M., et al, doc IIIB, 6.8.2-12). The results indicate a silver distribution pattern upon oral exposure of two different sizes of AgNPs and silver nitrate with highest amounts in liver and spleen, followed by the testis, kidney, brain, and lungs, without differences in the distribution pattern between the two different AgNPs, or the silver nitrate-exposed animals. The uptake of silver in blood and organs was higher in animals treated with silver nitrate compared to AgNP treated rats. However, when normalising the silver exposure dose in blood for soluble silver[27], the difference in blood was much smaller. This indicates that the major part of silver in plasma is ionic silver released from the nanoparticles. Normalising for soluble silver exposure dose in organs resulted in similar silver contents between Ag<20 and silver nitrate in all organs except for testis and spleen in which the silver dose was higher for Ag<20. The authors thus conclude that AgNP only contributes to the silver concentration in these two organs and to a much lower extent, a result stated to be in contrast with a different study indicating that not only soluble silver but a significant fraction of AgNPs contribute to silver in organs. Nevertheless, of significance for this assessment is the detection of silver from both nanoparticles of silver and silver nitrate in the testis meaning that it is reasonable to assume that effects discussed in this section are not specific to nanoparticles but also relevant for effects of ionic silver from salts administered via the oral route.

Results from the study by Philbrook et al show an increase in the number of non-viable foetuses (9.6% (stat sign), 6.1% and 5.5% in low, mid and high dose groups compared to 3.3 % in controls). The authors suggest that the lower frequency of effects at higher doses are due to agglomeration of nanoparticles after administration resulting in reduced toxicity and clearance by the animals. Nanoparticles are known to agglomerate unless stabilised e.g. by some type of surface-treatment. According to the information in the publication, particles were not surface-treated and thus not stabilised. Therefore, the explanation for the lack of dose-response seems plausible. There were no other developmental effects observed in the study and no effects were observed on the number of resorptions, birth weight and litter size in a rat study by Charehsaz.

[26] E.g. Cortese-Krott MM et al., (2009) Free Radic Biol Med., Shim I et al, (2017) J Appl Toxicol.

[27] "since the exposure dose of silver was not equal in all groups (90 mg/kg bw for the Ag < 20 and Ag < 15-PVP groups vs 9 mg/kg bw for the AgNO3 group), all results were normalized on the silver exposure dose and presented as the ratio between the measured silver concentration in blood or feces (μg silver/kg blood or feces) and the daily silver exposure dose (mg silver/kg body weight)."

However, it should be noted that exposure to the test substance was limited to gestation day 9 in mice and gestation days 6-19 in rats. No effects were observed in the study by Yu et al in which rats were administered nanoparticles between gestation days 6-20. The results of the study by Shavlovski et al indicate that the adverse effects on development observed with silver chloride manifest only if exposure is continuous during the entire gestation period (due to gradual decrease of active ceruloplasmin in plasma of dams). Therefore, it may be questioned whether or not it would have been possible to detect the type of effects noted with silver chloride in the studies with nano silver. This is also supported by the results of the two studies with silver acetate showing mild effects (i.e. slightly increased incidence of the percent litters with late foetal deaths) in the developmental study at a dose of 100 mg/kg bw/d (65 mg silver ion/kg bw/d) and exposure during GD 6-19 and more severe effects (reduced number of litters and implants and reduced male pup survival) at 40 mg/kg bw/d (26 mg silver ion/kg bw/d) in the one-generation study with continuous exposure to silver acetate during pre-mating, gestation and lactation. However, the observed pup brain damage, foetal mortality at the low dose, delay of onset of puberty and decrease in pup weight induced by silver NPs are considered to provide evidence on developmental toxicity. The observed large DNA deletions, micronucleus formation and DNA double strand breaks (DSB) in foetuses are considered under the classification proposal for germ cell mutagenicity.

Fatemi *et al*. (2013) tested the effects of prenatal exposure via pregnant dams with silver nanoparticles (20 ± 4 nm, sodium citrate buffer) on the developing brain in neonatal Wistar rats at 25 mg/kg bw/d from GD9 until parturition via intragastric administration. The offspring were sacrificed the day after birth. The effects in offspring consisted of an increase of silver and the number of microvacuolar structures in brain (612 vs 159 in controls), reduced antioxidant activity and increased peroxidation, statistically significant decrease in bw on PND0 and of a statistically significant decrease in the ratio of brain/body weight.

10.10.6 Comparison with the CLP criteria

There is no information specifically addressing the developmental toxicity of silver nitrate at non-corrosive concentrations. However, clear developmental toxicity has been observed with silver salts such as silver acetate, silver chloride, silver zinc zeolite (e.g. foetal/pup mortality) and to some extent with silver sodium zirconium hydrogen phosphate. Foetal/pup mortality is also indicated at the low dose in a study performed with nanosilver (Philbrook et al.) however due to the limited dosing period (a single dose on GD9) and the lack of surface coating which possibly prevents higher exposures to nanosilver and/or silver ions, the results from this study is not comparable. Likewise, the results from Yu et al in which rats were administered high doses of nanoparticles during gestation days 6-20 only are not comparable with the results from silver chloride (exposure over the entire gestation period) and silver acetate/silver zinc zeolite/silver zirconium hydrogen phosphate studies that were performed with continous exposure during pre-mating, gestation and lactation. Considering that the plausible mechanism of toxicity presented is a gradual decrease of active ceruloplasmin from plasma of dams, it can be questioned if silver toxicity would be detected in the standard developmental toxicity studies unless treatment is continuous during the entire gestation period. Therefore, one or two-generation studies with continuous exposure during pre-mating, gestation and lactation seem more appropriate to detect this type of toxicity in the foetus/pup. A possible explanation why effects seen in the two-generation study performed with silver sodium hydrogen zirconium phosphate were less pronounced despite a similar or even higher exposure to silver ions compared to silver acetate could be the difference in administration route (in drinking water and diet respectively) or due to normal variation between studies. In the case of silver zinc zeolite the presence of zinc could possibly also replace copper in ceruloplasmin and that could hypothetically contribute to the observed effects if this was the mechanism or one of the mechanisms causing the developmental effects. However, silver zinc zeolite is not the only silver- containing and –releasing substance that caused certain developmental effects and therefore zinc in not expected to be the critical contributor for the observed developmental effects by silver zinc zeolite.

Dietary administration of silver acetate at dose levels of 40, 80 and 120 mg/kg/day was associated with a number of effects including: F1 mortality at 120 mg/kg/day; F0/F1 red blood cell parameters at all dose levels; F1 offspring survival at 120 mg/kg/day; F1 offspring body weight at 80 or 120 mg/kg/day; F1 neurobehaviour/sensory function at 80 or 120 mg/kg/day; motor activity for F1 males and females at 80 or 120 mg/kg/day;

In addition to substance-related **increases in mortality rates of foetuses/pups** by silver acetate (EOGRTS), silver chloride (Doc IIIA, 6.8.1(03), Shavlovski, 1995), silver zinc zeolite (Doc IIIA 6.8.2 (04)), silver acetate (IIIA 6.8.2-06) and nanosilver (Doc IIIB 6.8.2-07, Philbrook et al., 2011) and to some extent by silver sodium zirconium hydrogen phosphate (Doc IIIA 6.8.2-03) as well as **increases in mortality rates of adolescences and young adults (possibly also being a consequence of developmental exposure**) by silver acetate (EOGRTS), silver zinc zeolite in the 2-generation study (Doc IIIA 6.8.2 (04), there were also other developmental effects including **significant brain damage** (by silver acetate in the EOGRTS, by nanosilver in Fatemi et al., 2013**), increase in relative brain weight** (by silver acetate in the EOGRTS, by silver zinc zeolite in Doc IIIA 6.8.2 (04), effects on neurobehaviour/sensory function (in the EOGRTS, DNT cohort), **enlargement of heart/increase in heart weight** (by silver acetate in the EOGRTS, by silver zinc zeolite in Doc IIIA 6.8.2 (04), **chryptorchidism** (by silver chloride in Doc IIIA, 6.8.1(03), Shavlovski, 1995), **hydronephrosis** (by silver zinc zeolite in IIIA 6.8.2 (04) and silver chloride in in Doc IIIA, 6.8.1(03)), **increased number of runts and/or reduced pup bodyweight** (by silver chloride Doc IIIA, 6.8.1(03), silver acetate in Doc IIIA 6.8.2-06, nanosilver in Fatemi et al., 2013, silver zinc zeolite in Doc IIIA 6.8.2 (04) and by silver sodium zirconium hydrogenphosphate in Doc IIIA 6.8.2-03. These effects are considered to provide clear evidence of developmental toxicity. Based on the severity assessment of maternal and developmental effects, the adverse effects on development are not considered to be secondary non-specific consequences of maternal toxicity.

Neurodevelopmental toxicity is also reported in the following published literature studies on silver nanoparticles. Wu *et al*. (2015) prenatally dosed offspring via mothers every two days from GD10 to GD18 ip to uncoated silver nanoparticles and showed histopathological changes with hippocampal neuronal cell loss along with impaired spatial learning and memory ability tested in Morris water maze test in rat offspring at postnatal day 35. In Ghaderi *et al*. (2015) NMRI mice had been treated subcutaneously once every three days from gestation day 3 until delivery, by 0, 0.2 and 2 mg/kg of bodyweight of silver nanoparticles. Spatial memory, passive avoidance learning, stress, anxiety-like behaviours and locomotor activities were assessed in adult offspring. Prenatal exposure to silver nanoparticles significantly impaired the cognitive behaviour in the Morris water maze. Also, the number of defecations and leanings in the open field assay and number of passages in the light-dark box were greater in groups prenatally exposed to silver nanoparticles. In Yin *et al.* (2015), an intranasal application of citrate stabilised silver nanoparticles to neonatal SD rats for 14 weeks showed that silver nanoparticles caused cerebellar ataxia like symptom in these rats, evidenced by dysfunction of motor coordination and impairment of locomotor activity. Observation of cerebellum sections revealed destruction of the cerebellum granular layer.

One plausible mechanism for silver developmental toxicity, i.e. silver interfering with copper binding to ceruloplasmin and thereby reducing the availability of copper, iron or perhaps both metals to the foetus, is supported by the copper analysis of F0 and F1 pups in the EOGRTS and in the F2 pups in the silver zinc zeolite study. Studies also show reduced ceruloplasmin activity. This information indicates that the developmental effects are caused by a specific mechanism rather than being a secondary non-specific consequence of toxicity in the mother. Based on the effects observed, pups seems to be more sensitive than dams to the adverse effects caused by silver. Since ceruloplasmin has the same function in humans, this potential mechanism cannot be considered irrelevant for humans. In addition, there is no evidence raising a doubt of human relevance of the adverse developmental effects for humans.

Substances with properties meeting criteria for classification are subcategorised into category 1A (known human reproductive toxicant), 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) depending on the strength of evidence.

Classification of a substance in category 1A is largely based on evidence from humans and since no such data is available, this criterion is not fulfilled. There was also a human case study Robkin *et al*. (1973), according to which the concentration of silver in 12 anencephalic human foetuses was higher (0.75±0.15 mg/kg) than the values from 12 foetuses obtained either through therapeutic abortions (0.23±0.05 mg/kg), or in 14 spontaneously aborted foetuses (0.21±0.05 mg/kg). The concentration in 9 premature infants was 0.68±0.22 mg/kg. The authors could not however determine if the malformation was associated with the higher concentration of silver in anencephalic foetuses or with foetal age.

Classification of a substance in category 1B is largely based on data from animal studies. According to CLP guidance, *"such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.“*

Substances are classified in Category 2 if there is *“some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.”*

As discussed in section 6 and in section 10.10.2, data on silver substances releasing silver ions is considered relevant for the assessment of silver nitrate. Substance-related **increases in mortality rates of foetuses/pups** by silver acetate (EOGRTS and IIIA 6.8.2-06) silver chloride (Doc IIIA, 6.8.1(03), Shavlovski, 1995), silver zinc zeolite (Doc IIIA 6.8.2 (04)), and nanosilver (Doc IIIB 6.8.2-07, Philbrook et al., 2011) and to some extent by silver sodium zirconium hydrogen phosphate (Doc IIIA 6.8.2-03), **increases in mortality rates of adolescences and young adults possibly also being a consequence of developmental exposure** by silver acetate (in the EOGRTS) and by silver zinc zeolite in the 2-generation study (Doc IIIA 6.8.2 (04), **significant brain damage** (by silver acetate in the EOGRTS and nanosilver in Fatemi et al., 2013**),), effects on neurobehaviour including sensory and motor functions** (in the EOGRTS, DNT cohort), **increase in relative brain weight** (by silver acetate in the EOGRTS and by silver zinc zeolite in Doc IIIA 6.8.2 (04), **enlargement of heart/increase in heart weight** (by silver acetate in the EOGRTS and by silver zinc zeolite in Doc IIIA 6.8.2 (04), **chryptorchidism** (by silver chloride in Doc IIIA, 6.8.1(03), Shavlovski, 1995), **hydronephrosis** (by silver zinc zeolite in IIIA 6.8.2 (04) and silver chloride in in Doc IIIA, 6.8.1(03)) as well as **increased number of runts and/or reduced pup bodyweight** (by silver chloride Doc IIIA, 6.8.1(03), silver acetate in Doc IIIA 6.8.2-06, nanosilver in Fatemi et al., 2013, silver zinc zeolite in Doc IIIA 6.8.2 (04) and by silver sodium zirconium hydrogenphosphate in Doc IIIA 6.8.2-03 are considered to provide clear evidence of major manifestation of developmental toxicity as given in Annex I: 3.7.1.4 of the CLP regulation: (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. As discussed above, the less obvious effects in the two-generation study performed with silver sodium hydrogen zirconium phosphate despite a similar or even higher exposure to silver ions compared to silver acetate and silver zinc zeolite may in the case of silver acetate be a consequence of a different administration route and thus bioavailability (diet compared to drinking water) and by the presence of zinc in silver zinc zeolite (dietary administered) possibly also replacing copper in ceruloplasmin.

Therefore, silver nitrate is considered to fulfil criteria for classification Repr. 1B, H360D.

10.10.7 Adverse effects on or via lactation

Table 59: Summary table of animal studies on effects on or via lactation

Summary table of published studies with nanosilver relevant for effects on or via lactation						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
Necropsy day 4 postpartum: liver, kidney, lung and brain OECD TG 422 No GLP	Rat (Sprague-Dawley)	Citrate-capped AgNPs (ABC Nanotech, Korea). 7.9 ± 0.95 nm Oral gavage, 62.5, 125 or 250 mg/kg bw/d Exposure: Males: 14 days before and during mating Females: 14 days before and during mating, during gestation, and 4 days after parturition	Not determined	AgNPs observed in livers, kidneys, brain and lungs of the offspring.	AgNPs were also identified in the brain of offspring, which means that AgNPs may reach the brain before the blood-brain barrier is formed in the foetus or they may directly pass the barrier.	IIIB 6.8.2-08 Lee, Y., Choi, J., Kim, P., Choi, K., Kim, S., Shon, W., and Park, K. (2012): A Transfer of Silver Nanoparticles from Pregnant Rat to Offspring. Toxicol. Res. Vol. 28 (3) 139-141
Examinations of foetuses: nanoparticle content of liver and brain Infant rats: gastrointestinal tract, liver, kidneys, spleen No guideline No GLP	Rat (Wistar), 3 females	110mAg radio-labelled silver nanoparticles, 34.9 ± 14.8 nm Pregnant alt. lactating female rats were dosed once at 1.69 and 2.11 mg/kg bw respectively Oral gavage	Not determined	Average NP level in foetuses: 0.085–0.147% of the administered dose. Accumulation of NP in female rats: Liver: 0.3–0.5% of the administered dose. Brain: 0.0035% of the administered dose . Thus the NP penetration in liver exceeded the penetration of NPs through the hematoencephalic	This study provides some evidence for the transfer of silver NPs from a mother to offspring through the placenta and breast milk; although the presence of silver NPs in milk was not directly investigated.	IIIA, 6.8.2-09 Melnik, E.A., Buzulukov, Y.P., Demin, V.F., Demin, V.A., Gmoshinski, I.V., Tyshko, N.V., and Tutelyan, V.A. (2013): Transfer of Silver Nanoparticles through the Placenta and Breast Milk during in vivo Experiments on Rats. Acta

Summary table of published studies with nanosilver relevant for effects on or via lactation						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
				barrier into the brain of female rats by at least 10-100 times (3.5.× 10-3 %). **Total inflow of [110mAg]-NPs into the milk: 1.94 ± 0.29% of the administered dose over a 48-hour period 25% of the amount was absorbed in the digestive tract of infant rats.**		Naturae Vol. 5 (3) 18; 107-115
Dams and offspring were, sacrificed on postnatal day 2. Determination of Ag in tissue and milk Biochemical and inflammatory analysis Measurement of oxidative stress Histopathology No guideline No GLP	Rat Sprague-Dawley 10/group	Citrate-capped silver nanoparticles, 55nm Pregnant female rats dosed orally once daily from Day 7 to Day 20 of gestation with 0, 0.2, 2, 20 mg/kg AgNPs or 20 mg Ag/kg as AgNO3	Not determined	Reduced bw gain in AgNO3 treated dams Administration of AgNO3 lead to higher tissue contents of Ag in dams than administration of Ag-NPs. **Ag content higher in all treated groups including milk from suckling pups** Accumulation of Ag in offspring confirms that Ag is able to cross the placenta. Kidney seems to be the main organ of fetal accumulation, followed by lung, liver and brain.	Prenatal exposure to Ag in both ionic and nanoparticle forms increase the levels of Ag in offspring tissues. The ionic Ag was associated with a higher degree of toxicity. The Ag in both nanoparticle and ionic forms induced oxidative stress in dams and pups, with the ionic form being more potent. Observation of hippocampal sclerosis even at the lowest dose level of 0.2 mg/ kg/day is observed,	IIIA, 6.8.2-10 Charehsaz, M., Hougaard, K.S., Sipahi, H., Ekici, A.I.D., Kaspar, C., Culha, M., Bucurgat, U.U., and Aydin, A. (2016): Effects of developmental exposure to silver in ionic and nanoparticle form: A study in rats. Journal of Pharmaceutical Sciences Vol 24:24

Summary table of published studies with nanosilver relevant for effects on or via lactation						
Method, Guideline, GLP status, Reliability	**Species, Strain, Sex, No/ group**	**Test substance Dose levels, Duration of exposure**	**NOAELs, LOAELs**	**Results**	**Remarks** *(e.g. major deviations)*	**Reference**
					as is observation of oxidative stress in offspring brain tissue.	
No guideline No GLP	Rats Wistar 10 males/group (5 sacrificed at PND 53, half at PND 90).	60 nm AgNPs suspended in aqueous solution. 0 µg/kg, 15 µg/kg, 30 µg/kg Oral Gavage Daily for 30 days (Post Natal Day (PND) 23 to PND 53). Post-exposure period: 37 days (PND 53 to PND 90)	Not determined	Delay onset of puberty (weight at puberty not affected) Numerical reduction in total and daily sperm production in the 50-µg/kg BW AgNP-treated group at PND53. At PND90, both AgNP-treated groups had significantly lowered total and daily sperm production AgNP exposure during the prepubertal period also decreased the sperm reserves in the caput, corpus, and cauda of the epididymis in both treatment groups at PND53 and PND90. Significant reduction of sperm transit time through the segments of the epididymis at PND53. No marked change of bw but reduced growth from the 50 µg/kg		IIIB, 6.8.2-17 Mathias, F. T.; Romano, R. M.; Kizys, M. M. L.; Kasamatsu, T.; Giannocco, G.; Chiamolera, M. I.; Dias-da-Silva, M. R.; Romano, M. A. (2015): Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters

Table 60: Summary table of human data on effects on or via lactation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No information available				

Table 61: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Please refer to section 10.10.2				

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Nanoparticles releasing silver ions have been detected in breast milk and in offspring following oral administration of ^{110m}Ag-labeled nanoparticles to dams (Doc IIIA 6.8.2-09). Silver was also detected in the milk of suckling pups (see table below).

Silver content (µg/g milk) in dams (from Charehsaz, M., et al, Doc IIIA 6.8.2-10)

Control	Ag-NP 0.2	Ag-NP 2	Ag-NP 20	AgNO3 20
0.25 ± 0.21	0.33 ± 0.25	0.32 ± 0.11	0.66 ± 0.57	0.76 ± 0.45

10.10.9 Comparison with the CLP criteria

According to CLP, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the human evidence and/or results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk and/or absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk. CLP Guidance states "*When the effect on the offspring is caused by the substance (or metabolite) after transport through the milk then the maternal toxicity has no relevance for classification. In general, positive data should usually be available to show that a substance leads to an adverse effect in offspring due to effects on lactation to support classification. However, in exceptional circumstances, if there are substantiated grounds for concern that the substance may have an adverse effect via lactation then it may be classified as such in the absence of direct evidence. This should be based on a quantitative comparison of the estimated transfer via the milk and the threshold for toxicity in the pups. This might apply in cases where the substance has the capacity to bioaccumulate which would lead to a potentially higher burden in the offspring, or where there is evidence that the offspring may be more sensitive to the substance's toxicity than adult.*

The mere presence of the substance in the milk alone, without a strong justification for a concern to offspring, would normally not support classification for effects on or via lactation."

Published studies indicate that nanoparticles of silver can be transferred in milk to the foetus. However, there are no studies clearly demonstrating that adverse effects in pups result from lactational exposure rather than exposure in utero and/or via food post weaning as the exposure is not limited to the lactation period in any of the studies . The delay in onset of puberty shown in the study by Mathias, F. T. et al (IIIB, 6.8.2-17) was observed in pups exposed only post-natally but after the lactational period (during post-natal days 23 to 53, i.e. after weaning) via food. It is thus not possible to conclude if the same effects would have been noted if exposure was limited to the lactational period. However, silver is known to accumulate in foetal and adult tissues and results by Charehsaz, et al (IIIA, 6.8.2-10) indicate that fetal accumulation occurs in kidney followed by lung, liver and brain. Histopathological changes in kidneys (pale, dilation, cyst) were noted in pups and hydronephrosis was observed in adult animals of both

generations in a two-generation study performed with silver zinc zeolite (see section 10.10.4) supporting that accumulation in tissues may lead to adverse effects in kidneys. However, it is not possible to exclude a contribution from the zeolite or zinc to kidney toxicity observed. Moreover, reduced bodyweight gains and post-natal mortalities were observed in pups exposed to silver acetate, silver zinc zeolite and silver zirconium hydrogen phosphate.

In conclusion, data on nanosilver and silver nitrate demonstrate that silver is transferred in milk and accumulates in foetal (and adult) tissues. However, existing data is not considered to provide sufficient evidence that lactational transfer results in adverse effects in pups since the developmental effects discussed in sections 10.10.5 may be consequences of in utero exposure only. Therefore, criteria for classification H362 are not considered fulfilled.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the data presented in section 10.10.1 and 10.10.4, silver nitrate is considered to fulfil criteria for classification in category 1B, H360FD.

10.11 Specific target organ toxicity-single exposure

Table 62: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28-day study OECD (407) GLP Rat Crl:WI(Han) 5/sex	Silver nitrate 20, 50, 100 mg/kg bw Gavage 28 days	LOAEL > 100 mg/kg bw Effects noted but not considered adverse: ↓ bw gain in males (14%) ↓ ALP m/f (149/97%	IIIA 6.3.1-07

Table 63: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No information available				

Table 64: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No information available				

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

There were no effects observed following the first dose in a 28-day study performed with doses up to 100 mg silver nitrate /kg bw/d. Additional information available is limited to published research that is fairly old and in general poorly reported. Moreover, most of effects described result from studies with repeated

exposure and are thus not considered relevant for this endpoint. Acute effects due to the corrosive properties including severe gastroenteritis, diarrhoea, reduced blood pressure, decreased respiration, spasms and paralysis which first affect the diaphragm musculature have been reported following ingestion (6.1.1(02)). However, a classification for skin corrosion and acute oral toxicity in Category 2 is warranted, thus these effects and death after a single dose are already covered.

As discussed in section 10.3 brief information describing respiratory effects in humans following acute exposure to an unknown level of silver nitrate dust can be found in different published reports (6.1.1(05) and 6.2(08)). The first summary (6.1.1(05)) refers to a study reporting acute irritation of the respiratory tract upon inhalation of silver nitrate dust. Irritation was only observed at doses also causing argyria. The other summary (6.2(08)) refers to a study of workers exposed to silver nitrate or silver oxide. Among workers, upper respiratory irritation was observed in 25 of 30 persons and cough, wheezing or chest tightness in 20 of 30 persons. There is no information on exposure levels and exposure duration but according to the summary, the eight hour time weighted average concentration analysed four months prior to the study indicated levels from 0.039 to 0.378 mg silver/m3. From this limited information, it is only possible to conclude that silver nitrate causes respiratory irritation and respiratory effects above a certain dose level.

10.11.2 Comparison with the CLP criteria

For corrosive substances, the CLP guidance states (in section 3.8.2.5) *"Classification as acutely toxic and/or corrosive is considered to cover and communicate the specific toxicological effect(s) adequately. An additional classification as specific target organ toxicant (single exposure, Category 1 or 2) is not indicated if the severe toxicological effect is the consequence of the local (i.e. corrosive) mode of action.*

It is a reasonable assumption that corrosive substances may also cause respiratory tract irritation when inhaled at exposure concentrations below those causing frank respiratory tract corrosion. If there is evidence from animal studies or from human experience to support this then Category 3 may be appropriate. In general, a classification for corrosivity is considered to implicitly cover the potential to cause RTI and so the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier. The Category 3 classification would occur only when more severe effects in the respiratory system are not observed."

The guidance also states *"Relevant information with respect to toxicity after single exposure may be available from case reports, epidemiological studies, medical surveillance and reporting schemes and national poisons centres."*

Cases of respiratory irritation in workers exposed via inhalation are described in the open literature. Although poorly reported, this indicate a potential for respiratory irritation at low doses. Considering that the substance is currently classified for corrosivity in category 1B and proposed to be changed to 1A, classification STOT SE in category 3 for respiratory tract irritation is considered superfluous.

10.11.3 Conclusion on classification and labelling for STOT SE

Silver nitrate is currently classified for corrosivity and can be expected to cause acute effects in tissues that are exposed to the substance. The corrosive properties may be the primary cause of other toxicological effects observed, including death, however due to the poor data base very limited information on effects following single exposure is available. The data on silver nitrate only allows for a conclusion that no effects following exposures to the first doses were described in the 28-day rat study at doses up to 100 mg/kg bw/d (see section 10.12). The, criteria for classification STOTSE in categories 1 or 2 are thus not fulfilled.

Cases of respiratory irritation at low doses are described in the open literature but the substance is currently classified for corrosivity in category 1B and proposed to be changed to 1A, thus classification STOT SE in category 3 for respiratory tract irritation is considered superfluous.

10.12 Specific target organ toxicity-repeated exposure

Table 65: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28-day study OECD (407) GLP Rat Crl:WI(Han) 5/sex Reliability 1	Silver nitrate 20, 50, 100 mg/kg bw Gavage 28 days	LOAEL > 100 mg/kg bw Effects noted but not considered adverse: ↓ bw gain in males (14%) ↓ ALP m/f (149/97% There were no treatment related effects on the FOB. Note: The OECD TG 407 includes the following investigations about the potential neurotoxicity: • At least one a week: changes in eyes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) • In the fourth exposure week sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli), assessment of grip strength and motor activity. (These investigations may be omitted when the study is conducted as a preliminary study to a subsequent sub-chronic (90-day) study which should then include these investigations). • Wet weight of brain Histopathological examination (gross lesions) of brain (representative regions including cerebrum, cerebellum and pons), spinal cord, eye, peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle	IIIA 6.3.1-07

Rat albino Reliability 3	0.1% silver nitrate “or silver chloride, maintained in suspension by sodium thiosulphate, at a concentration of 1:1000 as an alternative to drinking water”. (60 or 89* mg/kg bw/day Oral (drinking water) up to 30 months Rats were derived from Osborn Mendel stock; seventeen filial generations were bred by sibling matings over a period of 8-9 years. Termination of the rats occurred at various points and for diverse reasons including shortage of food during World War II – where possible silver-treated and water control littermates were terminated concurrently. Some rats survived to 30 months. A large number of rats died spontaneously, and these were excluded from the final study due to advanced pulmonary lesions. Most animals killed in good health had macroscopically normal lungs. Blood pressure measurements were attempted	↑increase in the incidence of ventricular hypertrophy ↑proteinuria The analyses showed a clear correlation between diseased lungs and relative hypertrophy. “Pulmonary disease was a more potent factor in inducing a high L/W value (ratio of left ventricular weight to bodyweight) than was administration of silver.”	Olcott, C.T. Experimental argyrosis. V. Hypertrophy of the left ventricle of the heart. Archives of Pathol. 49: 138-149, 1950.

	using a method that avoids anaesthesia, based on readings obtained from blood vessels in the tail. However, the influence of ambient temperature has a marked effect on the relaxation state of the rat or the vessels in the tail, resulting in inconsistent measurements. Cannulated aortas were used for obtaining reading from 25 rats, but the readings obtained showed more correlation with the plane of anaesthesia rather than any underlying pathological or physiological effects 433 rats were available for analysis following exclusions for macroscopic effects on bodyweight/ premature death/tumour incidence etc. Of these 245 rats had been given silver salts (131 males and 114 females) and 188 had been given water (89 males and 99 females). From this cohort young rats (less than 9 months old) were excluded, silver treated rats greater than 24		

	months old, for whom there were no water controls were excluded and 7 rats of unknown age were excluded. 296 rats were therefore analysed. *0.1% silver nitrate has been converted to a dose of 60 mg/kg bw in 6.5(01) and 89 mg/kg bw in 6.2(03).		

Oral (drinking water) Rat albino Wistar 40m Reliability 3-4	0.25% silver nitrate (stated to be 222 mg/kg bw/d in 6.5(04)) Daily exposure 9 months (after 10 weeks half of the animals were further exposed for 6 months, the rest for 12 months) After two weeks 2 rats were terminated, and the eyes were removed for electron microscopy. Remaining rats were divided into two groups where one group was kept on treatment for 6 months and the other received water. Then both groups received water for 6 months. At monthly intervals one rat from each group was terminated and eyes were examined for silver deposits by electron microscopy.	Rapid weight loss from week 23 onwards and eventually death. Rats surviving to 37 weeks had lost approximately 50% of their maximum weight (reversibility demonstrated) massive accumulation of silver particles in the outer aspect of the ciliary epithelium basement membrane.	IIIA 6.5(03) Matuk, Y. Gosh, M. and McCulloch, C. (1981): Distribution of silver in the eyes and plasma proteins of the albino rat. Handbook on the toxicology of Metals. Can. J. ophthalmol 16.

Table 66: Summary table of human data on STOT RE

Summary table of further human data				
Type of data/ report, Reliability	**Test substance**	**Relevant information about the study**	**Observations**	**Reference**
Published re-registration document US EPA (1992)		Summary	The document states that excessive industrial and/or medicinal exposures to silver have been associated with arteriosclerosis and lesions of the lungs and kidneys. Exposure to industrial dusts containing high levels of silver nitrate and/or silver oxide may cause breathing problems, lung and throat infections and abdominal pain. Skin contact with certain silver compounds may cause mild allergic reactions such as rash, swelling and inflammation in sensitive people.	IIIA 6.12.2(02)
Published report IRIS (US EPA) (1996)		Summary of published information.	The information contains a summary of underlying data for the oral reference dose set by the US EPA. Critical effects – Argyria (2- to 9-Year Human i.v. Study; Gaul and Staud, 1935) NOEL = None; LOAEL = 1 g (total dose); converted to an oral dose of 0.014 mg/kg/day* RfD = 3 mg/kg/day * Conversion Factors: Based on conversion from the total i.v. dose to a total oral dose of 25 g (i.v. dose of 1 g divided by 0.04, assumed oral retention factor; see Furchner et al., 1968 in Additional Comments section) and dividing by 70 kg (adult body weight) and 25,500 days (a lifetime, or 70 years).	IIIA 6.12.2(03)
Published article (1980)	Silver acetate	Case report, 47 year old woman exposed to silver acetate through anti-smoking lozenges.	According to this article, a constant fraction of 18 % was retained in a woman administered 4.5 mg silver acetate. The dry weight concentration of silver in her skin was increased by a factor of approximately 8000 compared to a historical value.	IIIA 6.12.2(04)

Summary table of further human data				
Published article (1986)	Silver nitrate	Case report, patient using a stick of silver nitrate (containing 0.53 g AgNO3) to treat the oral mucosa for suspected oral mycosis.	Discoloured mucous membranes in the oral cavity, among other clinical signs including taste and smell disorders, vertigo and hypaesthesia, Biopsy samples from the vestibulum oris, oral cavity and soft palate identified electron-dense mineral deposits in basal membranes for macrophages, in the pericurium of the peripheral nerves, along elastic and collagenous fibres and in the necrotic cells of the oral mucosa. The authors concluded that the affinity of silver for membrane and neuronal structures and the deposition of insoluble silver following extended high exposure on a daily basis had induced progression of the clinical condition of this patient.	IIIA 6.12.2(05)
Published article (2005)	Home-made colloidal silver solution.	Case report, 58-year-old man exposed to home-made colloidal silver solution.	Histopathological examination of a forearm biopsy revealed that fine, minute, round, brown/black granules were found to be deposited primarily in the basement membrane around the eccrine glands and to a lesser extent in the fibrous sheath of the pilo-sebaceous units, pilo-erector muscles, dermal elastic fibres and arteriolar walls.	IIIA 6.12.2(06)
Published article (2005)	Silver nitrate	Case report, fatal renal and hepatic failure in a patient following silver nitrate instillation in the renal pelvis	This report describes a case in which fatal hepatic and renal failure occurred following instillation of an unknown quantity of silver nitrate possibly absorbed systemically through larger lymphatic channels.	IIIA 6.12.2(07)
		Published report Oak Ridge Reservation Environmental Restoration Program (1992)	Summary of published information.	IIIA 6.12.2(08)
		Published article (1996) Center of Drug Evaluation and Research, Food and Drug Administration	Risk benefit assessment of silver products for medical indications	IIIA 6.12.5(01)
		Published re-registration document US EPA (1992)	Summary	IIIA 6.12.2(02)

Table 67: Summary table of other studies relevant for STOT RE

Oral route:

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Study designed based on: EC No 440/2008, B.26 Repeated Dose (90 days) Toxicity (oral), 2008. OECD 408, Repeated Dose 90-day Oral Toxicity Study in Rodents, 2018. OPPTS 870.3100, EPA 712-C-98-199, 90-Day Oral Toxicity in Rodents, 1998. Guideline on Bioanalytical Method Validation, European Medicines Agency (EMA), EMEA/CHMP / EWP/192217/2 009, 21 July 2011. Guidance for industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and	Silver acetate 64.58% Oral, in diet TK/main study: 0, 40, 120, 320 mg/kg bw/d	**320 mg/kg bw/d** Body weight (m, day 91): ↓ 15% Body weight gain (m, occasional weeks weeks): ↓ 27-82% Monocytes (m): ↑ 66Eosinophils (f): ↑ 80% RDWG (m/f): ↑ 11/14%' RBC (f): ↑ 6% HGB (m): ↑ 5% MCV (m/f): ↓ 7/5% MCH (m/f): ↓ 9/6% MCHC (m): ↓ 3% PT (m): ↓ 6% ALP (f) ↑ 84% ALT (m): ↑ 49% TBIL (f): ↓ 29% Urea (m): ↑ 34% Chol (m/f): ↑ 68/103% HDL (m/f): ↑ 61/114% Ca (f): ↑ 5% Epididymis (abs): ↓ 14% Testis (abs): ↑ 13% Brain (m, rel): ↑ 13% Heart (m, rel): ↑ 10% Liver (m/f, rel): ↑ 16/14% Pituitary gland (abs/rel): ↓ 24/10% **120 mg/kg bw/d** Monocytes (m): ↑ 101% Eosinophils (f): ↑ 60%	Draft report A 90-Day Study of Silver Acetate by Dietary Administration in Wistar Study No. 20274170

Research (CDER) and Center for Veterinary Medicine (CVM), May 2018. • OECD Guideline 417. Toxicokinetics. GLP, unaudited Rat, Wistar Han TK: 3/sex/dose Main: 10/sex/dose		RDWG (m): ↑ 8% RBC (f): ↑ 11% MCV (m/f): ↓ 7/5% MCH (m/f): ↓ 9/5% MCHC (m): ↓ 2% PT (m): ↓ 5% ALP (f) ↑ 75% TBIL (f): ↓ 29% Chol (m/f): ↑ 46/39% HDL (m/f): ↑ 49/42% Ca (f): ↑ 7% **40 mg/kg bw/d** Monocytes (m): ↑ 750% MCV (f): ↓ 3% ALP (f) ↑ 45% TBIL (f): ↓ 30% HDL (m): ↑ 22% Note: The study included the following investigations and results about the potential neurotoxicity: • Functional Observations: "*Hearing ability, pupillary reflex and static righting reflex were normal in all examined animals. Grip strength and motor activity was similar between control and the test item groups. All groups showed a similar motor activity habituation profile with a decreasing trend in activity over the duration of the test period*." • Tissues for this study were retained in 10% neutral buffered formalin, embedded in paraffin, sectioned, mounted on glass slides and stained with haematoxylin and eosin. In the brain, pigment (minimal or mild) was noted in the area postrema in all treated animals and in the subfornical organ of four animals (2 males at 40 mg/kg/day, and one female each at 40 and 320 mg/kg/day) for which these structures were present in the section (these areas are not routinely included in brain sections at Charles River). The presence of the pigment microscopically was not associated with any other tissue alterations and was considered by authors to be a non-adverse change. • Brain weight:	

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<tr><th colspan="2">Sex: Male

Day(s) Relative to Start Date</th><th>0
mg/kg
bw/day
Group 1</th><th>40
mg/kg
bw/day
Group 2</th><th>120
mg/kg
bw/day
Group 3</th><th>320
mg/kg
bw/day
Group 4</th></tr>
<tr><td>Terminal Body Weight (g) [G]</td><td>Mean</td><td>392.5</td><td>379.2</td><td>374.0</td><td>334.1 **</td></tr>
<tr><td></td><td>SD</td><td>38.0</td><td>18.4</td><td>24.5</td><td>28.0</td></tr>
<tr><td></td><td>N</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td></td><td>%Diff</td><td>-</td><td>-3.4</td><td>-4.7</td><td>-14.9</td></tr>
<tr><td>Brain Weight (g) [G1]</td><td>Mean</td><td>2.0850</td><td>2.0695</td><td>2.1047</td><td>2.0166</td></tr>
<tr><td></td><td>SD</td><td>0.0608</td><td>0.1083</td><td>0.0728</td><td>0.0793</td></tr>
<tr><td></td><td>N</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
</table>
<table>
<tr><th colspan="2">Sex: Female

Day(s) Relative to Start Date</th><th>0
mg/kg
bw/day
Group 1</th><th>40
mg/kg
bw/day
Group 2</th><th>120
mg/kg
bw/day
Group 3</th><th>320
mg/kg
bw/day
Group 4</th></tr>
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<tr><td></td><td>SD</td><td>18.4</td><td>15.1</td><td>19.7</td><td>16.3</td></tr>
<tr><td></td><td>N</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td></td><td>%Diff</td><td>-</td><td>-5.1</td><td>0.0</td><td>-5.3</td></tr>
<tr><td>Brain Weight (g) [G1]</td><td>Mean</td><td>1.9345</td><td>1.9132</td><td>1.9210</td><td>1.8668</td></tr>
<tr><td></td><td>SD</td><td>0.0759</td><td>0.0888</td><td>0.0570</td><td>0.0501</td></tr>
<tr><td></td><td>N</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td></td><td>%Diff</td><td>-</td><td>-1.1011</td><td>-0.6979</td><td>-3.4996</td></tr>
</table>
</td><td></td></tr>
<tr><td>Silver Acetate: Extended One Generation Reproductive Toxicity Study in the Sprague Dawley Rat by Dietary Administration</td><td>Sprague-Dawley [Crl:CD(SD)]

P0: 0, 40, 80, 120 mg/kg bw/d

25/sex</td><td>Abnormal colouration of tissues were observed in all treated animals

Parental P0 females:
120 mg/kg bw/d
Mortality: 1/25
↑ HCT:
w 10: 10%, st5%
↑ Hb, st* 4%
↑ RBC:
w 10: 14%, st*: 10%
↓ MCH:
w 10: 10%, st*: 5%
↓ MCHC:
w 10: 7%
↓ MCV:
w 10: 4%, st*: 4%
↑ RDW, st: 19%,
Plt:
↓w 10: 37% st*: 16%
↓ PT: w 10: 8%,
↑ ALP w 10: 77%
↑ AST w 10: 17%
↑ gGT st*:
1 compared to 0
↑ Chol
w 10: 77%, st*45%
↓ K:
w 10: 12, st*: 8%
↑ heart/rel bw: 10%
↑ epithelial degeneration of the glandular mucosa 5/23 (minimal), 5/23 (slight), 0 in cntrol

80 mg/kg bw/d:
↑ HCT: w 10: 9% st*: 6%
↑ Hb, st* 5%</td><td>Labcorp Study Number 8437234

Report Issue Date 24 January 2022</td></tr>
</table>

		↑ RBC: w 10: 13%, st* 10% ↓ MCH: w 10: 9%, st* 5% ↓ MCHC: w 10: 6% ↓ MCV: w 10: 3%, st* 4% ↑ RDW, st: 9% ↓ Plt: w 10: 42%, st: 21% ↑ ALP w 10: 39% ↑ Chol w 10: 63%, st*27% ↓ K: w 10: 11% ↑ heart/rel bw: 10% ↑ epithelial degeneration of the glandular mucosa 5/24 (minimal) **40 mg/kg bw/d:** ↑ HCT: w 10: 7%, st* 6% ↑ Hb, st* 6% ↑ RBC: w 10: 10%, st* 8% ↓ MCH: w 10: 8% ↓ MCV: w 10: 3% ↓ Plt: w 10: 43%, st* 17% ↑ RDW, st: 7% ↑ ALP w 10: 39% ↑ Chol w 10: 46%, st*19% ↓ K: w 10: 17% ↑ heart/rel bw: 5% ↑ epithelial degeneration of the glandular mucosa 3/25 (minimal) **Parental P0 males:** **120 mg/kg bw/d** ↑ Mortality 2/25 ↓ Overall bwg (d0-19): 7% ↓ HCT, st: 5% ↓ Hb, st* 11% ↑ RBC, st*: 7% ↓ MCH, st*: 18% ↓ MCHC, st*: 6% ↓ MCV, st*: 12% ↑ RDW, st: 35%, ↑ Plt, st*: 27% ↑ WBC, st*: 25% ↑ L, st*: 33%	

		↑ E, st*: 64% ↑ Plt, st*: 27% ↑ ALP, st*: 27% ↓ Creat, st*: 7% ↑ Chol, st*: 61% ↓ K, st*: 5% ↓ P, st*: 13% ↑ spleen/rel bw: 15% ↓Abs weight left testis: 5% ↓Abs weight left epididymis: 4% ↓Abs/rel prostate: 13% ↑ extramedullary hematopoiesis in spleen: Minimal: 15/23 (6/25 in control) Slight: 1/23 (0/25 in control) Marked: 0/23 (6/25 in control) **80 mg/kg bw/d** ↓ Overall bwg (d0-19): 8% ↓ Hb, st* 7% ↑ RBC, st*: 8% ↓ MCH, st*: 14% ↓ MCHC, st*: 5% ↓ MCV, st*: 10% ↑ RDW, st: 28%, ↓ Creat, st*: 4% ↑ Chol, st*: 57% ↓ K, st*: 6% ↓Abs weight left testis: 7% ↓Abs weight left epididymis: 7% ↓Abs/rel prostate: 17% ↑ extramedullary hematopoiesis in spleen Minimal: 12/24 (6/25 in control) Slight: 0/24 (0/25 in control) Marked: 2/24 (6/25 in control) **40 mg/kg bw/d** ↓ MCH, st*: 5% ↓ MCV, st*: 3% ↑ RDW, st: 11%, ↓ Creat, st*: 14% ↑ Chol, st*: 66% Minimal: 6/25 (6/25 in control) ↓Abs/rel prostate: 10%	

		Note: The study included the following investigations and results about the potential neurotoxicity: Standard brain sections, including cerebellum, cerebrum and midbrain were examined in F0 Generation animals. Both standard sections and extended coronal sections routinely include the hippocampus and thalamus. No histopathological findings in brain sections including the hippocampus and thalamus were observed in P0 Generation animals.	
*St = study termination			
Silver Acetate: Preliminary Reproductive Performance Study in the Sprague Dawley Rat by Dietary Administration	Sprague-Dawley [Crl:CD(SD)] F0: 0, 4, 40, 80, 160, 320 mg/kg bw/d 12/sex Silver acetate (AgAc) >99.5%	**Parental F0 females:** **320 mg/kg bw/d:** ↓ Bwg (pre-pairing): 62% ↓ Bwg (gestation, d 0-20): 58% 4/12 killed following total litter loss GD22-LD2; 8/12 killed for welfare reasons GD20-LD1 **160 mg/kg bw/d:** 2/12 killed following total litter loss GD22-LD1; 10/12 killed for welfare reasons GD21-LD4 Abnormal colour and content of GI tract, abnormal colour of Liver, pancreas, spleen: and mesenteric lymph nodes **80 mg/kg bw/d:** Abnormal colour of Pancreas: 11/12 Kidney: 10/12 Mesenteric lymph nodes: 8/12 **40 mg/kg bw/d:** ↑ ALP (40%) ↑ P (80%) ↑ gGT (1 vs 0 in control) ↑ A/G (13%) Abnormal colour of Pancreas: 11/12 Mesenteric lymph nodes: 2/12 **Parental F0 males:** **320 mg/kg bw/d:** ↓ Overall bwg (d1-64): 20% ↑ Platelet counts (35%) ↓ hematocrit (6%) ↓ hemoglobin (11%) ↓ MCH (12%) ↓ MCV (8%) ↓ MCHC (4%)	Covance Study Number 8436495 Report Issue Date 28 June 2021 (Draft 3)

		↑ ALP (27%) ↑ Cholesterol ↑ ALT (98%) Abnormal colour of Liver: 11/12 Pancreas: 12/12 Mesenteric lymph nodes: 5/12 Abnormal content of: Cecum: 11/12 Rectum: 4/12 **160 mg/kg bw/d:** ↑ Platelet counts (44%) ↓ hematocrit (9%) ↓ hemoglobin (14%) ↓ MCH (16%) ↓ MCV (11%) ↓ MCHC (5%) ↑ ALP (40%) ↑ Cholesterol (48%) Abnormal colour of Liver: 7/12 Pancreas: 10/12 Mesenteric lymph nodes: 5/12 Abnormal content of: Cecum: 7/12 Rectum: 4/12 **40 mg/kg bw/d:** ↑ Platelet counts (23%) ↑ ALP (40%) ↑ Cholesterol (47%) Note: Clinical signs in all dose groups were unremarkable, there was no evidence/no reporting of behavioural disturbances or lethargy. As confirmed by the study pathologist, there was no neurohistopathological examination performed in parental animals in this dose range finder to the main EOGRTS study.	
OECD TG 407 GLP Sprague-Dawley 10/dose Deviations: The applicant's version is	AgNPs (52.7-70.99 nm in size, average 60 nm) 0, 30, 300 or 1000 mg/kg bw/day in carboxymethyl-cellulose	1000 mg/kg bw/day ↑ brain weight (m) ↑ ALP (m/f) ↑ Cholesterol (m/f) ↑ MCV (m ↑ RBC, Hb and HCT (f) 300 mg/kg bw/day ↑ ALP (m) ↑ Cholesterol (f)	Kim, Y.S. et al (2008): Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhalation Toxicology 20, 575-

acceptable with following observations. No information is available on the stability of the test material. Animals were not mated and females were not pregnant in this study, therefore, it is an error to report that food consumption was recorded during pre-mating, pregnancy and lactation. The age of the animals at the study initiation was 6 weeks old (and not 8 weeks old as reported in the abstract of the published article and by the applicant above). Sensory reactivity and functional observations were not performed No details of body weights and food consumption measurements are available. Animals were fasted for 24 hours (instead of the recommended overnight fasting) prior to blood sampling for clinical	28 days	↑ RBC, Hb and HCT (f) ↑ dose-dependent incidences in the liver of bile-duct hyperplasia around the central vein to the hepatic lobule, with the infiltration of inflammatory cells, including eosinophils, in the hepatic lobule and portal tract, plus dilated central veins with the infiltration of inflammatory cells in and beneath the central veins. ↑ accumulation of silver in kidneys (twofold higher in females) Bile-duct hyperplasia also observed in low-dose animals. Sensory reactivity and functional observations were not performed.	583. Study summary in Annex I

biochemistry investigations. Optional urinalysis determination was not performed. Histopathological examinations of the following were lacking: spinal cord, lymph nodes, peripheral nerve and a section of bone marrow. Sensory reactivity and functional observations were not performed			
OECD TG 422 Deviations: The information on purity is lacking. performed. Histopathological examinations of the following were lacking: brain, spinal cord, small and large intestines, trachea, uterus, lymph nodes, peripheral nerve and a section of bone marrow. GLP Reliability 2 Sprague-Dawley 10/dose	AgNPs (7.9 ± 0.95 nm) 0, 62.5, 125 or 250 mg/kg bw/day (distilled water) 42 days (m) 52 days (f)	250 mg/kg ↑lung granulatomous lesions (2 females) ↑cholesterol granuloma (2 males) MTD was not reached. No treatment-related changes in detailed functional observations including auditory response, pupillary reflex, acute pain sensitivity, motor activity and passive avoidance were observed in any of the treatment groups. Functional observations were made in 5 males and 5 females from each group. The observations were made on the day before the final treatment in males, and on day 4 of lactation (after separating from their pups) for females. Auditory response, papillary reflex, hot-plate test, rotarod performance test and passive-avoidance test were. Note: The OECD TG 422 includes the following investigations about the potential neurotoxicity: • At least once a week detailed clinical observations should be made in all parental animals. These observations should be made outside the home cage in a standard arena and preferably at the same time, each day. Signs noted should include, but not be limited to changes in eyes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling), difficult	Hong, J-S., et al (2014) Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test. Nanotechnology 8(4): 349-362 Study summary in Annex I

[04.01-MF-003.01]

		or prolonged parturition or bizarre behaviour (e.g., self-mutilation, walking backwards) should also be recorded. • At one time during the study, sensory reactivity to stimuli of different modalities (e.g., auditory, visual and proprioceptive stimuli), assessment of grip strength and motor activity assessment should be conducted in five males and five females. ((These investigations may be omitted when the study is conducted as a preliminary study to a subsequent sub-chronic (90-day) or long-term study which should then include these investigations). • Brain wet weight. • Histopathological examination (gross lesions) of brain (representative regions including cerebrum, cerebellum and pons), spinal cord, eye, peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle.	
OECD TG 408 Deviations: There were 10 animals/sex/dose group. Justification for the choice of vehicle is missing. Animals were fasted for 24 hours before necropsy instead of the recommended overnight fasting. Histopathology of the following organs were lacking: spinal cord, parathyroid, salivary glands, stomach, aorta, female mammary gland, lymph nodes, peripheral nerve and a section of bone marrow.	Silver nanoparticles (count median diameter 56 nm and geometric standard deviation 1.46) 0, 30, 125 and 500 mg/kg bw/day in 0.5% aqueous carboxymethylcellulose 90 days	500 mg/kg bw/day ↑ ALP (f) ↑ Cholesterol (m/f) 125 mg/kg bw/day ↑ Cholesterol (m) ↑ Minimal bile-duct hyperplasia males: 4/10 control, 7/10 low, 8/10 middle and 6/10 high-dose females: 3/10 control, 7/10 low, 8/10 middle, and 7/10 high-dose. ↑ Focal, multifocal, or lobular liver necrosis males: 0/10 control, 4/10 low, 5/10 middle and 4/10 high-dose females: 0/10 control, 2/10 low, 2/10 middle, and 2/10 high-dose. ↑ Minimum or mild renal unilateral or bilateral mineralization females: 5/10 control, 8/10 low, 7/10 middle and 9/10 high-dose. Dose-dependent increase in the pigmentation of the villi (m/f) No investigations about the potential neurotoxicity were made	Kim, Y. S. et al (2010a): Subchronic oral toxicity of silver nanoparticles. Particle and Fibre Toxicology 7:20 Study summary in Annex I

GLP Reliability 2 SPF Fischer 344 10/dose			
Non guideline Non GLP Wistar Hannover Galas 8 females	Silver acetate Oral, gavage 14 mg Ag-acetate/kg bw/day group: twice daily (each time 7 mg Ag-acetate/kg bw/day) 2.25, 4.5 and 9.0 mg Ag/kg bw/d 28 days	Extract from REACH registration dossier (original data not assessed by DS): Observations and examinations performed and frequency. Cage side observations: Yes Time schedule: the animals were observed at least twice daily for abnormalities in clinical appearance. Detailed clinical observations: no data. Body weight: yes Time schedule for examinations: body weight was recorded throughout the study period. Food consumption and compound intake (if feeding study): Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: yes, feed intake was recorded throughout the study period. Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No data. Food efficiency: Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No data Water consumption and compound intake: Yes Time schedule for examinations: water intake was recorded throughout the study period. Ophthalmoscopic examination: No data Haematology: Yes Time schedule for collection of blood: at necropsy, rats were anaesthetised in CO2/O2 and decapitated. Blood sampes were obtained from the neck wound directly into heparinised tubes. Animals fasted: No data. Parameters examined: 1) white blood cells (WBC), erythrocytes (RBC), haemoglobin concentration (HGB), haematocrite (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), 2) lymphocytes (L), monocytes (M), neutrophilic granulocytes (N), eosinophilic granulocytes (E), basophilic granulocytes (B) and large unstained cells were counted in duplicate smears	REACH registration dossier: Hadrup, N. et al.(2012) Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats

		from vehicle Control and high-dose Ag-NPs Clinical chemistry: Yes Time schedule for collection of blood: at necropsy, rats were anaesthetised in CO2/O2 and decapitated. Blood sampes were obtained from the neck wound directly into heparinised tubes. - Animals fasted: No data - How many animals: all - Parameters examined: cholesterol, total protein, albumin, glucose, urea, creatinine, alanine aminotransferase (ALAT) and alkaline phosphatase (ALP) Urinalysis: Yes Time schedule for collection of urine: on day 18, Metabolism cages used for collection of urine: Yes, animals were placed individually in metabolic cages, with free access to water but not ot feed. Twenty-four-hour urine and twenty-four-hour faeces sampels were then collected on dry ice. Parameters examined: creatinine. Neurobehavioural examination: No data Ag-NP in doses up to 9.0 mg/kg bw/day did not affect body weight or body weight gain. In contrast, Ag-acetate treatment was associated with lower body weight gain, which however did not result in statistically significant differences in body weight at the end of the study as compared with the vehicle control group. Feed intake and energy expenditure assessed by the observation of locomotor activity were considered comparable in all the groups. Minor differences were recorded in haematological parameters. Ag-acetate at 14 mg/kg bw/day was associated with an increased ALP plasma concentration and a decreased urea concentration. Absolute weights of some organs were lower in Ag-NP- or Ag-acetate-treated groups compared with the vehicle controls. However, relative organ weights of Ag-NP-treated rats of both sexes were not different from vehicle controls. Neither Ag-NP nor Ag-acetate treatment affected circulating leucocyte subset numbers or infiltrating leucocytes in the liver or ileum. No macroscopic changes compared with vehicle controls along the digestive tract at necropsy and of histopathological changes in the ileum of Ag-NP- (or Ag-acetate) treated rats. The levels of apoptosis and leucocyte infiltration in the ileac sections were not different from the vehicle controls in neither Ag-NP- nor Ag-acetate-administered rats. No differences between the Ag-NP and vehicle control group in the urinary biochemistry, absolute and relative kidney weights, histological picture and apoptosis levels. In Ag-acetate group a lower plasma urea concentration. Neither Ag-NPs nor silver acetate affected the balance between the two main phyla of gastrointestinal tract bacteria, Firmicutes and Bacteriodetes.	

		Applicant´s conclusion in REACH registration dossier: *"According to the authors, the dose of silver in ionic form was associated with lower body weight gain, an increase in alkaline phosphatase and a decrease in urea concentrations in plasma and lower absolute and relative thymus weights."* No histopathological findings reported.	
Non guideline Non GLP Fischer 344 4/sex	Antismoking (A.S.) mouthwash (0.5 % silver nitrate) Swabbing of oral cavity 1.5 mg/kg (low dose) 30 days	Extract from REACH registration dossier (original data not assessed by DS): CLINICAL SIGNS AND MORTALITY - Mild diarrhea and teeth staining was observed in treated rats. - No mortality occured. BODY WEIGHT AND WEIGHT GAIN - Body weights at study termination were slightly lower (not significant) in mid and high dose groups (15 and 150 mg/kg bw/d). HAEMATOLOGY - Platelet counts showed a statistically significant increase ($p<0.05$) at 1.5, 15, 150 mg/kg bw/d and was most pronounced in high dose animals. - No other effects were observed. CLINICAL CHEMISTRY - No significant differences were observed. ORGAN WEIGHTS - Kidney weights were significantly reduced ($p<0.05$) in high dose females (150 mg/kg bw/d) compared to controls. HISTOPATHOLOGY: NON-NEOPLASTIC - High dose animals (150 mg/kg bw/d) showed mild inflammation in the gum, tongue and esophagus. - Teeth and tongue showed a dark staining in high dose animals. - Similar observations were found to a lesser extent in intermediate and low dose group animals (15 and 1.5 mg/kg bw/d). Applicant´s conclusion in REACH registration dossier: *"The main findings observed in the study were local effects (staining of teeth and tongue and mild inflammation of the gum, tongue and oesophagus) observed dose dependently in all dose groups. The pigmentation was most likely due to silver salt precipitation in the tissues. However, beside an effect on platelet counts, which can be regarded as of questionable toxicological relevance, no signs of systemic effects were observed at the high dose level of 150 mg/kg,*	REACH registration dossier: Tamimi, S.O.; et al. (1998) Toxicity of a new antismoking mouthwash in rats and rabbits

<table>
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<td></td>
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<td>which can be regarded as a NOAEL for systemic effects."

The route of administration is unusual and results in uncertainties about the actual systemic dose achieved.</td>
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<tr>
<td>Determination of Ag in tissue and milk

Biochemical and inflammatory analysis

Measurement of oxidative stress

Histopathology

No guideline

No GLP

Rat

Sprague-Dawley

10/group</td>
<td>Citrate-capped silver nanoparticles, 55nm

Pregnant female rats dosed orally once daily from Day 7 to Day 20 of gestation with 0, 0.2, 2, 20 mg/kg AgNPs

or 20 mg Ag/kg as AgNO3</td>
<td>Reduced bw in AgNO3 treated dams.

Administration of AgNO3 lead to higher tissue contents of Ag in dams than administration of Ag-NPs.

Ag in both nanoparticle and ionic forms induced oxidative stress in dams and pups, with the ionic form being more potent.

Increased frequency of histopathological findings in brain and liver (see table):

<table>
<tr><th></th><th>Control</th><th>0.2 AgNP</th><th>2 AgNP</th><th>20 AgNP</th><th>20 $AgNO_3$</th></tr>
<tr><td>No of dams with neuronal loss event (type 2 hippocampal sclerosis)</td><td>0/5</td><td>4/5</td><td>3/5</td><td>4/5</td><td>4/5</td></tr>
<tr><td>Hepatocellular vacuolation</td><td>0/5</td><td>2/5</td><td>1/5</td><td>2/5</td><td>3/5</td></tr>
</table>
</td>
<td>IIIA, 6.8.2-10

Charehsaz, M., Hougaard, K.S., Sipahi, H., Ekici, A.I.D., Kaspar, C., Culha, M., Bucurgat, U.U., and Aydin, A. (2016): Effects of developmental exposure to silver in ionic and nanoparticle form: A study in rats. Journal of Pharmaceutical Sciences Vol 24:24</td>
</tr>
<tr>
<td>Non guideline

Non GLP

Rat

Wistar

6 females</td>
<td>Silver acetate dissolved in polyvinylpyrrolidone.

Purity not stated

Gavage, 28 days</td>
<td>The original report has not been assessed.

↑ dopamine concentration in the brain (AgAc: 5.3 ± 0.2 nmol/g brain tissue, Control: 4.0 ± 0.2 nmol/g brain tissue, $p < 0.01$).

↑Control: (AgAc: 2.0 ± 0.05 nmol/g brain tissue, Control: 1.7 ± 0.08 nmol/g brain tissue, $p < 0.05$)</td>
<td>REACH registration dossier:

Hadrup, N. et al. (2012)

The similar neurotoxic effects of nanoparticulate and ionic silver in vivo and in vitro

NeuroToxicology 33, 416 - 423.</td>
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</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Rat albino Reliability 3	0.1% silver nitrate (60 or 89* mg/kg bw/day Oral (drinking water) 218 days *0.1% silver nitrate has been converted to a dose of 60 mg/kg bw in 6.5(01) and 89 mg/kg bw in 6.2(03).	↑increase in the incidence of ventricular hypertrophy ↑proteinuria	Olcott, C.T. Experimental argyrosis. V. Hypertrophy of the left ventricle of the heart. Archives of Pathol. 49: 138-149, 1950.
Oral (drinking water) Rat albino Wistar 40m Reliability 3-4	0.25% silver nitrate (stated to be 222 mg/kg bw/d in 6.5(04)) Daily exposure 9 months (after 10 weeks half of the animals were further exposed for 6 months, the rest for 12 months)	Rapid weight loss from week 23 onwards and eventually death. Rats surviving to 37 weeks had lost approximately 50% of their maximum weight (reversibility demonstrated) massive accumulation of silver particles in the outer aspect of the ciliary epithelium basement membrane.	IIIA 6.5(03) Matuk, Y. Gosh, M. and McCulloch, C. (1981): Distribution of silver in the eyes and plasma proteins of the albino rat. Handbook on the toxicology of Metals. Can. J. ophthalmol 16.
Oral (in diet) B6C3F1 mice (300/sex) Fischer 344 rats (350/sex)	Antibacterial Zeolite Zeomic Silver content 2.6% average zinc content 14.5%.	mice: 0.1%, 0.3% and 0.9% rats: 0.01, 0.03, 0.1 and 0.3% test material administered in diet. See 6.5(05) and 6.5(06) The document seems to be a published report of the study presented in 6.5(05) and 6.5(06). The document does not add any further information than what is presented below. IIIA	6.5(02) 6.7(03) Japanese Journal of Food Chemistry Vol 2 (1) 1995 Article in Japanese, only abstract available in English.

OECD TG 453 EPA 870.4300. DACO 4.4.4 GLP status unknown Reliability 2-3 Mouse B6C3F1 75/sex*	AgION Zeomic AJ 10N 0, 0.1, 0.3 and 0.9% "at least" 0, 67, 211 and 617 mg/kg bw/day 0, 0.67, 2.0 and 6.9 mg silver ion equivalents/kg bw LOAEL: 0.1%	0.9% ↓RBC, HCT, MCH, MCV, Hb ↑MCHC ↑ renal cysts (M, F; dose-response) ↑enlargement of Langerhan´s islands (M) ↓kidney (8%), liver (10%), brain, weight (10%) (F) ↑pancreas (19%, M) ↑pigmentation of liver and pancreas 0.3% ↓HCT, MCV, Hb ↑MCHC (F) ↑ ovarian cysts ↑pigmentation of liver and pancreas 0.1% ↑ ovarian cysts ↑pigmentation of liver and pancreas *Other effects;* *0.9%* *↓bodyweight gain <10% (M)* *↑severity of thrombi (M, F)* *↓spleen weight (37%, M)* *↓brain (10%, F)* *0.3%* *↓bodyweight gain <10% (M)* *↓spleen weight (31%, M)* *↓brain (6%, F)* *0.1%* *↓spleen weight (31%, M)* *↓brain (6%, F)*	IIIA 6.5-05 (1992a)

Combined chronic and carcinogenicity Oral Reliability 2-3 Rat 70/sex***	AgION Zeomic AJ 10N (2.3% Ag, 12.5% Zn) 0.01, 0.03, 0.1 and 0.3% ("at least" 0, 3, 9, 30 and 87 mg /kg bw/day)	0.1 % ↑Pigmentation of liver, kidneys, pancreas, stomach, lymph nodes choroid plexus ↑ALT (M/F 175/58%), AST (F 96%), ALP (M/F 25/39%), LDL-C (M/F 28/19%) ↑WBC (F 134%) ↓ HCT (10%), MCH (3/3%), MCHC (F 3%), Hb (F 12%) *Other effects:* *all dose levels* *↑endometrial polyps* *↑Severity of hepatic bile duct proliferation* *↓AST* *(M ≤42%, at 12 months)* *↑ALT* *(M ≤172%, at 24 months)* *↓LDH (F≤90%, at 24 months)* *0.3%* *↓thymus weight n.s.s(38%, F)* *0.1, 0.3%* *↓TP (M ≤10%, M* *ALB ≤10%*	6.5-06 (1992b)

Inhalation route:

OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day Study) Rat Sprague-Dawley 10/sex/dose Whole body	49 µg/m^3 133 µg/m^3 515 µg/m^3 Count median diameter: 18 nm	Extract from REACH registration dossier (original data not assessed by DS): CLINICAL SIGNS AND MORTALITY No gross effects were observed during the 90-day exposure period. One animal from the high-dose group died during the opthalmological examination. BODY WEIGHT AND WEIGHT GAIN There were no significant changes in body weights of male rats. Although female rats showed a significant body weight difference between high and middle dose groups, there were no significant dose-related changes. FOOD CONSUMPTION No significant differences were observed in food consumption between the exposed rats and the control groups. HAEMATOLOGY There were no significant dose-related differences in the haematology values among groups. To evaluate aggregation of red blood cells or blood coagulation attributable to silver nanoparticles, erythrocyte aggregation, activated	REACH registration dossier: Subchronic Inhalation Toxicity of Silver Nanoparticles / Sung, J.H. et al. Long-term Stability Characterisitics of Metal Nanoparticle Generator Using Small Ceramic Heater for ... Ji, J.H. et al. / Metal nanoparticle generation

		partial thromboplastin time, and prothrombin time were tested. Only the percent of aggregation in the high-dose females showed a statistically significant difference compared with the controls. CLINICAL CHEMISTRY There were no significant dose-related differences in the blood biochemical parameters. ORGAN WEIGHTS No significant organ weight changes were observed in either the male or female rats after the 90 days of silver nanoparticle exposure. HISTOPATHOLOGY: NON-NEOPLASTIC Liver: - minimal bile-duct hyperplasia was identified in 0/10, 0/10, 1/10, and 4/9 of the control, low, middle, and high dose males, respectively. - one high-dose male had minimal bile-duct hyperplasia with minimal portal mineralization. - the higher incidence of bile-duct hyperplasia in the high dose males, with or without mineralization - minimal bile-duct hyperplasia was present in 3/10, 2/10, 4/10, and 8/10 of the control, low, middle, and high dose females, respectively. - single-cell hepatocellular necrosis, characterised by increased cellular eosinophilia and shrunken condensed nucleo, was noted in 3/10 high dose females. - one high dose female exhibited moderate bile-duct hyperplasia with concurrent moderate centrilobular fibrosis, minimal single-cell hepatocyte necrosis, mild pigment accumulation, and moderate multifocal necrosis. - the higher incidence of bile-duct hyperplasia, with or without necrosis, fibrosis, and/or pigmentation, in high dose females, which was slightly more obvious than in the males. Lung: - examination revealed a high incidence of minimal alterations, including some chronic alveolar inflammation, a mixed cell perivascular infiltrate, and alveolar macrophage accumulation in high dose male and female animals when compared with the controls. Nasal pathways: - histopathologic findings in the nasal pathways (considered not to be treatment related in the REACH Reg. dossier) Kidneys: - incidence of minimal tubular basophilia was noted in all the groups, including the controls - tubular basophilia was more prevalent in males compared with the females.	using a small ceramic heater with a local heating area / Jung, J. H. et al. Summary Report: Derivation of a human equivalent concentration (HEC) for silver / EBRC / other company data

		- tubular dilatation, cast formation, mineralization, and inflammation were noted occasionally in the control and/or treated animals. Heart: - minimal degeneration/necrosis was observed in all the groups, including the controls - the change was more obvious in the males. - the finding is a common spontaneous background change. Harderian gland/prostate - inflammation was noted occasionally OTHER FINDINGS Silver distribution in tissue: - silver concentration in lung tissue from groups exposed were statistically significant ($p < 0.01$) and increased with dose. - a clear dose -dependent increase in silver concentration in the blood for both genders. - dose-dependent increase in the liver silver concentration for both genders. - silver concentration in the olfactory bulb was higher than in brain and increased in a dose dependent manner in both the male and female rats ($p<0.01$). - silver concentration in the kidneys showed a gender difference with the female kidneys containing two to three times more silver acculmulation than in male kidneys. - because gender difference in silver accumulation was noted in kidneys, the kidney function was measured based on the N-acetylglutamate and protein in urine. No significant difference was noted among the dose groups and between genders, except for an increase of protein in the urine from the high-dose male rats (high-dose group: 2.57± 0.13 g/g creatinine; control group: 1.89 ± 0.11 g/g creatinine; $p < 0.05$). Histopathological findings for male rats:	

Group				Control		Low		Middle		High	
Number of animals				10		10		10		9	
				N	%	N	%	N	%	N	%
Liver	No microscopic findings			10/10	100	10/10	100	9/10	90	5/9	55.6
	Abnormality*			0/10	0	0/10	0	1/10	10	4/9	44.4
	Necrosis	Multifocal	Minimum	0/10	0	0/10	0	0/10	0	1/9	11.1
	Hyperplasia*	Bile duct	Minimum	0/10	0	0/10	0	1/10	10	4/9	44.4
	Vacuolation	Hepatocellular	Minimum	0/10	0	0/10	0	0/10	0	1/9	11.1
	Mineralization	Portal	Minimum	0/10	0	0/10	0	0/10	0	1/9	11.1
Lungs	No microscopic findings			5/10	50	3/10	30	3/10	30	0/9	0
	Abnormality			5/10	50	7/10	70	7/10	70	9/9	100
	Accumulation	Macrophage, alveolar	Minimum	3/10	30	5/10	50	5/10	50	8/9	88.9
	Inflammation**	Chronic, alveolar	Minimum	2/10	20	3/10	30	2/10	20	8/9	88.9
	Infiltrate	Mixed cell perivascular	Minimum	3/10	30	4/10	40	6/10	60	7/9	77.8
	Hemorrhage	Alveolar	Minimum	1/10	10	0/10	0	0/10	0	0/9	0
	Osseous foreign body			0/10	0	0/10	0	0/10	0	1/9	11.1
	Hyperplasia	Respiratory epithelium level I		0/10	0	0/10	0	0/10	0	1/9	11.1

* $p < 0.05$, compared with control, ** $p < 0.01$, compared with control

Histopathological findings for female rats:

Group				Control		Low		Middle		High	
Number of animals				10		10		10		10	
				N	%	N	%	N	%	N	%
Liver	No microscopic findings			7/10	70	5/10	50	5/10	50	1/10	10
	Abnormality*			3/10	30	5/10	50	5/10	50	9/10	90
	Necrosis	Multifocal	Minimum	2/10	20	0/10	0	0/10	0	0/10	0
			Moderate	0/10	0	0/10	0	0/10	0	1/10	10
		Focal	Minimum	0/10	0	0/10	0	1/10	10	0/10	0
		Single-cell hepatocellular *	Minimum	0/10	0	0/10	0	0/10	0	3/10	30
	Hyperplasia*	Bile duct	Minimum	3/10	30	2/10	20	4/10	40	8/10	80
			Moderate	0/10	0	0/10	0	0/10	0	1/10	10
	Granuloma	Multifocal	Minimum	0/10	0	2/10	20	0/10	0	0/10	0
	Vacuolation	Hepatocellular	Minimum	0/10	0	1/10	10	0/10	0	0/10	0
	Fibrosis	Centrilobular	Mild	0/10	0	0/10	0	0/10	0	1/10	10
	Pigment	Centrilobular	Mild	0/10	0	0/10	0	0/10	0	1/10	10
Lungs	No microscopic findings			3/10	30	5/10	50	6/10	60	2/10	20
	Abnormality			7/10	70	5/10	50	4/10	40	8/10	80
	Accumulation	Macrophage, alveolar	Minimum	7/10	70	4/10	40	4/10	40	6/10	60
	Inflammation**	Chronic, alveolar	Minimum	3/10	30	2/10	20	0/10	0	8/10	80
	Infiltrate**	Mixed cell perivascular	Minimum	0/10	0	0/10	0	1/10	10	7/10	70

* $p < 0.05$, compared with control, ** $p < 0.01$, compared with control

Tissue content of silver in male rats (mean± SE) (Unit: ng/g tissue wet weight):

	Control	Low	Middle	High
Liver	0.70 ± 0.20 (3)	3.52 ± 0.98 (5)	13.75 ± 2.28 (5)	132.97 ± 22.87* (4)
Kidneys	0.85 ± 0.20 (5)	1.63 ± 0.33 (5)	3.58 ± 0.41** (5)	9.49 ± 0.86* (4)
Olfactory bulb	0.51 ± 0.38 (3)	6.44 ± 0.77 (5)	17.10 ± 1.61 (5)	30.48 ± 2.15*** (4)
Brain	1.12 ± 0.34 (2)	3.45 ± 0.73 (4)	7.89 ± 0.95** (5)	18.63 ± 1.24* (4)
Lungs	0.77 ± 0.25 (5)	613.57 ± 66.03† (5)	5450.29 ± 904.17** (5)	14645.42 ± 2630.24* (4)
Whole blood	0.09 ± 0.02 (7)	0.68 ± 0.08 (10)	1.82 ± 0.20** (10)	4.31 ± 0.37* (9)

Note. (): number of samples.

*p < 0.01, high-dose versus other groups.

** p < 0.01, middle-dose versus unexposed and low-dose groups.

*** p < 0.01, high-dose versus other groups (dose dependent).

†p < 0.05, male versus female in low-dose group.

Tissue content of silver in female rats (mean± SE) (Unit: ng/g tissue wet weight):

	Control	Low	Middle	High
Liver	0.90±0.31 (5)	4.55±1.40 (5)	12.07±2.50 (5)	71.08±24.50* (5)
Kidneys	0.94±0.18 (4)	2.61±0.57 (5)	11.81±4.27 (4)	37.66±7.04*†(5)
Olfactory bulb	2.26±0.74 (4)	7.43±0.75 (5)	13.75±1.32 (5)	32.84±2.74*** (5
Brain	0.66±0.26 (4)	4.09±0.46 (5)	10.22±1.19** (5)	19.97±2.41* (5)
Lungs	1.01±0.10 (3)	295.92±78.50 (5)	4241.17±641.10** (5)	20585.63±1880.3 (5)
Whole blood	0.05±0.01 (5)	0.85±0.14 (10)	2.10±0.22** (10)	6.86±0.60*,‡(10)

Note. (): number of samples.

*p < 0.01, high-dose versus other groups.

** p < 0.01, middle-dose versus unexposed and low-dose groups.

*** p < 0.01, high-dose versus other groups (dose dependent).

†p < 0.05, female versus male in low-dose group.

‡p < 0.01, female versus male in high-dose group.

Non guideline Non GLP Rat Sprague-Dawley 4/sex/dose Whole body	18 nm diameter 49, 133 and 515 µg/m^3 6 hours/day, 5 days/week 90 days.	Extract from REACH registration dossier (original data not assessed by DS): BRONCHOALVEOLAR LAVAGE (BAL) CELL EVALUATION When compared to the control group, all the exposed groups showed elevated total cell numbers, alveolar macrophages, polymorphonuclear cells (PMN), and lymphocytes in the male rats, while no elevation was noticeable in the female rats. Albumin, LDH, and total protein as inflammatory markers in the BAL were all increased in the female rats from the high-dose group (p < 0.01–0.05); however, there were no significant increases in the male rats. Please also refer to Table 1 in the field "Any other information on results incl. tables" below. LUNG FUNCTION TEST Among the pulmonary function test parameters, the tidal volume, minute volume, and peak inspiration flow showed significant changes during the 90 days of silver nanoparticle exposure (p < 0.01–0.05). The dose-dependent tidal volume decreases in the male rats led to minute volume and peak inspiratory flow decreases in the high-dose group. The tendency of a dose-dependent decrease in the tidal volume also appeared in the female rats. All the exposed groups of female rats exhibited decreased minute volumes and peak inspiration flows compared with the control.	Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparti... / Sung, J. H. et al. (2008)

HISTOPATHOLOGY: NON-NEOPLASTIC

The histopathological examinations of the lung samples from the rats exposed showed significantly increased incidence of mixed cell infiltrate perivascular and chronic alveolar inflammation, including alveolaritis, granulomatous lesions, and alveolar wall thickening and alveolar macrophage accumulation.

Parameters of lung inflammation:

Parameter	Control (n = 4)	Low (n = 4)	Middle (n = 4)	High (n = 4)
A. Male rats				
Total cell ($x10^6$)	0.45 ± 0.05	0.78 ± 0.15	0.65 ± 0.09	0.77 ± 0.09
Macrophage ($x10^6$)	0.43 ± 0.05	0.74 ± 0.14	0.62 ± 0.09	0.74 ± 0.09
PMN ($x 10^6$)	0.007 ± 0.001	0.012 ± 0.002	0.01 ± 0.001	0.012 ± 0.001
Lymphocyte ($\times 10^6$)	0.011 ± 0.001	0.019 ± 0.004	0.016 ± 0.002	0.029 ± 0.002
Albumin (µg/ml)	8.3 ± 0.6	16.5 ± 3.8	11.8 ± 3.5	1.8 ± 0.75[a]
LDH (IU/L)	55.3 ± 12.5	73.4 ± 5.7	69.1 ± 10.7	73.0 ± 10.0
Total protein (µg/ml)	13.3 ± 3.6	16.5 ± 1.2	15.1 ± 2.2	13.8 ± 1.3
B. Female rats				
Parameter	Control (n = 4)	Low (n = 4)	Middle (n = 4)	High (n = 4)
Total cell number ($x10^6$)	0.72 ± 0.08	0.51 ± 0.07	0.44 ± 0.07	0.76 ± 0.12
Macrophage ($x10^6$)	0.70 ± 0.08	0.50 ± 0.07	0.43 ± 0.07	0.74 ± 0.12
PMNs ($x 10^6$)	0.011 ± 0.001	0.008 ± 0.001	0.007 ± 0.001	0.011 ± 0.002
Lymphocyte ($x 10^6$)	0.004 ± 0.00	0.003 ± 0.00	0.002 ± 0.001	0.004 ± 0.00
Albumin (µg/ml)	9.3 ± 1.8	11.3 ± 1.5	11.8 ± 1.0	37.75 ± 20.1
LDH (IU/L)	57.3 ± 7.9	41.8 ± 4.5	41.8 ± 5.4	116.7 ± 20.0[b]
Total protein (µg/ml)	13.9 ± 1.9	10.4 ± 0.5	10.9 ± 1.0	20.33 ± 3.5[c]

a = Significantly different from high vs. low and middle, p < .05

b = Significantly different from high vs. other groups, p < .01

c = Significantly different from high vs. other groups, p > .05.

Incidence and severity of silver nanoparticle-related microscopic findings in lungs:

	Males				Female			
Tissue/finding	Control	Low	Middle	High	Control	Low	Middle	Hig
Number examined	10	10	10	10	10	10	10	1
Accumulation, macrophages: Alveolar minimal Minimal	3	5	5	8	7	4	4	6
Inflammation, chronic: Alveolar minimal	2	3	2	7	3	2	-	8
Infiltrate: mixed cells Perivascular minimal	3	4	6	8	-	-	1	7

Applicant´s conclusion in REACH registration dossier:

"Among the lung function test measurements, the tidal volume and minute volume showed a statistically significant decrease during the 90 days of silver nanoparticle exposure. Although no statistically significant differences were found in the cellular differential counts, the inflammation measurements increased in the high-dose female rats. Meanwhile, histopathological examinations indicated dose-dependent increases in lesions related to silver nanoparticle exposure, such as infiltrate mixed cell and chronic alveolar inflammation, including thickened alveolar walls and small granulomatous lesions. Therefore, according to the authors, when taken together, the decreases in the tidal volume and minute volume and other inflammatory responses after prolonged exposure to silver nanoparticles would seem to indicate that nanosized particle inhalation exposure can induce lung function changes, along with

		inflammation, at much lower mass dose concentrations when compared to submicrometer particles."	
OECD TG 412 GLP Rat Sprague-Dawley 10/sex/dose	0 µg/m^3, 0.48 µg/m^3, 3.48 µg/m^3 and 61.24 µg/m^3 Silver nanoparticles, average particle size 12-15 nm 6 h/day 5 days/week 4 weeks	Extract from REACH registration dossier (original data not assessed by DS): CLINICAL SIGNS AND MORTALITY No clinical signs of toxicity or mortality were observed during the 28-day exposure period. BODY WEIGHT AND WEIGHT GAIN The male and female rats did not show any significant changes in body weight according to the concentration of silver nanoparticles during the 28-day experiment. FOOD CONSUMPTION There were no significant differences in food consumption between male tratement groups and the control group. For the female rats, the middle-dose group showed a not treatment-related increased in food consumption ($p < 0.01$) from week 2–4 when compared with the control; however, no significant change in food consumption were observed in the high and low dose groups during the exposure period. HAEMATOLOGY There were no significant dose-related changes in the haematology values for the male rats and no treatment-related findings in female rats. However, the percentage of neutrophils and eosinopils increased significantly ($p < 0.05$) in female rats in the low-dose group when compared with the control. The mean corpuscular haemoglobin (MCH) in the female rats in the middle-dose group increased significantly ($p < 0.05$) when compared with the female rats in the high-dose group. CLINICAL CHEMISTRY The high-dose group revealed significantly increased ($p < 0.05$) calcium levels in both the male and female rats when compared with the control, and increased total protein ($p < 0.05$) in the male rats when compared with the control. However, the toxicological relevance of these differences remains unclear. In addition, the low-dose group of male rats showed increased gamma-glutamyl transpeptidase ($p < 0.05$) when compared with the control group. Since no effects were obseved in the mid and high dose group, this finding is not of toxicologicsl relevance. ORGAN WEIGHTS No significant organ weight changes were observed in either the male or female rats after the 28 days of silver nanoparticle exposure. HISTOPATHOLOGY: NON-NEOPLASTIC Histopathological examination of the male rat livers revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle and high dose groups, respectively. For female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high dose group and one case	Ji, J.H. et al. (2007)

among the female rats in the high dose group. These findings were not dose or treatment-related. The other organs, including the kidneys, spleen, lungs, adrenals, heart, reproductive organs, brain, and nasal cavity, were also examined histopathologically, with no distinct findings.

DETERMINATION OF TISSUE SILVER

The silver concentration in the lung tissue from the groups exposed to silver nanoparticles for 28 days revealed a statistically significant ($p < 0.01$) dose-dependent increase. Although no clear blood silver concentrations were detected for any of the dose groups, a clear increase ($p < 0.05$) was observed in the liver silver concentration for the high dose group, along with a statistically significant ($p < 0.01$) increase in the brain silver concentration. The olfactory bulb, which showed higher silver-concentration levels than the brain, also revealed a dose-dependent increase ($p < 0.01$) in both the male and female rats.

Serum biochemical values for male rats (mean ± SE):

Parameter	Control	Low	Middle	High
albumin (g/dl)	2.35±0.02	2.36 ± 0.04	2.39 ± 0.03	2.40 ± 0.06
alkaline phosphatase (IU/L)	318.20 ± 19.57	311.90 ± 20.10	303.40 ± 21.77	318.20 ± 10.68
calcium (mg/dl)	10.22 ± 0.19	10.87 ± 0.39	10.87 ± 0.26	11.58 ± 0.33*
cholesterol (mg/dl)	60.60 ± 4.22	62.10±3.94	58.60 ± 4.84	71.10 ± 4.66
creatinine (mg/dl)	0.81 ± 0.05	0.87 ± 0.04	0.82 ± 0.06	0.88 ± 0.05
Gamma-glutamyl transpeptidase (IU/L)	0.30 ± 0.15	0.90 ± 0.10*	0.30 ± 0.15	0.40 ± 0.16
glucose (mg/dl)	120.90 ± 5.64	121.50 ± 7.50	115.20 ± 7.88	139.60 ± 7.60
glutamic oxaloacetic transaminase (IU/L)	163.10 ± 16.55	182.90 ± 11.60	172.50 ± 14.93	168.10 ± 13.04
glutamic pyruvic transaminase (IU/L)	44.80 ± 3.21	40.90 ± 2.42	45.70 ± 5.94	45.20 ± 3.27
inorganic phosphorus (mg/dl)	9.90 ± 0.32	10.77 ± 0.27	10.56 ± 0.48	10.85 ± 0.49
lactate dehydrogenase (IU/L)	3213.30 ± 384.41	4002.90 ± 330.57	3542.50±351.92	3692.30 ± 367.70
magnesium (mg/dl)	2.94 ± 0.13	3.06 ± 0.09	3.06±0.14	3.10 ± 0.12
total protein (g/dl)	5.92 ± 0.05	5.87 ± 0.05	5.96±0.09	6.17 ± 0.10*
uric acid (mg/dl)	1.46 ± 0.16	1.43 ± 0.11	1.59 ± 0.14	1.54 ± 0.11
Blood urea nitrogen (mg/dl)	16.83 ± 1.12	15.82 ± 0.70	14.44 ± 0.66	16.62 ± 1.57
total bilirubin (mg/dl)	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
creatine phosphokinase (U/L)	1360.40 ± 148.51	1759.80 ± 159.14	1406.30 ± 162.76	1593.70 ± 184.72
sodium (mmol/L)	144.60 ± 0.64	145.00 ± 0.49	143.90 ± 0.60	144.10 ± 0.28
potassium (mmol/L)	7.34 ± 0.48	7.79 ± 0.34	8.24 ± 0.41	7.68 ± 0.21
chloride (mmol/L)	103.30 ± 0.45	102.80 ± 0.53	102.60 ± 0.58	102.00 ± 0.77
ratio of albumin and globulin	1.52 ± 0.03	1.49 ± 0.03	1.49 ± 0.02	1.58 ± 0.03
triglyceride (mg/dl)	25.10 ± 5.19	23.40 ± 4.59	27.10 ± 6.05	37.60 ± 5.75

Asterisk indicates significantly different from control value, p < .05

Serum biochemical values for female rats (mean ± SE):

Parameter	Control	Low	Middle	High
albumin (g/dl)	2.76 ± 0.05	2.73 ± 0.07	2.67 ± 0.03	2.77 ± 0.06
alkaline phosphatase (IU/L)	184.90 ± 13.70	151.90 ± 12.65	179.80 ± 14.68	146.80 ± 10.81
calcium (mg/dl)	10.82 ± 0.29	10.59 ± 0.31	10.82 ± 0.35	11.96±0.44*
cholesterol (mg/dl)	70.80 ± 4.51	75.20 ± 4.92	77.10 ± 7.84	85.00 ± 6.71
creatinine (mg/dl)	0.89 ± 0.05	1.04 ± 0.06	1.03 ± 0.05	0.92 ± 0.04
Gamma-glutamyl transpeptidase (IU/L)	1.10 ± 0.10	0.90 ± 0.10	1.10 ± 0.10	0.90 ± 0.10
glucose (mg/dl)	113.80 ± 5.64	110.20 ± 4.45	111.50 ± 6.41	116.50 ± 5.99
glutamic oxaloacetic transaminase (IU/L)	128.40 ± 11.66	111.50 ± 6.16	140.00 ± 8.62	130.90 ± 11.43
glutamic pyruvic transaminase (IU/L)	45.00 ± 2.70	52.00 ± 4.17	52.00 ± 5.65	43.30±2.72
inorganic phosphorus (mg/dl)	9.29 ± 0.26	9.05 ± 0.40	9.79 ± 0.63	9.30 ± 0.25
lactate dehydrogenase (IU/L)	2500.60 ± 312.58	1910.50 ± 241.05	2658.90 ± 236.37	2585.90 ± 341.63
magnesium (mg/dl)	2.92 ± 0.09	3.02 ± 0.08	3.20 ± 0.11	3.09 ± 0.08
total protein (g/dl)	6.35 ± 0.11	6.40 ± 0.09	6.27 ± 0.07	6.48 ± 0.10
uric acid (mg/dl)	1.47 ± 0.11	1.64 ± 0.13	1.61 ± 0.10	1.43 ± 0.08
Blood urea nitrogen (mg/dl)	17.04 ± 1.16	19.43 ± 1.18	17.63 ± 0.59	17.87 ± 0.71
total bilirubin (mg/dl)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
creatine phosphokinase (U/L)	1053.30 ± 130.48	793.30 ± 94.96	1116.90 ± 66.17	982.70 ± 125.04
sodium (mmol/L)	144.10 ± 0.48	145.70 ± 0.33	144.80 ± 0.36	144.60 ± 0.56
potassium (mmol/L)	6.28 ± 0.27	5.56 ± 0.29	5.92 ± 0.35	5.96±0.23
chloride (mmol/L)	104.20 ± 0.42	104.20 ± 0.25	103.50 ± 0.34	103.60 ± 0.56
ratio of albumin and globulin	1.30 ± 0.02	1.35 ± 0.03	1.35 ± 0.02	1.34 ± 0.02
triglyceride (mg/dl)	9.40 ± 3.24	7.50 ± 1.78	5.80 ± 0.96	7.50 ± 1.02

Asterisk indicates significantly different from control value, p <.05

Concentration of silver in tissue from male rats (mean ± SE, ng/g wet weight):

Tissue	Control	Low	Middle	High
Lung	0.89 ± 0.20 (5)	0.32 ± 0.20 (5)	1.25 ± 0.16 (5)	1180.76 ± 110.97**(5)
Liver	ND	0.49 (1)	0.61 ± 0.17 (2)	5.91 ± 2.61 (5)
Brain	0.15 ± 0.05 (5)	ND	0.15 ± 0.01 (2)	2.20 ± 0.14**(5)
Olfactory bulb	ND	0.20 (1)	0.93 ± 0.28 (5)	6.96 ± 0.23^a(5)
Blood	ND	2.59 (1)	ND	ND

Note. ND, not detected; n = 5 for each experiment; number of detected samples given in parentheses. Statistically significant difference indicated by double asterisk for p < .01 compared with control group and by a for p < .01 compared with low and middle group.

Concentration of silver in tissue from female rats (mean ± SE, ng/g wet weight):

Tissue	Control	Low	Middle	High
Lung	0.27 ± 0.21 (3)	0.45±0.16 (4)	1.19 ± 0.07 (5)	1496.64 ± 384.72**(5)
Liver	1.15 (1)	0.11 (1)	0.06 ± 0.03 (2)	6.89 ± 1.46 (5)
Brain	ND	0.56 ± 0.24 (3)	0.38 ± 0.13 (3)	3.10 ± 0.46^a(5)
Olfactory bulb	ND	0.12 (1)	1.12 ± 0.39 (5)	9.05 ± 2.93^b(4)
Blood	ND	0.24 ± 0.06 (2)	ND	1.58 ± 0.36 (4)

Note. ND, not detected; n = 5 for each experiment; number of detected samples given in parentheses. Statistically significant difference indicated by double asterisk for p < .01 compared with control group, by a for p < .01 compared with low and middle group, and by b for p < .05 compared with middle group.

Applicant´s conclusion in REACH registration dossier:

"The male and female rats did not show any clinical findings, significant changes in body weight and food consumption relative to the concentration of silver nanoparticles during the 28-day experiment. There were no significant treatment-related changes in the hematology and blood biochemical values, organ weights or histopathology in either the male or female rats. Therefore, the results indicated that exposure to silver nanoparticles at a concentration of 0.061 mg/m^3 did not appear to have any significant health effects."

Mice	3.3 mg/m3	Applicant´s conclusion in REACH registration dossier:	Stebounova, LV. et al.

C57Bl/6 Males (6 weeks old)	4h/day 10 days nanoparticles, 5 +- 2 nm	"*Minimal inflammatory response or toxicity was found following subacute exposure of mice to nanosilver. The median retained dose of nanosilver in the lungs measured by ICP-OES was 31 μg/g lung (dry weight) immediately after the final exposure. Bioaccessibiliyt testing indicated that nanosilver does not dissolve in solutions mimicking the intracellular or extracellular milieu.*"	(2011) Particle and Fibre Toxicology, 8(5) Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model /

Dermal route

Non guideline Non GLP Guinea pig Hartley albino 6 males/dose **Reliability 2**	Aqueous solution of 100, 1000 and 10000 µg/mL and an aqueous solution of 100 µg/mL **silver nitrate** 5 days/week 13 weeks	Significant dose-dependent histopathologic changes observed in the skin, liver, and spleen of all treated animals. **Skin:** Low dose: decreased epidermis and dermis thickness, increased levels of Langerhans cells, inflammation and decreased papillary layer with regular collagen fibres. Intermediate dose: decreased epidermis and dermis thickness, increased levels of Langerhans and round cells, inflammation and decreased papillary layer with regular collagen fibres and acidophilic cytoplasm in muscle fibres with inflammation also observed in the endomysium. Some muscle fibres surrounded by macrophages. High dose: acidophilic cytoplasm in muscle fibres with inflammation observed in the endomysium. Some muscle fibres were surrounded by macrophages. Degenerative fibres and increased levels of macrophages in endomysium. Silver nitrate: reduced dermis and papillary layer thickness and an increased number of Langerhans cells. **Liver**: Effects observed with silver nitrate and nanosilver; hepatocyte cords destroyed in all test groups, but the effect was magnified at the higher silver concentrations. Overproduction of Kupffer cells and degeneration of hepatocytes increased with increasing nanosilver concentrations. Necrosis only in the high dose. **Spleen:** Low dose: red capsules much thinner, inflammation, accumulation of red blood cells	Doc. IIIA 6.4.2-04

		and white pulp atrophy. Intermediate group: reactions similar to low dose. High dose: highest levels of red pulp inflammation, white pulp atrophy and thinnest capsules. Silver nitrate: thinner red capsules, inflammation and white pulp hypertrophy only observed in the silver nitrate group	
OECD TG 411 (Subchronic Dermal Toxicity: 90-Day Study) Hartley-albino Guinea pigs (only males; limited necropsy/histopathology GLP status not known	100, 1000, and 10000 ppm silver nanoparticles Particle size (TEM): < 100 nm	Extract from REACH registration dossier (original data not assessed by DS): CLINICAL SIGNS AND MORTALITY - all animals survived during this study - no clinical sign of toxicity was recorded in dose groups and control. BODY WEIGHT AND WEIGHT GAIN - no significant weight changes were detected during the study. FOOD CONSUMPTION - food consumption was not significantly different among control and treatment groups ($p = 0.085$) . WATER CONSUMPTION - water consumption was not significantly different among control and treatment groups ($p = 0.087$). HISTOPATHOLOGY: NON-NEOPLASTIC - Tissue levels of silver nanoparticles: A close correlation between dermal exposure and tissue levels of silver nanoparticles was found. Silver content in tissues increased dose-dependently and showed the following ranking: kidney>muscle>bone >skin>liver> heart> spleen. - Toxic responses of bone (bone samples of a total of 27 animals): Abnormal inflammatory responses were found in all treated groups and osteoclasts were formed in these animals in a dose dependent manner. Separated lines and marrow space narrow were observed in three different dose levels of silver nanoparticles when the alterations were compared with negative group.	REACH Registration dossier: Korani, M. et al. (2013): Iranian Journal of Pharmaceutical Research 12 (3): 511 - 519. Sub-chronic dermal toxicity of silver nanoparticles in guinea pigs: Special emphasis to heart, bone and kidneys toxicities

Please also refer to table 2 in the field "Any other information on results incl. tables" below.

- Toxic responses of heart (heart samples of a total of 26 animals):

Abnormal changes were detected in dose groups as well as silver nitrate group. However, 4 major signs of toxicity (inflammation, presence of clear zone around nucleus, cardiocyte deformities, congestion and hemorrhage) were magnified in the high dose group. Increased dermal dose of silver nanoparticles caused cardiocyte deformity.

Please also refer to table 3 in the field "Any other information on results incl. tables" below.

- Toxic responses of heart (kidney samples of a total of 28 animals):

Six major toxic responses were observed, and scoring was performed according to the following classification: Inflammation, gluomeral adhesion to Bowman's capsule, proximal convoluted tubule degeneration, capsular thickening, membranous thickening and increased mesangial cells. Inflammatory reactions and glomeral adhesion to Bowman's capsule were identified in all dose groups. These reactions were magnified in a dose-dependent manner. Besides these toxic reactions, increased mesangial cells, increased membranous thickening and increased capsular thickening were detected too. The highest levels of degeneration proximal convoluted tubule and distal convoluted tubule were seen in the middle and high-dose groups.

Tissue levels of silver nanoparticles in comparison to AgNo3 and negative control (ng/g):

Dose Groups	Heart (n=26)	Skin (n=22)	Kidney (n=28)	Bone (n=27)	Muscle (n=28)	Liver (n=27)	Sple (n=2
Control negative	8.46+ 6.85	9.36 ± 2.28	8.78 ±2.64	7.7736+1.96	6.109+1.06	11.5 ± 1.29	5.36+
Control Positive ($AgNO_3$)	14.34 + 6.85	18.84+ 7.72	16.6 ± 9.92	9.3377+2.75	7.124+1.39	24.06 ± 2.05	7.50+
100 ppm silver nanoparticles	9.09 +6.85	18.84+2.002	19.03 ± 9.92	12.025+1.97	14.316+5.68	24.06 ± 3.80	12.07+
1000 ppm silver nanoparticles	15.65+5.96	29.96+9.69	24.06 ± 15.36	12.557+2.75	14.026+7.84	26.148 ± 7.21	11.88+
10000 ppm silver nanoparticles	22.66+8.73	31.02+14.3	35.95 ± 12.94	32.325+9.1	33.63+3.31	30.324 ± 2.84	20.87+

Silver nanoparticles induced bone toxicity after dermal application in guinea pig:

Dose Groups	Inflammation	Osteoclasts	Separated Lines	Marrow narr
Control negative	-	-	-	-
Control Positive ($AgNO_3$)	+	+	+	+
100 ppm silver nanoparticles	+	+	+	+
1000 ppm silver nanoparticles	+	++	+	+
10000 ppm silver nanoparticles	+	+++	++	++

Severe (+++), moderate (++), mild (+), none(-)

Silver nanoparticles induced heart toxicity after dermal application in guinea pig:

Dose Groups	Inflammation	Cardiocyte deformity	Clear zones around nucleus	Congest hemorrh
Control negative	-	-	-	
Control Positive ($AgNO_3$)	+	+	-	
100 ppm silver nanoparticles	+	+	+	
1000 ppm silver nanoparticles	+	++	+	
10000 ppm silver nanoparticles	++	++	++	

Silver nanoparticles induced nephrotoxicity after dermal application in guinea pig:

Dose Groups	Inflammation	PCT Degeneration	Adhesion of glomerular epithelial cells to BC	Capsular thickening	Membranous thickening	in Me
Control negative	-	-	-	-	-	-
Control Positive ($AgNO_3$)	+	++	-	+	-	+
100 ppm silver nanoparticles	+	++	+	+	-	+
1000 ppm silver nanoparticles	+	++	+	+	+	+
10000 ppm silver nanoparticles	+	+++	++	+	++	++

BC: Bowman's capsule, DCT : Distal Convoluted Tubule, PCT: Proximal convoluted tubule, GL: Glomeru

The following references have been assessed based on the information available in abstracts. In case indicated by assumed relevance and reliability, the full article was retrieved and assessed.

Abstract	Reference
Abstract: Silver is a xenobiotic element with no recognized trace metal value in the human body. It is absorbed into the body through the lungs, gastrointestinal tract, mucus membranes of the urinogenital tract, and through the skin, mainly in the form of silver protein complexes. Although silver is metabolized throughout the soft tissues, available evidence from experimental animal studies and human clinical reports has failed to unequivocally establish that it enters tissues of the central nervous system or is a cause of neurotoxic damage. Argyria characterized by deposition of particles of silver sulfide or silver selenide is the principle contraindication for using silver in medical devices or occupationally. This presents discoloration of the skin but is not regarded as a health risk or manifestation of toxicity. No evidence is available to demonstrate the toxic risk of silver to the peripheral nervous system, although silver sulfide deposits have been identified in the region of cutaneous nerves. Transitory silver sulfide deposits seen in the tissues of the blood–brain and blood–CSF barriers are mostly lysosomally bound or deposited on basement membranes or collagen without toxic effect. Silver is mostly excreted from the body in the urine and feces. Further research is indicated to evaluate the role of metal binding proteins including metallothioneins as cytoprotectants for neurological tissue. **DS opinion:** This publication is approximately 15 years old and the information is not expected to invalidate or impact on the assessment of more recent data. The article is	Lansdown, A. B. G. (2007) Critical observations on the neurotoxicity of silver. Crit Rev Toxicol. 37(3):237-50. doi: 10.1080/10408440601177665.

thus not further considered for the assessment of neurotoxicity and classification STOT-RE.	
Abstract: Cutaneous argyria was diagnosed in a 59-year-old woman. Manic depressive psychosis developed at about the same or a short time thereafter. The patient died 6 years later from a ruptured aortic aneurysm. At autopsy silver deposits were seen in skin, mucous membranes, heart, kidney, and liver. In the central nervous system the leptomeninges and choroid plexuses contained silver granules. In addition, silver granules were visualized in the walls of many intraparenchymal vessels, particularly of the basal ganglia, hypothalamus, substantia nigra, and cerebellum. Progressive glial changes and cellular gliosis were evident in many areas of the brain. With the electron microscope the deposition of silver granules in basal membrane structures of the choroid plexus and intracerebral vasculature was amply confirmed. Furthermore, silver deposition was seen in brain parenchymal cells inside bodies of apparently lysosomal nature. The silver content of various brain regions was determined by absorption spectrophotometry. **DS opinion:** The case report is considered to provide evidence that silver can deposit in several brain structures but is not expected to provide robust and reliable information regarding the ethiology of other histopathological changes. The information is considered in a weight of evidence but it is noted that this information is based on a single case.	Dietl HW, Anzil AP, Mehraein P. (1984) Brain involvement in generalized argyria. Clin Neuropathol. 3(1):32-6. PMID: 6705320.
Abstract: Extensive incorporation of silver nanoparticles (AgNPs) into many medical and consumer products has raised concerns about biosafety. Since nanosilver accumulates persistently in the central nervous system, it is important to assess its neurotoxic impacts. We investigated a model of prolonged exposure of adult rats to a low environmentally relevant dose of AgNPs (0.2 mg/kg b.w.). Ultrastructural analysis revealed pathological alterations in mitochondria such as swelling and cristolysis. Besides, elongated forms of mitochondria were present. Level of adenosine triphosphate was not altered after exposure, although a partial drop of mitochondrial membrane potential was noted. Induction of autophagy with only early autophagic forms was observed in AgNP-exposed rat brains as evidenced by ultrastructural markers. Increased expression of two protein markers of autophagy, beclin 1 and microtubule-associated proteins 1A/1B light chain 3B (MAP LC3-II), was observed, indicating induction of autophagy. Expression of lysosome-related Rab 7 protein and cathepsin B did not change, suggesting inhibition of physiological flux of autophagy. Our results show that exposure to a low, environmentally relevant dose of AgNPs leads to induction of autophagy in adult rat brain in response to partial mitochondrial dysfunction and to simultaneous interfering with an autophagic pathway. The cell compensates for the defective autophagy mechanism via development of enhanced mitochondrial biodynamic. **DS opinion:** The article presents some evidence that autophagy in brain tissue of rats occurred in response to mitochondrial dysfunction. Authors states that dysfunctional mitochondria may further increase production of free radicals thereby inducing oxidative stress which the vast majority of in vitro and in vivo data provide strong support for being among the most important pathological events induced by AgNPs. Authors state that previous studies in the lab performed under the same conditions demonstrate increased production of ROS and peroxidation of lipids, as well as lowering of the reduced-to-oxidized glutathione ratio (GSH/GSSG) in brain of rats treated with a low dose (0.2 mg/kg b.w.) of AgNPs (Skalska et al. 2016). The autophagy pathway is regarded as the main mechanism for elimination of aberrant cell components and is persistently activated under different stress conditions, including toxic brain injury, and it is believed to be a central component of the integrated stress response (Kroemer et al.2010). Based on the results from this article, nanoparticles of silver could interfere with the protective mechanism. However, physiological levels of ATP were maintained and no AgNP-induced cellular death via the apoptotic pathway	Skalska J, Dąbrowska-Bouta B, Frontczak-Baniewicz M, Sulkowski G, Strużyńska L. (2020) A Low Dose of Nanoparticulate Silver Induces Mitochondrial Dysfunction and Autophagy in Adult Rat Brain. Neurotox Res. 38(3):650-664. doi: 10.1007/s12640-020-00239-4. Epub 2020 Jun 25. PMID: 32588355.

occurred thus compensatory mechanisms in mitochondria seems to counteract this. Therefore, this information is currently considered an interesting plausible mode of action but of limited value for the comparison with criteria for classification STOT-RE.	
Abstract: Due to their potent antibacterial properties, silver nanoparticles (AgNPs) are widely used in industry and medicine. However, they can cross the brain–blood barrier, posing a risk to the brain and its functions. In our previous study, we demonstrated that oral administration of bovine serum albumin (BSA)-coated AgNPs caused an impairment in spatial memory in a dose-independent manner. In this study, we evaluated the effects of AgNPs coating material on cognition, spatial memory functioning, and neurotransmitter levels in rat hippocampus. AgNPs coated with BSA (AgNPs(BSA)), polyethylene glycol (AgNPs(PEG)), or citrate (AgNPs(Cit)) or silver ions (Ag+) were orally administered at a dose of 0.5 mg/kg b.w. to male Wistar rats for a period of 28 days, while the control (Ctrl) rats received 0.2 mL of water. The acquisition and maintenance of spatial memory related to place avoidance were assessed using the active allothetic place avoidance task, in which rats from AgNPs(BSA), AgNPs(PEG), and Ag+ groups performed worse than the Ctrl rats. In the retrieval test assessing long-term memory, only rats from AgNPs(Cit) and Ctrl groups showed memory maintenance. The analysis of neurotransmitter levels indicated that the ratio between serotonin and dopamine concentration was disturbed in the AgNPs(BSA) rats. Furthermore, treatment with AgNPs or Ag+ resulted in the induction of peripheral inflammation, which was reflected by the alterations in the levels of serum inflammatory mediators. In conclusion, depending on the coating material used for their stabilization, AgNPs induced changes in memory functioning and concentration of neurotransmitters. **DS opinion:** Assessment by RAC in the opinion on elemental silver: *“In Dziendzikowska et al. (2021) AgNPs coated with BSA, polyethylene glycol or citrate or silver ions (Ag+) were orally administered at a dose of 0.5 mg/kg bw to 11.5-week-old male Wistar rats for a period of 28 days. Impairment of cognitive functions and behavioral disturbances which differed depending on the form of silver were reported”* Authors state that their previous results indicate that AgNPs redistributed from the initial retention organs, such as the liver, spleen, and lungs, and translocated and accumulated in the brain . Interestingly, after oral exposure, the concentration of silver was significantly higher in the hippocampus compared to the lateral and frontal cortex or cerebellum. Surprisingly, the study revealed that silver was found in an ionic form rather than as nanoparticles, suggesting the crucial role of silver ions (Ag+) in AgNPs-induced impairment of higher brain functions. Since it was still unclear whether AgNPs-induced dysfunction of memory and cognitive coordination is related to the coating material of the nanoparticles and/or the ability of the nanoparticles to release Ag+ after oral administration, the current study aimed to investigate the effect of AgNPs coating on the acquisition and maintenance of spatial memory tested in the active allothetic place avoidance task (AAPAT) and neurotransmitter levels in rat hippocampus. Additionally, the effects of particulate and ionic forms of silver on systemic toxicity and inflammatory marker levels were assessed. The study was claimed to be designed in accordance with OECD 407. Results showed an increased concentration of dopamine in the animals exposed to AgNPs(BSA) and Ag+, but not AgNPs(Cit) or AgNPs(PEG). The obtained results indicate that the action of Ag+ ions and AgNPs differs. The effect of nanosilver on cognitive functions depends on the coating material used for stabilization. A possible reason for the different behaviours of AgNPs coated with different types of materials can be the processes that the nanoparticles undergo in the gastrointestinal tract, which also include Ag+ release (probably in different amounts depending on the coating material used).Application of BSA- and PEG-coated AgNPs, as well as AgNO3 as a source of Ag+, was found to result in the impairment of cognitive functions. The substantial impairment of long-term memory, together with the impairment of memory acquisition, observed in rats	Dziendzikowska K, Węsierska M, Gromadzka-Ostrowska J, Wilczak J, Oczkowski M, Męczyńska-Wielgosz S, Kruszewski M. (2021) Silver Nanoparticles Impair Cognitive Functions and Modify the Hippocampal Level of Neurotransmitters in a Coating-Dependent Manner. Int J Mol Sci. 22(23):12706. doi: 10.3390/ijms222312706. PMID: 34884506.

from the AgNPs(BSA) group confirmed that memory formation in this group was disturbed during the formation of memory traces and their consolidation. Moreover, these rats did not improve their learning skills. Behavioural disturbances co-occurred with changes in the hippocampal concentrations of neurotransmitters as well as slight systemic changes, which indicates the pro-inflammatory nature of the orally administered AgNPs. The results of the study suggest that the mechanism of action of silver administered as ions (AgNO3 solution) differs from that of Ag+ release from AgNPs. Additionally, administration of Ag+ led to ineffective learning, causing debilitation of short- and long-term memory. On the other hand, the path length data showed no effect on locomotion. In conclusion, the article presents a study claimed to be designed according to OECD 407 and showing effects of AgNO3 on dopamine concentration and on learning and memory at a dose level of 0.5 mg/kg bw/day in Wistar rats during 28-days. This is taken into consideration in a WOE approach for the assessment of STOT-RE (see comparison with criteria).	
Silver nanoparticles (AgNPs) are one of the most widely used nanomaterials. The level of exposure to nanosilver is constantly raising, and a growing body of research highlights that it is harmful to the health, especially the nervous system, of humans. The potential pathways through which nanosilver affects neurons include the release of silver ions and the associated induction of oxidative stress. To better understand the mechanisms underlying the neurotoxicity of nanosilver, in this study we exposed male Wistar rats to 0.5 mg/kg body weight of AgNPs coated with bovine serum albumin (BSA), polyethylene glycol (PEG), or citrate, or to AgNO3 as a source of silver ions for 28 days and assessed the expression of antioxidant defense markers in the hippocampus of the exposed animals after 1 week of spatial memory training. We also evaluated the influence of AgNPs coating on neurosteroidogenesis in the rat hippocampus. The results showed that AgNPs disrupted the antioxidant system in the hippocampus and induced oxidative stress in a coating-dependent manner, which could potentially be responsible for neurodegeneration and cognitive disorders. The analysis of the influence of AgNPs on neurosteroids also indicated coating-dependent modulation of steroid levels with a significant decrease in the concentrations of progesterone and 17α-progesterone in AgNPs(BSA), AgNPs(PEG), and Ag+ groups. Furthermore, exposure to AgNPs or Ag+ resulted in the downregulation of selected genes involved in antioxidant defense (Cat), neurosteroid synthesis (Star, Hsd3b3, Hsd17b1, and Hsd17b10), and steroid metabolism (Ar, Er1, and Er2). In conclusion, depending on the coating material used for their stabilization, AgNPs induced oxidative stress and modulated the concentrations of steroids as well as the expression of genes involved in steroid synthesis and metabolism. **DS Opinion:** the abstract presents results indicating that AgNO3 influenced modulation of neurosteroid levels with a significant decrease in the concentrations of progesterone and 17α-progesterone and downregulates selected genes involved in antioxidant defense (Cat), neurosteroid synthesis (Star, Hsd3b3, Hsd17b1, and Hsd17b10), and steroid metabolism (Ar, Er1, and Er2).	Dziendzikowska K, Wilczak J, Grodzicki W, Gromadzka-Ostrowska J, Węsierska M, Kruszewski M. (2022) Coating-Dependent Neurotoxicity of Silver Nanoparticles-An In Vivo Study on Hippocampal Oxidative Stress and Neurosteroids. Int J Mol Sci. 23(3):1365. doi: 10.3390/ijms23031365. PMID: 35163290.
Over the last decade, silver nanoparticles have become an important class of nanomaterials utilized in the development of new nanotechnologies. Despite the fact that nanosilver is used in many commercial applications, our knowledge about its associated risks is incomplete. Although a number of studies have been undertaken to better understand the impact of silver nanoparticles on the environment, aquatic organisms and cell lines, little is known about their side effects in mammalian organisms. This review summarizes relevant data and the current state of knowledge regarding toxicity of silver nanoparticles in mammals, as well as the accumulated evidence for potent neurotoxic effects. The influence of nanosilver on the central nervous system is significant because of evidence indicating that it accumulates in mammalian brain tissue.	Skalska J, Strużyńska L. (2015) Toxic effects of silver nanoparticles in mammals--does a risk of neurotoxicity exist? Folia Neuropathol. 53(4):281-300. doi: 10.5114/fn.2015.56543. PMID: 26785363. Review article. The influence of nanosilver on the central nervous system is significant.
We compared the neurotoxic effects of 14 nm silver nanoparticles (AgNPs) and ionic silver, in the form of silver acetate (AgAc), in vivo and in vitro. In female rats, we	Hadrup N, Loeschner K, Mortensen A, Sharma AK,

found that AgNPs (4.5 and 9 mg AgNP/kg bw/day) and ionic silver (9 mg Ag/kg bw/day) increased the dopamine concentration in the brain following 28 days of oral administration. The concentration of 5-hydroxytryptamine (5-HT) in the brain was increased only by AgNP at a dose of 9 mg Ag/kg bw/day. Only AgAc (9 mg Ag/kg bw/day) was found to increase noradrenaline concentration in the brain. In contrast to the results obtained from a 28-day exposure, the dopamine concentration in the brain was decreased by AgNPs (2.25 and 4.5mg/kg bw/day) following a 14-day exposure. These data suggest that there are differential effects of silver on dopamine depending on the length of exposure. In vitro, AgNPs, AgAc and a 12 kDa filtered sub-nano AgNP fraction were used to investigate cell death mechanisms in neuronal-like PC12 cells. AgNPs and the 12 kDa filtered fraction decreased cell viability to a similar extent, whereas AgAc was relatively more potent. AgNPs did not induce necrosis. However, apoptosis was found to be equally increased in cells exposed to AgNPs and the 12kDa filtered fraction, with AgAc showing a greater potency. Both the mitochondrial and the death receptor pathways were found to be involved in AgNP- and AgAc-induced apoptosis. In conclusion, 14 nm AgNPs and AgAc affected brain neurotransmitter concentrations. AgNP affected 5-HT, AgAc affected noradrenaline, whereas both silver formulations affected dopamine. Furthermore, apoptosis was observed in neuronal-like cells exposed to AgNPs, a 12 kDa filtered fraction of AgNP, and AgAc. These findings suggest that ionic silver and a 14 nm AgNP preparation have similar neurotoxic effects; a possible explanation for this could be the release and action of ionic silver from the surface of AgNPs. **DS opinion:** According to the abstract, AgAc affected brain neurotransmitter concentrations (noradrenaline and dopamine). Furthermore, apoptosis was observed in neuronal-like cells exposed to AgAc. Similar findings in tests performed with14 nm silver nanoparticles suggest that ionic silver and a 14 nm AgNP preparation have similar neurotoxic effects; a possible explanation for this could be the release and action of ionic silver from the surface of AgNPs.	Qvortrup K, Larsen EH, Lam HR. (2012) The similar neurotoxic effects of nanoparticulate and ionic silver in vivo and in vitro. Neurotoxicology. 33(3):416-23. doi: 10.1016/j.neuro.2012.04.008 . Epub 2012 Apr 15. PMID: 22531227.
Silver nanoparticles (AgNPs) have been reported to penetrate the central nervous system (CNS) and induce neurotoxicity. However, there is a paucity of understanding of the toxicity of AgNPs and their effect on the blood-brain barrier (BBB) including the underlying molecular mechanism(s) of action. Such information is important for the formulation of new strategies for delivery of biological therapeutics to central nervous system (CNS) targets. Using an in vitro BBB model and mass spectrometry-based proteomics, we investigated alterations in the proteomes of brain endothelial cells and astrocytes at different time points after AgNPs exposure (24 and 48 h). Our data showed that several proteins involved in neurodisorders and neurodegeneration were significantly upregulated in endothelial cells (e.g. 7-dehydrocholesterol reductase, zinc transporters 1 and 6), while proteins responsible for maintaining brain homeostasis were significantly downregulated (e.g anti-oxidative proteins glutathione peroxidase 1 and glutathione peroxidase 4). Many inflammatory pathways were significantly upregulated at 24 h post-AgNPs exposure (C9 pathway), while at 48 h proteins involved in BBB damage and anti-inflammatory responses were upregulated (quinoneoxidoreductase1 and glutamate cysteine ligase catalytic subunit) suggesting that by the later time point, cellular protection pathways had been activated to rescue the cells from AgNPs-induced toxicity. Our study suggests that in the initial stage of exposure, AgNPs exerted direct cellular stress on the endothelial cells by triggering a pro-inflammatory cascade. This study provides detailed insight into the toxic potency of AgNPs on in vitro BBB model and adds to the understanding of the adaptive role of BBB with regards to AgNPs-mediated toxicity **DS opinion:** According to the abstract, this study investigates alterations in the proteomes of brain endothelial cells and astrocytes at different time points after AgNPs exposure (24 and 48 h). The overall conclusion is that AgNPs exerted direct cellular stress on the endothelial cells by triggering a pro-inflammatory cascade. No silver salt was included in this study thus it is difficult to conclude if effects are due to the nanoparticle itself or to silver ion released. However, it is noted that similar effects have been shown with silver salts in other studies included in this table.	Khan AM, Korzeniowska B, Gorshkov V, Tahir M, Schrøder H, Skytte L, Rasmussen KL, Khandige S, Møller-Jensen J, Kjeldsen F. (2019) Silver nanoparticle-induced expression of proteins related to oxidative stress and neurodegeneration in an in vitro human blood-brain barrier model. Nanotoxicology. 13(2):221-239. doi: 10.1080/17435390.2018.1540728. Epub 2019 Jan 9. PMID: 30623748. Using an in vitro blood-

The potent antimicrobial properties of nanoparticulate silver (AgNPs) have led to broad interest in using them in a wide range of commercial and medical applications. Although numerous in vivo and in vitro studies have provided evidence of toxic effects, rapid commercialization of AgNP-based nanomaterials has advanced without characterization of their potential environmental and health hazards. There is evidence that AgNPs can be translocated from the blood to the brain, regardless the route of exposure, and accumulate in the brain over time. As the brain is responsible for basic physiological functions and controls all human activities, it is important to assess the hazardous influence of AgNPs released from widely used nanoproducts and possible side effects of AgNP-based therapies. A number of studies have suggested that the size, shape and surface coating, as well as rates of silver ion release and interactions with proteins are the key factors determining the neurotoxicity of AgNPs. AgNPs target endothelial cells forming the blood-brain barrier, neurons and glial cells and leads finally to oxidative stress-related cell death. In this chapter, we review in detail current data on the impact of AgNPs on the central nervous system and discuss the possible mechanisms of their neurotoxic effects. **DS opinion:** According to the abstract, this is a review of current data on the impact of AgNPs on the central nervous system and the possible mechanisms of their neurotoxic effects. Secondary information on nanoparticles is not considered to add substantial information to aid in the comparison with criteria for classification STOT-RE of silver nitrate and is thus not considered further in this assessment.	Lidia Strużyńska, Joanna Skalska. (2018) Mechanisms Underlying Neurotoxicity of Silver Nanoparticles. Adv Exp Med Biol. 1048:227-250. doi: 10.1007/978-3-319-72041-8_14.
Earlier we showed that chronic administration of engineered nanoparticles (NPs) from metals, e.g., Cu, Ag, or Al (50-60 nm, 50 mg/kg, i.p. daily for 1 week) alter blood-brain barrier (BBB) disruption and induce brain pathology in adult rats (age 18 to 22 weeks). However, effects of size-dependent neurotoxicity of NPs in vivo are still largely unknown. In present investigation, we examined the effects of different size ranges of the above-engineered NPs on brain pathology in rats. Furthermore, the fact that age is also an important factor in brain pathology was also investigated in our rat model. Our results showed that small-sized NPs induced the most pronounced BBB breakdown (EBA +480 to 680 %; radioiodine +850 to 1025 %), brain edema formation (+4 to 6 %) and neuronal injuries (+30 to 40 %), glial fibrillary acidic protein upregulation (+40 to 56 % increase), and myelin vesiculation (+30 to 35 % damage) in young animals as compared to controls. Interestingly, the oldest animals (30 to 35 weeks of age) also showed massive brain pathology as compared to young adults (18 to 20 weeks old). The Ag and Cu exhibited greater brain damage compared with Al NPs in all age groups regardless of their size. This suggests that apart from the size, the composition of NPs is also important in neurotoxicity. The very young and elderly age groups exhibited greater neurotoxicity to NPs suggests that children and elderly are more vulnerable to NPs-induced brain damage. The NPs-induced brain damage correlated well with the upregulation of neuronal nitric oxide synthase activity in the brain indicating that NPs-induced neurotoxicity may be mediated via increased production of nitric oxide, not reported earlier. **DS opinion:** According to the abstract, results suggest that apart from the size, the composition of NPs is also important in neurotoxicity and that very young and elderly age groups are more vulnerable to NPs-induced brain damage. The NPs-induced brain damage correlated well with the upregulation of neuronal nitric oxide synthase activity in the brain indicating that NPs-induced neurotoxicity may be mediated via increased production of nitric oxide. Ag and Cu exhibited greater brain damage compared with Al NPs in all age groups regardless of their size. No silver salt was included in this study thus it is difficult to conclude if effects are due to the nanoparticle itself or to silver ion released. However, it is noted that brain pathology has been shown with silver salts in other studies included in this table.	Sharma A, Muresanu DF, Patnaik R et al., (2013) Size- and age-dependent neurotoxicity of engineered metal nanoparticles in rats. Mol Neurobiol 48:386–396.
Silver nanoparticles (Ag-np) are very promising engineered nanomaterials which play an important role in the world biomedical, healthcare and in general nanotechnology applications. With the most impressive effect in antibacterial and many other broad-spectrum biotechnological advantages, Ag-np in real applications is still a controversial issue. This study investigated effects of the Ag-np on hippocampal	Liu Y, Guan W, Ren G et al., (2012) The possible mechanism of silver nanoparticle impact on hippocampal synaptic

synaptic plasticity and spatial cognition in rats and followed with the research on their possible mechanism. In this study, twenty-four adult male Wister rats were randomly divided into 3 groups: control group, low-dose group (Ag-np, 3 mg/kg) and high-dose group (Ag-np, 30 mg/kg). After two-week exposure to Ag-np through the nasal administration, Morris water maze (MWM) test was performed for the spatial cognition, followed by the long-term potentiation (LTP) recording and reactive oxygen species (ROS) detection in hippocampal homogenate. Results showed that compared with the control group, both LTP and MWM were abnormal in low-dose group and high-dose group. The quantity of ROS in hippocampal homogenate was increased significantly in low-dose group and high-dose group, which may be the reason of the neural damage caused by Ag-np. **DS opinion:** According to the abstract, results showed that compared with the control group, both long-term potentiation and Morris water maze were abnormal in low-dose group and high-dose group. The quantity of ROS in hippocampal homogenate was increased significantly in low-dose group and high-dose group, which may be the reason of the neural damage caused by Ag-np. No silver salt was included in this study thus it is difficult to conclude if effects are due to the nanoparticle itself or to silver ion released. However, it is noted that brain pathology and indications of oxidative have been shown with silver salts in other studies included in this table.	plasticity and spatial cognition in rats. Toxicol Lett 209:227–231.
Use of silver nanoparticles (AgNPs) for their antimicrobial properties is widespread. Much of the previous work on the toxicity of AgNPs has been conducted in vitro or following oral or intravenous administration in vivo. Intranasal (IN) instillation of AgNPs mimics inhalation exposure and allows further exploration of the toxicity of these particles via respiratory tract exposure. The present study involved 1) single-dose exposures to assess tissue distribution and toxicity and 2) repeated exposures to assess behavioral effects of IN AgNP exposure (nominally uncoated 25 nm AgNP). AgNP deposition was localized in the liver, gut-associated lymphoid tissue, and brain. Decrease cellularity in spleen follicles was observed in treated mice, along with changes in cell number and populations in the spleen. The splenic GSH:GSSG ratio was also reduced following AgNP exposure. Expression of the oxidative stress-responsive gene Hmox1 was elevated in the hippocampus, but not cortex of treated mice, as was the level of HMOX1 protein. Mice receiving 7 days of IN exposure to 50 mg/kg AgNPs exhibited similar learning- and memory-related behaviors to control mice, except that treated mice spent significantly less time in the target quadrant of the Morris Water Maze during the acquisition phase probe trial. These findings indicate systemic distribution and toxicity following IN administration of AgNPs. **DS opinion:** Single dose (10–500 mg/kg) and repeated dose (50 mg/kg/d for 7 d, followed by a 7d wait period) studies were performed with male C57BL/6 mice via intranasal instillation. AgNP deposition was systemic including the brain. Expression of the oxidative stress-responsive gene Hmox1 was elevated in the hippocampus, but not cortex of treated mice. There was only limited evidence for effects on learning- and memory-related behaviours. No silver salt was included in this study thus it is difficult to conclude if effects are due to the nanoparticle itself or to silver ion released. However, it is noted that similar findings have been shown with silver salts in other studies included in this table.	Davenport LL, Hsieh H, Eppert BL et al., (2015) Systemic and behavioural effects of intranasal administration of silver nanoparticles. Neurotoxicol Teratol 51:68–76.
It is known that the biological half-life of silver in the central nervous system is longer than in other organs. However, the potential toxicity of silver nanoparticles (NPs) on brain tissue and the underlying mechanism(s) of action are not well understood. In this study, neurotoxicity of silver NPs was examined in rat after intragastric administration. After a two-week exposure to low-dose (1 mg/kg, body weight) or high-dose (10 mg/kg) silver NPs, the pathological and ultrastructural changes in brain tissue were evaluated with H&E staining and transmission electron microscopy. The mRNA expression levels of key tight junction proteins of the blood-brain barrier (BBB) were analyzed by real-time RT-PCR, and several inflammatory factors were assessed in blood using ELISA assay. We observed neuron shrinkage, cytoplasmic or foot swelling of astrocytes, and extra-vascular lymphocytes in silver NP exposure groups. The cadherin 1 ($2^{(-\Delta\Delta Ct)}$: 1.45-fold/control) and Claudin-1 ($2^{(-\Delta\Delta Ct)}$: 2.77-	Xu L, Shao A, Zhao Y et al., (2015) Neurotoxicity of silver nanoparticles in rat brain after intragastric exposure. J Nanosci Nanotechnol 15:4215–4223.

fold/control) were slightly increase in mRNA expression levels, and IL-4 significantly increased after silver NP exposure. It was suggest that silver NP can induce neuronal degeneration and astrocyte swelling, even with a low-dose (1 mg/kg) oral exposure. One potential mechanism for the effects of silver NPs to the nervous cells is involved in inflammatory effects. **DS opinion:** According to the abstract, AgNP can induce neuronal degeneration and astrocyte swelling via proinflammatory mechanisms. No silver salt was included in this study thus it is difficult to conclude if effects are due to the nanoparticle itself or to silver ion released. However, it is noted that neuropathology has been shown with silver salts in other studies included in this table.	
Silver nanoparticles (AgNPs) are currently used widely, however, their impact on central nervous system still remains ambiguous and needs to be elucidated. This study is performed to investigate the neurotoxicity of AgNPs and illustrate the potential molecular mechanism. Neonatal Sprague-Dawley (SD) rats are exposed to AgNPs by intranasal instillation for 14 weeks. It is demonstrated that AgNPs exposure causes cerebellar ataxia like symptom in rats, evidenced by dysfunction of motor coordination and impairment of locomotor activity. Observation of cerebellum section reveals that AgNPs can provoke destruction of cerebellum granular layer with concomitant activation of glial cells. AgNPs treatment decreases calcium channel protein (CACNA1A) levels in cerebellum without changing potassium channel protein (KCNA1) levels. The levels of silver in rat cerebellum tissue are correlated with exposure doses. In vitro experiments reveal that AgNPs treatment significantly reduces the protein and mRNA levels of CACNA1A in primary cultured cerebellum granule cells (CGCs). These findings suggest that AgNPs-induced rat motor dysfunction is associated with CACNA1A expression decrease, which reveals the underlying molecular mechanism for the neurotoxicity of AgNPs. Possible counteractions may accordingly be suggested to attenuate the unexpected harmful effects in biological applications of AgNPs. **DS opinion:** According to the abstract, intranasal instillation of AgNPs leads to cerebellar ataxia like symptoms, evidenced by dysfunction of motor coordination and impairment of locomotor activity. No silver salt was included in this study thus it is difficult to conclude if effects are due to the nanoparticle itself or to silver ion released.	Yin N, Zhang Y, Yun Z, Liu Q, Qu G, Zhou Q, Hu L, Jiang G. (2015) Silver nanoparticle exposure induces rat motor dysfunction through decrease in expression of calcium channel protein in cerebellum. Toxicol Lett. 237:112–120.

Additional oral RDT studies summarised by the Registrant or taken from the abstracts related to REACH registration dossier on Silver EC number: 231-131-3 CAS number: 7440-22-4

Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats, Williams, K. et al. 2014; Nanotoxicology, Early Online: 1 - 11.

In this study, the effects of silver exposure on intestinal microbiota were reported as examined by measuring the antimicrobial activity, real-time PCR analyses, and intestinal mucosa-associated immune responses. Sprague-Dawley rats (both male and female) were gavaged orally with discrete sizes of silver nanoparticles (10, 75 and 110 nm) and silver acetate. Doses of silver nanoparticles (9, 18 and 36 mg/kg bw/day) and silver acetate (100, 200 and 400 mg/kg bw/day) were divided and administered to rats twice daily (10 hours apart) for 13 weeks. Vehicle control groups were run concurrently. Changes in rat microbiome caused by Ag+ ions or silver nanoparticles indicate that ingestion of silver affects the gastrointestinal tract function adversely. All male rats (10/10) and eight female rats gavaged with 400 mg/kg bw silver acetate were moribund. Most of the animals dosed with 200 mg/kg bw silver acetate had severe gastroenteritis.

Dietary exposure to silver nanoparticles in Sprague-Dawley rats: Effects on oxidative stress and inflammation, Ebabe Elle, R. et al. 2013 ; Food and Chemical Toxicology 60: 297 - 301.

Subchronic toxicity of commercial silver nanoparticles (20 nm) was investigated in Sprague Dawley rats (92±3 g). Groups of 16 male rats received orally by gavage the following: tap water (control) or an aqueous solution Collargol (500 mg silver nanoparticles/kg bw/day) for 81 days. Biochemical assays included measurement of plasma lipids, liver and heart superoxide anion production ($O2^{-}$) and liver malondialdehyde levels (MDA). Antioxidant status was appraised using plasma paraoxonase activity (PON), plasma antioxidant capacity (PAC) and liver superoxide dismutase activity (SOD). Liver inflammatory cytokines TNFα and interleukin-6 (IL-6) levels and plasma alanine aminotransferase (ALT) were assayed. Orally delivered silver nanoparticles induced deleterious effects in rats that target liver and heart and led to oxidative stress and inflammation.

Details of the results:

Body weight gain were significantly lower in silver nanoparticles-fed rats (~20 %; treatment group: 4.38 ± 0.19 g/day; control group: 5.53 ± 0.16 g/day; $p < 0.05$). Food intake was significantly lower in silver nanoparticles-fed rats (~18 %; treatment group: 23.1 ± 0.3 g/day; control group: 28.1 ± 0.4 g/day; $p < 0.05$). In clinical chemistry, HDL-cholesterol was unchanged (p = 0.0931) whereas a significant increase (9.5 %; 2.31 ± 0.05 mmol/L; p = 0.0133) in plasma total cholesterol and LDL-cholesterol (30 %; 1.24 ± 0.06 mmol/L; p = 0.0028) concentrations compared with those in control group (total cholesterol: 2.11 ± 0.06 mmol/L; LDL-cholesterol: 0.95 ± 0.07 mmol/L) was observed. Triglyceridee levels were strongly reduced (41 %; treatment group: 0.55 ± 0.05 mmol/L, control group: 0.93 ± 0.07 mmol/L; p = 0.0003). The ratio of paraoxonase activity to HDL was reduced by about 15 % (treatment group: 47.4 ± 3.1; control group: 55.6 ± 2.0; p = 0.0293) in comparison to the standard group. When expressed as Units/mL of plasma, this drop is similar (16 %) but more significantly different (p = 0.0032). Plasma alanine aminotransferase activity rised (12 %, treatment group: 40.0 ± 1.6 U/L; control group: 35.7 ± 1.2 U/L; p = 0.0390) while plasma antioxidant capacity was not modified. No significant organ-weight changes of liver, heart, spleen, and kidneys were observed after 81 days. Liver superoxide anion production increased 30 % in the experimentals when compared to controls (p = 0.0067), and 41 % in the heart (p = 0.0265), whereas liver malondialdehyde levels and superoxide dismutase activity did not change. Concentrations of interleukin-6 and liver tumour-necrosis-factor-alpha in the liver of experimental rats were significantly increased by 12 % (p = 0.0344) and 9 % (p = 0.0001) respectively, in comparison with concentrations in control rats.

Toxic effects of silver nanoparticles on liver and some hematological parameters in male and female mice (Mus musculus), Heydrnejad, M.S., et al. (2015) Biol Trace Elem Res 165: 153 - 158.

In this study the toxic effect of silver nanoparticles (mean diameter: averaged 40 nm) on liver function (aspartate aminotransferase and alanine aminotransferase) and some blood parameters (total red blood cell count, haematocrit, haemoglobin concentration, total white blood cell count, and percent differential leukocytes) of male and female Balb/c mice was evaluated. Also, a histopathology of the liver was conducted. The treatment groups (18 mice/group) were given the following dose levels of the test item by gavage: 20 or 50 ppm. A control group was run concurrently. Values of red blood cells, haemoglobin and haematocrit did not vary significantly in the silver nanoparticles-treated and control groups. Serum aspartate aminotransferase level in male and female mice with 20 and 50 ppm silver nanoparticles was significantly increased compared to controls. Alanine aminotransferase level was increased in 50 ppm females only. Histological studies of liver tissue showed time-dependent increasing changes in both sexes including necrosis, hepatocyte inflammation and lymphocytic aggregation. All treated mice exposed to silver nanoparticle had minimal to moderate lymphocyte aggregation in hepatic area. The changes were more severe in mice exposed to 50 ppm AgNPs than to 20 ppm. Histopathological examinations of the male and female mice livers revealed that while there was a dose-dependent deposition of silver nanoparticles, the effect of the silver nanoparticles on male was more prominent than female.Taken together, increased serum aspartate aminotransferase and alanine aminotransferase levels indicate damage of liver tissue which was confirmed by histological examination.

Silver nanoparticles infulences rat serum metabolites and tissue morphology, Adeyemi et al. (2015); J Basic Clin Physiol Pharmacol 26(4): 335 - 361.

Groups of 5 male Wistar rats received silver nanoparticles at the dose level of 100, 1000, and 5000 mg/kg via gavage for a duration of 7 or 14 days. A vehicle control group was run concurrently. In addition, two groups also received the vehicle control and 5000 mg/kg for a duration of 21 days, respectively. Biochemical parameters (inorganic phosphate levels, sodium, potassium, creatinine, and urea) were measured, and a histopathological examination (liver and kidney) was conducted.

LOAEL (rat; male): 100 mg/kg bw/day: silver nanoparticles caused increased levels of serum creatinine at all doses and of urea (at 5000 mg/kg bw/d for 21 days) which may be attributed to impaired kidney function (LOAEL: 100 mg/kg bw/d).

Administration of silver nanoparticles to rats for 14 days produced a dose-dependent increase of serum potassium relative to the control, while sodium was decreased at 1000 and 5000 mg/kg bw/d. The inorganic phosphate level was significantly decreased at 5000 mg/kg bw/d.

Experimental Argyrosis:ultrastructural localization of silver in rat eye. Rungby, J. 1986 Experimental and molecular pathology 45, 22-30

Silver solutions were administered orally and intraperitoneally to rats and the distribution in the various tissues of the eye was studied by a combination of autometallography and light or electron microscopy. Following doses were used: oral 0.02 % silver lactate or silver nitrate solution as drinking water supply for 45 days or 0.02 % silver nitrate as drinking water supply for 3 months. IP injections totaling 12 mg silver lactate (0.66 mL of a 6 mg/mL aqueous solution daily over 3 successive days). Average daily water intake in groups 1 and 2 was 20 mL per rat (equivalent to the intake of the controls). The amount of silver salt ingested was thus apporx. 180 mg per rat (of which about 10 % was absorbed).

The results of the present study show that systemic doses of silver insufficient to produce macroscopically evident argyrosis in the rat treated for 45 days resulted in rapid and lasting deposition in many occular structures. The intensity of silver stain was most pronounced in animals treated for 3 months follwed by 15 months of recovery. These animals exhibited macroscopically visible argyrosis.

Morphofunctional cell assessment of dynamics of silver nanoparticles exposure on the rat liver Belyaeva, N.N. et al. (2014); Gigiena I Sanitariia no.1: 50-54.

Groups of 6 male outbred rats received different concentrations of silver nanoparticles stabilized with gum arabic (14.3 ± 0.05 nm)(0.0006, 0.0028, 0.023, and 0.3 mg/kg) via drinking water and different concentrations of silver sulfate 0.0005, 0.0028, 0.03 and 0.28 mg/kg . The animals were exposed 1, 3, and 6 months. A control group receiving arabic gum was run concurrently. The effect on the liver was investigated by looking at 13 cell morphofunctional indices. Out of 13 morphofunctional liver indices, the number of polyploid hepatocytes, micronecroses and discomplexation of hepatic beams was increased by the 6-month exposure to silver nanoparticles and that of cells of reticulo-endothelial system was decreased. Based on increased formation of micronecroses during 3-month exposure, the NOAEL was set at 0.0028 mg/kg for silver nanoparticles and 0.0005 mg/kg bw/d for silver sulfate. According to registrant, no conclusion can be drawn from this publication due to lack of quality, reliability and adequacy of the experimental data for the fulfilment of data requirements under REACH.

OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents), GLP study: Toxicity, distribution, and accumulation of silver nanoparticles in Wistar rats, Espinosa-Cristobal, L.F. et al. (2013) : J Nanopart Res 15: 1702

10 female Wistar rats (24 days old) / group were exposed orally via drinking water ad libitum to 0, 535 µg/mL (14 nm silver nanomaterial) and 535 µg/mL (36 nm silver nanomaterial) test substance for 55 days.

The authors stated that the silver concentrations were higher in small intestine, followed by kidney, liver, and brain. Furthermore, they stated that clinical chemistry and haematology showed altered values in blood urea nitrogen, total proteins, and mean corpuscular hemoglobin, concentration values had statistical difference in both groups (14 and 36 nm) ($p<0.05$). According to the authors, histopathology, silver concentration in tissues, clinical chemistry, and haematology tests suggest that the administration way, concentration, shape, size, presentation, administration time of silver nanoparticles used in this study, do not change significantly these values.

Details of the results

No statistical difference were found among the initial and final body weights of the treatment and control groups ($p > 0.05$). Any alteration in food consumption was not observed.

HAEMATOLOGY

- 14 nm silver nanoparticle group (day 25 after treatment): differences were found when compared treatment group and basal group in white blood cells, lymphocytes, granulocyte percent, red blood cells, haemoglobin concentration, and haematocrits ($p < 0.05$).
- 36 nm silver nanoparticle group (day 25 after treatment): differences were found when compared treatment group and basal group in white blood cells, lymphocytes, granulocytes, lymphocytes percent, red blood cells, haemoglobin concentration, haematocrits, mean corpuscular volume, and platelets ($p < 0.05$).

- silver nanoparticles of 14 and 36 nm groups were compared among haematology values and statistical differences were found in white blood cells, lymphocytes, hemoglobin concentration, and haematocrits ($p < 0.05$).

- 14 nm silver nanoparticle group (day 55 after treatment): statistical differences were obtained in haemoglobin concentration and mean corpuscular haemoglobin concentration when the treatment group was compared to the control group

- 36 nm silver nanoparticle group (day 55 after treatment): group showed differences in mean corpuscular haemoglobin concentration ($p<0.05$) when the treatment group was compared to the control group.

- 14 and 36 nm silver nanoparticle groups (day 55 after treatment): no statistical differences were found when 14 and 36 nm groups were compared at the end of treatment ($p>0.05$).

CLINICAL CHEMISTRY

- 14 nm silver nanoparticle group (day 25 after treatment): group showed alterations in the middle of the treatment when comparisons were carried out with basal values regarding to glucose, urea, total bilirubin, indirect bilirubin, pyruvic glutamic transaminase, and total protein values ($p<0.05$).
- 36 nm silver nanoparticle group (day 25 after treatment): group showed alterations in the middle of the treatment when compared with basal group in glucose, total bilirubin, indirect bilirubin, and oxaloacetic glutamic transaminase ($p < 0.05$).

- 14 and 36 nm silver nanoparticle groups (day 25 after treatment): groups were compared among them in the middle of the treatment and statistical differences were found only in uric acid ($p < 0.05$).
- 14 and 36 nm silver nanoparticle groups (day 55 after treatment): most of the clinical chemistry values in both treatment groups showed a constant concentration obtaining statistical differences when were compared with final values of control group.

- 14 nm silver nanoparticle group (day 55 after treatment): group showed a statistical significant difference in urea and total proteins

- 36 nm silver nanoparticle group (day 55 after treatment): group presents a difference in total protein ($p < 0.05$).

- 14 and 36 nm silver nanoparticle groups (day 55 after treatment): statistical difference was found in blood urea nitrogen when both treatment groups were compared in final values ($p < 0.05$).

HISTOPATHOLOGY: NON-NEOPLASTIC

- determination of silver concentration in tissues: distribution of silver in tissues was higher in small intestine, followed by kidney, liver, and finally brain. Silver concentration in small intestine was higher in the 36 nm silver nanoparticle treatment group (49.71 µg Ag/g) than in the 14 nm silver nanoparticle treatment group (41.73 µg Ag/g). Kidney showed higher concentration in 36 nm silver nanoparticle treatment group (25.57 µg Ag/g) than 14 nm silver nanoparticle treatment group (15.49 µg Ag/g). Silver concentration in liver was higher in 14 nm silver nanoparticle treatment group (18.77 µg Ag/g) than 36 nm silver nanoparticle treatment group (12.02 µg Ag/g). Finally, brain had higher silver concentration in 36 nm silver nanoparticle treatment group (5.73 µg Ag/g) than 14 nm silver nanoparticle treatment group (3.0 µg Ag/g), having a significant statistical difference among silver nanoparticle groups only in this tissue ($p < 0.05$).

- no histopathological alterations were found in the silver nanoparticle treatment groups and control groups.

- only one alteration in small intestine from one rat in 36 nm silver nanoparticle treatment group showed an infiltration of inflammatory cells into submucosal layer. Light and scanning electron micrographs of inflammatory cells from small intestine in 36 nm silver nanoparticle treatment group were obtained and the authors identified a limited area where inflammation cells were found.

Remarks from the registrant: According to the authors the study was compliant to OECD guideline 407 (1995). Regardless this claim the study is considered not valied for human health risk assessment. The study suffers from severe shortcomings with respect to the test item composition and administration, and not yield information relevant for human health risk assessment. Shortcomings are further detailed below. The authors employed self-synthesised silver nanoparticles, which they characterised by by DLS and TEM. Whereas they analysed the initial silver concentration in their drinking water solution, there is no purity analysis whatsoever, and no analytical verification during the 55 day administration, neither for particle size nor for silver concentration. Further, while the chosen route of administration may be of relevance for toxicological considerations, it is not considered conducive of a precise quantitative assessment of silver uptake. Furthermore, the study suffers of the following shortcomings: - only single dose - animals too young - females tested only (n =10) - dose administeration unclear - rationale for dose selection not clear - some raw data missing - silver tissue concentrations in exposed animals were determined, but not in controls. - time schedule of clinical observations missing - body weight and water consuption were not measured weekly, but at the start and the end of the treatment period - time schedule of food consumption measurement was not clear - determination of organ weights was incomplete - incomplete histopathology - following parameters were not completely missing from examination: detailed clinical observations, neurobehavioural examination, blood clotting time/potential, and gross pathology.

21 day oral toxicity study in rats. Rathore, M. et al. 2014: J. Nanopart. Res. 16, 2338/1-2238/12

Repeated oral dosing of silver nanoparticles (3 mg/kg bw/d) and distilled water to groups of six Wistar male animals each once a day for 21 days. Test material self-synthesized nanoparticles, diameter within the range from 10 to 25 nm (TEM). Biochemical parameters estimated: creatinine phosphokinase-MB (CPK-MB), urea, BUN, alkaline alanine transferase (ALT) and aspartate transaminase (ASP). Histopathological examination of liver, heart, brain, lungs and kidney. A significant decline in hepatic and renal function in the GNP treated group was observed as compared to SNP. GNP was found to be relatively more toxic on the lungs and SNP on the myocardial tissue as compared to SNP and GNP treatments, respectively. Interestingly, neither SNP nor GNP adversely affected the basal architecture of the brain as compared to sham. The present study demonstrated that GNP was significantly more noxious on the liver and kidney as compared with SNP.

Remarks from the registrant: The references contained in the summary entry for oral repeated dose toxicity are of no value for risk assessment purposes. All references do not fulfil the criteria for quality, reliability and adequacy of experimental data for the fulfilment of data requirements and hazard assessment purposes under REACH. Authors used self-synthesised AgNP, without further identification of additives or impurities. It remains therefore unclear whether any adverse effects were a true substance induced adverse effect, or whether adverse effects were caused by reaction products. Further shortcomings: number of animals per dose too low; only one dose group, which does not allow any does-response relationship correlation; only selected organs/parameters investigated.

21 day oral toxicity study in mice, Shahare, B. et al. (2013) Toxicol Mech Methods 23(3): 161 - 167.

Groups of 5 male Swiss mice received silver nanoparticles at different concentrations (5, 10, 15, and 20 mg/kg bw/day silver nanoparticles solution) via gavage daily for a duration of 21 days. A vehicle control was run concurrently. Test material self-synthesized nanoparticles, average diameter 10.15 nm (range from 3 to 20 nm, shape was either oval or circular (TEM). The effect of silver nanoparticles on mucosa of small intestine was investigated using light microscopy and TEM. There was a significant decrease ($p < 0.05$) in the body weight of mice in all the AgNPs-treated groups. Mice treated at a dose of 10 mg/kg showed the maximum weight loss. Effects were noted by using light microscopy as well as transmission electron microscopy. It was found that AgNPs damage the epithelial cell microvilli as well as intestinal glands. It may be hypothesized that loss of microvilli reduced absorptive capacity of intestinal epithelium and hence weight loss.

Remarks from the registrant: The references contained in the summary entry for oral repeated dose toxicity are of no value for risk assessment purposes. All references do not fulfil the criteria for quality, reliability and adequacy of experimental data for the fulfilment of data requirements and hazard assessment purposes under REACH. Authors used self-synthesised AgNP, without further identification of additives or impurities. It remains therefore unclear whether any adverse effects were a true substance induced adverse effect, or whether adverse effects were caused by reaction products. Further shortcomings: number of animals per dose too low; sex not identified; only selected organs/parameters investigated.

Oxidative Stress Following Exposure to Silver and Gold Nanoparticles in Mice, Shrivastava, R. et al. (2016): Toxicol. Ind. Health

This study investigated the toxic effects of Ag and Au NPs (1 µM and 2 µM, oral) on mouse (Swiss albino male) erythrocytes and tissues after 14 consecutive days' oral gavage exposure.

Prior to dosing, the AgNPs suspension was sonicated for 30 min in order to avoid formation of nanoparticle aggregates. Groups of 8 mice each received orally by gavage the following doses of silver nanoparticles (AgNPs): 1 µM/kg (0.1 mg/kg/2 ml) and 2 µM (0.2 mg/kg/2 ml) daily for 14 days. Control animals received drinking water. nanoparticles, Ag dispersion NP, 20 nm in size.

The results demonstrate significant increase in reactive oxygen species (ROS) and depletion of antioxidant enzyme status in erythrocytes and tissues. Hepatic and renal toxicity was evident from liver and kidney function tests. Inflammatory markers, interleukin-6 and nitric oxide synthase increased in plasma on administration following exposure to these NPs at both the doses. A more pronounced increase was noted in kidney metallothionein (MT) compared to liver MT on exposure to these NPs. Toxic potential of these NPs was further confirmed by increased 8-hydroxy-2'-deoxyguanosine levels in urine, a biomarker of DNA damage. Among the two NPs, Ag NP was more toxic at 2 µM dose compared to lower dose of 1 µM. The

study suggests oxidative stress as the major mechanism responsible for the toxic manifestations induced by Ag and Au NPs.

Silver nanoparticle-induced oxidative stress-dependent toxicity in Sprague-Dawley rats, Patlolla, A.K. et al. (2015) Mol. Cell. Biochem. 399, 257-268

Four groups of 5 rats each were orally via gavage administered silver nanoparticles (AgNPs) in water, diameter 10 nm with daily doses of 5, 25, 50, and 100 mg/kg bw for 5 days. The control group received deionized water. Blood and liver were collected 24 h after the last treatment following standard protocols. Ag-NPs exposure increased the induction of ROS, activities of the liver enzymes (ALT, AST, ALP), concentration of lipid hydroperoxide (LHP), tail migration, and morphological alterations of the liver tissue in exposed groups compared to control. The highest two doses, 50 and 100 mg/kg showed statistically significant ($p < 0.05$) increases in ROS induction, ALT, AST, ALP activity, LHP concentration, DNA damage, and morphological alterations of liver compared to control. Based on these results, it is suggested that short-term administration of high doses of Ag-NP may cause organ toxicity and oxidative stress.

Experimental argyria: a model for basement membrane studies, Walker, F. (1971): The British Journal of Experimental Pathology, 52(6), 589-593

Mechanistic study on argyria in rats. Continuous exposure to silver via drinking water (12 mmol/L) for 81 and 10 weeks in two separate experiments. Silver permanently labels many basement membranes *in vivo* and is readily detectable both by light and electron microscopy. Investigations aimed at defining this situation, with a view to developing a standard experimental model, are described. The results indicate that a suitable and convenient animal for basement membrane studies is a male Sprague-Dawley rat which has ingested 12mM-$AgNO_3$ drinking fluid for 10 weeks and is then given ordinary drinking water for a further 4 weeks. After this time no more silver is deposited and the distribution and density of the silver, with certain noted exceptions, conform to a standard pattern.According to registrant, outdated mechanistic study on argyria deposits in the rat; methodology and dosing regime poorly described in comparison with current standards.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Animal data:

The only robust information available on repeated dose toxicity of silver nitrate is a 28-day study performed in rats at doses up to 100 mg/kg bw/d. The study was performed according to the principles of GLP and according to the recommendations in OECD YTG 407 including a functional observational battery (FOB). The FOB assessments were undertaken in a manner, so the observer did not know the dose group of animals during testing. Observations were made once during the pre-dose phase (Day 7) and once weekly thereafter during (Days 3, 9, 16, and 28). Where possible, observations were performed at approximately the same time on each occasion. Animals were observed in order, from the lowest numbered animal in each cage to the highest, until all animals had been subject to assessment. This regime was employed in an effort to minimize the disparity in arousal states between animals, due to recent cage activity by the assessor. Animals were observed in the home cage for the following parameters:

Activity, aggression to cage mate, alertness, behaviour (including stereotypic behaviour and circling), excretion, gait, involuntary movements (including convulsions and tremors), posture, respiration (initially in the home cage, then throughout the observation period), restlessness, tail, vocalization.

Animals were observed upon removal from the home cage for the following parameters:

Abnormal skin colour, body tone, excessive lacrimation, excessive salivation, excretion, extensor thrust (elicited response), eyes (colour and/or protruding/exophthalmos), reactivity to handling (upon transfer to the hand and during handling, included touch response, ease of removal, and ease of handling), involuntary movements (including convulsions and tremors), respiration (initially in the home cage, then throughout the observation period), tail, vocalization

Open field measurements. Animals were placed in a circular arena for 2 minutes and assessed for the following parameters:

Activity, alertness, behaviour (including stereotypic behaviour and circling), excretion, eye closure, eyes (protruding, rubbing, squinting and/or blinking), gait, involuntary movements (including convulsions and tremors), latency to first step (sec), number of faecal boli, number of rears, number of urine pools, pelage, posture, respiration (initially in the home cage, then throughout the observation period), tail, vocalization.

Sensory reactivity to stimuli and grip strength. Qualitative observations of elicited responses were made upon removal from the home cage and in the open field. The following parameters were measured:

Approach response (a required open field measurement), auditory startle response, bar test, grasping loss, grip strength (subjective), pain response (toe pinch), palpebral (corneal tactile) reflex, pinna reflex, proprioception test (right hind leg), pupil status, pupillary response (to light), reactivity to handling (upon transfer to the hand and during handling, included touch response, ease of removal, and ease of handling), righting reflex, visual response, waxy rigidity test

Additionally, quantitative assessments were performed on Day 28 of the dosing phase. Hind limb foot splay (mm), fore limb grip strength (kg), and hind limb grip strength (kg) were recorded, as well as the average of each parameter (average of two recordings for foot splay and three recordings for grip strength).

There were no treatment related effects on the FOB and no other adverse effects observed at any dose level. The corrosive properties of the substance are apparently not expressed at or below 100 mg/kg bw/d during this exposure time.

Additional information on silver nitrate includes two published studies in rat that are old and poorly reported. In these studies, pigmentation of organs and tissues were noted at doses of 0.1% silver nitrate (60 or 89 mg/kg bw/day) and 0.25% silver nitrate (stated to be 222 mg/kg bw/d), respectively. This effect is commonly observed following repeated exposure to different silver compounds (see below). The study by Olcott (1950) also reported an increased left ventricular hypertrophy rate in rats administered silver nitrate in drinking water at 60 or 89 mg/kg bw/day. It was postulated (but not verified) that the effect was caused by hypertension. Since only a few scattered granular deposits were observed in the heart, it was further suggested that the hypertension was due to a thickening of the basement membrane of kidney glomeruli following silver deposition. The exact exposure duration is not clear from the publication but animals up to 30 months age were analysed. Extrapolating guidance values using Haber´s rule (a factor of 12) would result in very low guidance values indicating that criteria for classification and labelling would not be fulfilled. However, any conclusion based on such extrapolation must be considered with caution, especially considering the uncertain exposure duration. The Agency for Toxic Substances and Disease Registry (ATSDR) dismissed the study based on poor experimental design and inadequate reporting of methods and did not consider the study useful to predict equivalent exposure levels in humans. The DS agrees with this conclusion and the study is thus not considered for this assessment.

Data obtained with other silver substances:

The data include studies performed with nanosilver, silver acetate, silver zinc zeolite and silver sodium zirconium hydrogenphosphate.

<u>Silver acetate:</u>

According to data in the registration dossier submitted under REACH, administration of 14 mg silver acetate/kg bw/d (equal to 9 mg Ag/kg bw/d and 14 mg silver nitrate/kg bw/d) for 28 days increased the

brain levels of dopamine and noradrenaline in female Wistar rats[28]. The level of 5-hydroxytryptamine and the brain weight was not affected. The study was not performed according to any guideline or the principles of GLP and the report contains no information on clinical signs, bodyweight, water and compound intake and other investigations such as neurobehavior. Since the original report is not available to the dossier submitter, the reliability cannot be assessed. Although the effects described were noted at a dose below the guidance value for category 1 (adjusted for 28-day exposure), the information is not considered sufficient to allow for a robust comparison with criteria. Moreover, the effects noted i.e. increased brain levels of dopamine and noradrenaline, are not in isolation considered to fulfil criterion (b) in section 3.9.2.7.3 of Annex 1; *"significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell)."*, this is further discussed in 10.12.2.

There were no effects seen in the neurohistopathological investigation of mature animals in an EOGRTS which recently became available (according to the study neuropathologist the investigated brain structures included both the hippocampus and thalamus) but the study showed clear indications of developmental neurotoxicity. This as well as other effects on reproductive toxicity are discussed in detail in section 10.10. The EOGRTS was preceded by a dose-range finding study but examinations of neurohistopathological changes in parental animals were not included.

The preliminary study was performed to assist dose selection for the EOGRTS and included groups of F0 rats (12/sex) receiving doses of 4, 40, 80, 160 and 320 mg AgAc/kg bw/day via the diet. Males were treated for 29 days before pairing and until necropsy after litters had weaned (after at least 65 days of treatment) whereas females were treated for 29 days before pairing, throughout pairing, gestation and until necropsy on Day 21 of lactation. The F0 generation was checked for clinical condition, body weight, food consumption, estrous cycles, pre-coital interval, mating performance, fertility, gestation length, exposure assessment and biomarkers (ceruloplasmin activity, glutathione peroxidase (GPx) activity, copper, selenium and silver levels), organ weights and macroscopic and microscopy pathology investigations. In addition, haematology and blood chemistry investigations were made in all treated males and in females treated with 4 and 40 mg/kg bw/day. The results obtained are discussed below (parameters considered adversely affected by treatment are marked in bold text) and further details are given in section 10.10.

Biomarkers:
<u>Ceruloplasmin:</u> except for animals administered 80 mg/kg bw/day, activity decreased with increasing doses of silver acetate in F0 male and female animals on Day 12, in males on Day 43 and in females on Gestation Day (GD) 6.

<u>Glutathione Peroxidase:</u> (GPx) activity decreased in high dose female rats (37-42% lower than controls) at Day 12 and GD6. Both males and females administered 80 mg/kg bw/day showed low GPx activity.

<u>Silver:</u> blood levels generally increased with increasing dose levels. In males, mean values at Day 43 were generally similar to Day 12 values, except at 320 mg/kg/day where Day 43 was ~2-fold the Day 12 value. Values for females at Day 12 were ~2-fold higher than males at 4, 40, 80 and 160 mg/kg/day, and ~2.5 fold at 320 mg/kg/day. In females, mean values at GD 6 were generally similar to Day 12, with the exception of a ~ 2-fold increase at 320 mg/kg/day. In late gestation increases of approximately 1.6 to 3.7 fold were observed in all groups, but were not proportionate to dose.

<u>Copper</u> <u>and selenium:</u> serum levels tended to decrease with increasing dose and was more pronounced for copper. On Day 12 of treatment, mean serum copper levels were 3, 5, 8 and 12-fold lower than controls in males at 40, 80, 160 and 320 mg/kg/day, and 3, 5, 4 and 15-fold lower in females. Mean values for males on Day 43 and for females on Day 6 of gestation were broadly similar to Day 12 values for each sex, with female values remaining higher than males. For selenium, male values were generally higher than females on Day 12 of treatment, and there were dose-related reductions of up to 50% at 40, 80, 160 and 320 mg/kg/day. Values for males on Day 43 and females on Gestation Day 6 were broadly similar to Day 12. There was evidence for a dose-related decrease in copper levels in the testes for F0 males and a

[28] Hadrup, N. et al.(2012) Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats.

non-dose related increase in selenium levels in the ovaries for F0 females. There was no clear effect of treatment on the ovarian copper levels or the testis selenium levels. The significance of these changes in biomarkers are further discussed in section 10.10.5.

Four females at 320 mg/kg bw/day and two at 160 mg/kg bw/day were killed between completion of parturition and Day 2 of lactation following total litter loss with offspring either found dead, missing (presumed cannibalized) or killed for welfare reasons. Routine physical examination of males from the start of treatment up to termination and for females before pairing and during gestation did not reveal any findings that could be attributed to administration of silver acetate at any dose level. The mean **body weight gain** for males administered 320 mg/kg bw/day was markedly low (approximately 20% of controls), resulting in low mean body weight at the end of the treatment period. The overall body weight gain of high-dose F0 females during the four-week pre-pairing treatment period was low (approximately 38% of controls) with an overall **gestational body weight gain** approximately 42% of controls. High dose F0 males showed statistically significantly low mean values during the majority of **food consumption** phases and high-dose females showed periods of low food consumption during prepairing and consistently throughout gestation. At scheduled termination, mid and high-dose males showed statistically significant **changes in hematological parameters** (low hematocrit, hemoglobin level, mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume) but without dose response. **Platelet counts** for males receiving 40, 160 or 320 mg/kg bw/day were slightly but statistically significantly high. **Alkaline phosphatase activity and cholesterol** levels were increased in males and females administered 40 mg/kg bw/day and statistically significantly high in males at 160 and 320 mg/kg bw/day (except female cholesterol); at 4 mg/kg bw/day the mean cholesterol levels for males was also slightly but statistically significantly high. Without any accompanying histopathological changes besides test substance-related dark coloration of several organs (i.e., liver, cecum. rectum, pancreas, mesenteric lymph nodes,uterus, kidney, thymus, urinary, bladder and salivary gland) in males and females of several dose groups, the toxicological significance of these changes is unclear. Based on the results of this study it was concluded that dose levels of 160 and 320 mg/kg bw/day were not tolerated and thus unsuitable for the subsequent OECD 443 study.

Main study: In the extended one generation study claimed to be performed according to OECD TG 443 and the principles of GLP (unsigned version available to the DS), F0 animals were administered doses of 40, 80 or 120 mg silver acetate/kg bw/day orally in diet. Males were treated for ten weeks before pairing and up to necropsy made after litters were weaned. Females were treated for ten weeks before pairing, throughout pairing up to necropsy on Day 28 of lactation. The data for the F0 generation included clinical observations, body weight, food consumption, water consumption (by visual assessment), estrous cycles, mating performance and fertility, gestation length and parturition observations and reproductive performance. Clinical pathology (hematology and blood chemistry), analyses of thyroid hormones, blood silver, serum copper/selenium, sperm assessment, organ weight and macroscopic and microscopic pathological investigations were performed.

One male administered 80 mg/kg/day and two males administered 120 mg/kg/day died of unclear reason and one female administered 120 mg/kg/day was killed owing to a mammary lesion which was unrelated to treatment. **Body weight gain for males** administered 80 and 120 was significantly reduced at termination whereas female body weight was unaffected by treatment. Intermittent, transient effects on food intake were observed but the efficiency of food utilization for animals before pairing and for females during gestation was unaffected by treatment. At Week 10 (females) and at termination, differences in erythrocyte count, mean cell haemoglobin and mean cell volume, red cell distribution width, mean cell heamoglobin, mean haemocrit, haemoglobin concentration, platelet count were observed in males and females and white blood cell count with variable dose response and with males more affected than females. **Females administered 120 mg/kg/day showed high counts for eosinophils, monocytes and large unstained cells**. At Week 10 (females) and at termination (both sexes) the biochemistry analyses showed **high alkaline phosphatase activity, high plasma cholesterol and low potassium levels**, in part but not fully according to a clear dose-response. The only possible correlation with pathlogical findings was a higher incidence of increased extramedullary hematopoesis in males administered 120 or 80 mg/kg bw/day and an increased weight of spleen in males administered 120 mg/kg bw/d.

There were no statistically significant differences in thyroid stimulating hormone or thyroxine serum concentrations levels in any group or generation of males or females after dietary administration of silver acetate administered 40, 80 and 120 mg/kg/day when compared with controls.

In general **serum copper** and selenium levels decreased with increasing doses of silver acetate. The decreases in serum levels were not dose proportionate. The consequences of silver-induced copper deficiency for development are further discussed in section 10.10.5.

Copper Serum levels (ng/mL)

Group/ sex	F0 Week 10		F1 PND22 offspring		F0 Males Term		F1 Cohort 1A Term		F1Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1M	1287	13.8	666	21.8	1515	18.8	1091	21.3	1211	13.1
2M	470	32.6	550	47.1	390	21.6	368	16.2	398	21.5
As % Control	*37*		*83*		*26*		*34*		*33*	
3M	316	23.4	337	30.1	255	19.3	245	43.2	254	26.5
As % Control	*25*		*51*		*17*		*22*		*21*	
4M	245	39.4	383	41.8	176	19.5	217	77.0	161	33.5
As % Control	*19*		*58*		*12*		*20*		*13*	

Group/ sex	F0 Week 10		F1 PND22 offspring		F0 Females PND28		F1 Cohort 1A Term		F1Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1F	1800	12.9	875	59.7	1840	9.73	1623	16.8	1578	19.2
2F	719	22.6	458	29.4	811	29.8	869	21.5	1020	14.3
As % Control	*40*		*52*		*44*		*54*		*65*	
3F	544	21.2	363	22.1	562	30.9	576	15.8	650	27.9
As % Control	*30*		*30*		*31*		*35*		*41*	
4F	374	33.2	449	78.4	448	28.5	286	29.1	378	36.1
As % Control	*21*		*51*		*54*		*18*		*24*	

Selenium Serum levels (ng/mL)

Group/ sex	PND22		F0 Males Term		F1 Cohort 1A Term		F1 Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1M	184	13.3	456	6.90	274	21.3	320	18.4
2M	127	9.73	253	15.0	166	15.6	233	17.6
As % Control	*69*		*55*		*61*		*73*	
3M	110	21.3	219	14.6	136	13.3	224	16.2
As % Control	*60*		*48*		*50*		*70*	
4M	111	15.2	219	8.61	108	24.6	169	11.7
As % Control	*60*		*48*		*39*		*53*	

Group/ sex	F1 PND22 offspring		F0 Females PND28		F1 Cohort 1A Term		F1 Cohort 1B Term	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
1F	192	6.53	381	5.04	216	19.3	252	10.0
2F	129	17.7	315	11.6	156	12.3	221	17.1
As % Control	*67*		*83*		*72*		*88*	
3F	115	20.9	268	7.33	132	13.2	210	32.0
As % Control	*60*		*70*		*61*		*95*	
4F	109	17.1	266	9.97	98.2	13.7	154	12.4
As % Control	*57*		*70*		*45*		*61*	

Body weight-relative heart weight was high for both males and females at all dose levels and all treated groups of males had low mean absolute pituitary weights. High mean body weight-relative spleen weight was observed in animals administered 120 mg/kg/day. Females receiving 120 mg/kg/day showed low absolute and body weight-relative mean **thymus weight**. The majority of macroscopic findings at scheduled termination included abnormal colouration of tissues, including the gastrointestinal tract, kidneys, lacrimal glands, liver, harderian glands, mesenteric lymph nodes, pancreas, salivary glands, thymus, thyroids and urinary bladder. At histopathology, extracellular pigment was observed in various organs/tissues and was considered to represent deposition of test item at these sites. In general, pigment was more prominent in females, and there was not always an apparent dose response. Pigment was not associated with any inflammatory or degenerative changes. Other histopathological findings included increased extramedullary hematopoiesis observed in the spleen of males administered 80 or 120 mg/kg/day and epithelial degeneration of the glandular mucosa of the stomach in females receiving 40, 80 or 120 mg/kg/day.

White blood cell parameters at either scheduled or premature termination were high in treated females. Mean platelet counts were low in females administered 80 or 120 mg/kg/day, prothrombin times were

shorter in all treated groups of females and activated partial thromboplastin time was shorter for females administered 120 mg/kg/day.

At premature termination, the **blood chemistry** analyses in high dose animals principally showed high alkaline phosphatase activity, high alanine amino-transferase activity, high aspartate amino-transferase activity (females only), high cholesterol, high glucose, high urea (males), low creatinine, low albumin/A:G ratio, high potassium, low phosphorous levels and low sodium(females).

At scheduled termination of animals at 40 or 80 mg/kg/day the blood chemistry investigations revealed high alkaline phosphatase activity (males), high alanine amino-transferase activity (males), high cholesterol and low creatinine. Cardiac troponin levels were unaffected in females at all dose levels. For further details regarding the results above, please refer to section 10.10).

In summary, treatment with silver acetate resulted in

Parental NOAEL F0: could not be set since degeneration in stomach mucosa was observed in all treated females.

Parental NOAEL F1 adults: 40 mg/kg/day based on effects observed at 80 or 120 mg/kg/day including mortality, neurobehavioural changes (reduced activity and rearing in the arena, reduced reactivity, abnormal motor movement/gait, intramyelinic edema and neuronal and/or glial cell necrosis and F1 brain morphometry (low mean hippocampus). Changes in haematological and biochemical parameters and changes in organ weights (heart, thymus, spleen) at 120 mg/kg bw/d. It is noted that the study author did not include mortality and changes in hematological and biochemical parameters in the NOAEL set at 40 mg/kg bw/day.

There is also a new 90-day study performed with silver acetate in a different rat strain, the Wistar Han. The study was designed according to recognized guidelines and claimed to follow the principles of GLP although the version available was not yet signed. The study also included a subgroup of animals for toxicokinetic investigations.

Groups of 10 animals/dose/sex were administered doses of 0, 40 (41-42, M-F), 120 (122-126, M-F) and 320 (287-319, M-F) mg silver acetate/kg bw/day. All animals survived treatment. The bodyweight and bodyweight gain in males administered 320 mg/kg body weight/day were reduced throughout the study (14.9% and 33% lower than control at the end of the administration period). The body weight and body weight gain in low and mid dose males were mildly reduced during the first month (<5%) but recovered thereafter. The same pattern was observed in females administered 320 mg/kg body weight/day with a mean body weight up to 6.1% lower than control on Day 85 (not statistically significant) and a 14.5% lower mean body weight gain than control at the end of the administration period. There were no clear effects on body weight and body weight gain in low and mid dose females. Food consumption was reduced in high dose animals during several weeks of the study period. There were no clinical signs observed and hearing ability, pupillary reflex and static righting reflex were normal in all examined animals. Grip strength and motor activity was similar between treated groups and controls.

Some changes in absolute and/or relative organ weights were observed (epididymis, testis, brain, heart, liver, pituitary gland) but without any correlation with any histopathological findings except for deposition of silver. Black-brown pigment (ranging from minimal to moderate degree) was present in tissues from all animals at all dose levels. Affected organs included the brain (area postrema and subfornical organ), kidney, liver, lymph nodes (mesenteric, mandibular), pancreas, skin, stomach, small and large intestines, thymus, urinary bladder, harderian gland, preputial/clitoral gland, and lacrimal gland. The increased pigment observed in the spleens of animals administered 320 mg/kg body weight/day was not associated with any other tissue alterations and thus considered a non-adverse change. In the brain, pigment (minimal or mild) was noted at all dose levels in the area postrema in all treated animals for which this structure was present in section, and in the subfornical organ of four animals (2 males at 40 mg/kg body weight/day, and one female each at 40 and 320 mg/kg body weight/day) for which this structure was present in section. According to the pathologist, both structures are circumventricular organs and thus have a specialized capillary structure necessary for their function and are considered to be outside the blood brain barrier which likely explains the selective presence of the pigment at these locations.

Pigment in the area postrema was characterized by the presence of black-brown pigment in/around the blood vessel walls, highlighting these structures. In the subfornical organ, the pigment was less obvious, and was sparce and finely granular black-brown and not clearly associated with the interstitium/blood vessels.

The NOAEL is considered by the study author to be 120 mg/kg bw/d based on adverse effects on bodyweight, bodyweight gain, food consumption in males and females administered 320 mg/kg bw/d. The study author considered effects on clinical parameters minimal and non-adverse. The DS disagrees with this conclusion since levels in ALP, ALT and cholesterol are considerably increased and is an effect seen across repeated dose toxicity studies with different silver substances. Since the effects on clinical parameters in isolation are not considered a sufficient basis for the LOAEL the NOAEL at 120 mg/kg bw/d is agreed by the DS however the DS also includes the changes in clinical parameters in the justification for the LOAEL. However, these effects are not, in isolation and without accompanying clinical symptoms or histopathological findings, considered to represent “significant or severe toxicity” and were generally observed at dose levels above guidance values thus criteria for classification and labelling STOT-RE are not considered fulfilled.

Studies with nanosilver:

Specific target organ toxicity-oral route: four studies in the open literature investigating effects in rats following 28-, 42-52 and 90-days of exposure (Kim, Y.S. et al (2008), Hong, J-S., et al (2014) and Kim, Y. S. et al (2010a), respectively) and findings in dams in a study of developmental toxicity Charehsaz, M. et al (2016), exposure during days 7-20) were considered relevant for this endpoint. Bile-duct hyperplasia was observed both in the 28-day and the 90-day study both performed in the same lab and the same study author. However, bile-duct hyperplasia is an effect also seen following exposure via inhalation (below) and in the chronic toxicity/carcinogenicity study performed with silver zinc zeolite. Increased levels of ALP and cholesterol noted are effects also seen with other silver substances. Moreover, in the 90-day study observations of focal, multifocal, or lobular liver necrosis were made in males and females, but incidences were not dose-related and not observed at similar doses in the 28-day study performed by the same laboratory or in the other study performed with nanoparticles of silver (Hong, J-S et al).

According to the article, the necrosis observed was characterised as presented in the table below. Based on this information it seems that multifocal necrosis was only observed in 1/10 medium-dose males. This is further discussed in the next section.

Table 10.12.1-1

	Control	Low	Medium	High
Necrosis focal minimum	0/10 (m) 0/10 (f)	4/10 (m) 2/10 (f)	3/10 (m) 2/10 (f)	4/10 (m) 1/10 (f)
Necrosis lobular moderate	0/10 (m)	0/10 (m)	1/10 (m)	0/10 (m)
Necrosis multifocal moderate	0/10 (m)	0/10 (m)	1/10 (m)	0/10 (m)
Necrosis central vein	0/10 (f)	0/10 (f)	1/10 (f)	1/10 (f)

In the combined repeated-dose toxicity and reproduction/developmental toxicity screening test (Hong, J-S et al) a marked accumulation of silver was observed in lungs of animals administered 250 mg/kg bw. The histopathological findings included granulomatous lesions in 2 out of 10 females and cholesterol granuloma in 2 out of 10 males. According to the study authors the association between silver in the lung and granulomatous lesions is unclear and further investigations would be necessary. Although the LOAEL

and NOAEL are set at 250 mg/kg bw and 125 mg/kg bw, respectively, it is noted that the maximum tolerated dose was not reached in this study. The top dose was chosen based on salivation observed in a few (unknown number) pregnant rats during a 7-day treatment period in a dose-range finding study. However, the effect was not reproduced in the main study except for one high dose female showing transient salivation the day after gestation.

Measurements of enzyme levels (SOD, CAT, GPx and MDA) in liver and brain tissues of rats indicate that both nanoparticular and ionic forms of silver induce oxidative stress in dams (and pups) at all dose levels, with the ionic form being more potent (Charehsaz et al, IIIA, 6.8.2-10). Moreover, a mild to moderate neuronal loss and mild gliosis was observed in dams, predominantly in CA1 sector, which was categorized as a type 2 hippocampal sclerosis according to the ILAE (International League against Epilepsy) classification. Hippocampal sclerosis was observed even at the lowest dose level of 0.2 mg/kg/day. Bile duct hyperplasia was not observed in any of the animals.

The REACH registration dossier contains some additional references but considering robustness and reliability these do not seem to add further information that would be crucial for the assessment of this hazard class.

90-day studies with silver zinc zeolite:

Dogs: All dogs survived doses of 10, 50 and 250 mg/kg bw/day of silver zinc zeolite type AgION Antimicrobial Type AK . This corresponds to mg/kg/day (~ 0.2, 1.0 and 5.1 mg silver ion equivalents /kg bw). Clinical signs such as head shaking, salivation and vomiting were observed in dogs administered 250 mg/kg bw and the haematological and clinical chemistry analyses made indicated a decreased level of hemoglobin (20/8%) and an increased levels of cholesterol, phospholipids and ALP. The histopathological examinations made revealed discoloration of the pancreas and gastro-intestinal tract and histopathological changes in the kidney (increased severity of corticomedullary tubular basophilia and lymphoid infiltration, interstitial fibrosis and hyaline/cellular casts) of high dose animals. The clinical signs observed in all high dose animals throughout the study period (i.e. occasional salivation, shaking of head and vomiting) were claimed to be related to administration route (capsules) or taste or irritancy rather than to the test substance. Since these types of effects are commonly noted in dogs following capsule administration it seems realistic to assume that they represent an unspecific response to a high local concentration of the active substance. However, vomiting brings an uncertainty regarding the dose actually achieved. The level of hemoglobin was 20 % lower in high dose males compared to controls. Occasional changes in blood parameters were noted also in high dose females (reduced MCV (3%) and prolonged partial thromboplastin time (10%)) but they were not considered toxicologically significant. The effects on haematological parameters indicative of anemia such as decresed Hb, haema-, MCV, MCH, MCHC and increased synthesis of erythrocytes were also noted in the rat study (see below). According to the study author of the rat study 6.4.1(06), alterations in erythropoietic parameters (haemoglobin, haematocrit, MVC, MCH, MCHC and platelet counts) are suggestive of possible zinc toxicity. Zinc toxicity may include inhibition of heme synthesis and/or acute erythrocytic destruction but it is not possible to exclude a similar effect of silver. According to the document “Guidance on the application of the CLP criteria”, a reduction of 20 % or more in Hb concentration is considered a stand-alone criterion for haemolytic anaemia. However, since the 20% reduction was observed at a dose level of 250 mg AgION Type AK/kg bw (10% Hb reduction at 50 mg/kg bw) which is 2.5 times above the guidance values (10<C>100 mg/kg bw) for STOT-RE, category 2, it is not considered necessary to classify silver zinc zeolite for this effect. Assuming that the effect is ascribed the silver ion, this corresponds to an effect level of 5.1 mg silver ion/kg bw/d which is within the guidance value for STOT category 1 (i.e. ≤ 10 mg/kg bw/d). Nevertheless, since it is not possible to exclude the influence of other constituents in this zeolite, estimated effect levels of silver ions and extrapolated doses of silver nitrate are considered of little relevance. Especially considering that only minor effects on hematological parameters were observed with silver acetate. Therefore, this information is only included for completeness.

Rats: All rats survived treatment with 1000, 6250 and 12500 ppm AgION Antimicrobial Type AK (6.4.1(06)) except for a few single rats in each dose group that died during blood sampling. This

corresponds to estimated doses of 0.65, 2.0 and 6.0 mg silver ion/kg bw/d. The bodyweights of high dose males were reduced at 5 of the 14 study weeks but only to an extent of ± 8%. The bodyweight gain was reduced by 10% but this parameter was not statistically analysed. The bodyweights and bodyweight gains of high dose females were not affected. Administration of 6250 ppm (278/366 mg/kg bw) or higher doses resulted in effects on behaviour/activity (hypersensitivity to touch, vocalization, increased activity, aggressive behaviour), pigmentation of pancreas, thymus, the mandibular lymph node and an increase in cholesterol and alkaline phosphatase (ALP). Increased levels of erythrocytes (M) and platelets (M) were observed in high dose males and decreased levels of Hemoglobin (Hb) (15/10%), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were observed in high dose males and females. There were no statistically significant differences between the animals in the neurobehaviour, FOB or motor activity evaluations performed except for an increased touch response in high dose animals and a few minor effects observed in the neurological examinations. Nevertheless, since it is not possible to exclude the influence of other constituents in this zeolite, estimated effect levels of silver ions and extrapolated doses of silver nitrate are considered of little relevance. Therefore, this information is only included for completeness.

Silver sodium zirconium hydrogenphosphate:

Dogs: AlphaSan RC2000, a type of silver sodium zirconium hydrogenphosphate, was administered to dogs in gelatin capsules containing doses of 200, 400 and 700/1000 mg/kg bw/day during 90 days. This corresponds to estimated doses of 5, 10 and 18/20 mg silver ion equivalents /kg bw). One male and one female dog administered the highest dose died or were humanely killed prior to termination (on the day of the scheduled sacrifice and on day 42, respectively). Both dogs were emaciated. Autopsy showed enlarged salivary glands, engorged gall bladder, thickened stomach and small intestine in the male dog. Observations in the female dog included pale liver, stomach and intestines, a dark and shrunken spleen, a discolored area and dark gel on the occipital region of the brain. Both dogs had discolored contents in the intestinal tract but in the absence of histopathological changes, the study author did not consider the findings to be of toxicological significance. It is noted that similar observations were made in a four week rat study performed with a different SCAS, i.e. the reaction mass of titanium dioxide and silver chloride (JMAC powder) at a dose of 750 mg/kg bw/d. In this study the brown discoloration observed along capillary basement membranes within caecum and the small intestine (ileum) was assumed to be silver accumulation (see core dossier). The food consumption was reduced in high dose animals during the entire study period and was most pronounced in females (by approximately 30-70%). One high dose male and two high dose females, including the female sacrificed on day 42, stopped eating and had to be force-fed and/or fed moist food to stimulate the appetite. Due to the reduced food consumption, the highest dose was reduced to 700 mg/kg bw on day 43 for females and day 71 for males. Bodyweights were reduced in females and males from approximately days 14 and 49 respectively and throughout the study. Despite that the mean starting and mean final weights were the same in high dose males (compared to a weight gain of 2.7 kg in controls) and that the mean final weight of high dose females was 1.6 kg less than the mean weight at start (mean weight gain in controls was 4 kg), statistical significance was only achieved at one of the readings. Due to the few number of animals in each group, the non-statistical significant effects on bodyweight gain are yet considered toxicologically significant. The pathological examinations revealed pigmentation of intestine, liver, kidneys and hepatic inflammation in animals treated with 400 or 1000/700 mg/kg bw/day mg/kg bw. In animals treated with 1000/700 mg/kg bw/day (estimated to correspond to 28 mg silver nitrate/kg bw/d), the hepatic inflammation was accompanied with hepatic vaculolisation and necrosis, increased level of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT). The histopathological evaluation also revealed renal tubular dilation and necrosis. Thymic atrophy/reduced thymus weight was observed in 5/8 high dose animals, an effect also noted in the two generation study (see section 10.10.2) and in studies performed with other SCAS (i.e. 6.3.1(02), 6.5(06), and 6.8.2(0410.10.2)). The effects described above are considered treatment-related whereas single observations made among high dose animals (i.e. cerebral hemorraghes with thrombosis, bronchointerstitial pneumonia and thymic atrophy with lymphoid depletion) are considered to be of unclear significance. According to the study author, these findings (and also the renal effects) are likely to be secondary to dogs being debilitated. It is noted however that thrombosis (atrial) was observed also

in studies with silver zinc zeolite (6.4.1(02) and 6.5(05). Nevertheless, since it is not possible to exclude the influence of other constituents in this ion-exchange substance, estimated effect levels of silver ions and extrapolated doses of silver nitrate are considered of little relevance. Therefore, this information is only included for completeness and is not used for the comparison with criteria.

Rats: The repeated dose toxicity of a different type, AlphaSan RC5000, was investigated in CD rats. All rats survived treatment with 30, 300 or 1000 mg AlphaSan RC5000/kg bw/day and there were no clinical signs observed. This corresponds to estimated doses of 0.29, 2.9 and 9.5 mg silver ion equivalents /kg bw). Increased ALP levels, discoloration of pancreas and the Harderian gland were observed in both high and mid dose animals. According to the study author, the discoloration and effects on the Harderian gland (congestion, fibrosis and inflammatory cells) in females administered 300 or 1000 mg/kg bw was due to the blood sampling procedure. It is noted though that results of a rat study performed with silver lactate/silver nitrate (6.3.1 (04) indicate that deposition of silver in many structures of the eye may occur at systemic doses of silver that are insufficient to cause visible agyria in rats. It thus seems possible that the discoloration observed in the Harderian gland in females administered 300 and 1000 mg/kg bw respectively is due to deposition of particulate silver. Other effects noted among high and mid dose animals included an increase in red blood cells and cholesterol (males only) and changes in organ weights. The absolute weight of spleen was reduced in mid and high dose males but increased in mid and high dose females. Due to the inconsistency between sexes, this difference is not considered to be of toxicological significance. The relative heart weight was increased in high dose animals but the increase was only statistically significant in males (cardiac effects are discussed further in the section below). The absolute weights of testes and epididymides were reduced in mid and high dose animals (for epididymides this reduction was only statistically significant for the right organ) (assessed and concluded under sexual function and fertility). In the absence of histopathological findings the significance of these effects are unclear. Nevertheless, since it is not possible to exclude the influence of other constituents in this ion-exchange substance, estimated effect levels of silver ions and extrapolated doses of silver nitrate are considered of little relevance. Therefore, this information is only included for completeness and is not used for the comparison with criteria.

Chronic toxicity:

The study with silver zinc zeolite type AJ suffers from several deficiencies including lack of GLP, lack of statistical analyses for some parameters and some deficiencies in reporting (e.g. tables seem to be missing from the study report). Nevertheless, results in this study are in line with those obtained in sub-chronic toxicity studies performed with silver zinc zeolite and these deficiencies are thus not considered to invalidate use of the study for assessing chronic toxicity.

Results mice: AgION Zeomic AJ was administered in diet at daily doses of 0, 0.1, 0.3 and 0.9% corresponding to intake of “at least” 0, 67, 211 and 617 mg/kg bw/day (stated to be the minimum drug intake). This corresponds to estimated doses of 0.65, 2.0 and 6.0 mg silver ion/kg bw/d. The cumulative survival rate and the mean survival time were similar between treated and control mice. Clinical signs were not tabulated and the information on this parameter is restricted to a sentence stating that abdominal masses and corneal clouding was reported in all mice (including controls) whereas pigmentation of skin was noted in treated animals. The body weight gain was reduced in the two highest dose groups but the difference was below 10% at all measurements except for weeks 18-65 when body weight gain was reduced by 18% in high dose males compared to controls. Thereafter, the bodyweight gain was higher in high-dose animals compared to controls and at terminal sacrifice (24 months) it was within 10% of the bodyweight gain in female and male control mice. Effects on hematological parameters (decrease in HCT, Hb, MCV and increase in MCHC) were observed at the two highest dose levels. The gross pathological examinations showed decreased weights of spleen, brain and pancreas as well as pigmentation of liver and pancreas in all treated mice (see table). Thymus was not weighed. The histopathological examination revealed a statistically significant dose-response of renal cysts in males and females and increased kidney weights of high dose females and enlarged Langerhan´s islands in males. Although the frequency of renal cysts was low and no statistical significance was achieved in pair-wise comparisons, the effect is considered toxicologically significant as the increase was observed in both sexes and effects on kidneys

have been observed in other studies (6.4.1 (05-07), 6.4.2(01)). The total number of cardiac thrombi was identical between control and high dose males but it is noted that the proportion of severe cardiac thrombi was increased in high dose males. Considering that no statistical significance was achieved and that there was no similar effect in females, the observation is not given further significance in this assessment. However, it is noted that an increased frequency of thrombi was observed also in studies 6.4.1(02) and 6.4.1(05). Nevertheless, since it is not possible to exclude the influence of other constituents in this zeolite, estimated effect levels of silver ions and extrapolated doses of silver nitrate are considered of little relevance. Therefore, this information is only included for completeness.

Results rats: Rats received daily doses of 0, 0.01, 0.03, 0.1 and 0.3% corresponding to an intake of “at least” 0, 3, 9, 30 and 87 mg/kg bw/day (minimum drug intake). This corresponds to estimated doses of 0.03, 0.09 and 0.84 mg silver ion/kg bw/d. The cumulative survival rate and the mean survival time in treated animals and controls were similar. Clinical signs were not tabulated and the only information given is a sentence stating that abdominal and subcutaneous masses and corneal clouding was observed in all rats (including controls) whereas pigmentation of skin was noted in treated animals. Increased levels of liver enzymes (AST, ALT and LDH) and hepatic bile duct proliferation were observed in all treated rats indicating the liver being a target organ. The total count of white blood cells was 2-5 times higher in high dose males and females at 24 months. Effects on hematological parameters (decrease in HCT, Hb (12%), MCH and MCHC) were observed at 24 months in the two highest dose levels in females but there were no effects in males. There were no effects noted in any of the treated animals at 6 and 12 months or among animals in the lower dose groups at 24 months. The pathological examination revealed pigmentation of liver, kidneys, pancreas, stomach, lymph nodes and the choroid plexus in high-dose rats.

Comparing the effects noted among studies with different SCAS, it becomes clear that pigmentation of organs and tissues is observed in all repeated dose toxicity studies performed via the oral route. Undoubtedly, this effect is associated with the silver ion and can be expected for all silver substances releasing silver ions at a certain rate. Nevertheless, since it is not possible to exclude the influence of other constituents in this zeolite, estimated effect levels of silver ions and extrapolated doses of silver nitrate are considered of little relevance. Therefore, this information is only included for completeness.

Human data:

According to a pesticide re-registration document for silver prepared by US EPA (1992), excessive industrial and/or medicinal exposures to silver have been associated with arteriosclerosis and lesions of the lungs and kidneys.

Exposure to industrial dusts containing high levels of silver nitrate and/or silver oxide may cause breathing problems, lung and throat infections and abdominal pain. Skin contact with certain silver compounds may cause mild allergic reactions such as rash, swelling and inflammation in sensitive people (6.12.2(02)).

Another document on silver prepared by US EPA Integrated Risk Information System (IRIS) (6.12.2(03) refers to a publication by Gaul and Staud (1935) reporting 70 cases of generalized argyria following organic and colloidal silver medication, including 13 cases of generalized argyria following intravenous silver arsphenamine injection therapy. The authors concluded that argyria may become clinically apparent after a total accumulated i.v. dose of approximately 8 g of silver arsphenamine. The document states that the authors of a 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 body accumulates silver throughout life, it is theoretically possible that amounts less than this (for example, 4 g silver arsphenamine) can result in argyria. Therefore, based on cases presented in 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 to be a minimal effect level.

Another reference included is Blumberg and Carey (1934) who reported argyria in an emaciated chronically ill (more than 15 years) 33-year-old female (32.7 kg) who had ingested capsules containing 16 mg silver nitrate three times a day over a period of 1 year (about 30 mg silver/day) for alternate periods

of 2 weeks. The authors noted that this marked argyremia was striking because even in cases of documented argyria, blood silver levels are not generally elevated to the extent observed (0.5 mg/L). Normal levels for argyremic patients were reported to range from not detected to 0.005 mg Ag/l blood. Heavy traces of silver in the skin, moderate amounts in the urine and feces, and trace amounts in the saliva were reported in samples tested 3 months after ingestion of the capsules was stopped. However, despite the marked argyremia and detection of silver in the skin, the argyria at 3 months was quite mild. No obvious dark pigmentation was seen other than gingival lines which are considered to be characteristic of the first signs of argyria. The authors suggested that this may have been the case because the woman was not exposed to strong light during the period of silver treatment. The US EPA concludes that this study is not suitable to serve as the basis for a quantitative risk assessment of silver because it is a clinical report on only one patient of compromised health. Furthermore, the actual amount of silver ingested is based on the patient's recollection and cannot be accurately determined.

The last case referred to in the IRIS document was reported by East et al. (1980) and is also presented in 6.12.2(04). The article describes argyria diagnosed in a 47-year previously healthy woman (58.6 kg) who had taken excessively large oral doses of anti-smoking lozenges containing silver acetate over a period of 2.5 years. No information was provided as to the actual amount of silver ingested. Symptoms of argyria appeared after the first 6 months of exposure. Based on whole body neutron activation analysis, the total body burden of silver in this female was estimated to be 6.4 (plus or minus 2) g. Both the total body burden and concentration of silver in the skin were estimated to be 8000 times higher than normal. In a separate 30-week experiment, the same subject retained 18% of a single dose of orally-administered silver, a retention level much higher than that reported by other investigators. East et al. (1980) cited other studies on this particular anti-smoking formulation (on the market since 1973) which demonstrated that "within the limits of experimental error, no silver is retained after oral administration." However, this may not hold true for excessive intakes like that ingested by this individual. The US EPA concludes that the study is not suitable to serve as the basis for a quantitative risk assessment.

The article presented in 6.12.2(05) describes a case where clinical signs including taste and smell disorders, vertigo and hypaesthesia occurred in a patient using a stick of silver nitrate (containing 0.53 g $AgNO_3$) daily over a nine year period to treat the oral mucosa. This study is further discussed in the section on neurotoxicity.

Another case report describes blue-gray discoloration of skin in a 58 year old man who had treated himself with a colloidal silver solution that was made at home using a 38000 Volt generator, 100% pure silver coins and distilled water (6.12.2(06)). The man drank 8 fluid ounces (~2.4 dl) every hour from 8 AM to 8 PM for four days without any intake of any other food or beverages. Four weeks after self-treatment, a bluish appearance to the oral mucosa that progressed to involve the face, trunk and extremities. Examination of the patient revealed a diffuse blue-grey coloration of the skin which was most pronounced in the sun-exposed areas of forearm, hands, face, neck and the “V” of the chest. Discoloration was also noted in the lunulae, sclera, and conjunctivae of the eyes and spotty blue macules were evident on the oral mucosa of the soft palate. Histopathological examinations of biopsies from the forearm revealed fine, minute, round, brown/black granules deposited primarily in the basement membrane around the eccrine glands and to a lesser extent in the fibrous sheath of the pilo-sebaceous units, pilo-erector muscles, dermal elastic fibres and arteriolar walls. The increased discoloration in the sun exposed tract is explained by the combined effect of sun-induced reduction of colorless silver compounds to elemental silver and an increased melanin production due to silver stimulated melanocyte tyrosinase activity.

A case of fatal renal and hepatic failure is described in 6.12.2(07). The article describes the course of disease in a patient that underwent silver nitrate instillation in the renal pelvis for treatment of chyluria. Since the instillation was completed at a separate hospital, the authors could not confirm the dose administered to this patient. Within 24 hours of dosing the patient developed severe renal and hepatic failure despite given N-acetyl cysteine in view of acute toxic hepatitis and placed on haemodialysis for renal failure. The case was further complicated by development of epistaxis that required post-operative ventilation support. Although the patients’ general condition and liver function tests improved by the type of dialys used, the patient died from cardiorespiratory arrest (probably caused by pulmonary embolism or aspiration pneumonia) approximately 48 hours after extubation and beginning oral feeding.

A summary of the toxicity of silver has been prepared for the Oak Ridge Reservation Environmental Restoration Program. It is stated in the document that besides cases of localised or generalised forms of argyria, accidental or intentional ingestion of large doses of silver nitrate caused corrosive damage to the gastrointestinal tract, abdominal pain, diarrhea, vomiting, shock, convulsions and death. The estimated fatal dose of silver nitrate is ≥ 10g, but recoveries have been reported following ingestion of larger doses.

Acute irritation of the respiratory tract can occur from inhalation of silver nitrate dust, but generally only at concentrations that produce argyria. One case report described severe respiratory effects in a worker who had become ill 14 hours after working with molten silver ingots. In a study referred to (Rosenman 1979), 30 workers were exposed to silver nitrate and silver oxide dusts for periods of less than one year to greater than ten years. Twenty five individuals experienced respiratory irritation (sneezing, stuffiness, running nose or sore throat) at some time during their employment. Twenty of thirty workers reported coughing, wheezing, chest tightness and abdominal pain; the latter finding was closely correlated with blood silver levels. Granular silver-containing deposits, observed in the conjunctiva and cornea of 20/30 workers, correlated with duration of employment. Some of the workers reported decreased night vision. The eight hour time weighted average exposure (determined 4 months prior to the study) was in the range 0.039 to 0.378 mg silver/m3 for this subpopulation.

Decreased night vision was also reported in a group of workers manufacturing metal silver powder (Rosenman et al 1987). Increased excretion of the renal enzyme N-acetyl-β-D-glucosaminidase and decreased creatinine clearance seen in these workers may indicate an impaired kidney function however since the same workers were exposed to cadmium which is a known nephrotoxin, the effect cannot with certainty be ascribed to silver. Chronic exposure to silver for reclamation workers exposed to silver and insoluble silver compounds, revealed conjunctival and corneal argyria in 21 and 25% of the workers respectively. Many also exhibited internal nasal-septal pigmentation. Examination of liver enzyme levels for silver-exposed and non-exposed workers revealed no significant differences. Ocular damage has been reported from application of solutions containing >2% silver nitrate. Corneal opacification may be so severe as to cause blindness.

Application of silver nitrate to gingival may result in necrotizing ulcerative gingivitis. The document further states that case histories indicate that dermal exposure to silver or silver compounds for extended periods can lead to generalised skin discoloration and that mild allergic responses attributed to dermal contact with silver or silver compounds have been reported (6.12.2(08)).

A risk benefit assessment of silver products for medical indications was performed by the US Food and Drug Administration (6.12.5(01)). It is stated in the article that burn treatment with silver nitrate can cause methemoglobinemia, hydrochloridemia, hyponatremia and eschars that adhere to dressings. Silver suladiazine used to replace silver nitrate in this type of treatment may cause leucopenia and nephrotic syndrome rarely. It also states that there is a potential risk for the developing fetus when pregnant women use silver products.

The results of a case-control epidemiology study suggested (after adjustment for confounding factors) some association between maternal exposures to 0.001 mg/L of silver in drinking water and some increase in fetal developmental anomalies (ear, face and neck). However, the authors of the epidemiologic study recognized that there are inferential limitations to epidemiologic studies and that further research is needed to explore these findings. The authors of the risk-benefit assessment concluded that the lack of established effectiveness and potential toxicity of these products should be emphasized. The risk was considered to exceed the unsubstantiated benefit for over the counter silver-containing products.

Argyria is a permanent discoloration of skin and so far, antidote treatment (such as depigmentation creams, hydroquinone, dermal abrasion or chelation therapy with British antilewisite or D-penicillamnine) appears to be without effect (6.12.2(06)).

According to the CLP guidance *"Generally, adequate, reliable and representative data on humans (including epidemiological studies, scientifically valid case studies as specified in this Annex or statistically backed experience) shall have precedence over other data."*. The data referred to above is not considered to represent reliable information. The information available in each report is very limited

and reliable information on the exact exposure level and exposure situation is rarely available. Moreover, most reports include single or a few cases and there is no information on co-exposures to confounding factors. Therefore, this information is considered to support the observation in animal studies that silver can deposit in different organs and tissues, but it is not considered sufficiently robust to allow for a meaningful comparison with classification criteria.

Specific target organ toxicity-dermal route: two 90-day studies are available from the same study author (Korani 2011 and 2013, respectively) both investigating effects in guinea pigs dermally exposed to 100, 1000, and 10000 ppm (µg/ml) nanoparticles of silver or the positive control silver nitrate (100 µg/ml).

In the first study methods similar to those described in OECD Test Guideline 411 were used but no details are given regarding body weight, food/water consumption, ophthalmoscopic examination, haematology, clinical chemistry, urinalysis, sacrifice and organ weights. The histopathological analyses made on samples from skin, liver and spleen showed significant dose-dependent changes (inflammation in skin, muscle, liver and spleen, increased levels of Langerhans cells as well as decreased epidermis and dermis thickness) in all treated animals as well as in animals treated with silver nitrate. The effects were noted at all dose levels, i.e. from 100 µg/ml (according to the article corresponding to 0.1 mg/kg), which is far below the guidance value of 20 mg/kg bw/d and effects are thus compared with criteria for classification in the next section.

Korani (2013) study is claimed to be performed according to OECD TG 411 and with a focus on heart, kidney and bone. According to information stated in the registration dossier (original study not available to the DS), there were no mortalities, no clinical signs, no effects on bodyweight or food/water consumption but non-neoplastic histopathological observations were made. No other parameters were reported. The registration dossier states that there was a close correlation between dermal exposure and tissue levels of silver nanoparticles increasing dose-dependently with the following ranking: kidney>muscle>bone >skin>liver> heart> spleen. Toxic responses of bone (based on samples from 27 animals) included abnormal inflammatory responses in all treated groups and osteoclasts were formed in these animals in a dose dependent manner. Separated lines and marrow space narrow were observed in three different dose levels of silver nanoparticles when the alterations were compared with negative group. Toxic responses of the heart (based on samples from 26 animals) included abnormal changes in dose groups as well as silver nitrate group. However, four major signs of toxicity (inflammation, presence of clear zone around nucleus, cardiocyte deformities, congestion and hemorrhage) were magnified in the high dose group. Increased dermal dose of silver nanoparticles caused cardiocyte deformity. Toxic responses of heart (based on samples from 28 animals) was stated to include six major toxic responses classified as inflammation, gluomeral adhesion to Bowman’s capsule, proximal convoluted tubule degeneration, capsular thickening, membranous thickening and increased mesangial cells. Inflammatory reactions and glomeral adhesion to Bowman's capsule were identified in all dose groups. These reactions were magnified in a dose-dependent manner. Besides these toxic reactions, increased mesangial cells, increased membranous thickening and increased capsular thickening were also detected. The highest levels of degeneration proximal convoluted tubule and distal convoluted tubule were seen in the middle and high-dose groups. The effects above occurred at doses below the guidance values for STOT-RE and are thus compared with criteria for classification in the next section.

Specific target organ toxicity-inhalation route: Three studies are available in the REACH registration dossier (Sung, J. H. et al. (2008), Ji, J.H. et al. (2007) and Stebounova, LV. et al. (2011)). The information is summarised by the registrant and original data has not been assessed by DS.

The 90-day study by Sung et al, performed with doses 49, 133 and 515 µg silver nanoparticles/m^3 did not show any clinical signs, treatment-related mortality, significant dose-related changes in body weight or weight gain, no significant differences in food consumption, haematology (except for the percent of aggregation in the high-dose females), in the blood biochemical parameters or in organ weights.

Minimal bile-duct hyperplasia was identified in 0/10, 0/10, 1/10, and 4/9 of the control, low, middle, and high dose males, respectively and in 3/10, 2/10, 4/10, and 8/10 of the control, low, middle, and high dose females, respectively. Moreover, single-cell hepatocellular necrosis, characterised by increased cellular eosinophilia and shrunken condensed nucleo, was noted in 3/10 high dose females and one high dose female exhibited moderate bile-duct hyperplasia with concurrent moderate centrilobular fibrosis, minimal single-cell hepatocyte necrosis, mild pigment accumulation, and moderate multifocal necrosis. A high incidence of minimal alterations was observed in lungs including some chronic alveolar inflammation, a mixed cell perivascular infiltrate, and alveolar macrophage accumulation in high dose male and female animals when compared with the controls. Histopathological changes in kidneys and in the heart were noted both in treated animals and in controls.

The 28-day study by Ji, et al. (2007) was conducted in rats exposed to 0.48 µg/m^3, 3.48 µg/m^3 or 61.24 µg/m^3 silver nanoparticles for 28 days. According to information in the REACH registration dossier, there were no clinical signs of toxicity or mortality, no significant treatment-related changes in body weight, food consumption, haematology values (except for an increase of neutrophils and eosinopils in low-dose females and an increase in mean corpuscular haemoglobin (MCH) in middle-dose females), no clear effects on clinical chemistry parameters (including alkaline phosphatase, gamma-glutamyl transpeptidase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase), organ weights and no histopathological changes considered related to treatment. It is noted, though, that the liver analyses revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle and high dose groups, respectively. For female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high dose group and one case among the female rats in the high dose group. Since the guidance values (adjusted for 28-day exposure) for classification is ≤ 0.06 mg/litre/6h/day for category 1 and 0,06 < C ≤ 0,6 for category 2 (dust/mist/fume), the high dose of 61.24 µg/m^3 (6.1 × 10^{-5} mg/dm^3 and 9.6× 10^{-5}) is below the guidance value for category 1. This is further discussed in the next section.

The third study by Stebounova, LV. et al. (2011) investigated effects in mice exposed to 3.3 mg nanoparticles/m^3 for 10 days. According to the registrant, results showed minimal inflammatory response or toxicity and bioaccessibility testing indicated that nanosilver does not dissolve in solutions mimicking the intracellular or extracellular milieu.

Table 68: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days [if adequate, otherwise please delete]

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Not relevant, see section 10.12.2				

10.12.2 Comparison with the CLP criteria

The most robust substance-specific data available for silver nitrate is a 28-day study in rat. There were no adverse effects observed at doses up to 100 mg/kg bw/d but several of the parameters required for a robust assessment of repeated dose toxicity are not included in this type of study and since the top dose of 100 mg/kg bw/d is below the upper guidance value 300 mg/kg bw/d (adjusted for 28-day exposure), the negative study is not conclusive for classification. In a published study investigating developmental toxicity of nanoparticles of silver and of silver nitrate, minimal hepatocellular vacuolation was seen in the livers of treated adult dams (IIIA, 6.8.2-10) and similar effects were noted in studies with nanoparticles (i.e., bile duct hyperplasia in two studies from the same laboratory and focal, multifocal or lobular liver necrosis in a 90-day study).

Annex 1: 3.9.2.7.3 states *"Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, haematology, clinical chemistry, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:*

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites.

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell).

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters.

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration."

Of the effects seen in liver and bile duct, only necrosis is considered to fulfil the CLP criterion (e) for STOT RE however as shown in Table 10.12.1-1 in the previous section, multi-focal necrosis was only observed in a medium-dose male, otherwise almost all incidences of necrosis were characterised as minimal focal and were observed in males without dose-response. Consequently, although indicative of liver injury, the adversity of the histological changes is not considered sufficient to fulfil criteria for classification despite occurring at doses below the guidance value for STOT-RE.

Pigmentation of organs and tissues are observed in published studies with silver nitrate and in repeated dose toxicity studies performed with other silver substances. This well-known effect of silver ions was discussed in the context of classification and labelling during the 35th meeting in the Committee for Risk Assessment (RAC) since the DS considered classification and labelling justified on the basis that irreversible deposition of a heavy metal in organs and tissues should be regarded as an undesirable effect. However, the meeting did not consider the effect to fulfil criteria for classification:

"The precipitation of a heavy metal in organisms is an irreversible bioaccumulative process. Since the human health consequences are not known in the case of silver, it is uncertain whether this effect fulfils the severity criterion described in the CLP Guidance."

Argyria was again discussed in RAC for the classification and labelling of silver, now with a particular focus on a more recent review by Mota, L.and Dinis-Oliveira, R.J. (2021)[29]. This document reviewed the state of the art regarding pathophysiology, diagnosis, treatment, and relevant clinical and forensic features of argyria. It refers to argyria as an inert silver deposition in tissues. Discolouration develops following ultraviolet exposure since silver ions undergo photoreduction to atomic silver (Ag^0), which can be oxidised to low-solubility and chemically stable compounds such as silver sulfide (Ag_2S) and silver selenide (Ag_2Se). In the eye, silver deposits exhibit a clear preference for Descemet's membrane along with many other ocular structures. There was no firm consensus that ocular argyrosis resulted in a

[29] Mota, L.; Dinis-Oliveira, R.J. Clinical and Forensic Aspects of the Different Subtypes of Argyria. J. Clin. Med. 2021, 10, 2086. https://doi.org/10.3390/jcm10102086

functional disturbance of the eye, silver deposition would seem to be innocuous in most cases though the reviewers acknowledged that more data is required to fully substantiate this claim. Therefore, the previous conclusion remained. Since no additional data is available for this CLH report, the DS respects the recent opinion on the data and no classification is proposed for silver deposition.

Mortality was observed in females in both the preliminary and the main study with silver acetate in Sprague Dawley rats. However, effects occurred almost exclusively at levels above guidance values (i.e., at doses of 160 and 320 mg/kg bw/d in the preliminary study and at 120 mg/kg bw in the main study (one male died at 80 mg/kg bw/d[30]). All animals survived treatment with 40, 120 and 320 mg silver acetate/kg bw/d in the 90-day study performed with Wistar Han rats. Overall, the DS does not consider the mortality observed to fulfil criteria for classification STOT-RE.

Changes in haematological and biochemical parameters (both dose-related and non-dose related) were observed in the preliminary and the main EOGRTS as well as in the 90-day study. In the main EOGRTS and in the 90-day study, a slightly higher incidence and/or increased incidence of extramedullary haematopoiesis was observed in males (at 120 and 80 mg/kg bw/d in the EOGRTS) and in females (at 320 mg/kg bw/day in the 90-day study). There was no reduction in Hb at or above 20% in any of the studies. Considering that effects were generally mild and observed mainly at doses comparable to or above the upper guidance value for STOT RE category 2, the effects on haematological parameters and the effects on spleen noted at 80 mg/kg bw/d in males (12 minimal/24 compared to 6/25 in controls, 2/24 marked compared to 0 in controls) are not considered sufficient to fulfil criteria for classification. Some changes in organ weights were observed in all three studies with silver acetate but changes were generally not dose-related and/or changes above 10% were only observed at doses above guidance values. Degeneration in stomach mucosa was observed in all treated females in the EOGRTS performed with silver acetate. Since it was classified as minimal at doses below guidance values and only considered slight in females treated with 120 mg/kg bw/d, this effect is not considered to fulfil criteria for classification.

Reduced haemoglobin levels were also noted in dogs treated with silver zinc zeolite. In the guidance document on haemaolytic anemia prepared within the European Chemicals Bureau (document ECBI/07/03 Add. 11) and in the Guidance to Regulation (EC) No 1272/2008, a reduction of 20 % or more in Hb concentration is considered to be a sufficient stand-alone criterion for haemolytic anaemia. Based on silver content and the estimated release of silver ions at conditions assumed to mimic physiological conditions, this dose corresponds to 5.1 mg silver ion equivalents/kg bw (corresponding to 7.9 mg silver nitrate/kg bw) which is below the guidance value for STOT-RE 1 ($C \leq 10$ mg/kg bw/d). Nevertheless, it is not possible to conclude from the data available if the effect is due to the presence of Zn, Ag, the zeolite or a combination of the individual constituents. Therefore, the effect cannot be unequivocally assigned the silver ion and is not used as evidence to classify silver nitrate for STOT RE.

Likewise, other effects noted in studies with silver zinc zeolite, (i.e. hematoloical parameters, histopathological changes in the kidneys, effects on behaviour/activity) and in studies with silver sodium hydrogen zirconium phosphate (i.e. hepatic inflammation with vacuolisation and necrosis, increased liver enzymes, histopathological changes in kidneys with renal tubular dilation and necrosis, thymic atrophy/reduced thymus weigh) occurred at estimated doses of silver nitrate within guidance value range for classification. However, it is not possible to exclude a toxicological impact of other constituents in silver zinc zeolite and silver sodium hydrogen zirconium phosphate (e.g zeolites are known to cause kidney effects[31] and Zn has effects on haematological parameters) thus effects cannot be unequivocally assigned the silver ion and are not used as evidence to classify silver nitrate for STOT RE.

Nanoparticles and silver nitrate induce oxidative stress and hippocampal neuronal loss in dams already at a low dose level of 0.2 mg/kg (nanoparticles) and 20 mg/kg (silver nitrate) after a 14-day exposure during pregnancy (Charehsaz et al., 2016). Neuronal loss is permanent and thus considered to be a significant

[30] Since silver acetate and silver nitrate have similar molar mass, doses are comparable.

[31] Human & Environmental Risk Assessment on ingredients of European household cleaning products, Zeolite A represented by CAS Number 1344-00-9 (Sodium Aluminium Silicate) and by CAS Number 1318-02-1 (Zeolites), Version 3.0, January, 2004.

effect fulfilling criterion (g) as it occurred within the Guidance value range for STOT RE 1. Clinical signs indicative of neurotoxicity (i.e., hypersensitivity to touch, vocalization, increased activity, aggressive behaviour) were observed in rats exposed to silver zinc zeolite (corresponding to a dose of 3.1 mg silver nitrate/kg bw) but they cannot be unequivocally ascribed the silver ion (and not other constituents such as zinc) and is not used as evidence to classify silver nitrate for STOT RE.

There were no findings in the histopathological examinations made on brain tissue, neither in the 28-day study (Kim 2008) nor in the 90-day study (Kim 2010a) on silver nanoparticles, therefore the dossier submitter did not consider the effects noted in a single publication (Charehsaz et al., 2016) sufficient evidence that criteria for classification are fulfilled. Increased levels of dopamine and noradrenaline were noted in a non-guideline, non-GLP study in female rats at a dose of 14 mg silver acetate /kg bw/d (equal to 9 mg Ag/kg bw/d and 14 mg $AgNO_3$/kg bw/day). Although the effects were noted at a dose below the guidance value, the information available is not considered sufficient for a robust comparison with criteria. Moreover, the effects are not considered stand-alone to fulfil criterion (b) in section 3.9.2.7.3 of Annex 1; *"significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell)."*

However, from a brief investigation of the published scientific literature, there are several studies (1-14 below) suggesting that the size, shape and surface coating, as well as rates of silver ion release and interactions with proteins are some of the key factors determining the neurotoxicity of AgNPs. AgNPs target endothelial cells forming the blood-brain barrier, neurons and glial cells leading to oxidative stress-related cell death. Since these studies were performed with a specific scientific aim, each study focusing on certain aspects of brain pathology such as up or downregulation of certain genes, the studies rather contribute to an understanding of the mode of action behind brain pathology than provide clear evidence of neurotoxic effects. Moreover, since most studies only include nanoparticles of silver without any concurrent investigation of silver salts, it is difficult to conclude in some studies if effects are specific for the nanoparticle or due to silver ions released. Nevertheless, the overall picture is considered to indicate that nanosilver as well as different silver compounds are distributed to the brain and cause damage in different brain structures and this is also clinically manifested in test animals in some of the studies (for short summaries of the studies, please refer to table 67).

1. Lansdown, A. B. G. (2007) Critical observations on the neurotoxicity of silver. Crit Rev Toxicol. 37(3):237-50. doi: 10.1080/10408440601177665. Available evidence from experimental animal studies and human clinical reports fail to unequivocally establish that silver enters tissues of the central nervous system or is a cause of neurotoxic damage.

2. Dietl HW, Anzil AP, Mehraein P. (1984) Brain involvement in generalized argyria. Clin Neuropathol. 3(1):32-6. PMID: 6705320. Single case report. Cutaneous argyria in a 59-year-old woman with manic depressive psychosis. At autopsy progressive glial changes and cellular gliosis were evident in many areas of the brain. Silver deposits noted in the brain.

3. Skalska J, Dąbrowska-Bouta B, Frontczak-Baniewicz M, Sulkowski G, Strużyńska L. (2020) A Low Dose of Nanoparticulate Silver Induces Mitochondrial Dysfunction and Autophagy in Adult Rat Brain. Neurotox Res. 38(3):650-664. doi: 10.1007/s12640-020-00239-4. Epub 2020 Jun 25. PMID: 32588355. Adult rats were exposed to a low dose (0.2 mg/kg b.w.) oral administration (once daily via a gastric tube) of AgNPs (citrate-stabilised, 10nm) or silver citrate (as a source of Ag+) for 14 days. Analysis of brain sections taken from cerebral cortex and hippocampus was performed by TEM. Some evidence presented that autophagy in brain tissue of rats occurred in response to mitochondrial dysfunction.

4. Dziendzikowska K, Węsierska M, Gromadzka-Ostrowska J, Wilczak J, Oczkowski M, Męczyńska-Wielgosz S, Kruszewski M. (2021) Silver Nanoparticles Impair Cognitive Functions and Modify the Hippocampal Level of Neurotransmitters in a Coating-Dependent Manner. Int J Mol Sci. 22(23):12706. doi: 10.3390/ijms222312706. PMID: 34884506. AgNPs coated with BSA (AgNPs(BSA)), polyethylene glycol (AgNPs(PEG)), or citrate (AgNPs(Cit)) or silver ions (Ag+) were orally administered at a dose of 0.5 mg/kg b.w. to 11.5-week-old male Wistar rats for a period of 28 days. This study evaluated the effects on cognition, spatial memory functioning, and neurotransmitter levels in rat hippocampus. It found

impairment of cognitive functions and behavioral disturbances which differed depending on the form of silver investigated.

5. Dziendzikowska K, Wilczak J, Grodzicki W, Gromadzka-Ostrowska J, Węsierska M, Kruszewski M. (2022) Coating-Dependent Neurotoxicity of Silver Nanoparticles-An In Vivo Study on Hippocampal Oxidative Stress and Neurosteroids. Int J Mol Sci. 23(3):1365. doi: 10.3390/ijms23031365. PMID: 35163290. 11-week-old male Wistar rats were exposed to 0.5 mg/kg body weight of AgNPs coated with bovine serum albumin (BSA), polyethylene glycol (PEG), or citrate, or to AgNO3 as a source of silver ions for 28 days. Results showed that AgNPs disrupted the antioxidant system in the hippocampus and induced oxidative stress in a coating-dependent manner.

6. Skalska J, Strużyńska L. (2015) Toxic effects of silver nanoparticles in mammals--does a risk of neurotoxicity exist? Folia Neuropathol. 53(4):281-300. doi: 10.5114/fn.2015.56543. PMID: 26785363. Review article. The influence of nanosilver on the central nervous system is significant.

7. Hadrup N, Loeschner K, Mortensen A, Sharma AK, Qvortrup K, Larsen EH, Lam HR. (2012) The similar neurotoxic effects of nanoparticulate and ionic silver in vivo and in vitro. Neurotoxicology. 33(3):416-23. doi: 10.1016/j.neuro.2012.04.008. Epub 2012 Apr 15. PMID: 22531227. Compared the neurotoxic effects of 14 nm silver nanoparticles (AgNPs) and ionic silver, in the form of silver acetate (AgAc), in vivo over 28 days and in vitro. There were perturbations in neurotransmitters (dopamine, 5-hydroxytryptamine, noradrenaline). Apoptosis was observed in neuronal-like PC12 cells exposed to AgNPs and AgOAc.

8. Khan AM, Korzeniowska B, Gorshkov V, Tahir M, Schrøder H, Skytte L, Rasmussen KL, Khandige S, Møller-Jensen J, Kjeldsen F. (2019) Silver nanoparticle-induced expression of proteins related to oxidative stress and neurodegeneration in an in vitro human blood-brain barrier model. Nanotoxicology. 13(2):221-239. doi: 10.1080/17435390.2018.1540728. Epub 2019 Jan 9. PMID: 30623748. Using an in vitro blood-brain barrier (BBB) model this study investigated alterations in the proteomes of brain endothelial cells and astrocytes at different time points after AgNPs exposure (24 and 48 h). The overall conclusion was that AgNPs exerted direct cellular stress on the endothelial cells by triggering a pro-inflammatory cascade.

9. Lidia Strużyńska, Joanna Skalska. (2018) Mechanisms Underlying Neurotoxicity of Silver Nanoparticles. Adv Exp Med Biol. 1048:227-250. doi: 10.1007/978-3-319-72041-8_14. A book chapter review on current data on the impact of AgNPs on the central nervous system and the possible mechanisms of their neurotoxic effects.

10. Sharma A, Muresanu DF, Patnaik R et al., (2013) Size- and age-dependent neurotoxicity of engineered metal nanoparticles in rats. Mol Neurobiol 48:386–396. Results showed that small-sized NPs induced the most pronounced blood-brain barrier (BBB) disruption, brain oedema formation, and neuronal injuries (+30 to 40 %), glial fibrillary acidic protein upregulation (+40 to 56 % increase), and myelin vesiculation (+30 to 35 % damage) in young animals as compared to controls. The older animals (30 to 35 weeks of age) also showed brain pathology as compared to young adults (18 to 20 weeks old). Ag and Cu exhibited greater brain damage compared with Al NPs in all age groups regardless of their size.

11. Liu Y, Guan W, Ren G et al., (2012) The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats. Toxicol Lett 209:227–231. Adult male Wister rats were exposed to silver nanoparticles through nasal administration for two weeks: negative control group, low-dose group (Ag-np, 3 mg/kg) and high-dose group (Ag-np, 30 mg/kg). After exposure, the Morris water maze (MWM) test was performed to investigate effects on spatial cognition, followed by long-term potentiation (LTP) recording and reactive oxygen species (ROS) detection in hippocampal homogenates. Results showed both LTP and MWM were abnormal in low-dose and high-dose groups relative to controls. The quantity of ROS in hippocampal homogenate was increased significantly in both dose groups.

12. Davenport LL, Hsieh H, Eppert BL et al., (2015) Systemic and behavioural effects of intranasal administration of silver nanoparticles. Neurotoxicol Teratol 51:68–76. Single dose (10–500 mg/kg) and repeated dose (50 mg/kg/d for 7 d, followed by a 7d wait period) studies were performed with male C57BL/6 mice via intranasal instillation. AgNP deposition was systemic including the brain. Expression

of the oxidative stress-responsive gene Hmox1 was elevated in the hippocampus, but not cortex of treated mice. There was only limited evidence for effects on learning- and memory-related behaviours.

13. Xu L, Shao A, Zhao Y et al., (2015) Neurotoxicity of silver nanoparticles in rat brain after intragastric exposure. J Nanosci Nanotechnol 15:4215–4223. Silver nanoparticles were examined in rat after intragastric administration. After a two-week exposure to low-dose (1 mg/kg bw) or high-dose (10 mg/kg bw) AgNPs, neuronal shrinkage, cytoplasmic or foot swelling of astrocytes, and extra-vascular lymphocytes were obseved in silver NP exposure groups. The authors concluded that AgNP can induce neuronal degeneration and astrocyte swelling via proinflammatory mechanisms.

14. Yin N, Zhang Y, Yun Z, Liu Q, Qu G, Zhou Q, Hu L, Jiang G. (2015) Silver nanoparticle exposure induces rat motor dysfunction through decrease in expression of calcium channel protein in cerebellum. Toxicol Lett. 237:112–120. Neonatal Sprague-Dawley (SD) rats were exposed to AgNPs by intranasal instillation for 14 weeks. This leads to cerebellar ataxia like symptoms, evidenced by dysfunction of motor coordination and impairment of locomotor activity.

Moreover, further information shedding light on neurotoxic effects of silver acetate recently became available in a new EOGRTS in the context of classification and labelling of silver (May 2022)). The reprotoxic effects are described in detail in section 10.10 and the developmental toxicity observed includes brain myelinopathy, cell loss, and diminution of the size of the hippocampus along with deficits in motor function and neurobehavioural parameters in the F1 generation. Among parental P0 generation, relevant for the assessment of STOT RE, neurohistopathological investigation was performed but no effects were observed in brain structures, including hippocampus and thalamus. There is also a dose range-finding study for the EOGRTS and a 90-day rat dietary study on silver acetate. There were no indications of behavioural or brain abnormalities in these studies however only limited neurobehavioral tests were performed in the 90-day study and the brain was not investigated in the dose-range finding study. However, as effects were seen in hippocampus of F1 offspring in EOGRTS (assessed and concluded under developmental toxicity), it increases the confidence that hippocampal neuronal cell loss seen also after exposure of mature rats in Charehsaz et al., 2016 and that the other effects indicating nervous system toxicity by silver nitrate and silver nanoparticles in adult animals in the published studies are true positive findings.

Therefore, based on the data on silver nitrate, silver acetate and nanosilver, criteria for classification STOT RE 2 (nervous system) are considered fulfilled. Effects were observed among studies at doses both within guidance values for category 1 and category 2. Category 1 was not proposed by RAC in the discussion on classification and labelling of silver[32] because *“the overall database on the lowest dose levels where hippocampus toxicity and neurofunctional deficits involving learning and memory started to occur was considered insufficiently robust or inconsistent to support the more severe category”.* Although category 1 and category 2 are distinguished by the effect levels rather than strength of evidence, the guidance values are as implied only for guidance and not strict cut-off values. Considering the limitations in the published data the DS agrees that these effect levels should not be used for categorisation, and it is thus considered appropriate to propose category 2 based on the neurotoxic effects observed in several studies with silver nitrate and nanosilver that are supported also by the neurodevelopmental effects seen amongst F1 offspring.

In the combined repeated-dose toxicity and reproduction/developmental toxicity screening test (Hong, J-S et al) a marked accumulation of silver was observed in lungs of animals administered 250 mg/kg bw. The histopathological findings included granulomatous lesions in 2 out of 10 females and cholesterol granuloma in 2 out of 10 males. According to the study authors the association between silver in the lung and granulomatous lesions is unclear and further investigations would be necessary. Nevertheless, the effect was observed at a dose of 250 mg/kg bw which is above the guidance values for classification.

Specific target organ toxicity-dermal route: two 90-day studies available from the same study author (Korani 2011 and 2013, respectively) showed significant dose-dependent inflammatory responses in

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[32] RAC Opinion proposing harmonised classification and labelling at EU level of Silver CLH-O-0000007152-82-01/F

tissues from Guinea pigs dermally exposed to 100, 1000, and 10000 ppm (µg/ml) nanoparticles of silver or silver nitrate (100 µg/ml). The effects were noted at all levels, i.e., as low as 100 µg/ml (according to the article corresponding to 0.1 mg/kg), which is far below the guidance value of 20 mg/kg bw/d. However, inflammation is not considered to fulfil any of criteria (d, e, f or g) of section 3.9.2.7.3 in Annex I:

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity.

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver).

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

Additionally, necrosis (mild) and severe hepatocyte degeneration were observed in the liver at doses of 10 000 µg/ml (Korani 2011) and cell degeneration was stated in the REACH registration dossier (Korani 2013) to be observed in the proximal convoluted tubules in the kidneys at all dose levels (including the control AgNO3), thus below the guidance value for STOT-RE 1. Necrosis is considered in criterion (e) (multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity) and the liver is indeed a vital organ. However, in the absence of further information on the necrosis besides the grading "mild", it is not considered unequivocally demonstrated that criterion (e) is fulfilled. Cell degeneration is considered in criterion (g) however since the liver (2011 study) and the kidney (2013 study) are organs capable of or partly capable of regeneration[33], effects are not considered to fulfil criterion (g).

Specific target organ toxicity-inhalation route:

Three repeated-dose studies performed via inhalation are available in the REACH registration dossier. The original data has not been assessed by the dossier submitter. In the Sung, J. H. et al. (2008) study, some liver effects were observed that need to be considered for classification. The liver effects included a dose-related increase of minimal bile-duct hyperplasia in both males and females, single-cell hepatocellular necrosis in 3/10 high dose females and moderate bile-duct hyperplasia with concurrent moderate centrilobular fibrosis, minimal single-cell hepatocyte necrosis, mild pigment accumulation, and moderate multifocal necrosis in a single high-dose female. Considering that the bile duct proliferation observed was mild, this is not considered to indicate an effect fulfilling criteria (d), i.e., *"significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination"* or criterion (f) *"morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver)."* Although the *"moderate multifocal necrosis"* observed is considered to fulfil criterion (e), i.e., *"multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity",* it was only observed in a single high-dose female and the finding is thus not considered as sufficient evidence to fulfil criteria for classification. The biochemical parameters indicative of hepatic injury showed no clear effect of treatment but the liver analyses in the 28-day study by Ji, et al. (2007) in rats revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle and high dose groups, respectively. For female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high dose group and

[33] E.g. Duccio Lombardi,1 Francesca Becherucci,2 and Paola Romagnani. How much can the tubule regenerate and who does it? An open question, Nephrol Dial Transplant. 2016 Aug; 31(8): 1243–1250.

Rinkevich Y, Montoro DT, Contreras-Trujillo H et al. In vivo clonal analysis reveals lineage-restricted progenitor characteristics in mammalian kidney development, maintenance, and regeneration. Cell Rep 2014; 7: 1270–1283

one case among the female rats in the high dose group. Since the guidance values (adjusted for 28-day exposure) for classification is ≤ 0.06 mg/litre/6h/day for category 1 and 0,06 < C ≤ 0,6 for category 2 (dust/mist/fume), the high dose of 61.24 µg/m^3 (6.1 × 10^{-5} mg/dm3 and 9.6× 10^{-5}) is below the guidance value for category 1. Nevertheless, in the absence of original data, it is not possible to further analyse the hepatic focal necrosis to clarify if it was restricted to single cells or multifocal. Therefore, the severity of effects cannot be adequately assessed and the data available is not considered sufficient to fulfil criteria for classification.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the indications of neurotoxicity in the data on silver nitrate (hippocampal neuronal loss), silver acetate (increased levels of dopamine and noradrenaline) and nanosilver (hippocampal neuronal loss, increased levels of dopamine and noradrenaline, impairment of cognitive functions and behavioural disturbances), criteria for classification STOT RE 2 (nervous system) are considered fulfilled. Identification of the nervous system as a target organ is also supported by the neurodevelopmental effects seen amongst F1 offspring discussed in section 10.10.

Dermal route: the histopathological changes observed in the liver and kidneys of Guinea pigs exposed to nanoparticles of silver are not considered to fulfil criteria for classification.

Inhalation route: the histopathological changes observed in the liver of rats exposed to nanoparticles of silver are not considered to fulfil criteria for classification.

10.13 Aspiration hazard

Table 69: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Silver nitrate is not a liquid of low viscosity and does not need to be classified for aspiration hazard.

10.13.2 Comparison with the CLP criteria

Not relevant.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Not relevant.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Silver nitrate is an inorganic solid substance containing the metal silver, and falls under the classification scheme for metals and metal compounds in the CLP-guidance Annex IV, chapter 5 (ECHA, 2017). The hazard classification schemes, according to the guidance, is based on the acute and long-term hazards posed by metals and metal compounds in the form they are available to pelagic aquatic organisms, i.e. exist as dissolved metal ions. The hazard classification does not take into account exposures to metals and metal compounds that are not dissolved in the water column, but may still be bioavailable, such as metals in foods. Only environmental hazards connected to conventionally dissolved silver (< 0.45 µm PSE filter) is considered in the present hazard assessement.

In the present CLH dossier the ecotoxicity tests presented have been performed with highly soluble silver salts of which most is silver nitrate. Some studies for chronic toxicity to algae were performed

with silver chloride. Chloride in test medium has both shown to lead to decreased silver toxicity for some fish species like rainbow trout and less protection or even higher silver toxicity for other species. This indicates that the protection against silver toxicity by chloride is species dependent and not simply due to water chemistry and silver speciation. The mechanisms behind the differences in protection between species remain unclear (Bielmyer et al. 2008). The key studies for acute and chronic toxicity are however performed with silver nitrate.

According to the CLP guidance (ECHA, 2017) the rate and extent to which ions from silver nitrate can be generated needs to be considered if the acute and/or chronic ecotoxicity reference value (ERV) for the metal ions, corrected for the molecular weight of the compound ($ERV_{compound}$), is less than or equal to 1 mg/l. Silver nitrate has an acute $ERV_{compound}$ of 0.00034 mg/l and a chronic $ERV_{compound}$ of 0.00016 mg/l and therefore the rate and extent of the generation of silver ions from silver nitrate needs to be considered.

A metal compound is considered readily soluble if the water solubility is greater or equal to the acute ERV of the dissolved metal ion. The readily soluble criterion is fulfilled by silver nitrate, which is readily soluble in water (approximately 2340 g/l at 25 ^{o}C), see chapter 7 in the present report.

The resulting classification proposals are presented in section 11.7.

11.1 Rapid degradability of organic substances

Not applicable, as silver is not an organic substance.

For further information see section 11.2.1 in the present report.

11.1.1 Ready biodegradability

Not applicable, as silver is not an organic substance.

For further information see section 11.2.1 in the present report.

11.1.2 BOD_5/COD

Not relevant - see 11.1.1.

11.1.3 Hydrolysis

Silver nitrate is an inorganic compound that dissociates in water, but it does not have any chemical bonds prone to hydrolysis. Hence, hydrolysis is not considered a relevant pathway.

11.1.4 Other convincing scientific evidence

Not relevant - see 11.1.1.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant - see 11.1.1.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Not relevant - see 11.1.1.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Not relevant - see 11.1.1.

11.1.4.4 Photochemical degradation

There is no quantitative data available for the effects of photolysis processes on silver in water. Photo-reduction and photo-oxidation may affect the rate at which silver-ions (and other non-hazardous ions) are released from the molecule and the speciation of these ions in the water compartment. However, photolysis processes are not considered relevant for the environmental hazard assessment of silver nitrate as such.

11.2 Environmental transformation of metals or inorganic metals compounds

See summary below in section 11.2.1

11.2.1 Summary of data/information on environmental transformation

Silver is a natural element and, thus, not degradable. It is therefore not relevant to assess degradation rate as is usually done for organic compounds.

Chemical speciation of silver is governed by complexation with both inorganic ligands and natural organic matter. As for all equilibrium, there is a concentration-dependent binding constant between silver and the available ligands. It is recognised that sulphide, normally present at low concentrations in natural waters, forms a strong complex with silver ions. However, the potential for the reverse change to occur cannot be ruled out (Paquin and Di Toro, 2008).

Depending on the levels of sulphide and silver ions present in water, other speciation reactions with varying binding constants e.g. binding with chloride and natural organic matter may occur (Paquin and Di Toro, 2008). Complexing ligands such as chloride (Cl-), DOC and sulphide plus competing cations decrease the toxicity by reducing the bioavailability of silver to toxic sites at fish gills (Morgan and Wood 2004). However, in a paper funded by Water Environment Research Foundation a review of studies performed to refine an acute biotioc ligand model for silver, is provided. The protectiveness of the chloride ion against silver toxicity was investigated in a series of physiological and toxicological studies using *P. promelas* (4 days old), *Danio rerio* and *Fundulus heteroclitus*. The results showed uncertainty regarding the protectiveness of chloride as a ligand to silver as no significant protective benefit of the chloride ion against silver toxicity for fish was found (Paquin and Di Toro, 2008).

Furthermore, in the principal REACH-dossier for silver a total of five transformation/dissolution tests (T/D-tests) were submitted of which two were performed with silver powder. The results from the tests with silver powder (loading of 1 mg/L for 28 days at pH 6 and 8) demonstrate an increase in the dissolved metal concentrations with time up to day 28, please see graphs and summaries for the two tests below. This occurred despite the fact that the T/D medium contained a high concentration of chloride, which is expected to remove some of the silver due to formation of insoluble silver chloride. Consequently, there is no evidence of rapid environmental transformation of silver from soluble to insoluble forms.

Study report by CIMM (2009). Metallic Silver and Silver Oxide: Full Transformation/dissolution tests in OECD media at pH 6 and pH 8.

The silver dissolution from a silver powder (D_{50} = 1.9 µm) in pH 6 buffer (prepared in accordance with OECD 203 with reconstituted water and further diluted 10 times) and pH 8 buffer (prepared in accordance with OECD 203) were tested at 1 mg/l, 10 mg/l and 100 mg/l corresponding to a SAL of 3 x 10^3 mm2/l, 3 x 10^4 mm2/l and 3 x 10^5 mm2/l respectively. For the purpose of using the data for acute and chronic classification the test at 1 mg/l was performed during 28 days whereas for the other two loadings the tests were only conducted for 7 days. Three replicates were used for each test. At each sampling point, dissolved silver was analysed using ICP-MS (detection limit 0.04 µg/l) on filtered solutions (0.2 µm syringe filters). The release pattern were similar at all loadings and pH, with an initial rapid increase in silver concentration followed by a slower increase towards the end of the testing period.
At 1 mg/l and 28 days the silver dissolution was 3.55 µg/l for pH 6. For pH 8 the silver dissolution was 5.71 µg/l at 1 mg/l loading and 28 days. The results indicated similar silver dissolution with 1 mg/l loading at both pH with slightly higher levels at pH 8.

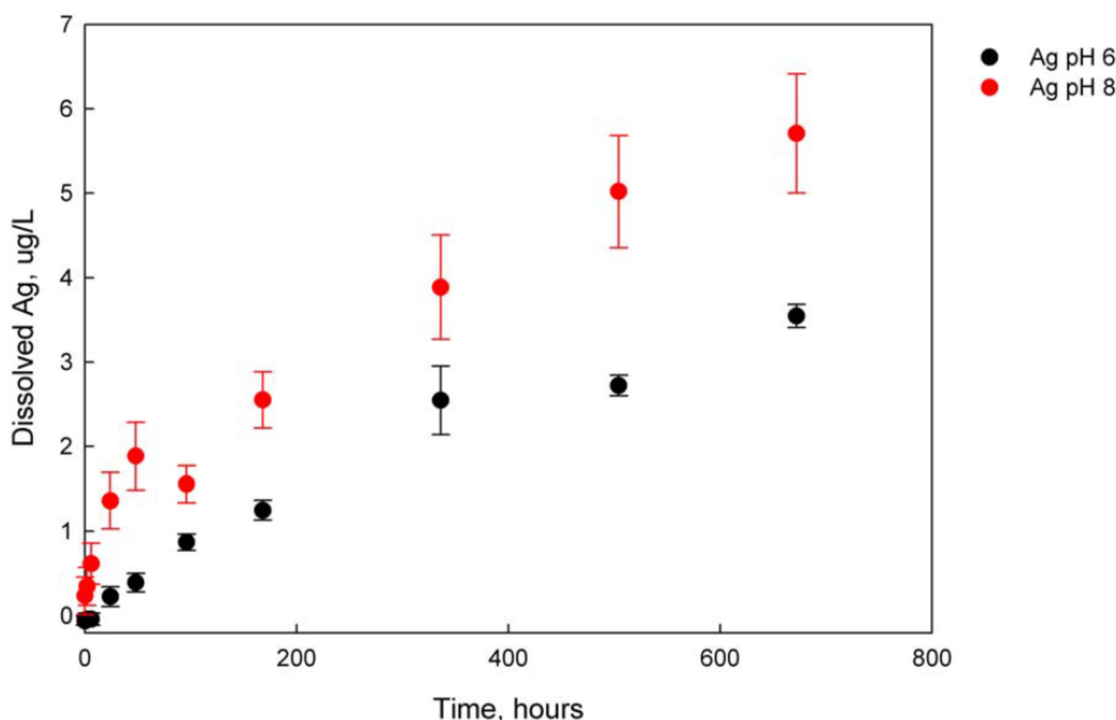

Figure 1. Dissolved silver at pH 6 and pH 8 over 28 days and 1 mg/l loading. Graph copied from the study report on T/D-test by CIMM (2009)

Study report by ECTX (2010). Transformation/Dissolution test of_Silver flake at a 1, 10 and 100 mg/l loading in a standard aqueous medium at pH 6.

The silver dissolution from silver flakes (D90=7.83 µm, D50=2.61 µm and D10=1.07 µm) in pH 6 buffer (prepared as in OECD 203 with reconstituted water and further diluted 10 times) were tested

at 1 mg/l, 10 mg/l and 100 mg/l corresponding to a surface area loading (SAL) of 1.17 x 10^3 mm2/l, 1.17 x 10^4 mm2/l and 1.17 x 10^5 mm2/l respectively.
For the purpose of using the data for acute and chronic classification the test at 1 mg/l was performed during 28 days whereas for the other two loadings the tests were only conducted for 7 days. Three replicates were used for each test. At each sampling point, dissolved silver was analysed using ICP-MS (reporting limit 0.01 µg/l) on acidified and filtered solutions (0.2 µm syringe filters). The release pattern was similar at all loadings, with an initial rapid increase in silver concentration followed by a slower increase towards the end of the testing period. The silver dissolution ranged between 1.79-38.1 µg/l at 1-100 mg/L loading and 7 days. At 1 mg/L and 28 days the silver dissolution was 3.60 µg/l.

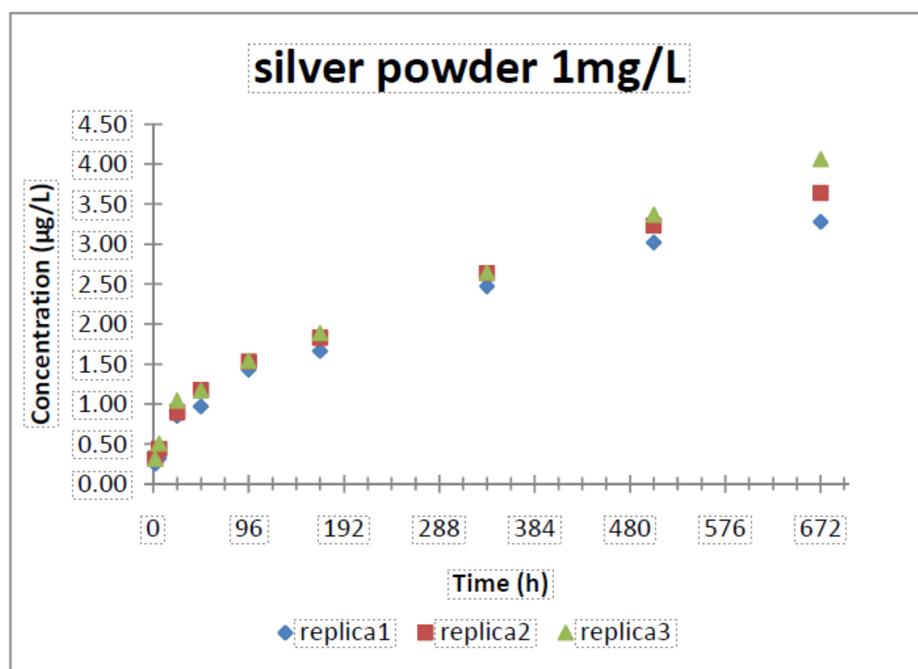

Figure 2. Dissolved silver at pH 6 over 28 days and 1 mg/l loading. Graph copied from the study report by ECTX (2010).

The media used in both studies summarised above contains a high concentration of chloride, please see table 70 below.

Table 70: Chloride content of T/D-testing media (in accordance with OECD 203)

	pH 6	pH 8
KCl (mg/l)	0.58	5.75
$CaCl_2$.2HCl (mg/l)	29.4	294

<u>CLP-guidance</u>
Regarding environmental transformation to non-available forms of the substance the following is stated in the CLP-guidance on application of the CLP criteria, annex IV.1, introduction:

"However, partitioning of the metal ion from the water column to other environmental media does not necessarily mean that it is no longer bioavailable, nor does it necessarily mean that the metal has been made permanently unavailable."

Furthermore, in the next paragraph: "In the first instance it should be assumed that the metal ions, once in the water, are 'not rapidly partitioned' from the water column. Underlying this is the assumption that, although speciation can occur, the species will remain available under environmentally relevant conditions."

The above points to that that evidence is needed to show that a substance is rapidly transformed, however, more evidence to prove the opposite default assumption is not required.

In summary, it is not possible to conclude that silver is rapidly transformed to permanently non-available forms.

The dossier submitters conclusion is supported by the recent RAC opinion for elemental silver.

11.3 Environmental fate and other relevant information

Silver nitrate dissolves and releases silver-ions (Ag^{+}) under the use envisaged. Dissolved silver is considered the active species of the active substance. For the environment, it is thus reasonable to focus on the fate, behaviour and effects of silver and not on the substance itself. For the purpose of this classification the nitrate ion is not considered of environmental concern.

11.4 Bioaccumulation

Bioconcentration: Silver may be released into the water and taken up by organisms through ion transport channels.

Bioaccumulation: In the aquatic environment silver nitrate will most likely dissolve quickly and it is possible that silver bound to suspended particles are taken up via ingestion, especially by particle filtrating organisms. In the gastrointestinal tract, silver ions may enter the organisms. Especially in the case of particle-reactive silver ions, the major route of uptake is via ingestion of silver associated to organic particles.

A large body of published literature exists concerning bioaccumulation of metals. Uptake is species specific and mainly controlled by physiological mechanisms.

In the course of the risk evaluation under Biocide legislation, literature surveys were carried out for bioaccumulation and biomagnification of silver. The literature reviews are attached to this CLP report.

Annex II: Literature reviews regarding aquatic bioaccumulation and biomagnification of silver

a) Review prepared by RMS Sweden under the Review Programme of the Biocide Directive 98/8/EC, Draft May 2012

b) Position paper submitted by the applicant under the Review Programme of the Biocide Directive 98/8/EC in 2011, Biomagnification of silver in aquatic environments (European Silver Task Force via TSGE).

The bioaccumulation of metals according to the classification criteria should be evaluated on a case-by-case basis using expert judgement as far as the mechanisms for uptake and depuration rates of metals are very complex and variable and there is at present no general model to describe. Silver is a non-essential element. The ability of dissolved silver to accumulate varies widely between species, depends on silver chemical species and external conditions (specific active transport systems, ligand binding and competitive interactions at the receptor site). Aquatic organisms have evolved mechanisms to regulate, store, detoxify or remove silver as a consequence of exposure to natural sources of the metal. As far as it can be generalised, it appears that fish have physiological mechanisms to keep silver levels low, whereas in invertebrates, storage of silver in metabolically unavailable forms is a means of detoxification.

Based on valid experimental results for fish, supported by valid experimental results for invertebrates it is concluded that silver has a low potential for bioaccumulation for classification purposes. The recent RAC opinion on elemental silver provides further support for this conclusion.

11.5 Acute aquatic hazard

Silver nitrate is a metal compound and a silver salt and the compound dissolves readily in aqueous media. Silver nitrate is considered readily soluble and 100 % of silver nitrate is expected to be dissolved in water. It is the silver ions released that is considered to be the environmentally relevant species and the species that cause the toxicity observed in the ecotoxicity tests. Therefore, only the presence of dissolved silver in the ecotoxicity tests is taken into account in the present CLH-report.

The released amount of silver is related to the loading rate of silver nitrate. In order to relate the toxicity from the dissolved silver to silver nitrate the concentrations obtained in the ecotoxcicty tests needs to be recalculated to represent silver nitrate based on the molecular mass. The content of silver in silver nitrate based on molecular mass is 64 %. This percentage is used to re-calculate ecotoxicity results obtained for dissolved silver, to represent silver nitrate.

For example: The most conservative acute toxicity value for *Oncorhynchus mykiss* and dissolved silver is LC_{50} = 3.5 µg/L.

3.5 x 1/0.64 = 5.5 µg/l = 0.0055 mg silver nitrate/L

In Table 71, data on silver ecotoxicity evaluated under BPR (EU) 528/2012 during the substance evaluation and/or reported in the Reach registration dossier are presented. The table includes the test results that resulted in the lowest LC/EC50 values and additional supportive data are discussed in narrative form below.

In addition, Dossier Submitter has performed an extensive screening of available data concerning silver toxicity in the aquatic and marine environment in collaboration with Stockholm University, the Swedish Agency for Marine and Water Management and the Swedish Environmental Protection Agency. The data was mainly collected from a report published by RIVM on environmental risk limits for silver (C.T.A. Moermond and R. Herweijnen, 2012) and the Reach registration dossier for silver and silver nitrate. From the initial data set studies given a reliability score of 3 and 4 (Klimisch et al., 1997) in the RIVM-report were not considered crucial for the derivation of endpoints for classification of silver nitrate.Studies given a score of 3 or 4 were poorly described and/or did not investigate the toxicity of dissolved silver. Study results for total silver, unknown silver concentration or free ionic silver were disregarded as only environmental hazards connected to conventionally dissolved silver (< 0.45 µm PSE filter) is considered in the present hazard assessement. Therefore, there are studies in the Reach registration dossier that are not presented/discussed further in the

present CLH-report. The reduced data set (data set containing data based on dissolved silver <0.45 µm filter) is included appendix 1 to this CLH-report.

Freshwater species are more sensitive to silver than marine species according to the available ecotoxicity data. However, for completeness, the most conservative and also sufficiently reliable results for aquatic organisms in the marine environment that were found in the screening of available data, are presented in separate tables in Annex I to the present CLH-report.

Table 71: Summary of relevant information on acute aquatic toxicity.
Where total silver is measured and reported the dissolved silver concentration is reported within brackets.

Method, Guideline, GLP status, Reliability (Klimisch et al., 1997)	Species	Endpoint	Exposure		Results		Recalculated value for silver nitrate (~ 64 % silver content, see identity in CAR chapter 1.1)	Remarks	Reference
			Design	Dura-tion	NOEC	LC/EC_{50}			
Fish									
ASTM E729-80; published peer-reviewed research Reliability. 2	*Oncorhynchus mykiss* Steelhead trout	Mortality	$AgNO_3$ Flow-through	96h		9.2 (3.8) µg/L#	Calculation example: 3.5 µg/L / 0.64 = 0.0055mg/L 0.0055 mg/L (dissolved)	Measured total silver concentration (values in parentheses are estimates based on dissolved <0.45µm silver)#	IIIA 7.4.1.1-01 Nebeker 1983, published
	Oncorhynchus mykiss Rainbow trout	Mortality	$AgNO_3$ Static	96h		4 tests: 8.5 – 72.9 (3.5-29.9) µg/L#			
		Mortality	$AgNO_3$ Flow-through	96h		2 tests: 8.6, 9.7 (3.5, 4.0) µg/L#			
	Pimephales promelas Fathead minnow	Mortality	$AgNO_3$ Static	96h		2 tests: 9.4, 9.7 (3.9, 4.0) µg/L#	0.0036 mg/L (dissolved)		
		Mortality	$AgNO_3$ Flow-through	96h		2 tests: 5.6, 7.4 (2.3, 3.0) µg/L#			
No guideline, published peer-	*Pimephales promelas*	Mortality	$AgNO_3$	96h		2.3 µg/L*	0.0036 mg/L (dissolved)	Estimated dissolved silver (< 0.45 µm) concentration	IIIA 7.4.1.1-02 Erickson 1998, published

reviewed research Reliability. 3	Fathead minnow		Flow-through and static						
Invertebrates									
No guideline, published peer-reviewed research Reliability. 2	*Daphnia magna*	Mortality	$AgNO_3$ Static renewal	48h		**0.22 µg/L**	**0.00034 mg/L** (dissolved)	Mean measured dissolved silver (0.45 µm/L). This is the most sensitive end-point for acute aquatic toxicity.	**IIIA 7.4.1.2-03, Bianchini et al. 2002** **Published**
ASTM E729-80; published peer-reviewed research Reliability. 2	*Daphnia magna*	Mortality	$AgNO_3$ Static	48h		0.6 µg Ag/L (0.25 µg/L)#	(0.00039 mg/L dissolved)	Mean total silver concentration (values in parentheses are eCA estimates based on dissolved <0.45µm silver)#	IIIA 7.4.1.2-01, Nebeker 1983 Published
No guideline, published peer-reviewed research Reliability. 3	*Daphnia magna*	Mortality	$AgNO_3$ Static	48h		0.58 µg Ag/L (0.52 µg Ag/L)***	0.00081 mg/L (dissolved)	Measured dissolved silver (< 0.45 µm) concentration.	IIIA 7.4.1.2-02, Erickson 1998 Published
Algae (growth inhibition)					**NOE_rC**	**E_rC_{50}**			
OECD 201 Reliability: 2	*Pseudokirchneriella subcapitata*	Growth rate	$AgNO_3$ static	72 h		0.96 µg/L (dissolved)	0.0015 mg/L (dissolved)	Based on mean measured conventional dissolved Ag concentrations (0.45 µm PSE filter)	Reach registration dossier for silver: 008 key. Schlich et al., 2017 (Mertens et al., 2019 as published)

Dissolved silver (<0.45µm) has been determined at least once. The article states that 59% of the silver was lost after well water was filtered.

* The lowest 96h LC_{50} value is approximately 3 µg Ag/L (at pH 7.17). The mean dissolved silver concentration was 78% of measured total silver concentration resulting in a corresponding 96h LC_{50} value of 2.34 µg dissolved Ag/L.

*** Dissolved silver was reported to be 89 % of total silver based on a laboratory study.

11.5.1 Acute (short-term) toxicity to fish

IIIA 7.4.1.1-01 Nebeker 1983 (Published peer-reviewed research)

A series of acute toxicity studies, both flow-through and static were conducted using silver nitrate employing fathead minnow and steelhead and rainbow trout as test organisms. Toxicity results are based on the measured total silver concentrations of the test media. Dissolved silver concentration (< 0.45 µm) has been determined at least once in the mentioned study. The article states that 59 % of the silver was lost after well water was filtered. Using this information, a LC_{50} of 0.0036 mg/L can be estimated for fathead minnow, and a LC_{50} of 0.0055 mg/L can be estimated for steelhead/rainbow trout (re-calculated to silver nitrate).

The study has been assigned a reliability of 2 and can be used for classification. (However, a more conservative value has been obtained for invertebrates in the study by Bianchini *et al.* 2002)

IIIA 7.4.1.1-02 Erickson 1998 (Published peer-reviewed research)

A series of studies conducted with fathead minnows investigated the effect of manipulating water hardness, pH and alkalinity, and organic carbon. The effects of adding sodium sulphate and sodium chloride were determined. Finally, the effect of ageing the test media and use of natural versus laboratory test media were investigated. The acute toxicity of silver to juvenile fathead minnows was substantially reduced by increasing hardness with the addition of calcium and magnesium sulphate, and by increasing dissolved organic carbon with the addition of humic acid. Toxicity was also inversely related with pH and alkalinity when these were jointly altered by the addition of a strong base or acid. Silver was much less toxic in natural river water (106 µg total Ag/L) compared to laboratory water (10.4 µg total Ag/L), probably due to the higher organic carbon content of the river water. The LC_{50}:s for flow-through exposure with fathead minnows were approximately two-fold lower than for static exposure with fresh test solutions, but not significantly lower than for static exposure with aged solutions. The lowest endpoint was obtained from the unfed flow-through study and was a 96-hour LC_{50} of 2.3 µg dissolved Ag/L (recalculated to 0.0036 mg/L silver nitrate). The decrease in LC_{50} for aged test solutions was unexpected according to the authors of the study, since ageing was supposed to result in formation of complexes with low bioavailability. The authors do not further discuss this finding. It could indicate that complexed silver may, indeed, be bioavailable, and/or ionic and complexed silver have different toxicologically relevant targets within the organism.

The study has some weaknesses and the study has been assigned a reliability score of 3. It is not clear if the test concentration was maintained throughout the study and the survival in the controls was only 80 %. In conclusion, with the information given it was not possible to determine the exact value for the 96 h LC_{50}. The study can be included as supportive information. However, the most sensitive species with regards to acute toxicity is an invertebrate, not a fish, in the present evaluation.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

IIIA 7.4.1.2-03 Bianchini et al. 2002 (Published peer-reviewed research)

The aim of the study was to investigate the influence of sulphide (as ZnS) on the toxicity of silver to Daphnia magna. LC_{50} for sulphide-free exposure was 0.22 µg/L, recalculated to silver nitrate 0.00034

mg/L. Mortality to *Daphnia magna* was reduced in the presence of sulfide only when results are based on total measured silver concentrations. This might include particulate silver and it cannot be excluded that the daphnids took up silver by ingestion of particles. However, when measured filtered silver was considered, the toxicity curves were virtually identical, indicating that the dissolved fraction was the source of available silver.
The present study produced the most conservative value with sufficient reliability for the acute toxicity in the aquatic environmental compartment based on dissolved silver. **The LC_{50} of 0.00034 mg/L for silver nitrate is taken forward for classification of acute aquatic toxicity.**

Two studies that were part of the screening of available data concerning silver toxicity in the aquatic environment show results in the same range as the study by Bianchini et al., 2002. In the study by Kolts et al., 2006 an EC_{50} of 0.1 µg Ag/l was obtained (test organism: *Ceriodaphnia dubia*) and in the study by Glover et al., 2005 an EC_{50} of 0.23 µg Ag/l was observed (test organism: *Daphnia magna*). The studies are not considered as reliable as the study by Bianchini, but can still be used as supportive data and are included in the table presented in Annex I to the present CLH-report.

IIIA 7.4.1.2-01, Nebeker 1983 (Published peer-reviewed research)

Several acute tests were conducted with Daphnia magna. One test was carried out with addition of food and the remaining three without food. Toxicity results are based on measured total silver concentrations of the test acute (short-term) toxicity to algae or other aquatic plants media, leading to an underestimation of silver toxicity. Dissolved silver concentration (< 0.45µm) has been determined at least once in the mentioned study. Addition of food decreased toxicity to LC50 = 12.5 µg/L based on measured total silver. Considering that 89 % of the silver was lost by filtration when food was added, the estimated LC_{50} based on dissolved fraction is 1.4 µg Ag/L. The article states that 59 % of the silver was lost after filtration when no food was added. Using this information, a LC_{50} of 0.25 dissolved µg Ag/L can be estimated and recalculated to 0.00039 mg/L silver nitrate. The study is assigned a reliability score of 2 and can be used for classification. (However, a more conservative value has been obtained in the study by Bianchini *et al.* 2002).

IIIA 7.4.1.2-02, Erickson 1998 (Published peer-reviewed research)

This study concerns the effects of different test regimes such as feeding or no feeding and ageing or no ageing of test solutions before exposure on the acute toxicity of silver (as silver nitrate) to *Daphnia magna*. A 48-hour LC_{50} value of 0.58 µg Ag/L for *Daphnia magna* was obtained in a static test in non-aged laboratory water without feeding. Dissolved silver is reported to be 89 % in the tests with *Daphnia magna* and hence a LC50 of 0.52 µg/L for dissolved silver can be calculated and recalculated to 0.00081 mg silver nitrate/L. The toxicity of silver in natural water was found to be much lower than in laboratory water, by a factor of 60. The major difference between the two waters is the concentration of organic matter, the organic content of the river water being more than an order of magnitude higher.

The study is assigned a reliability score of 3. Important information such as dose-response curves and number of dead/immobile individuals per test concentration was not reported. Furthermore, it was not clear if the test concentrations had been maintained throughout the study. The aim of the study was to investigate different laboratory conditions and how it will affect toxicity of silver. Therefore, most of the results of the tests are given as trends and percentage differences related to other treatments. The study can be used as supportive information.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Reach registration dossier for silver. Study referenced as key 008, Schlich et al., 2017 (Mertens et al., 2019 as published)

The 72 hour toxicity of silver nitrate to the uni-cellular green alga *Pseudokirchneriella subcapitata* was determined in a static system and the study was reported to be performed according to GLP and OECD 201. The alga was exposed to nominal concentrations of 0.316, 1.00, 3.16, 10.0 and 31.6 µg Ag/L.

The nominal test concentrations were prepared in sterile modified AAP growth medium under sterile conditions. The medium was prepared with reduced EDTA concentrations and nitrate compounds replaced chloride compounds. The concentrations of the test item in the test media were determined by chemical analysis of silver in the aqueous phase of all treatment levels by ICP-MS at test initiation, after 24 h, 48 h and at the test termination of the growth test (LOQ = 0.001 µg/L). Three different types of measurements were conducted: total Ag, conventional dissolved Ag after filtration of a subsample through 0.45 µm PSE filters and truly dissolved Ag after filtration with centrifugal filters at 3000 x g. The particle size and the zeta potential were measured from samples of an extra analytical vessel without algae to characterise the test item in test media at test initiation and test termination. The evaluation of the results was based on the geometric mean measured concentrations of total Ag, conventional dissolved Ag and truly dissolved Ag.

Dose-response was shown both for inhibition of yield as well as inhibition of growth rate. For conventionally dissolved silver (< 45 µm) an ErC_{50} of 0.96 µg/l was determined. To reflect ErC_{50} for silver nitrate the value was recalculated to 0.0015 mg/l.

The study is assigned a reliablilty of 2 and can be used for classification. (However, a more conservative value for acute aquatic toxicity has been obtained for invertebrates in the study by Bianchini *et al.* 2002.)

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No further data available

11.6 Long-term aquatic hazard

Silver nitrate is a metal compound and a silver salt and the compound dissolves readily in aqueous media. Silver nitrate is considered readily soluble and 100 % of silver nitrate is expected to be dissolved in water. It is the silver ions released that is considered to be the environmentally relevant species and the species that cause the toxicity observed in the ecotoxicity tests. Therefore, only the presence of dissolved silver in the ecotoxicity tests is taken into account in the present CLH-report.

The released amount of silver is related to the loading rate of silver nitrate. In order to relate the toxicity from the dissolved silver to silver nitrate the concentrations obtained in the ecotoxicity tests needs to be recalculated to represent silver nitrate based on the molecular mass. The content of silver in silver nitrate based on molecular mass is 64 %. This percentage is used to re-calculate ecotoxicity results obtained for dissolved silver, to represent silver nitrate.

For example: The chronic toxicity value from the study by Dethloff, Naddy et al., 2007, based on the endpoint growth, for the species *Oncorhynchus mykiss* and dissolved silver is NOEC = 0.21 µg/L µg/L.

0.21 x 1/0.64 = 0.33 µg/L = 0.00033 mg silver nitrate/L.

In Table 72, data on silver ecotoxicity evaluated under BPR (EU) 528/2012 during the substance evaluation and/or reported in the Reach registration dossier are presented.

In addition, Dossier Submitter has performed an extensive screening of available data concerning silver toxicity in the aquatic and marine environment in collaboration with Stockholm University, the Swedish Agency for Marine and Water Management and the Swedish Environmental Protection Agency. The data was mainly collected from a report published by RIVM on environmental risk limits for silver (C.T.A. Moermond and R. Herweijnen, 2012) and the Reach registration dossier for silver and silver nitrate. From the initial data set studies assessed as 3 and 4 (Klimisch et al., 1997) in the RIVM-report were not considered crucial for the derivation of endpoints for classification of silver nitrate . Studies assessed as 3 or 4 were poorly described and/or did not investigate the toxicity of dissolved silver. Study results for total silver, unknown silver concentration or free ionic silver were also eliminated. Therefore, there are studies in the Reach registration dossier that are not presented/discussed further in the present CLH-report. The reduced data set (data set containing data based on dissolved silver (<0.45 µm filter)) is included in appendix I to this CLH-report.

The most sensitive species to silver between marine and freshwater species are the freshwater species according to the available ecotoxicity data. However, for completeness, the most conservative and also sufficiently reliable results for aquatic organisms in the marine environment that were found in the screening of available data, are presented in separate tables in Annex I to the present CLH-report.

Table 2: Summary of relevant information on chronic aquatic toxicity. Data normalised to silver and re-calculated for silver nitrate.

Method, Guideline, GLP status, Reliability (Klimisch et al., 1997)	**Species**	**Test compound End point Type of test**	**Exposure**		**Results**	**Recalculated value for silver nitrate (64 % silver content, see identity in CAR chapter 1.1)**	**Remarks**	**Reference**
			Design	**Duration**	**NOEC/EC$_{10}$**			
Fish								
ASTM 1241-98; GLP Reliability: 2	*Oncorhynchus mykiss*	$AgNO_3$, Mortality, hatching success, time to hatch, growth, deformations		73-77 (30d post swim-up)	NOEC growth 0.38 µg/L (total) 0.21 µg/L (dissolved) NOEC mortality 1.48 µg/L (total) 1.09 µg/L (dissolved)	Calculation example: 0.21 µg/l / 0.64 = 0.00033 mg/l Growth: 0.00033 mg/L (dissolved) Mortality: 0.0017 mg/L (dissolved)	Four replicates according to private communication with the authors in 2018.	IIIA 7.4.3.2-05 (Dethloff, Naddy et al. 2007) , published
- Reliability: 3	*Oncorhynchus mykiss*	$AgNO_3$, Mortality, time to swim-up, growth		60d	NOEC growth 0.05 (= ½ of LOEC 0.1) (total) 0.02* µg/L (dissolved) NOEC mortality 0.36 µg/L (total) 0.15 µg/L (dissolved)	Growth; 0.000031 mg/L (dissolved) Mortality: 0.00023 mg/L (dissolved)	No information about number of replicates. The nominal and measured dissolved concentrations are not specified. No dose-response curves presented.	IIIA 7.4.3.2-01 (Nebeker, McAuliffe et al. 1983), published
- Reliability: 3	*Oncorhynchus mykiss*	$AgNO_3$, Mortality, percent hatch, percent swim-up, degree of yolk sac absorption, growth		51d	NOEC growth 0.14 (total) 0.13** µg/L (dissolved)	Growth: 0.00020 mg/L (dissolved) Mortality: 0.00020 mg/L (dissolved)	Number of replicated test chambers = 2. Insufficient information about substance concentration (are the reported values mean values?)	IIIA 7.4.3.2-03 (Brauner and Wood 2002), published

Method, Guideline, GLP status, Reliability (Klimisch et al., 1997)	Species	Test compound End point Type of test	Exposure		Results	Recalculated value for silver nitrate (64 % silver content, see identity in CAR chapter 1.1)	Remarks	Reference
			Design	Duration	$NOEC/EC_{10}$			
					NOEC mortality 0.14 µg/L (total) 0.13 µg/L (dissolved)			
- Reliability: 3	*Oncorhynchus mykiss*	$AgNO_3$, Mortality, time to hatch, growth		37d	NOEC growth 0.1*** (total) NOEC mortality 0.1 µg/L (total)	Growth: 0.00016 mg/L(total) Mortality: 0.00016 mg/L (total)	Number of replicated test chambers = 3. Insufficient information about substance concentration (are the reported values mean values?)	IIIA 7.4.3.2-04 (Brauner and Wood 2002), published
- Reliability: 3	*Oncorhynchus mykiss*	$AgNO_3$, Mortality, time to hatch, growth, physiological parameters		58d	NOEC growth 0.09 (total) NOEC mortality (< 0.09)# µg/L (total)	Growth: 0.00014 mg/L (total) Mortality: < 0.00014 mg/L (total)	Number of replicated test chambers = 2 (mortality) and 1 (growths)	IIIA 7.4.3.2-06 (Brauner, Wilson et al. 2003), published
- Reliability: 2	*Pimephales promelas*	$AgNO_3$, mortality, growth (weight), hatching success		30d	NOEC growth 0.35 µg/L (dissolved) NOEC mortality 0.35 µg/L (dissolved)	Growth: 0.00055 mg/L (dissolved) Mortality: 0.00055 mg/L (dissolved)		Naddy et al. 2007 (ref in IIIA 7.4.3.2-02; Moermond, C. and van Herwijen, R. 2012), published
Invertebrates								
ASTM E729-80; published peer-reviewed research Reliability: 2	*Daphnia magna*	$AgNO_3$, Survival and reproduction	Static-renewal	21d	1.6 µg/L (total) (0.7 µg/L) (dissolved)*	0.0011 mg/L (dissolved)	Mean measured silver concentration.	IIIA 7.4.3.4 Nebeker 1983 Published

[04.01-MF-003.01]

Method, Guideline, GLP status, Reliability (Klimisch et al., 1997)	Species	Test compound End point Type of test	Exposure		Results	Recalculated value for silver nitrate (64 % silver content, see identity in CAR chapter 1.1)	Remarks	Reference
			Design	Duration	NOEC/EC_{10}			
No guideline, published peer-reviewed research Reliability: 3	*Ceriodaphnia dubia*	$AgNO_3$, Survival and reproduction	Static	10d	NOEC 0.53 µg/L (dissolved)	0.00083 mg/L (dissolved)	Not entirely clear whether results are based on mean measured “dissolved” silver	IIIA 7.4.1.2-03 Rodgers et al. 1997a Published
	Daphnia magna				NOEC 0.8 µg/L (dissolved)	0.0013 mg/L (dissolved)		
	Hyalella azteca	$AgNO_3$, Survival			NOEC 4.0 µg/L (dissolved)	0.0063 mg/L (dissolved)		
	Chironomus tentans				NOEC 125 µg/L (dissolved)	0.20 mg/L (dissolved)		
Algae								
OECD 201 Reliability: 2	Pseudokirchneriella subcapitata	AgNO3 Growth rate	static	72 h	**ErC_{10} 0.1 µg/l (dissolved)**	**0.00016 mg/L (dissolved)**	Based on mean measured conventional dissolved Ag concentrations (<0.45 µm PSE filter). This is the most sensitive end-point for chronic aquatic toxicity. The value is taken forward for classification.	Reach registration dossier: 008 key. Schlich et al., 2017. (Mertens et al., 2019 as published)

* The article states that 59% of the silver was lost after well water was filtered
** Small (<10%) but significant weight gain was observed at 0.02 µg/L dissolved silver in the presence of a higher level of dissolved organic carbon. A small difference in length and weight was also observed at 0.13µg/L. However, since the difference is very small and due to the suspected flaw in statistics, it is doubtful whether these are real effects. See discussion in text.
*** Based on weight increase in newly hatched larvae. A slight (<10%) but significant increase in growth of newly hatched larvae was also observed at 0.1 µg. However, due to the suspected flaw in statistics, this is probably not a real effect. See discussion in text.
# Visually assessed from graph

11.6.1 Chronic toxicity to fish

At a Technical Meeting under the Biocides Directive (TM II 2013), it was decided that available information in the report from RIVM on environmental risk limits for silver in water should be collected and used to support the PNEC derivation (IIIA 7.4.3.2-02; Moermond, C. and van Herwijen, 2012). The applicant provided the requested study summaries. Finally, the eCA evaluated these studies and added further information. The BPC-working group (WGIII 2015) agreed to the approach, as it is presented here:

The RIVM report is considered to provide a recent comprehensive overview of the ecotoxicological endpoints available within public domain literature for dissolved silver.

The RIVM report searched data sources between 1998 and 2012, identifying the lowest chronic fish endpoint of 0.1 µg Ag/L from Brauner et al. (2003). However, a study conducted by Nebeker in 1983 reported a NOEC of < 0.1 µg Ag/L, which was contained in the dossier.

Based on the information provided in the table above, endpoints based on growth parameters can be considered the most sensitive with values ranging between 0.02 and 0.35 µg Ag/L (Nebeker 1983 and Naddy 2007). In this respect, *Oncorhynchus mykiss* appears to be more sensitive than *Pimephales promelas*. Four independent studies testing the same sensitive species are mentioned in the report, whereby fertilized *Oncorhynchus mykiss* eggs and larvae have been exposed to silver nitrate and dissolved silver, have been measured. During re-evaluation of the mentioned studies, the eCA found out that in one of the studies the dissolved concentration was actually not measured at the relevant concentration, thus ending up with three useable studies (see table). The durations of the studies ranged between 58 and 77 days with the most conservative end-point derived following 60 days of exposure and the least conservative following 77 days of exposure.

Study summaries for studies in the dossier and Nebeker 1983 and the 4 published research studies identified in the RIVM report investigating larval growths of *Oncorhynchus mykiss* were prepared by the applicant, under the BPR (528/2012) substance evaluation, (IIIA 7.4.3.2-01, IIIA 7.4.3.2-03, IIIA 7.4.3.2-04, IIIA 7.4.3.2-05). An additional study was identified and included in this report (IIIA 7.4.3.2-06). Further details on the studies are provided in text below.

Common for all studies (except Dethloff et al. 2007) is a low number of replicates (n = 1-3) for each tested concentration, if stated at all, which makes the application of the statistical analysis of length and weight data questionable (see explanation in the study descriptions above). Some small but significant deviations, both increase and decrease, in growth could possibly be explained by the artificially high statistical power, i.e. falsely identifying small differences as significant, whereas true differences related to treatment may remain undetected. At the time the studies were conducted, ASTM and corresponding OECD 210 (Fish, Early-life Stage Toxicity) guidelines contained requirements of minimum two replicates, meaning at least two test chambers per concentration. The current OECD 210 (2013) prescribes at least four replicate test chambers per concentration to be used per concentration. Thus, although they conform to guidelines at their time, the studies were assigned a reliability indicator of 3. Only the study by Dethloff et al. 2007 is in accordance with the requirement of OECD 210 (2013) and, thus has higher reliability.

In addition, two studies by Davies et al., 1998, are reported in the Reach registration dossier. The study performed with the fish species *Oncorhynchus mykiss* resulted in a LC_{10}-value of 0.17 µg/l and the study performed with the fish species *Salmo trutta* resulted in a EC_{10} value of 0.19 µg/l. The

studies bring further support to the results presented in Table 72. More information about the studies are presented in the summary table in Annex I to the present CLH-report. The DS did not have access to the full study reports.

Taken all fish studies together, the general picture is that growth of *Oncorhynchus mykiss* larvae is the most sensitive endpoint. There is no obvious explanation for the difference in results, in relation to differences in the test conditions. For the purpose of classification, the lowest endpoint (derived from a study with sufficient reliability) is chosen.

IIIA 7.4.3.2-01; Nebeker 1983 (Published peer-reviewed research)

A 60-day embryo-larval study was conducted with steelhead trout. For the endpoint of growth (fish length), the LOEC value is 0.1 µg Ag/L. At LOEC the length reduction is 12 % and the curve does not show clear dose-response relation. According to TGD 3.2.2. Table 16, NOEC can be calculated from LOEC. If LOEC > 10 % and < 20 % effect, NOEC can be calculated as LOEC/2. That makes in this case a NOEC of 0.05 µg Ag/L (total silver). Toxicity results are based on the measured total silver concentrations of the test media, leading to an underestimation of silver toxicity. Dissolved silver concentration (< 0.45 µm) has been determined at least once in the mentioned study. The article states that 59 % of the silver was lost after well water was filtered. Using this information, the eCA has estimated a NOEC of 0.02 µg/L for inhibition of growth, based on dissolved silver concentrations, or 0.000031 mg/L re-calculated to silver nitrate. Due to that e.g. no information about number of replicates and no dose-response curves were presented, the study is assigned a reliability of 3.

IIIA 7.4.3.2-03; Brauner and Wood 2002a

Oncorhynchus mykiss embryos were exposed to silver nitrate (nominal of 0, 0.1, and 10 mg/L total silver). Exposures were conducted in Hamilton hard water, in the presence or absence of dissolved organic carbon at a concentration of 12 mg/L in control and reference treatments. Each day, mortality, percent hatch, and percent swim-up were determined, and degree of yolk sac absorption was visually estimated. At 51 days post fertilization mortality, percentage hatch, percentage swim-up, ion regulation, and degree of yolk sac absorption were examined. Fish were sampled for the determination of whole embryo/larval Na+, K+ -ATPase activity levels, extractable protein and Na+, Cl-, and total silver concentrations and whole embryo/larval unidirectional Na+ uptake. Total and dissolved silver concentrations were analysed. It is not clear whether the results presented are mean values and how they were derived. Throughout development, there was a large increase in percentage daily mortality at 10 µg/l total silver. The protective effects of DOC (in the form of humic acid) during chronic silver exposure appear to be less than that observed during acute exposure. Exposure to 0.13 µg/l total silver (filtered/dissolved, in the absence of DOC) resulted in a small reduction in growth at Day 51 compared to the corresponding control. The reduction was reported to be statistically significant. When the Day 51 weight data are compared, for the exposures including DOC, a statistically significant decrease in weight is observed at 10 µg/l total silver. However, a statistically significant increase in weight is observed at 0.11 µg/l total silver (unfiltered), 0.02 µg/L total silver (filtered/dissolved) when compared to the control. This conclusion is supported by the results of extractable protein that indicate no statistically significant difference at 0.1 µg silver/L in the presence of DOC, when compared to the corresponding control. However, it appears that the statistical method was not appropriate for weight and length data. Only two test chambers were applied per concentration. The presentation of results indicates pseudoreplication when ANOVA and Dunnet's post-hoc test was applied (i.e. ±-values, not even specified whether standard deviation or standard error and small differences in growths appear to be significantly different) and comparisons were

based on single fish as statistical unit. This will lead to an artificially high statistical power, i.e. falsely identifying small differences as significant, whereas true differences related to treatment may remain undetected. Since the differences are small (<10%) and due to the suspected flaw in statistics, it is doubtful whether these are real effects. It is therefore suggested that a NOEC of 0.13 µg/L filtered, dissolved silver is used as supportive data for the purpose of classification, or 0.00020 mg/L re-calculated to silver nitrate. The study is assigned a reliability of 3.

IIIA 7.4.3.2-04; Brauner and Wood 2002b

Oncorhynchus mykiss embryos were randomly distributed to one of three flow-through silver exposure conditions, nominally of 0, 0.1 µg/L, and 1 µg/L total silver as $AgNO_3$ in Hamilton hard water. 37 days post fertilization mortality, time to hatch, growth, ion regulation, and ammonia and cortisol levels were examined. Total and dissolved silver concentrations were analysed. It is not clear whether the results presented are mean values and how they were derived. Exposure to 1.0 µg/L total silver resulted in a small but statistically significant increase in mortality, however exposure to 0.1 µg/L total silver (0.098 µg/L measured silver) was not significantly different to the control. There were no significant differences in the time to 50 % hatch at 1.0 and 0.1 µg/L total silver. At Day 30 and 37, a statistically significant increase in weight and size was observed at 1.0 µg/L total silver when compared to the control, but corresponding changes observed at 0.1 µg/L were not significant. Assuming that weight gain is considered an adverse effect, the lack of statistically significant effects on body weight and length at 0.1 µg/L total silver supports the proposed NOEC of 0.1 µg/L. A slight (< 10%) but significant increase in growth of newly hatched larvae was also observed at 0.1 µg. It appears that the statistical method was not appropriate for weight and length data. Although three test chambers were applied per concentration, the presentation of results indicates pseudoreplication when ANOVA and Dunnet's post-hoc test was applied and comparisons were based on single fish as statistical unit. (I.e. the standard error was small and small differences in growths appear therefore to be significantly different. SE CA also noted that there seem to be similar circumstances in other reports from the same research group.) This will lead to an artificially high statistical power, i.e. falsely identifying small differences as significant, whereas true differences related to treatment may remain undetected. Due to this, only effects larger than 10 % of the control are considered relevant, which in this state only regards the weight increase in newly hatched larvae at 1.0 µg/L. Since no dissolved silver concentrations were reported, this study is not considered for classification.

IIIA 7.4.3.2-05; Dethloff et al. 2007

A GLP study (complying with US EPA principles) following ASTM Method 1241-98 conducted with *Oncorhynchus mykiss* embryos and larvae. The animals were exposed to silver nitrate (5 concentrations of nominal 0.12-2.0 µg/L silver). Exposure in unmodified dilution water continued for 73 d, and that in dilution water amended with chloride (nominal 30 mg/L) continued for 77 d (30 d beyond the mean day to swim-up of the control for each). The parameters examined in the test were hatching, post hatch survival, and growth during the test. Fish were sampled for analysis of whole-body sodium and silver concentrations. Total and dissolved silver concentrations were analysed. Weight gain was decreased at dissolved silver concentrations of 0.21 µg/L and above in chloride-amended water. No effect on weight was observed in unmodified water at the tested concentrations up to 1.25 mg/L dissolved silver. The lowest-observed-effect concentrations were greater than 1.25 µg/L of dissolved silver for survival, mean day to hatch, mean day to swim-up and whole-body sodium content for both unmodified dilution waters and waters amended with NaCl. Four replicates were tested at each concentration level, according to private communication with the authors in 2018. Furthermore, due to the clear dose-responsea and the tight concentration intervals, the results are

considered sufficiently reliable. No effects were observed in the test without chloride amendment (dissolved concentrations up to 1 µg/l tested).

The NOEC based on mean dry weight was 0.5 µg/L of nominal silver (0.21 µg/L of dissolved Ag) will be used as supportive data for the purpose of classification, or 0.00033 mg/L re-calculated to silver nitrate.

However, the most sensitive species for chronic aquatic toxicity is an algae not a fish.

IIIA 7.4.3.2-06; Brauner et al. 2003

Rainbow trout embryos were exposed to silver nitrate (0, 0.1, and 1.0 µg/L nominal silver) at three water chloride levels (nominal of 30, 300, and 3000 µM). Exposures were conducted in a synthetic soft water until 58 days post fertilization. Mortality, time to hatch, silver accumulation, ion regulation, and growth and extractable protein were examined. Effects on embryo/larvae wet weight after 58 days in the presence of 30 µM chloride were significant for 1.0 µg/L total silver with a lowering of body weight observed. Effects were not significant for the 0.1 µg/L total silver treatment over the same period. It appears that the statistical method was not appropriate for weight and length data. The test chamber should be the statistical unit, since test chambers can be considered independent from each other. The number of replicated test chambers was 2 (mortality) and 1 (growth), respectively. The presentation of results indicates pseudoreplication when ANOVA and Dunnet’s post-hoc test was applied (i.e. small standard error; and small differences in growths appear to be significantly different) and comparisons were based on single fish as statistical unit. This will lead to an artificially high statistical power, i.e. falsely identifying small differences as significant, whereas true differences related to treatment may remain undetected. This could explain the apparent significant increase in body weight, at 0.1 µg/L in the test with 3000 µM chloride. Since no dissolved silver concentrations were reported, this study is not considered for classification.

11.6.2 Chronic toxicity to aquatic invertebrates

IIIA 7.4.3.4, Nebeker 1983 (Published peer-reviewed research)

The applicant, under the BPR (528/2012) substance evaluation, provided one published peer-reviewed scientific article, describing three *Daphnia magna* reproduction studies conducted with silver nitrate in which water hardness was varied. Survival and reproductive success (as young/survived adult) were equally sensitive endpoints. The statement made that water hardness did not affect the survival or reproduction of D. magna has not been statistically verified in this study. Dissolved silver concentration (<0.45µm) has been determined at least once in the mentioned study. The article states that 59% of the silver was lost after filtration. Using this information, values can be estimated for survival and reproduction to 0.7 µg Ag/L, and recalculated to silver nitrate 0.0019 mg/L. The study is afflicted with some shortcomings like high mortality in controls as well as lacking or inconclusive information about silver concentrations, and purity of the test substance. The study is assigned a reliability of 2.

IIIA 7.4.1.2-03 Rodgers et al. 1997 (Published peer-reviewed research, added by eCA)

Static toxicity tests over 10 d were conducted with several invertebrate species of which *Ceriodaphnia dubia* was the most sensitive with the lowest NOEC 0.53 µg Ag/L (dissolved), and recalculates to silver nitrate 0.00083 mg/L. Test species and further results are presented in Table 72. The studies are assigned a reliability of 3.

Two studies (Kolts et al., 2009 and Diamond et al.,1990) that were part of the screening of available data concerning silver toxicity in the aquatic environment, see Annex I to the present CLH-report, show results in the same range as the study by Nebeker et al., 1983 and Rodgers et al., 1997. In the study by Kolts et al., 2009, a NOEC of 0.37 µg Ag/l was obtained (test organism: *Ceriodaphnia dubia*) and in the study by Diamond et al., 1990, a NOEC of 0.58 µg Ag/l was observed (test organism: *Hyalella azteca*). These results further supports the outcome of the two studies discussed above.

11.6.3 Chronic toxicity to algae or other aquatic plants

Reach registration dossier for silver. Study referenced as 008 key, Schlich et al., 2017 (Mertens et al., 2019, as published).

The 72hour-toxicity of silver nitrate to the uni-cellular green alga *Pseudokirchneriella subcapitata* was determined in a static system and the study was reported to be performed according to GLP and OECD 201. The alga was exposed to nominal concentrations of 0.316, 1.00, 3.16, 10.0 and 31.6 µg Ag/L.

The nominal test concentrations were prepared in sterile modified AAP growth medium under sterile conditions. The medium was prepared with reduced EDTA concentrations and compounds including chloride were replaced by nitrate compounds. The concentrations of the test item in the test media were determined by chemical analysis of silver in the aqueous phase of all treatment levels by ICP-MS at test initiation, after 24 h, 48 h and at the test termination of the growth test (LOQ = 0.001 µg/L). Three different types of measurements were conducted: total Ag, conventional dissolved Ag after filtration of a subsample through 0.45 µm PSE filters and truly dissolved Ag after filtration with centrifugal filters at 3000 x g. The evaluation of the results was based on the geometric mean measured concentrations of total Ag, conventional dissolved Ag and truly dissolved Ag.

Dose-response was shown for both inhibition of yield as well as inhibition of growth rate. For conventionally dissolved silver an ErC_{10} of 0.10 µg/l was determined for growth rate. The present study produced the most conservative, and sufficiently reliable (reliability score of 2) value for chronic toxicity in the aquatic environmental compartment. Compared to the study by Manson P.S. (2000), where the same test species (*Pseudokirchneriella subcapitata*) is used, the results in the present study are considered more relevant as they are based on dissolved silver (< 0.45 µm) instead of total silver.

The ErC_{10} was recalculated to 0.00016 mg/l in order to reflect silver nitrate and taken forward for classification of chronic toxicity in the aquatic environment.

11.6.4 Chronic toxicity to other aquatic organisms

No further data available

11.7 Comparison with the CLP criteria

Silver is a metal compound and falls under the classification scheme for metals and metal compounds in the CLP-guidance Annex IV, (ECHA, 2017).

According to the already available harmonised classification ((EC) No 1272/2008, Annex VI) silver nitrate is classified as Aquatic Acute 1 – H400 and Aquatic Chronic 1 – H410. The same conclusion has been reached in the present CLH-report. See below for more information. Please also see below the derived multiplying factors (M-factors) that were not included in the previous harmonised classification.

11.7.1 Acute aquatic hazard

Solubility

According to CLP Appendix IV Chapter 5.3 ”Classification strategies for metal compounds”, a metal compound will be considered as readily soluble if the water solubility is greater or equal to the acute ecotoxicity reference value (ERV) of the dissolved metal ion. This criterion is fulfilled by silver nitrate, which is readily soluble in water (approximately 2340 g/l at 25 ^{o}C), see chapter 7 in the present report.

For readily soluble metal compounds the CLP classification strategy IV.5.3.1 proposes: “Classify the metal compound as Category Acute 1 if the acute ERVcompound ≤ 1 mg/L.”

Acute aquatic hazard
The most conservative value (0.22 µg/l) from three studies with *Daphnia magna* is used to derive the ERVsilver nitrate = 0.00034 mg/L.
ERVsilver nitrate is below 1 mg/L and silver nitrate can be classified as Category Acute 1. An M-factor needs to be determined, see below.

M-factor (acute aquatic hazard):

For soluble metal compounds, M-factors are applied as for organic substances (CLP Guidance Annex IV 5.4.; CLP, (EC) No 1272/2008, Annex I: Table 4.1.3).

The Table 4.1.3 in CLP regulation 1272/2008, Annex I for acute aquatic hazards presents M-factors related to ecotoxicity intervals based on L(E)C50 for acute toxicity and NOEC for chronic toxicity.

From the Table 4.1.3 mentioned above, an M-factor of 1000 is generated from the $ERV_{silver\ nitrate}$ of 0.00034 mg/L.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Approach based on available toxicity reference data (CLP guidance on the application of the CLP criteria, IV.5.3.2.1)

Solubility:

According to CLP Appendix IV Chapter 5.3 ”Classification strategies for metal compounds”, a metal compound will be considered as readily soluble if the water solubility is greater or equal to the acute ecotoxicity reference value (ERV) of the dissolved metal ion. This criterion is fulfilled by silver nitrate, which is readily soluble in water (approximately 2340 g/l at 25 ^{o}C), see chapter 7 in the present report.

Transformation (evidence of rapid transformation/degradation):

Silver is a natural element and, thus, not degradable by definition. It is therefore not relevant to assess degradation rate as is usually done for organic compounds. There is also no evidence of rapid environmental transformation of silver to non-available forms, please see further information in section 11.2.

In summary, it is not possible to conclude that silver is rapidly transformed to permanently non-available forms.

Chronic aquatic hazard

The most conservative value for chronic toxicity for silver (0.1 µg/l) is from a study performed with an algae, *Pseudokirchneriella subcapitata,* and is used to derive the $ERV_{silver\ nitrate}$ = 0.00016 mg/L.

For readily soluble metal compounds for which there is no evidence of rapid environmental transformation, the metal compound is classified as Category Chronic 1 if the chronic ERVcompound ≤ 0.1 mg/l according to the CLP guidance on the application of the CLP criteria.

The chronic $ERV_{silver\ nitrate}$ < 0.1 mg/l and consequently silver nitrate is classified as Category Chronic 1. An M-factor needs to be determined, see below.

M-factor (chronic aquatic hazard)

For soluble metal compounds, M-factors are applied as for organic substances (CLP Guidance Annex IV 5.4.; CLP, (EC) No 1272/2008, Annex I: Table 4.1.3).

The Table 4.1.3 in CLP regulation 1272/2008, Annex I for acute aquatic hazards presents M-factors related to ecotoxicity intervals based on L(E)C50 for acute toxicity and NOEC for chronic toxicity.

From the Table 4.1.3 mentioned above, an M-factor of 100 is generated from the $ERV_{silver\ nitrate}$ of 0.00016 mg/L (no rapid environmental transformation assumed).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

For the purpose of this classification, silver nitrate is considered as readily soluble. According to CLP guidance chapter IV.5.3.1, the metal compound is classified as Category Acute 1 if the acute ERVcompound ≤ 1 mg/l. An M-factor is established as part of this classification.

Acute $ERV_{silver\ nitrate}$ = 0.00034 mg /L -> short-term aquatic hazard: **Category Acute 1, M-factor: 1000.**

According to CLP guidance chapter IV.5.3.2.1, the metal compound is classified as Chronic 1 if the chronic ERVcompound ≤ 0.1 mg/l and there is no evidence of rapid environmental transformation.

Chronic $ERV_{silver\ nitrate}$ = 0.00016 mg/L -> long-term aquatic hazard: **Category Chronic 1, M-factor: 100** (no rapid environmental transformation assumed).

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not applicable – silver nitrate is a high temperature melting inorganic salt and the vapour pressure is anticipated to be negligible. Please see chapter 7 regarding vapour pressure in the present CLH-report.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

Reference list over studies not included in the CAR is found here below:

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Morgan, T. P. and C. M. Wood (2004). "A relationship between gill silver accumulation and acute silver toxicity in the freshwater rainbow trout: support for the acute silver biotic ligand model." Environ Toxicol Chem **23**(5): 1261-1267.

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