Agency technical report on the classification and labelling of: silver nitrate

EC Number: 231-853-9
CAS Number: 7761-88-8

March 2026

Contents

Brief summary

The conclusion of the Agency technical report is that silver nitrate meets the classification criteria for:

Ox. Sol. 1; H271 (May cause fire or explosion; strong oxidiser)

Met. Corr. 1; H290 (May be corrosive to metals)

Repr. 1B; H360FD (May damage fertility. May damage the unborn child)

Acute Tox. 2; H300 (Fatal if swallowed), with an ATE of 50 mg/kg bw

Skin Corr. 1A; H314 (Causes severe skin burns and eye damage)

Eye Dam. 1; H318 (Causes serious eye damage)

STOT RE 1; H372 (Causes damage to the nervous system through prolonged or repeated exposure)

Carc. 2; H351(Suspected of causing cancer)

Aquatic Acute 1; H400 (Very toxic to aquatic life), with an M-factor of 1000

Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects), with an M-factor of 100

Supplemental hazard statement: **EUH071** (Corrosive to the respiratory tract)

Is this in agreement with the RAC opinion? NO

The Agency is aware of new information on silver nitrate for the assessment of germ cell mutagenicity so has not proposed classification for this hazard class in this technical report. A targeted Article 37A report will be produced for the assessment of germ cell mutagenicity. Additionally, the Agency disagrees with RAC for the hazard class skin sensitisation and has proposed no classification. The Agency agrees with RAC for all other hazard classes.

At the time of publication, this mandatory classification and labelling (MCL) has not been agreed and/or adopted in Great Britain.

Introduction

Under Article 37 of the GB CLP Regulation[1], the Agency[2] is required to produce a technical report for each substance on which the Committee for Risk Assessment (RAC) of the European Chemicals Agency produces an opinion[3].

This technical report documents an independent scientific assessment, conducted by HSE technical specialists with support from the Environment Agency for the environmental hazard classification, of the classification and labelling of silver nitrate.

Table 1. Information considered in the scientific assessment

Document	**Included in assessment**
EU CLH report	Yes
Annexes to the EU CLH report	Not applicable
RAC opinion	Yes
Background document	Yes
Information submitted during the EU public consultation process (RCOM table, including attachments)	Yes
RAC minority opinion(s)	Not applicable
Other information:	Yes – for Germ Cell Mutagenicity, HSE is aware of further data being generated for the GB active substance assessment. Therefore, this will not be addressed in this technical report and will instead be

[1]The retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

[2] HSE acting in its capacity as the GB CLP Agency

[3] Under Article 37(4) of Regulation (EU) No 1272/2008 on classification, labelling and packaging of substances and mixtures

	considered under Article 37A of the GB CLP Regulation.

This information has been evaluated against the classification and labelling criteria set out in the GB CLP Regulation.

Overview of current and proposed classification and labelling

Table 2. Current and proposed classification and labelling

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
GB MCL List 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	H272 H314 H400 H410	-	-	-
EU dossier submitter's proposal	047-001-00-2	silver nitrate	231-853-9	7761-88-8	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 1B Acute Tox. 2 Eye Dam. 1 Skin Sens. 1 STOT RE 2 Muta. 2 **Modify** Ox. Sol. 1 Skin Corr. 1A	**Retain** H314 H400 H410 **Add** H360FD H300 H318 H317 H373 (nervous system) H341 **Modify** H271	**Retain** GHS03 GHS05 GHS09 **Add** GHS08 GHS06	**Retain** H314 H410 **Add** H360FD H300 H317 H373 (nervous system) H341 **Modify** H271	**Add** EUH071	Oral ATE: = 29 mg/kg bw M=1000 M=100	
EU RAC opinion	047-001-00-2	silver nitrate	231-853-9	7761-88-8	**Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H314 H400 H410 **Add**	**Retain** GHS03 GHS05 GHS09 **Add**	**Retain** H314 H410 **Add** H360FD	**Add** EUH071	Oral ATE: = 50 mg/kg bw M=1000 M=100	**Add:** T

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)**		
					Add Met. Corr. 1 Repr. 1B Acute Tox. 2 Eye Dam. 1 Skin Sens. 1 STOT RE 1 Muta. 2 Carc. 2 **Modify** Ox. Sol. 1 Skin Corr. 1A	H290 H360FD H300 H318 H317 H372 (nervous system) H341 H351 **Modify** H271	GHS08 GHS06	H300 H317 H372 (nervous system) H341 H351 **Modify** H271			
Agency technical report conclusion	047-001-00-2	silver nitrate	231-853-9	7761-88-8	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Met. Corr. 1 Repr. 1B Acute Tox. 2 Eye Dam. 1 STOT RE 1 Carc. 2 **Modify** Ox. Sol. 1 Skin Corr. 1A	**Retain** H314 H400 H410 **Add** H290 H360FD H300 H318 H372 (nervous system) H351 **Modify** H271	**Retain** GHS03 GHS05 GHS09 **Add** GHS08 GHS06	**Retain** H314 H410 **Add** H360FD H300 H372 (nervous system) H351 **Modify** H271	**Add** EUH071	Oral ATE: = 50 mg/kg bw M=1000 M=100	**Add:** T
Resulting MCL entry on GB MCL list	047-001-00-2	silver nitrate	231-853-9	7761-88-8	Ox. Sol. 1 Met. Corr. 1 Repr. 1B Acute Tox. 2 Skin Corr. 1A	H271 H290 H360FD H300 H314	GHS03 GHS05 GHS09 GHS08 GHS06	H271 H290 H360FD H300 H314	EUH071	Oral ATE: = 50 mg/kg bw M=1000 M=100	T

	Index No.	International Chemical Identification	EC No.	CAS No.	Classification		Labelling			Specific Concentration 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)		
					Eye Dam. 1 STOT RE 1 Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H318 H372 (nervous system) H351 H400 H410	Dgr	H372 (nervous system) H351 H400 H410			

Note T: *This substance may be marketed in a form which does not have the physical hazards as indicated by the classification in the entry in Part 3. If the results of the relevant method or methods in accordance with Part 2 of Annex I of this Regulation show that the specific form of substance marketed does not exhibit this physical property or these physical hazards, the substance shall be classified in accordance with the result or results of this test or these tests. Relevant information, including reference to the relevant test method(s) shall be included in the safety data sheet.*

Background

Active substance in Plant Protection Products: ☐

Active substance in Biocidal Products: ☒

Chemical registered under REACH: ☒

Background information

Silver nitrate is a highly soluble crystalline solid at room temperature with a molecular weight of 169.9 g/mol and a melting point of 212°C (ECHA, 2025).

Uses for silver nitrate involve disinfection/hygiene across biocidal and REACH-related activities (ECHA, 2025). The active chemical moiety is the silver ion (Ag^+), which has biocidal activity (ECHA, 2025). The silver ion is considered to be the relevant toxicophore so differences in bioavailability across multiple silver sources is important to consider when relying on read-across from other silver sources. This is further discussed below.

Read-Across

The dataset relied upon for the assessment of silver nitrate is similar to that used for the CLH dossier submitted for elemental silver (EC: 231-131-3, CAS: 7440-22-4). The Agency technical report for elemental silver agreed with RAC that the silver ion is the toxicophore with toxicity differences, between sources, driven by the bioavailability of the ion (HSE, 2023). Thus, the suitability of different sources for the assessment of silver nitrate is discussed below.

Silver acetate (AgAc)

In an aqueous solution, both silver nitrate and silver acetate will dissociate into the silver ion and their relevant counter ions (nitrate/acetate). It was noted by RAC that the counter ions are ubiquitous in a physiological environment or known to be of no relevant toxicological (systemic) concern.

During the public consultation, arguments were raised against the proposed read-across. It was argued that the corrosive properties of silver nitrate had not been taken into account. This was particularly emphasised for endpoints such as reproductive toxicity where no data on silver nitrate were available and data on silver acetate were relied upon. It was speculated that silver nitrate would have more pronounced general toxicity than silver acetate, suggesting that concentrations of silver nitrate would show generalised toxicity before reaching levels needed to see effects relevant for classification. RAC concluded

that whilst the corrosivity/acute toxicity of silver nitrate should be considered in dedicated endpoints, they noted that the corrosive properties of silver nitrate are not a reason to dismiss read-across from silver acetate for endpoints such as reproductive toxicity etc.

The Agency concludes that read-across from silver acetate is acceptable, especially for hazard classes where there is an absence of data on silver nitrate. The arguments suggesting that general toxicity would preclude effects relevant for classification is not accepted because there is little evidence presented that silver nitrate would be overtly toxic at doses relevant for classification. This has been discussed internally, where the Agency has compared the toxicity of nitrate to doses where relevant effects for carcinogenicity and reproductive toxicity occur. It was concluded that silver ion-related toxicity occurs at lower concentrations than that of nitrate. It is also agreed that the systemic toxicity of the silver ion would not cease to occur in the presence of corrosivity. Furthermore, a 28d oral gavage study using silver nitrate (CLH report ref: IIIA 6.3.1-07) was well tolerated in Wistar rats with no signs of GI tract effects up to the maximum dose of 100 mg/kg bw/d. The argument that gavage dosing is not relevant due to bypassing the corrosivity effect of silver nitrate is not accepted. This is because gavage dosing will introduce a large bolus dose of silver nitrate directly into the stomach so it would be expected that this would represent a worst case for corrosivity, if any were to be seen at this dose (i.e. 100 mg/kg bw/d). Based on these reasons, the Agency considers read-across from silver acetate to be justified.

Silver nanoparticles (AgNPs)

RAC considered the read-across from silver nanoparticles to be valid for the assessment of STOT RE, germ cell mutagenicity, reproductive toxicity and carcinogenicity, particularly in the absence of reliable data on silver nitrate and/or silver acetate. A summary of the key points from the RAC opinion, which were considered relevant for the read-across assessment, are listed below:

- Silver is widely distributed after silver nanoparticle exposure, and thus partly bioavailable.
- The organ/tissue distribution pattern is similar between silver nanoparticles and silver nitrate.
- Silver concentration in blood/organs is lower following exposures of equivalent doses of silver nanoparticles vs silver nitrate or silver acetate.
- Silver nanoparticle bioavailability/kinetics are impacted by size, coating, silver ion availability, vehicle, sex and other variables.
- Silver nanoparticles have a complex oxidative dissolution.
- Silver nanoparticles are able to be absorbed intact (Boudreau *et al*., 2016).
- Silver granules were detected in the tissues of animals exposed to either nanoparticles or silver acetate (Boudreau *et al.,* 2016, Van der Zande *et al.,* 2012; Loeschner *et al.,* 2011)

- A possible distinction between where silver-containing granules are deposited. Granules following silver nanoparticle exposure appear to be found within the cells whereas they are deposited extracellularly in animals exposed to silver nitrate (Boudreau *et al*., 2012). However, RAC noted that this conflicts with other literature, so this remains speculative.
- The role of free ionic silver in silver nanoparticle suspensions are not solely responsible for tissue silver levels; some is due to the silver nanoparticle. RAC considers the systemic exposure of silver ions after silver nanoparticle exposure is intermediate compared with silver nitrate/acetate.
- Oxidative stress is not unique to silver nanoparticles and thus disregarding effects resulting from this mode of action is not appropriate.
- Silver nanoparticle agglomeration may influence toxicity, but available literature is conflicting.
- The genotoxicity dataset indicates that citrate-coated nanoparticles may lead to micronucleus formation but positive genotoxicity data from non-coated nanoparticles is also available suggesting coating alone doesn't explain genotoxicity.
- Silver nanoparticles, following IV administration, can form a biomolecular corona in the blood which reduces extracellular dissolution and promotes cellular uptake. Within the cells, acidic lysosomes trigger the release of silver ions causing toxicity (Trojan horse effect) (Recordati *et al*., 2016).
- Silver nanoparticles can also dissolve extracellularly when administered orally. Using <20nm silver nanoparticles (size used for all germ cell mutagenicity data), Bove *et al.* (2017) demonstrated that 90-95% dissolve completely in gastric juice, and lose their primary nanoparticle properties.
- Silver nanoparticles show a similar set of effects to silver nitrate/acetate across fertility, developmental toxicity, mutagenicity and STOT RE, where data were available.

The Agency considers that silver nanoparticles may be able to inform on the assessment of silver nitrate, as supporting evidence. However, it is concluded that a decision on classification for all hazard classes, except Germ Cell Mutagenicity, can be concluded based on evidence from silver nitrate itself or read-across of data from simple silver salts, particularly silver acetate. For hazard classes such as STOT RE and reproductive toxicity, silver nanoparticle data provides a large body of evidence which does not contradict the conclusion of data on silver salts. Therefore, the data increases the justification to classify for these hazard classes, rather than serve as the sole reason to classify.

For Germ Cell Mutagenicity, the Agency is aware of new data on silver nitrate which will be assessed in a targeted Article 37A report. Thus, the Agency has not assessed this hazard class under this technical report and therefore reliance on read-across from silver nanoparticles is not required.

Other Silver Containing Active Substances (SCAS)

There were additional SCAS data available in the CLH report including those on silver chloride, silver sulphate, silver zeolites, silver/zinc zeolites and silver sodium zirconium hydrogen phosphate.

For the zeolites and silver sodium zirconium hydrogen phosphate, the Agency agrees with RAC that the other constituents within these complexes may influence the results due to them having their own toxicity. Therefore, they are not considered further in this report.

However, for studies using silver chloride and silver sulphate, the Agency considers these to be suitable for read-across to silver nitrate. This is because the toxicity of the counter ions (chloride and sulphate) are low, hence silver is the main toxicophore. However, these studies carry less weight than those with silver acetate as there was no bioavailability assays reported in the CLH report or RAC opinion to determine how much silver is absorbed from these compounds. They are therefore considered as supportive evidence only.

Scientific assessment of the physical, human health and environmental hazard classes

Physical Hazards

Classification agreed by RAC:

RAC considered the following physical hazard classes in their opinion on silver nitrate: explosives, substances which in contact with water emit flammable gases, oxidising solids and corrosive to metals.

Explosive

RAC noted that nitrate salts are not considered as explosives themselves but are usually oxidisers in explosive mixtures. Other nitrogen-containing groups such as nitrites, or other readily oxidised groups are associated with explosive properties. In this case, RAC concluded that silver nitrate does not contain any chemical groups associated with explosive properties. Furthermore, RAC highlighted that the UN Model Regulations on the Transport of Dangerous Goods (UNTGs Rev. 22) does not consider silver nitrate as an explosive but instead an oxidising substance.

Overall, RAC concluded that classification was not warranted for silver nitrate.

Substance which in contact with water emit flammable gases

Silver nitrate is commonly available in aqueous solutions. There is no reaction with water that would release flammable gases. Thus, based on experience with handling and use, **RAC concluded that classification was not warranted for silver nitrate.**

Oxidising solids

The results of a study by Michael-Schulz (2023) were relied upon in the RAC opinion. The test following the protocol of UN O.1. Six samples of silver nitrate (from the same manufacturer) were assessed, with 3 samples being ground before testing and 3 samples tested as received. The ground samples met the criteria for classification as Ox. Sol. 1 whereas the samples tested as received met the criteria for classification as Ox. Sol. 2.

With this in mind, RAC referenced the CLH guidance (v6.0, 2024, Section 2.14.2) which discusses differing conclusion on hazard for different particle sizes. RAC concluded that silver nitrate does meet the criteria as Ox. Sol.1 but that the data indicate that silver nitrate

with a particle size of >250µm meet the criteria as Ox. Sol.1. For this situation, RAC proposed Note T[4] which gives permission for different classification for form of silver nitrate with larger size than those for which Ox. Sol.1 is relevant

Overall, RAC concluded that **classification as Ox. Sol. 1 was warranted, with the addition of Note T.**

Corrosive to metals

RAC noted that the silver cation acts as an oxidiser towards metal ion in aqueous solution, due to a very high electrode potential Ag/Ag^{+}. In air, silver nitrate will oxidise and will be corrosive to all metals with a lower electrode potential such as copper, zinc, and aluminium due to humidity. This was supported by industry who mentioned during the public consultation that based on experience in handling and use, corrosivity of this nature is considered a potential hazard for the solid substance.

Overall, RAC concluded that **classification as Met. Corr. 1 was warranted.**

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification. **Silver nitrate meets the criteria for Ox. Sol. 1 and Met. Corr. 1, with the addition of Note T.**

Health Hazards

Acute Toxicity

Classification agreed by RAC:

Acute toxicity – oral route

RAC acknowledged the poor dataset available to determine the acute oral toxicity of silver nitrate. The only silver nitrate result available in the CLH report was a report from an old textbook (Venugopal and Luckey, 1978) but this lacked information such as substance purity, number of animals tested and dosing regimen. The LD_{50} value reported for mice was 129 mg/kg bw and the LD_{100} value for humans was 140 mg/kg bw. (WHO, IPCS, ATSDR and Lansdown (2010)) further information provided by the DS suggested that the oral lowest lethal dose in humans is approximately 28.6 mg/kg bw, although RAC noted that this figure was obtained from material safety data sheets from an unknown source.

[4] Note T - This substance may be marketed in a form which does not have the physical hazards as indicated by the classification in the entry in Part 3. If the results of the relevant method or methods in accordance with Part 2 of Annex I of this Regulation show that the specific form of substance marketed does not exhibit this physical property or these physical hazards, the substance shall be classified in accordance with the result or results of this test or these tests. Relevant information, including reference to the relevant test method(s) shall be included in the safety data sheet.

During the CLH consultation, a non-GLP acute oral toxicity study performed on mice was highlighted (Goldberg *et al*., 1950), and this study proposed an LD_{50} of 50 mg/kg bw. Key information was missing/unclear such as doses tested, concentration/purity of test item and information on the animals. Additionally, reference to a textbook (Arena, 1970, 3rd edition) was brough to the DS and RAC's attention. The textbook suggested that a dose of silver nitrate <2.5 grams is generally harmless, that ingestions of 2.5-10 grams may be fatal and that doses exceeding 10 grams are almost always fatal. This means that doses of 36 mg/kg bw/d and higher may be fatal in humans.

It was noted that silver nitrate is well tolerated in rats at doses of 100 mg/kg bw/d over 28 days, or 222 mg/kg bw/d over 23 weeks (IIIA 6.3.1-07 and Matuk et al., 1981, respectively). RAC could not establish whether this difference in toxicity between mice and rats is due to differences in corrosivity of the preparations tested. This is because the corrosive properties can be neutralised. It was further highlighted that LD_{50} values proposed in safety data sheets on the internet are vastly different between mice and rats (i.e. 50 mg/kg bw in mice and 1173 mg/kg bw in rats). Overall, RAC considered that there is a difference in sensitivity amongst species, suggesting that corrosivity is not the only cause of death.

In conclusion, RAC explained that the LD_{50} should be selected from the most detailed study performed from the most sensitive species. This was considered to be 50 mg/kg bw based on the study performed by Goldberg *et al* (1950). RAC further noted that this LD_{50} value in mice is within the same range as doses suggested to be fatal in humans (Arena., 1970). Therefore, RAC proposed a classification of silver nitrate as **Acute Tox. 2; H300 (Fatal if swallowed) with an ATE of 50 mg/kg bw.**

Acute toxicity – dermal route

There were no robust data available on the acute dermal toxicity of silver nitrate. Therefore, RAC concluded that classification for acute dermal toxicity was not warranted, owing to inconclusive data.

Acute toxicity – inhalation route

There were no robust data available on the acute inhalation toxicity of silver nitrate. Therefore, RAC concluded that classification for acute inhalation toxicity was not warranted, owing to inconclusive data.

RAC also proposed to apply the supplemental hazard statement EUH071, 'corrosive to the respiratory tract', owing to the lack of acute inhalation toxicity data and corrosive nature of the substance. This is in line with Section 1.2.6., Annex 2 of the CLP Regulation.

Classification proposed by the Agency:

Acute toxicity – oral route

The Agency agrees with RAC's conclusion on classification. Silver nitrate meets the criteria for classification as Acute Tox. 2; H300 (Fatal if swallowed)) with an ATE of 50 mg/kg bw.

Acute toxicity – dermal route

The Agency agrees with RAC's conclusion on classification. Classification is not warranted for acute dermal toxicity.

Acute toxicity – inhalation route

The Agency agrees with the RAC opinion. Classification is not warranted for acute inhalation toxicity.

The Agency also agrees with the addition of the supplemental hazard statement, EUH071 (Corrosive to the respiratory tract).

Specific target organ toxicity – single exposure (STOT SE)

Classification agreed by RAC:

There was only one study available in the CLH report under STOT SE. Five rats/sex/group (3/sex in controls) were exposed to 20, 50 and 100 mg AgNO3/kg bw/d for 28 days via gavage. RAC did not note any effects within this study to be relevant for STOT SE.

RAC also considered effects seen across studies from the acute toxicity section. Although effects such as gastroenteritis, diarrhoea, low blood pressure, decreased respiration, spasms and paralysis were described following oral exposure, there was no indication as to what dose these occurred at, with RAC suggesting some could have coincided with mortality. Overall, RAC did not consider there to be enough evidence to conclude whether silver nitrate should be classified for STOT SE. Therefore, RAC concluded that classification was not warranted for STOT SE, due to inconclusive data.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification. Silver nitrate does not warrant classification for STOT SE.

Skin corrosion/irritation

Classification agreed by RAC:

There were two *in vitro* studies described in the CLH report. The first study was OECD TG 431 and GLP-compliant, exposing 3D human skin model (EpiDerm™) to 1%, 2.5%, 5%, 10% and 25% w/v of silver nitrate in purified water. Following 60 minutes of exposure, skin viability was 90%, 93%, 79%, 66% and 7%, respectively. The substance was therefore considered corrosive from a concentration of 25% (i.e. < 15% viability after 60-minute exposure). However, sub-categorisation was not possible as this requires an exposure time of 3 minutes as per OECD TG 431.

The second study was described in both the original REACH registration dossier and an EPMF document received during the public consultation. The DS determined this study to be authored by Heppenheimer (2009). Human skin model (epiCS®) was exposed to 25 mg of silver nitrate in 25 µL of deionised water for 3 minutes and 1 hour. Relative absorbance (viability) was reduced to 8.4% after 3 minutes which is below the threshold for corrosivity. However, relative absorbance was reduced to only 25.1% after exposure for 60 minutes, which does not meet the threshold for corrosivity. It was noted in the 60 minute group that the test item precipitated, affecting the absorbance values, and the cells were unable to reduce MTT, suggesting complete cell death. Therefore, corrosivity was considered to be confirmed across both exposure groups. According to OECD TG 431, when viability is reduced below 15% following 3 minutes of exposure (when using the epiCS® model), the prediction is classification in sub-category 1A.

Additional data (not presented in detail in the RAC opinion) was made available to RAC after the public consultation. RAC considered this data supportive of Skin Corr. 1A.

Considering the available data, RAC concluded that silver nitrate met the criteria for **Skin Corr. 1A; H314 (Causes severe skin burns and eye damage).** No SCL was set as there was limited information to propose a reliable concentration limit. RAC considered the GCL of 1% to be potentially conservative.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification. Silver nitrate meets the criteria for classification for **Skin Corr. 1A; H314 (Causes severe skin burns and eye damage)**.

Serious eye damage/irritation

Classification agreed by RAC:

One published study was reported in the CLH report (Calvery *et al*., 1941) which described a dose-related increase in eye damage in young albino rabbits as the administered concentration of AgNO3 increased from 1-12%. At the highest doses, coagulation and corneal injury were mentioned alongside scar tissue and one case of blindness. RAC noted that the severe and irreversible effects reported in this study alongside the skin corrosive properties of silver nitrate indicate that silver nitrate should be **classified as Eye Dam. 1; H318 (Causes serious eye damage)**. RAC considered that the GCL was appropriate based on the available data.

Classification proposed by the Agency:

The Agency agrees with the RAC opinion. Silver nitrate meets the criteria for classification for **Eye Dam. 1; H318(Causes serious eye damage)**.

Respiratory sensitisation

Classification agreed by RAC:

There were no studies available to assess respiratory sensitisation in the CLH report. However, two human cases of 'reported allergy' following exposure to colloidal silver were described. The first case reported swelling of the face, generalised urticaria and state of near collapse whereas the second case reported severe asthma. RAC concurred with the DS that this evidence was weak with no details on medical history, a possible reaction to other components in the colloidal silver and no available animal data. Therefore, RAC concluded that **no classification was warranted for respiratory sensitisation, based on inconclusive data**.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification. Silver nitrate does not warrant classification for respiratory sensitisation.

Skin sensitisation

Classification agreed by RAC:

The data available for the assessment of skin sensitisation comes from a mix of animal data, mainly on AgNP's and human data on silver nitrate from the CLH report and public consultation.

Animal data

A summary of the animal data considered by RAC is presented in the table below:

Table 3: RAC summary of the animal skin sensitisation data, taken from pages 24-25 of the RAC opinion (ECHA, 2025)

Study	Results	Uncertainties	RAC Evaluation
Hultman *et al.,* 1994; Non-GLP	AgNO3 induces antinuclear antifibrillarin antibodies	Exposure via drinking water	Low relevance for skin sensitisation (ss) properties
Kim *et al.,* 2012 (GPMT); OECD TG 402	Exposure to AgNP (10 nm) induces discrete and patchy erythema in 1/20 animals	No information regarding a positive control study or any preliminary dose-setting investigations. Ag+ release after topical/dermal exposure to AgNP unknown.	Overall TK studies show that small sized NP in acidic solutions tend to release more Ag+, but no TK data available for dermal route. In addition, no positive control and dose-setting investigation is missing. Taken together, negative results are to be taken with caution.
Doc IIIA 6.1.5-02 (Buehler); GLP study, OPPTS 870.2600	Exposure to Axenohl induces reaction graded 0.5 in 16/20 exposed and 6/10	This score is not considered positive but the historical data for the positive	These effects could be considered as positive, considering the

Study	Results	Uncertainties	RAC Evaluation
	naive animals (24h); in 14/20 exposed and 5/10 controls animals (48h).	control (DNCB) includes reactions from 0.5 to 2. Animals challenged at doses inducing irritation in 50% of the animals in preliminary irritation tests. Unknown if effects could be assigned to Ag+.	values in HCD and the description of grade 0.5 but the specificity could be discussed, and the dose may be inadequate. Taken together, positive results to be taken with caution.
IIIA 6.1.5-08 (Buehler); GLP study, US EPA 870.2600	Exposure to zeolites induced reactions graded 0.5 in 7/20 exposed and 2/10 naive animals (24h); 2/10 exposed animals still positive at 75h.	Positive control (HCA) only 3/10 animals showed positive response (score 1–2). Unknown if effects assigned to Ag+. Release of Ag+ from zeolites unknown.	These effects could be considered as equivocal, considering the grade noted in positive control (1-2). Nevertheless, only 3/10 positive controls are considered positive. As above, the specificity could be discussed and the dose may be inadequate. Taken together, results to be taken with caution. In addition, read-across from zeolites is not

Study	Results	Uncertainties	RAC Evaluation
			supported by RAC.
Prinsen, 1995 (GPMT); GLP, OECD TG 406	Sodium silver thiosulphate did not induce skin sensitisation at concentration tested.	Ag+ concentration could be considered low (1% silver in sodium silver thiosulfate + 10% dilution). No robust information on dermal absorption. Results not determinable for controls (positive/negative) due to methodological limitations.	It cannot be excluded that the results would be positive at higher doses. Negative results to be taken with caution.
Haist, 2007 (LLNA); GLP and OECD TG 429	AgNP did not induce skin sensitisation at concentration tested.	Concentration very low (0.008%, 80 ppm Ag0). LLNA sensitivity could be reduced for metals and salts. No positive control.	It cannot be excluded that the results would be positive at higher doses. Negative results to be taken with caution.
SCCP/1196/08, 2009 (LLNA)	Silver citrate did not induce skin sensitisation at concentration tested.	Citric acid and silver citrate tested up to 25%; silver citrate contains 2400 ppm Ag+ (0.24%). LLNA sensitivity may be reduced for metals.	It cannot be excluded that the results would be positive at higher doses. Negative results to be taken with caution.

RAC concluded that the animal data does not fulfil the criteria for skin sensitisation as no appropriate positive result was reported. Although the two Buehler assays may fulfil criteria (e) of Section 3.4.2.2.1.4. of the CLP Regulation (i.e. positive results from a structural analogue), there still remains uncertainty in the mix of positive and negative results from the dataset plus the fact that read-across of data from silver zeolites was not considered appropriate.

Human data

RAC considered nine case reports and one survey investigating silver allergies, taken from the CLH report and consultation.

Six of these cases were from individuals patch tested with silver nitrate, for which clear positive results were reported. Four were described in the CLH report, as included in a publication by A. B. G. Lansdown (2010). Two were provided during the CLH consultation (Ozkaya (2009) and Iliev and Elsner (1998)). These are summarised in the table below:

Table 4: Human individual patch tests

Study	Test Substance	Relevant information	Observations
A.B.G Lansdown (2010) – Gaul, L. E. and Underwood G.B, 1948	10% Silver Nitrate	White male of 26 years, a machinist with dermatitis pedis.	An increase of pain and dermatitis when silver nitrate was applied to an area of the skin (heel) but also a reaction in healthy skin (arm) after exposure to 5/10% silver nitrate. Silver chloride and silver iodide were also tested with no reaction.
A.B.G Lansdown (2010) - Agarwal, S, and	0.5% silver nitrate	30-year old man exposed to metals and precious	Positive allergic reaction in test stated to be consistent with

Study	Test Substance	Relevant information	Observations
Gawkrodger D, J, 2002		stones for many years	European standards
A.B.G Lansdown (2010) – Fisher, A. A, 1987	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.
A.B.G Lansdown (2010) – Fisher, A. A, 1987	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
Ozkaya., 2009 (Provided during CLH consultation)	Silver nitrate	42-year old man who has been working as a machine operator. Had eczema of 10 months in duration. The patient had used Silverdin® cream containing silver sulfadiazine at 1% on his left foot for a thermal burn. Redness and weeping had developed on the foot spreading to the legs, trunk, arms, and face during treatment.	Positive patch test reaction to marking fluid containing Silver nitrate at 10%. Subsequent testing with ingredients of the marking fluid confirmed sensitisation to silver nitrate (++ reaction to 1% in pet.)

Study	Test Substance	Relevant information	Observations
Iliev and Elsner, 1998 (Provided during CLH consultation)	Phenylmercuric silver acetate and silver nitrate	A 58-year old man with a history of dermatitis in both hands for 3 years. Worked as a locksmith, reporting improvements during vacations.	Positive reactions for phenylmercuric silver acetate. Additionally, a sharply demarcated erythematous infiltration on the edge of the field covered with 1% silver nitrate in water.

Additionally, RAC noted a population based study by Jankićević *et al* (2007), where patients with chronic venous leg ulcers (CVLU) vs controls with clinical symptoms of contact dermatitis were tested against silver nitrate. The results showed a higher sensitisation rate in CVLU patients (i.e. 6.7% of CVLU patients vs 1.2% in controls) where it was considered that these patients were likely exposed to silver nitrate through topical drugs.

A publication titled ‘Frequency of skin sensitisation to specific substances and in specific occupational groups’ by J. Geler and S. Schubert (2021) was also discussed by RAC. In this publication, members of the Information Network of Departments of Dermatology (IVDK) gathered information on unselected dermatitis patients between 2007-2016. From a total of 1029 cases, 8 were positive following silver nitrate exposure. RAC considered this supporting evidence that silver nitrate can induce skin sensitisation.

Based on the above data, RAC observed that the tests were consistently positive when tested against silver nitrate.

RAC also considered two cases following exposure to colloidal silver (Argyrol) via nasal exposure. In these two cases, swelling of the face and asthma was reported and RAC considered these to be supportive of the positive results in silver nitrate. Finally, a survey reporting no reaction in 93 workers after silver exposure was reported. However, RAC considered this to be of low reliability as very little information was available.

Overall, RAC highlighted that less than 100 patch test cases were available and that this corresponded to a low/moderate frequency according to table 3.2 of the CLP guidance

(Page 129, Part 3, Version 5.0, ECHA 2024b). In regards to the population study, RAC considered the frequency of occurrences to be high even from the lowest dose of 1% silver nitrate. Unfortunately, in both cases, exposure to silver nitrate/silver forms was not known or unquantifiable. When considering the criteria outlined in Section 3.4.2.2.4.1 of the CLP Regulation, RAC concluded that in a weight of evidence approach, criteria (a)[5] and (e)[6] were fulfilled. Additionally, for the criteria outlined in Section 3.4.2.2.4.3. of the CLP Regulation, RAC concluded that at the very least criteria (a)[7] and (b)[8] were fulfilled. Since the occurrence frequencies between case studies and patch testing were not consistent for two groups of dermatitis patients and because exposure could not be determined, RAC considered sub-categorisation unnecessary.

In conclusion, RAC proposed to classify **silver nitrate as Skin Sens. 1; H317 (May cause an allergic skin reaction).**

Classification proposed by the Agency:

The Agency has noted the available data presented by RAC and arguments for classification. However, the Agency does not agree that the criteria for classification have been met for skin sensitisation for the reasons outlined below.

Firstly, the Agency does agree with RAC that the animal data are insufficient to conclude on the classification. The two results indicating a potential positive response (Doc IIIA 6.1.5-02 (Buehler); GLP study, OPPTS 870.2600 and IIIA 6.1.5-08 (Buehler); GLP study, US EPA 870.2600) were considered to be not reliable by the Agency. In the OPPTS study, Axenhohl was used as the test substance. It is noted that this is a mixture (i.e. silver ions, citric acid, water and sodium lauryl sulfate) and mixtures are not usually used for classifying substances. Furthermore, the reactions in this study were more characteristic of irritation (i.e., fewer animals responding at 48h compared to 24h) than sensitisation. In the EPA study, silver zeolite was used which, as noted in the read-across discussion above, is not considered acceptable for the assessment of silver nitrate. Overall, the animal data are insufficient to conclude on the skin sensitisation potential of silver nitrate.

Secondly, whilst the human data provide some evidence of skin sensitising potential (6 positive patch test cases, 8 positive cases in survey (J. Geler and S. Schubert (2021)) and increased reactions in CVLU patients (Jankićević et al (2007)), the Agency considers the number of cases to be too low to meet the criteria for classification (only 6 cases over the time period of 1948-2009 and 8/1029 positive cases in the survey). Furthermore, there is very little information in the study by J. Geler and S. Schubert (2021) as to how these

[5] Positive data from patch testing, normally obtained in more than one dermatology clinic

[6] Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic

[7] Isolated episodes of allergic contact dermatitis

[8] Epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence

cases were sensitised in the first place, so there is no information to determine the potency. The CVLU patients are considered to represent a group of individuals where the skin barrier has been severely compromised, hence it was considered to not represent a normal exposure situation. Given that silver nitrate is also corrosive (See assessment performed in this TR), it is possible that some reactions represent irritancy/corrosivity rather than a sensitisation reaction.

For classification in Category 1, the GB CLP criteria state that a substance should cause sensitisation by skin contact in a substantial number of persons. Although 'substantial' is undefined in the Guidance on the Application of the CLP Criteria (Part 3, Version 5, ECHA 2024b), given the long history of use and availability of silver nitrate, the Agency has concluded that the human dataset does not represent a substantial number of persons.

Therefore, the Agency concludes that silver nitrate does not meet the criteria for classification for skin sensitisation.

Specific target organ toxicity – repeated exposure (STOT RE)

Classification agreed by RAC:

There is a large dataset outlined in the CLH proposal and subsequent RAC opinion to address STOT RE. Some of these studies were discussed within the CLH report but additional published literature was also available to RAC, which was not considered in the original report. For conciseness, the technical report has summarised RAC's conclusions. Therefore, to see detailed study summaries, please see the CLH report and RAC opinion. For a full breakdown of all studies available to RAC, please see pages 160-171 of the RAC opinion (ECHA, 2025).

Oral exposure

RAC considered that from the available dataset, only the neurotoxic effects after exposure to silver nitrate, silver acetate, other silver salts and silver nanoparticles were relevant for STOT RE. Effects reported on other organs (e.g. heart, bone, zymbal gland) occurred at doses outside the guidance value (GV) range for classification and thus will not be discussed further in this technical report as the Agency agrees with this conclusion. It is important to note that there are no guideline studies presenting neurotoxic effects relevant for STOT RE classification. However, neurotoxicity investigations are usually limited in guideline studies and thus a lack of evidence from guideline studies is not a reason to dismiss the effects in the presence of a large body of evidence from the external literature.

Two important considerations were presented in the RAC opinion. The first of which explains that the degree of systemic exposure to silver ions is considered higher for silver nitrate and silver acetate than for silver nanoparticles, hence studies on silver

nanoparticles may underestimate the hazards of silver nitrate. Secondly, RAC emphasised that no study alone provides clear and unequivocal evidence of neurotoxicity and is instead based on a weight of evidence assessment, with some studies showing a strong case for neurotoxicity of silver nanoparticles and silver ions.

As part of the dataset, some studies addressed neurodevelopmental toxicity, and RAC viewed these as supportive of STOT RE. A study by Wu *et al* (2015) demonstrated histopathological changes, including hippocampal neuronal cell loss and impaired spatial learning and memory in rats following intraperitoneal exposure to silver nanoparticles of their mothers, during gestation days 10-18. Similarly Ghaderi *et al* (2015) reported impaired cognitive behaviour in the Morris water maze test, following prenatal exposure to silver nanoparticles. Yin *et al* (2015) observed cerebellar ataxia-like symptoms in neonatal rats exposed intranasally for 14 weeks. These symptoms were supported by evidence of 'destruction of the cerebellum granular layer'.

RAC highlighted a range of studies demonstrating that the brain, and more specifically the hippocampus is a target tissue of silver. These studies included Charehsaz *et al* (2016) and Chang *et al* (2022) who found neuronal damage in the hippocampus of test animals. Studies by Dziendzikowska *et al* (2021 and 2022), Liu *et al* (2012) and Davenport *et al* (2015) noted effects in the hippocampus, such as oxidative stress and modulation of neurosteroids. RAC further noted additional studies where effects (oxidative stress, modulation of neurotransmitters, or mitochondrial dysfunction) were reported in the brain without specifying the exact location (Hadrup *et al*., 2012, Skalska *et al.,* 2020, Skalska *et al.,* 2016, Strużyńska and Skalska, 2018, Sharma *et al.,* 2013, Xu *et al.,* 2015, Yin *et al.,* 2015, Zhai *et al.,* 2024). Additionally, RAC considered the developmental neurotoxicity evidence from the extended one generation reproductive toxicity study (Anon, 2022b) and Wu *et al* (2015) as supporting evidence for an adverse impact on the hippocampus for STOT RE, since these studies noted neuronal damage in the hippocampus.

RAC provided a summary table of all studies where neurological effects in adult rodents, other than on memory and learning, were reported. Those with a non-oral route of exposure were considered to be of less weight but still considered supportive, and are highlighted in grey. In the RAC opinion table, they also specified in red where effect levels were within the range of STOT RE 1. However, RAC did this for studies using non-relevant routes of exposure (e.g. intranasal, intraperitoneal etc.) which the Agency considers to be inappropriate. Therefore, in Table 5 below only the studies using oral exposure are highlighted in red where effects occur at doses within the GV for STOT RE 1. RAC noted that, in most cases, the hippocampal structures were affected across these studies potentially indicating a higher sensitivity for neurotoxicity in this structure. In some studies, the hippocampus was not specifically considered (Sharma *et al.,* 2013, Yin *et al*., 2015, Xu *et al*., 2015, Skalska *et al.,* 2016, 2020, Anonymous, 2024).

Table 5: Studies showing neurological effects in adult rodents, adapted from pages 96-97 of the RAC opinion (ECHA, 2025).

Studies with neurological effects other than learning and memory	Effect level within GV range for STOT RE 1	Effects
Liu et al. (2012), 14-d, rat, intranasal	AgNP: 3 mg/kg bw/d	↑ ROS in hippocampus, altered pyramidal cells
Sharma et al. (2013), 7-d, rat, i.p.	AgNP: 50 mg/kg bw/d	cortical oedema, blood brain barrier breakdown, neuronal damage
Bagheri-abassi et al. (2015), 28-d, rat, oral	AgNPs: 30 mg/kg bw/d	↑ dark + apoptotic neurons in hippocampus
Davenport et al. (2015), 7-d, mouse, intranasal	AgNP: 50 mg/kg bw/d	↑ oxidative stress-response in hippocampus
Yin et al. (2015), 14-w, rat, intranasal	AgNP: 1 mg/kg bw/d	↑ damage in cerebellum granular layer
Xu et al. (2015), 14-d, rat, oral	AgNPs: 1 mg/kg bw/d	↑ neuronal shrinkage, swelling of astrocytes
Charehsaz et al. (2016), 14-d, rat, oral	AgNO3, NP: 31.5, 0.2 mg/kg bw/d	↑ neuronal cell loss, hippocampal sclerosis
Skalska et al. (2016), 14-d, rat, oral	AgNP/Silver citrate: 0.2 mg/kg/d	↑ ROS, lipid peroxidation, GPx in brain
Dan et al. (2018), 24-h, rat, i.v.	AgNP: 5 mg/kg	↑ hippocampal astrocyte foot swelling, neuronal shrinkage
Dalfardi et al. (2019), 28-d, rat, oral	AgNPs: 30 mg/kg bw/d	↑ dark + apoptotic neurons in hippocampus
Skalska et al. (2020), 14-d, rat, oral	AgNP/Silver citrate: 0.2 mg/kg/d	↑ mitochondrial dysfunction and autophagy in brain

Antsiferova et al. (2021), 180-d, mouse, drinking water	AgNPs: 2 mg/kg bw/d	↑ hippocampal cell integrity
Recordati et al. (2021), 28-d, oral	AgAOC, NP: 1.55, 1 mg/kg bw/d	↑ hippocampal astrocyte foot swelling
Chang et al. (2022), 10-d, mouse, i.v.	AgNP: 12 mg/kg bw every 3rd day	↑ hippocampal neuronal damage
Dziendzikowska et al. (2022), 28-d, rat, oral	AgNO3, NP: 0.79, 0.5 mg/kg bw/d	↑ altered hippocampal neurosteroid levels
Zhai et al. (2024), 12-w, mouse, intranasal	AgNP: 4 mg/kg bw/d	↑ hippocampal pyramidal cells loosely arranged
Anonymous, (2024), 104-w rat, diet	AgAc: 20 mg/kg bw/d (after 52 weeks)	↑ nerve fibre degeneration in spinal cord, degeneration in the brain

The combined chronic/carc study (Anon., 2024) was performed in accordance with OECD TG 453 and GLP and used silver acetate as the test substance. Rats were exposed to 0, 20, 80 or 240/320 mg/kg bw/d (males/females) for 52 weeks (chronic phase) and 104 weeks (carcinogenicity phase). An increased incidence of nerve fibre degeneration in the spinal cord was reported at the end of the chronic phase in males and females of all dose groups. It was reported that the spinal cord changes were no different to controls at the end of the carcinogenicity phase. RAC noted that spinal cord nerve degeneration is a common spontaneous change in ageing rats but the fact that it happened earlier in life was indicative of a neurotoxic effect. Furthermore, a slightly higher incidence of sciatic nerve degeneration was reported in males at the mid (12/50) and high dose (16/50) compared to concurrent controls (7/50). Degeneration of the brain was also highlighted in the RAC opinion. This was highly anatomically specific, bilaterally symmetrical and consistent in dorsolateral striatum and thalamus regions. This mainly occurred at the top dose of 240/320 mg/kg bw/d (males/females) but also occurred at 80 mg/kg bw/d in males only. However, these changes in the brain were only reported in the late stages of the carcinogenicity phase of the study meaning that they occurred at doses outside of the GV range for STOT RE 2. From the combined chronic/carc study (Anon, 2024), only the effects in the spinal cord, occurring at ≥20 mg/kg bw/day, were considered to meet the criteria for STOT RE and were within the GV range for category 2. According to RAC, since an assessment was performed only at the 1-year timepoint, category 1 cannot be ruled out since effects may have occurred earlier, if they had been evaluated.

RAC also highlighted a range of studies where effects on learning and memory were reported, mainly from silver nanoparticles. These are summarised in the table below. Studies highlighted in grey have been performed using a non-oral route of exposure.

Table 6: Studies reporting effects on learning and memory

Studies with neurological effects other than learning and memory	**Effect level within GV range for STOT RE 1**	**Effects**
Ursu et al. (2010), 7-d, rat, i.p.	AgNPs: 5 and 10 mg/kg bw/d	↓learning, memory (Y-, radial arm-maze)
Hritcu et al. (2011), 7-d, rat, i.p.	AgNPs: 50 mg/kg bw/d	↓memory (Y-, radial arm-maze)
Liu et al. (2012), 14-d, rat, intranasal	AgNPs: 3 and 30 mg/kg bw/d	↓learning, memory (Morris Water Maze LTP)
Davenport et al. (2015), 7-d, mouse, intranasal	AgNPs: 50 mg/kg bw/d	↓learning (Morris Water Maze)
Wesierska et al. (2018), 28-d, rat, oral	AgNPs: 1 and 10 mg/kg bw/d	↓learning, memory (Carousel Maze)
Antsiferova et al. (2018), 180-d, mouse, drink	AgNPs: 2 mg/kg bw/d	↓learning, memory (Video Fear Cond. Syst.)
Dalfardi et al. (2019), 28-d, rat, oral	AgNPs: 30 mg/kg bw/d	↓learning, memory (Morris Water Maze)
Greish et al. (2019), 3-w, mouse, i.v. (1x/w)	AgNPs: 80 µg/kg bw/w	↓learning, memory (Morris Water Maze)
Antsiferova et al. (2021), 180-d, mouse, drink	AgNPs: 2 mg/kg bw/d	↓memory (Video Fear Conditioning System)
Dziendzikowska et al. (2021), 28-d, rat, oral	AgNO3, NP: 0.79, 0.5 mg/kg bw/d	↓learning, memory (Carousel Maze)
Chang et al. (2022), 10-d, mouse, i.v. (4x)	AgNP: 12 mg/kg bw every 3rd day	↓learning, memory (Morris Water Maze)

Studies with neurological effects other than learning and memory	Effect level within GV range for STOT RE 1	Effects
Tarbali et al. (2022), 21-d, rat, i.p.	AgNPs: 100 ppm	↓learning, memory (Morris Water Maze + other tests)
Zhai et al. (2024), 13-week, mouse, intranasal	AgNPs: 4 mg/kg bw/d	↓learning, memory (Morris Water Maze)

As highlighted in table 5, the hippocampus was affected across many studies. The hippocampus plays a major role in learning and memory and the literature highlights that it is a plastic and vulnerable structure, able to be damaged by a variety of stimuli (Anand and Dhikav (2012)). Overall, since effects in the hippocampus are expected to affect learning and memory, the pattern of effects seen in the above studies are considered to support this.

In terms of classification for STOT RE, RAC highlighted that the oral studies by Charehsaz *et al* (2016), Dziendzikowska (2021), Xu *et al* (2015), Wesierska *et al* (2018) all showed severe neurotoxic effects at doses within the range of STOT RE category 1. They also considered that two intranasal studies were supportive of neurotoxic effects at low doses (Liu *et al* (2012) and Zhai *et al* (2024)). These two studies were supportive only because the intranasal route is not compatible with the oral guidance values.

Nonetheless, RAC did highlight numerous inconsistencies with the data:

- Impaired cognitive functions and neurotoxic effects were not seen in all studies (e.g. Liu *et al* (2013) which dosed, intraperitoneally, mice with silver nanoparticles for 7 days).
- Effects seen in silver nanoparticles were dependent on degree of exposure, particle size, surface coating, agglomeration state and the type of cell/organism used. Hence the data on silver nanoparticles did reveal neurotoxic effects all of the time. Nonetheless, a large proportion of the studies support neurotoxic effects.
- Chang *et al* (2022) reported neurotoxicity for silver nanoparticles (12 and 120 mg/kg bw/d) but not for silver nitrate (5 mg/kg bw/d) following IV administration. RAC considered this to be of less weight as the route of exposure is less relevant and other oral studies on silver nitrate did show neurotoxic effects (Dziendzikowska *et al* (2021) and Charehsaz *et al.,* (2016)).

Overall, RAC gave the highest weight in the weight of evidence assessment to studies performed on silver nitrate via the oral route. However, they did not fully dismiss studies using other routes of exposure (IV, IP, intranasal etc.) and other forms of silver (silver

nanoparticles) but rather considered them to contribute to a large body of evidence. RAC explained that the very specific neurotoxic observations reported across the dataset are not always a part of the OECD TG studies, and since a high number of studies consistently reported severe neurotoxic effects in the range of category 1, RAC considers that there is strong and robust evidence to conclude STOT RE 1 for silver nitrate. This is supported by the CLP guidance (Guidance on the Application of the CLP Criteria, Part 3, Version 5.0, ECHA, 2024b) which states that the study giving the most severe classification will be used unless there are good reasons that it is not the most appropriate.

RAC touched on the previous opinion on silver, adopted on June 2nd, 2022, which was supported by the Agency in our associated technical report. In this assessment, it was agreed that STOT RE category 2 was most appropriate for neurotoxicity effects. However, RAC explained that since the opinion on silver, new evidence has become available, not previously considered, and that this has increased the weight of evidence supporting severe neurotoxicity at low doses. Additionally, RAC concluded that since the degree of systemic exposure is higher for silver nitrate, studies by silver nanoparticles, which constitute a large proportion of the dataset, may underestimate its toxicity. These together support the higher classification in this opinion.

In conclusion, **RAC considered that silver nitrate met the criteria for classification as STOT RE 1; H372 (Causes damage to the nervous system).**

Inhalation exposure

Four studies were available in the CLH report on silver nanoparticles only (Sung *et al*., 2009; Sung *et al*., 2008; Ji *et al*., 2007; Stebounova *et al*., 2011). In the study by Ji *et al* (2007), hepatic focal necrosis was reported in 2 males and 1 female at the top dose of 61.24 µg/m³ but due to the lack of original data, it was unclear if this was single cell or multifocal necrosis. In the study by Sung *et al* (2008), effects on lung function were reported (i.e. significantly decreased tidal volume and minute volume). However, an argument made during the CLH consultation noted that the physical nature of silver nitrate would mean that it would deposit in the upper respiratory tract and translocate rapidly to the GI tract. In the absence of TK data, RAC noted that it is not possible to compare the effects of silver nanoparticles and silver nitrate through inhalation exposure. Therefore, RAC concluded that inhalation studies on silver nanoparticle cannot be considered further for classification of silver nitrate due to insufficient data.

Dermal exposure

Two studies were available (Korani *et al*., 2011; Korani *et al*., 2013) to assess the repeated dose toxicity effects of silver nitrate administered dermally.

In the 2011 study (non-guideline, non-GLP), guinea pigs were exposed to doses of silver nanoparticles up to 10000 µg/mL for 13 weeks. Silver nitrate (100 µg/mL) was used as a

positive control. Histopathological changes were reported at all dose groups (inflammation in the skin, muscle, liver and spleen, increased levels of Langerhans cell and decreased epidermis/dermis thickness). Necrosis of the liver was reported at the highest dose for silver nanoparticles.

Similarly, the 2013 study dosed guinea pigs with similar doses of silver nanoparticles and silver nitrate for 90d and was performed in accordance with OECD TG 411. Abnormal inflammatory responses were reported in the bones, signs of toxicity in the heart (inflammation, presence of clear zone around nucleus, cardiocyte deformities, congestion and haemorrhage) and toxic responses in the kidney (inflammation, glomeral adhesion to Bowman's capsule, proximal convoluted tubule degeneration, capsular thickening, membranous thickening and increased mesangial cells).

Overall, the DS considered the necrosis in the liver and cell degeneration in the liver/kidney as possible effects for STOT RE classification. However, the necrosis was graded as 'mild' without further information and because the liver/kidney can partly regenerate, the cell degeneration of these organs was not considered to fulfil the classification criteria by the DS. RAC agreed that this study did not unequivocally demonstrate that the classification criteria had been met.

Human data

In the CLH report, there were human studies and reports available. However, the data were described as very limited and lacked reliable information on exposure level and situation. Furthermore, co-exposures to confounding factors were not described. RAC agreed with the DS that this information was not sufficiently robust to allow for a meaningful comparison with the classification criteria.

RAC also highlighted a recent review by Bonura *et al* (2024). The review highlighted 15 cases of patients with argyria and neuropsychiatric manifestations such as epilepsy, neurodegenerative syndromes, multiple sclerosis, peripheral neuropathy and psychiatric disorders. The authors concluded that argyria is a potentially preventable, rare systemic condition with neuropsychiatric complications which support silver affecting the brain. Nonetheless, RAC considered the human data inadequate to evaluate low dose silver effects on learning and memory.

Classification proposed by the Agency:

Overall, the evidence presented in the RAC opinion points overwhelmingly to an adverse effect on the brain from silver substances. Whilst there is little evidence from guideline studies amongst the dataset, the evidence from published literature consistently reported effects in the brain (most notably the hippocampus and learning/memory) with low doses (within the range of STOT RE 1) of silver substances such as silver nitrate and silver nanoparticles. One issue with the dataset is the number of studies discussed which used

non-oral routes of exposure. These studies are not usually relied upon for the assessment of STOT RE via the oral route. However, they do not contradict the effects seen in oral studies using silver nitrate (e.g. Dziendzikowska *et al* (2021) and Charehsaz *et al.,* (2016)), so can be considered supportive of a neurotoxic effect.

In conclusion, the Agency agrees with RAC that silver nitrate meets the criteria for classification as STOT RE 1; H372 (Causes damage to the nervous system).

Germ cell mutagenicity

The Agency are aware of new data, requested during the current GB active substance assessment, relating to silver nitrate. Therefore, The Agency has agreed to postpone the assessment of Germ Cell Mutagenicity until the new data are available. Therefore, no classification has been proposed within this technical report. A targeted assessment will be performed under the Article 37A process, once the new data has been received and assessed.

Carcinogenicity

Classification agreed by RAC:

Studies presented in the CLH report

No robust carcinogenicity data on silver nitrate were available. The DS presented some published studies from the literature, along with chronic/carcinogenicity studies performed using silver zinc zeolite type AJ in rats and mice. Whilst the RAC opinion was in development, industry provided an OECD combined chronic toxicity (52 weeks) and carcinogenicity (104 weeks) study in rats, performed using silver acetate; this study was taken into consideration in RAC's assessment.

The first literature study was by Schmahl and Steinhoff (1960). Rats (strain unspecified) were administered subcutaneous injections of colloidal silver for 14 months (doses not stated). Fibrosarcomas were observed in 8 of the 26 animals (23%), with 6/8 tumours located at the injection site. No vehicle controls were included in the study, but the spontaneous tumour frequency at any site was stated by the authors to be 1-3%. Therefore, the frequency of tumours located at other sites (2/26; 7.7%) was considered by the DS to be above the spontaneous frequency.

In contrast, no fibrosarcomas were observed at injection sites in a study by Furst and Schlauder (1977), in which F344 rats received intramuscular injections of silver metal powder for 20 months (dosing schedule: 5 months at 5 mg/dose, then 5 months at 10 mg/dose, then 5 months at 5 mg/dose, and finally 5 months at 10 mg/dose. A few cases of mild local inflammation were observed at the injection sites in the latter stages of the

study. Necropsy revealed several cases of encapsulation of the vehicle or injected metal, but no muscular atrophy.

The DS referred to an IRIS background document which stated that the occurrence of local sarcomas has been observed after subcutaneous implantation of silver foil. The document referred to a publication by Furst (1979), who suggested that such tumours may arise via solid state carcinogenesis, and therefore their relevance for exposure via ingestion is difficult to interpret. RAC additionally noted that an Agency for Toxic Substances and Disease Registry (ATSDR) publication reported that fibrosarcomas developed earlier and more frequently in rats after subcutaneous embedding of silver foil, compared to other metal foils, but that these were preliminary results.

The DS considered the open literature data to be of poor quality, and determined that no conclusion on classification could be drawn based on these reports.

One OECD TG 453 combined chronic/carcinogenicity study was available with B6C3F1 mice, performed using silver zinc zeolite type AJ (2.3% Ag, 12.5% Zn; Anon., 1992a)). Seventy-five animals/sex/group were administered the test substance via the oral route at concentrations of 0, 0.1, 0.3 and 0.9%; these were reported to be equivalent to ‘at least’ 0, 67, 211 and 617 mg/kg bw/d, with silver ion equivalents of 0, 0.67, 2.0 and 6.9 mg/kg bw/d. There were no statistically significant increases in tumours in any treated animals; in fact, the total number of tumours per animal was lower in top dose males compared to controls (1.00 vs 1.26), and comparable between top dose females and controls. There was a statistically significant increase in the incidence of ovarian cysts, with no dose-response. AgION AJ was considered not carcinogenic to mice based on the results of this study.

A combined chronic/carcinogenicity study (guideline not stated) on silver zinc zeolite type AJ (2.3% Ag, 12.5% Zn) was also available with F344 rats (Anon., 1992b). Seventy animals/sex/dose were administered the test substance via the oral route for 105 weeks at concentrations of 0.01, 0.03, 0.1 and 0.3%; these were reported to be equivalent to ‘at least’ 0, 3, 9, 30 and 87 mg/kg bw/d, with silver ion equivalents of 0.03, 0.09, 0.9, 0.3 and 0.9 mg/kg bw/d. At the top dose, the total number of tumours per animal was lower in males compared to controls (1.86 vs 1.96), but higher in females compared to controls, though without statistical significance (2.11 vs 1.37). A statistically significant increase was noted in the frequency of leukaemia and infiltration of leukaemia cells into different tissues in males and females (Cochran-Armitage trend test, one sided: 0.026 and 0.019 for females and males, respectively); the time point at which leukaemia developed was not clear. Statistically significant, dose-dependent increases were also observed in pituitary adenomas and endometrial polyps in females. The study author dismissed the findings of leukaemia, pituitary adenomas and endometrial polyps on the basis that the incidences fell within the HCD range. The DS did not consider this dismissal appropriate, but did acknowledge that the positive trend for endometrial polyps was dismissed by a Technical

Meeting for Biocides in June 2013, and on this basis, gave this finding no further significance.

The DS additionally referred to some *in vitro* data from the literature, which suggested that nanosilver may have tumour-promoting properties, but these were not considered sufficient evidence to inform classification.

RAC noted that at the highest concentrations in the rat and mouse studies, the silver ion equivalent doses were 0.9 and 6.9 mg/kg bw/d, respectively, and therefore the studies were not sufficient to assess the carcinogenic potential of silver nitrate up to the MTD. Overall, they agreed with the DS' conclusion that the carcinogenicity data provided in the CLH report was inconclusive and could not be used to determine classification.

Additional study submitted for *ad hoc* public consultation: EPMF, 2024

RAC based their carcinogenicity assessment on the combined chronic/ carcinogenicity silver acetate study provided by industry (EPMF, 2024), noting that they supported read-across between silver nitrate and silver acetate (see 'Read-Across' section).

Study design

Wistar Han rats were administered silver acetate (>99.9% purity; 64.68% Ag content) via the diet for either 52 weeks (chronic phrase) or 104 weeks (carcinogenicity phase). Both phases of the study were conducted according to GLP, and the carcinogenicity phase was compliant with OECD TG 453. A toxicokinetic evaluation was also performed in accordance with OECD TG 417. The target dose levels in all three parts of the study were 0, 20, 80 and 240/320 mg/kg bw/d (M/F). The numbers of animals per group are shown in Table 7.

Table 7: experimental design of the combined chronic toxicity and carcinogenicity study, taken from page 49 of the RAC opinion

Group No.	Test Item Id.	Target dose level (mg/kg body weight)/day)	Subsets	Number of Animals		Animal Numbers	
				Males	Females	Males	Females
1	Control	0 (Vehicle)	Main[b]	50	50	1-50	261-310
			Chronic[a]	10	10	51-60	311-320
			TK[a]	5	5	61-65	321-325
2	Silver Acetate	20	Main[b]	50	50	66-115	326-375
			Chronic[a]	10	10	116-125	376-385
			TK[a]	5	5	126-130	386-390
3		80	Main[b]	50	50	131-180	391-440
			Chronic[a]	10	10	181-190	441-450
			TK[a]	5	5	191-195	451-455
4		Males: 240 Females: 320	Main[b]	50	50	196-245	456-505
			Chronic[a]	10	10	246-255	506-515
			TK[a]	5	5	256-260	516-520

Id. = Identification, TK= Toxicokinetic animals
[a] Received the test material via diet for 52 weeks.
[b] Received the test material via diet for at least 104 weeks.

Mortality and clinical signs

One male in the chronic 80 mg/kg bw/d group was found dead on day 263 of the study, with the cause of death presumed by the study authors to be a spontaneous histiocytic sarcoma with widespread metastases.

In the carcinogenicity phase of the study, male mortality incidences were 10/50 (20%), 11/50 (22%), 5/50 (10%) and 17/50 (34%) in the control, low, mid and top dose groups. Statistical analysis detected a significant ($p < 0.05$) trend in death rate as the dose increased, but pairwise group comparisons did not give statistically significant results. Of the 17 top dose deaths, 9 were attributed to neoplastic causes (including 3 animals with malignant histiocytic sarcoma), 6 were attributed to non-neoplastic causes (including 4 animals with cardiovascular dysfunction), and for the remaining 2, the cause was undetermined. In the control group 5 of the 10 deaths were attributed to neoplastic causes (including 1 animal with malignant histiocytic sarcoma), 3 were attributed to non-neoplastic causes (no cases of cardiovascular dysfunction) and for the remaining 2, the cause was undetermined. None of the deaths at the low or mid dose were attributed to histiocytic sarcoma, but RAC noted that one mid-dose animal died from cardiomegaly.

Female incidences of mortality were comparable across the groups (15/50 (30%), 14/50 (28%), 12/50 (24%) and 16/50 (32%) in the control, low, mid and top dose groups). Of the 16 deaths at the top dose, 5 were attributed to treatment-related nephropathy and for 4 deaths, no cause could be determined.

In the carcinogenicity phase of the study, clinical signs included observations of poor health condition at the top dose, which occurred without clear dose-dependency. These observations consisted of hunched posture (12 males vs 7 in control; 16 females vs 8 in control), decreased activity (8 males vs 3 in control; 13 females vs 4 in control), fur erected (17 males vs 12 in control; 26 females vs 11 in control) and fur loss (14 males vs 8 in control; 19 females vs 10 in control).

All treated animals were found to have grey discolouration of the body/mouth. The authors of the study summary provided by industry noted that argyria (presence of silver pigment in the organs) is an ‘expected outcome of feeding high concentrations of the silver compound for an extended duration’ (EPMF, 2024).

Reductions in body weight gain were observed throughout the first year in top dose animals in both the chronic and carcinogenicity phases of the study, beginning on day 29 in males and day 16 in females. This led to body weight reductions at the end of the first year of 16% and 14% in males and females, respectively, compared to the control group. Animals in the carcinogenicity phase continued to show reduced body weight gain through the second year of the study, resulting in body weight reductions at the end of treatment of 32% in males and 30% in females at the top dose, and 11% in males at the mid dose. RAC noted that food consumption was reduced through the second year of the study at

the top dose compared to controls (by 18% in males and 17% in females), but that overall mean food consumption over the whole study period was comparable to controls in all treatment groups.

Based on these findings, the study author considered the maximum tolerated dose (MTD) to be the mid dose of 80 mg/kg bw/d. RAC concurred with the study author that the large decreases in weight at the top dose were indicative of excessive toxicity.

Haematology, clinical chemistry and urinalysis

RAC noted statistically significant changes in all red blood cell parameters investigated in chronic males at the top dose, but only with low magnitude, ranging from -12% for MCH at week 13/52 and +27% for reticulocytes at week 13/26. They considered that changes in red and white blood cell parameters in all other groups were of low magnitude, not statistically significant or lacked a clear dose-response. Prothrombin time was statistically significantly decreased in males from the low dose at weeks 13 and 52, and in females at the top dose in weeks 26 and 52; RAC noted that the decrease was ~10% in males and females in week 52.

Dose and time-dependent reductions in copper concentrations were observed in both sexes at all dose levels in weeks 26 and 52 (TK animals) and week 104 (carcinogenicity animals). Reductions ranged from -39% and -22% at week 26 in low dose males and females (not statistically significant), to -83% at week 104 in both sexes at the top dose. The study author considered these findings to be adverse from the mid dose.

Clinical chemistry findings in the chronic animals included increases in total cholesterol and high density lipoprotein (HDL) in both sexes. The increase was statistically significant in males at all doses and in females from the mid dose. In week 52 at the top dose, the total cholesterol increases reached +58% and +78% and HDL increases reached +69% and +109% in males and females, respectively. ALP levels increased in a dose-dependent and statistically significant manner from the mid dose in females, reaching +159% at week 26 in the top dose group. Triglycerides decreased in a dose-dependent and statistically significant manner in males from the mid dose, reaching -36% at week 26 in the top dose group.

Other notable clinical chemistry and urinalysis findings in top-dose chronic animals consisted of: a 2x increase in ALT in both sexes; a +51% increase in AST in males at week 52 (statistically significant); increased urea at week 26 (+26% and +19% in males and females, respectively) which remained statistically significant in females at week 52; decreased creatinine (-15% in at week 52 in males and -15% at week 26 in females); a non-dose-dependent but statistically significant decrease in glucose in females at all doses, which became more pronounced with exposure duration, ranging from -14% at week 13 to -21% at week 52); and slight but statistically significant lower mean urinary pH in top-dose males (7.05 vs 7.95 in controls).

Macroscopic pathology and organ weights

Macroscopic pathology of chronic-phase animals revealed one top-dose male with bilaterally enlarged testes; the right was found to have interstitial oedema, whilst the left had dilatation of seminiferous tubules along with degeneration/atrophy consistent with an outflow obstruction and sperm granuloma in the epididymis. Similar findings were reported in one control male, and therefore RAC gave this finding minimal weight. In the carcinogenicity phase, macroscopic pathology revealed a dose-related increase in enlarged testes (uni-/bilateral incidences of 1/2, 6/2, 9/22 and 11/24 in the control, low, mid and top dose groups). This finding was observed in early death animals in 1, 2 and 6 cases at the low, mid and top doses. RAC additionally noted a dose-dependent increase across testis abnormalities in general (enlarged size, unpalpable/undescended testis, swollen scrotum), with incidences of abnormally sized testes (side not recorded) of 6, 6, 17 and 36 (control, low, mid and top dose). This effect was also dose-dependent when the side was recorded (incidences 1/1, 2/4, 21/24 and 22/24 (left/right) in the control, low, mid and top dose groups). Incidences of swollen scrotum were 4, 4, 32 and 39 animals in the control, low, mid and top doses.

In the carcinogenicity phase, macroscopic enlargement of the heart was observed in 3 mid-dose and 8 top-dose males. This effect was deemed treatment-related by the study author.

Fifteen carcinogenicity-phase females at the top dose were found to have irregular surface of the kidney. At this dose, the finding was considered treatment-related by the study author; other occurrences of this finding in males and low/mid-dose females lacked a dose-response and were therefore considered to be background variation.

Organ weights were only measured in chronic animals. Statistical significance was only recorded at the top dose, in variations in organ weight relative to body weight. In males, variations consisted of increases in the following relative organ weights: brain (+25%), adrenal (+29%), prostate (+41%), seminal vesicle (+40%), heart (+23%), kidney (+30%), liver (+28%), spleen (+14%) and testis (+57%). Mean absolute testes weight increased by 30% at the top dose, but the study author attributed this to the male with bilateral testes enlargement, described above, combined with one male with bilaterally small testes in the control. Increases in relative prostate (+28%), seminal vesicle (+30%), heart (+12%) and liver (12.5%) were also observed in mid-dose males. In females, statistically significant changes in organ weights at the top dose consisted of increases in relative brain (+17%), heart (+14%), liver (29%) and spleen (16%) weights. Mid-dose females also showed an 11% increase in relative liver weight.

Microscopic pathology of chronic animals

Microscopic pathology of chronic animals revealed one incidence of epididymis sperm granuloma in a top dose male, and one mid-dose male with histiocytic sarcoma. No other treatment-related neoplastic findings were observed in the chronic phase of the study.

RAC highlighted several non-neoplastic microscopic findings in the chronic study. Extramedullary haematopoiesis was observed in the spleen of males at all dose levels, increasing in severity and occurrence as the dose increased (incidences of 0/10, 2/10, 6/9 and 9/10 in the control, low, mid and top dose groups). There were no associated macroscopic/spleen weight changes in the males, and in females, only one incidence of this finding was reported at the top dose. Nerve fibre degeneration was observed in the spinal cord in males and females at all doses, again increasing in severity and occurrence with dose; male incidences were 1/10, 7/10, 9/9 and 9/10, and female incidences were 1/10, 5/10, 8/10 and 10/10 in the control, low, mid and top dose groups.

At the top dose, non-neoplastic findings were reported in bone (sternum and femur), mesenteric lymph nodes, ovaries and kidneys. Increased bone, in the form of a greater number/thickness of trabeculae formed from lamellar bone in the medullary cavity, was reported, with 4 incidences in the sternum and 3 incidences in the femur (all ranging from minimal to mild) among the 10 top dose males. In the 10 top dose females, there were 6 incidences of increased bone in the sternum (ranging from mild to moderate) and 4 incidences in the femur (ranging from minimal to mild). No associated macroscopic findings in bone were reported in either sex. Diffuse angiomatous hyperplasia was observed in the mesenteric lymph nodes of 2/10 males, with no associated macroscopic findings. Nine of the 10 females at the top dose were found to have diffuse hypertrophy/hyperplasia of interstitial cells in the ovary, ranging in severity from minimal to moderate with no associated macroscopic or organ weight changes. Kidney findings in males and females included 5/10 males and 3/10 females with calculus in the renal pelvis (minimal-mild in males and minimal in females), occasionally alongside urothelial hyperplasia (3/10 males and 1/10 females). Minimal multifocal dilatation of renal cortical tubules was observed in 2/10 males and 4/10 females. In males, there was also 1 incidence of kidney fibrosis, 2 incidences of tubular basophilia, 1 incidence of tubular inflammation and 1 incidence of angiectasis of the papilla.

Other top-dose findings highlighted by RAC consisted of single incidences of lobuloalveolar hyperplasia in the mammary gland, focal hyperplasia in the parathyroid gland and hyperplasia of endocardial Schwann cells, all in females.

Neoplastic findings in the carcinogenicity phase

The study author concluded that the number of animals with neoplastic findings was generally comparable across all groups, with female treatment groups actually showing a slightly reduced incidence of neoplasia compared to the control. RAC highlighted the

following table comparing overall neoplastic incidences across groups, noting that no statistical analysis was performed on these comparisons.

TABLE 8: overall tumour incidence in the carcinogenicity phase (taken from page 56 of the RAC opinion, ECHA, 2025)

Overall Tumour Incidence: Carcinogenicity Study: Main Animals

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Removal Reason: All of those selected	MALE				FEMALE			
	0 mg/kg	20 mg/kg	80 mg/kg	240/320 mg/kg	0 mg/kg	20 mg/kg	80 mg/kg	240/320 mg/kg
Animals Examined	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Tumour Bearing Animals	30	35	30	33	43	41	43	42
Animals with Malignant Tumours	11	15	10	16	15	15	5	17
Animals with Benign Tumours	24	28	27	21	41	36	42	33
Animals with Multiple Tumours	14	16	15	13	29	25	23	21
Animals with Single Tumours	16	19	15	20	14	16	20	21
Animals with Multiple Malignant Tumours	0	3	2	2	2	1	2	0
Animals with Multiple Benign Tumours	11	8	11	9	22	16	20	17
Animals with Metastasising Tumours	6	6	2	10	8	4	2	6
Total Tumours	54	57	52	52	93	74	79	68
Total Malignant Tumours	11	18	12	18	18	16	8	17
Total Benign Tumours	43	39	40	34	75	58	71	51
Total Metastasising Tumours	6	6	2	10	8	5	2	6

However, silver acetate treated groups did show an increase in the incidence of renal neoplasia in the kidneys, without metastasis (incidences of neoplastic findings discussed by RAC are provided in Table 9 below). The increased incidence was statistically significant in males at the top dose (5/50 animals with benign adenoma or malignant carcinoma, compared to 0/50 in controls) when analysed using a Peto's pairwise comparison test. A Peto's trend test also revealed a statistically significant trend in the incidences of renal adenoma and incidences of adenoma and carcinoma (combined) across all treated groups. Although not statistically significant, the incidence of renal neoplasms also increased in top dose females (3/50 with benign adenoma compared to 0/50 in the control group). The incidences of renal neoplasms in the treated groups fell outside of the HCD range for the laboratory (males: carcinoma range = 0-2, mean = 0.8, no adenoma reported; females: no adenoma or carcinoma reported) and those provided from literature reports. Therefore, the study author concluded that the increased incidence in females at the top dose was also likely to be treatment-related; this was supported by the similar histologic appearance of the adenomas observed in both sexes and the occurrence of similar non-neoplastic findings. RAC additionally noted that one of the adenomas observed at the top dose in males was associated with early death (day 601), and that one case of kidney haemangioma was also described in the same group.

One incidence of adenoma was reported in males at the mid dose and in females at the low dose. The study author dismissed these findings, concluding that they were not treatment-related owing to their low incidence, lack of dose-response and lack of associated findings in the renal parenchyma/cortex. However, RAC noted that, in the carcinogenicity phase, both sexes showed dose-dependent increases in calculus, males showed dose-dependent increases in incidences and severity of urothelial hyperplasia,

and females showed tubular hypertrophy from the mid dose (see 'Pre-/non neoplastic findings in the carcinogenicity phase').

Other neoplastic findings included epididymis mesotheliomas with metastasis, mammary gland fibroadenomas, hepatocellular neoplasia and endometrial adenocarcinomas. The study author did not consider these findings to be treatment-related. With regards to the epididymis mesotheliomas, they noted that although the incidence at the top dose was outside of the HCD range (4/50 (8%) compared to 1/50 in the concurrent control and a HCD mean of 1.3% and range of 0-2%), the increase compared to the concurrent control was only statistically significant in the Peto trend test and not in a pairwise comparison. However, RAC considered that the epididymis mesotheliomas were treatment-related, citing that 1 of the 4 observed in the top dose group was associated with early death (day 722), whereas the single incidence in the control group was observed at study termination. They further noted that one other incidence of mesothelioma was reported in the abdominal body cavity in mid-dose females, despite only a small number of animals being examined for this effect. RAC therefore concluded that epididymis mesotheliomas were a rare tumour, and showed a highly statistically significant trend increase in top dose males, above the HCD range.

The study authors highlighted a reduction in the incidence of mammary gland fibroadenomas at the top dose (1/48 compared to 7/50 in the control group), noting that the control incidence for this finding was above the HCD range of 2-12%. RAC considered this finding to be of low relevance for classification.

Males at the mid and top dose showed an increased incidence of hepatocellular neoplasia (at the mid dose, 4/50 had adenomas and at the top dose, 2/50 had adenomas and 1/50 had carcinomas. Neither tumour was observed in the control group). Top-dose females showed a similar increase in adenomas (3/50 compared to 1/50 in the control, low and mid dose groups). The increased incidences fell outside of the HCD range, which reported no cases of carcinoma in either sex, and adenoma ranges of 0-4% in males and 0-2% in females. These findings were not statistically significant in a Peto's pairwise comparison test and were therefore considered by the study author to be unrelated to treatment. However, RAC noted that 1 adenoma in each of female treatment groups, as well as 1 adenoma in the low dose male group, was visible at early death, with the single incidences in the low and mid dose groups appearing earlier than in the control (day 574 and 597 at the low and mid dose compared to day 696 in the control). Additionally, some top dose animals showed associated non-neoplastic findings such as eosinophilic or basophilic focus cellular alteration (see Pre-/non neoplastic findings in the carcinogenicity phase'). Therefore, despite a lack of statistical significance, RAC considered that the increase in hepatocellular neoplasia may have been treatment-related.

There was an increase in the incidence of endometrial adenocarcinomas (uterus/cervix) in top-dose females (11/50 compared to 6/49 in the controls). This finding was statistically

significant according to a Peto trend test but not statistically significant according to pairwise comparison. The study author considered the adenocarcinomas observed at the top dose to be less often fatal than in the control group (1/11 compared to 3/6 in the control) and therefore concluded that the findings were not treatment-related. RAC did not agree with this conclusion, noting that whilst the control incidences did appear 'more fatal', the adenocarcinomas in treated animals were more often observed with early deaths, and were associated with non-neoplastic findings including a dose-dependent increase in cystic endometrial hyperplasia.

Several other neoplastic findings were acknowledged by RAC but showed no dose-response or statistical significance.

Haemangiosarcomas were observed in the mesenteric lymph nodes of males (3/49, 1/50, 2/50 and 2/50 in the control, low, mid and top doses) and were associated with angiomatous hyperplasia (see Pre-/non neoplastic findings in the carcinogenicity phase').

Malignant schwannomas were observed in the heart in 2/50 low-dose males (1 with metastasis) and 1/50 top dose males, and in the uterus/cervix in 2/50 low-dose females (1 with metastasis) and 2/50 top dose females (1 with metastasis). Other malignant glial cell tumours consisted of 1/50 females at the top dose with oligodendroglioma in the brain, associated with early death, and astrocytomas in the brain (2/50 mid dose and 1/50 top dose males, with 1 of the mid dose incidences being associated with early death) and spinal cord (1/50 top dose males, associated with early death).

Parathyroid gland adenomas were observed in 0/46, 2/48, 1/46 and 2/48 males and 0/49, 0/48, 0/43 and 1/46 females in the control, low, mid and top dose groups.

The incidence of histiocytic sarcoma increased in top dose males compared to the control group (3/50 vs 2/50). RAC noted that all three top dose incidences of this finding occurred alongside early deaths, compared to only one in the control group. Incidences of histocytic sarcoma were comparable in top dose and control females (1/50 vs 1/50), but again, RAC noted that the top dose incidence of this finding appeared earlier than the one in the control (day 331 vs day 569).

One mid-dose male was found to have malignant sarcoma in the stomach without metastasis; this finding was not observed in any other group.

TABLE 9: Neoplastic incidences highlighted in RAC's assessment. Numbers of tissues examined presented next to each organ.

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Kidney	50	50	50	50	50	50	50	50
Adenoma	0	0	1	4	0	1	0	3
Carcinoma	0	0	0	1	0	0	0	0
Adenoma + carcinoma	0	0	1	5	0	1	0	3
Epididymis	50	50	50	50	-	-	-	-
Mesothelioma with metastasis *Early death*	1* 	0 	0 	4 *1*	-	-	-	-
Mammary gland	45	8	4	33	50	50	50	48
Fibroadenoma	0	0	0	0	7	10	9	1
Liver	50	50	50	50	50	50	50	50
Hepatocellular adenoma *Early death*	0 	2 *1*	4 	2 	1 	1 *1*	1 *1*	3 *1*
Hepatocellular carcinoma	0	0	0	1	0	0	0	0
Uterus/cervix	-	-	-	-	49	50	50	50
Endometrial adenocarcinoma *Early death*	-	-	-	-	3* 	6 	2 *1*	8 *1*
Endometrial adenocarcinoma with metastasis *Early death*	-	-	-	-	3 *3*	0 	0 	3 *1*

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Mesenteric lymph nodes	49	50	50	50	48	-	50	49
Haemangiosarcoma	3	1	2	2	1	-	1	2
Early death	*1*						*1*	*1*
Glial cells (heart)	50	50	50	50	49	50	50	49
Schwannoma	0	1	0	1	0	0	0	0
Early death				*1*				
Schwannoma with metastasis	0	1	0	0	0	0	0	0
Early death								
Glial cells (uterus/cervix)	-	-	-	-	49	50	50	50
Schwannoma	-	-	-	-	0	1	0	1
Early death						*1*		*1*
Schwannoma with metastasis	-	-	-	-	0	1	0	1
Early death						*1*		*1*
Glial cells (brain)	50	50	50	50	50	50	50	50
Astrocytoma	0	2	1	0	0	0	0	0
Early death		*1*						
Oligodendroglioma	0	0	0	0	0	0	0	1
Early death								*1*
Glial cells (spinal cord)	50	50	50	50	50	50	50	50
Astrocytoma	0	2	1	0	0	0	0	0

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Parathyroid gland	46	48	46	48	49	48	43	46
Adenoma	0	2	1	2	0	0	0	1
Stomach	50	50	50	50	50	16	-	-
Sarcoma	0	0	1	0	0	0	-	-
Histiocytic sarcoma of haemolymphoreticular tissue	50	11	5	50	50	15	15	50
With metastasis *Early death*	2 *1*	0	0	3 *3*	1 *1*	1	0	1 *1*

*Asterix on control = statistically significant Peto's trend test
- Not applicable

A Peto's pairwise test also revealed the following statistically significant tumour incidences:

- Skin fibroma in mid dose males (1/50, 4/50, 5/50* and 2/50 in the control, low, mid and top dose). This finding was given low weight in the assessment owing to a lack of dose response and a similar magnitude of incidence in control females (5/50).
- Thymoma in low dose male and mid dose females (males: 2/46, 3/12*, 0/4 and 0/50; females: 4/48, 2/16, 4/15* and 4/47; both in control, low, mid and top dose). This finding was also given low weight owing to a similar magnitude of incidence being observed at the top dose compared to the control, and comparable total numbers of individuals being observed with tumours despite differing numbers of animals assessed.

**statistically significant according to Peto's pairwise test.*

Pre-/non neoplastic findings in the carcinogenicity phase

Pre- and non-neoplastic findings were observed in several organs in the carcinogenicity phase of the study. Full incidences of findings discussed by RAC are provided in Table 10 below.

In the kidneys, findings in the renal papilla consisted of urothelial hyperplasia (incidences of 9/50 and 36/50 males at the mid and top doses, compared to 5/50 in the control; and

14/50 in females at the top dose, compared to 9/40 in the control) and angiectasis (6/50 and 10/50 males and females at the top-dose, compared to 0/50 in the control). The study author considered these findings to be a response to the presence of calculi in the pelvis, which was observed in 14/50 and 43/50 males at the mid and top dose, compared to 3/50 in the control, and 14/50, 19/50 and 33/50 females at the low, mid and top doses, compared to 12 in the control. Findings in the renal parenchyma and cortex included tubular basophilia, which was observed in 1/50 and 29/50 males at the low and mid dose, compared to 1/50 in the control, and in 2/50 and 40/50 females at the mid and top dose, compared to 2/50 control females. At the top dose, 7 of the male incidences and 9 of the female incidences of tubular basophilia were observed in early death animals, whereas only 1 case was identified in early death in the male control group. Multifocal tubular dilatation was observed in 35/50 males and 44/50 females at the top dose; of these, 9 male cases and 11 female cases were seen in early death animals. There were no incidences of multifocal tubular dilatation in the control. Multifocal tubular inflammation was observed in 24/50 males and 29/50 females at the top dose, compared to 0/50 in the control group, with 8 of the female cases being identified in early death animals. Tubular hypertrophy was observed in 10/50 males at the top dose compared to 0/50 in the control, and in 4/50 and 8/50 females at the mid and top dose, compared to 1/50 in the control. Lastly, mononuclear cell interstitial infiltrates were observed in 17/50 females at the top dose compared to 0/50 in the control, with no increased incidence in males. RAC could not exclude whether the parenchymal/cortex findings were pre-neoplastic lesions, as the study report did not always specify the precise location of the kidney adneomas. However, they noted that the carcinoma identified in a top-dose male was indicated to be in the cortex, and one adenoma was indicated to be in the corticomedullary junction.

An increased incidence of angiomatous hyperplasia was reported in the mesenteric lymph nodes of top-dose males, ranging from mild to severe (16/50 animals compared to 1/50 in the control group). One of these incidences was observed in early death. No such increase was observed in females, with 2/49 incidences (1 observed in early death) reported at the top dose, compared to 0/48 in the control. There was a slight increase in the number of incidences of angiomatous focal hyperplasia in females (2/50 and 3/49 at the mid and top doses, compared to 0/48 in the control), but this did not exceed the HCD range of 2-6%. The study author did not consider the effects in the lymph nodes to be treatment-related, but RAC noted that haemangiosarcomas were observed in the lymph nodes of males, although with no dose-response (3/49, 1/50, 2/50 and 2/50 in the control, low, mid and top doses). They additionally noted that 2/10 top-dose males in the chronic phase of the study were found to have mesenteric lymph node angiomatous hyperplasia. Therefore, RAC concluded that the findings in the lymph nodes were likely to be treatment-related in males.

Focal hyperplasia of the parathyroid gland was noted in both sexes at the top dose (29/48 males and 17/46 females, with 4 male and 3 female cases observed in early deaths), ranging in severity from minimal to marked, based on the size and multiplicity of foci. The

study author considered this finding to be adverse, owing to the focal nature of the lesions, which excluded the likelihood that they developed secondary to renal lesions as a result of renal secondary hyperparathyroidism. RAC agreed that the effect was likely to be treatment-related, and additionally highlighted that the same effect was identified in 1 top-dose female in the chronic phase of the study (see 'Microscopic pathology of chronic animals'*)*.

In the liver, the incidence of eosinophilic foci increased in males (1/50, 0/50, 3/50 and 16/50 in the control, low, mid and top dose) and females (2/50, 3/50, 10/50 and 17/50 at the control, low, mid and top dose). Additionally, there was an increase in basophilic foci (non-tigroid but otherwise unspecified) in both sexes (4/50, 9/50, 7/50 and 19/50 in males and 9/50, 10/50, 12/50 and 21/50 in females at the control, low, mid and top doses). RAC considered these increases to be treatment-related. The incidence of non-regenerative hepatocellular focal hyperplasia also increased in mid-dose males (4/50 animals compared to 1/50 at all other doses) and in females (1/50, 2/50, 5/50 and 5/50 at the control, low, mid and top doses). The study author did not consider the hyperplasia to be treatment-related in males, owing to a lack of dose-response, but RAC considered that the finding was treatment-related in females, citing that 1/10 mid-dose female in the chronic phase showed the same effect.

A dose-dependent increase in glandular focal hyperplasia was observed in the stomach in males at all doses, ranging in severity from minimal to moderate (incidences of 4/50, 7/50, 10/50 and 17/50 in the control, low, mid and top dose groups). Two of the top-dose incidences of hyperplasia were observed in early death animals. The effect was not associated with ulceration/inflammation, altered gastric function or progression to neoplasia, and the study author therefore considered it to be non-adverse. RAC noted that one male in the low dose group was reported to have a secondary stomach adenocarcinoma, and that one mid-dose male had malignant sarcoma without metastasis (see 'Neoplastic findings in the carcinogenicity phase').

RAC also noted a dose-dependent increase in diffuse interstitial cell hypertrophy/ hyperplasia in the ovary (incidences of 3/48, 4/50, 7/50 and 29/49 in the control, low, mid and top dose; 1, 2, 3 and 8 of these cases were associated with early death). After two years, top dose females also showed an increased incidence of cystic endometrial hyperplasia in the uterus/cervix (30/50 vs 14/49 in the control; in both groups, 4 incidences were associated with early death). RAC considered that increases in cystic endometrial hyperplasia and endometrial adenocarcinoma may represent a continuum.

Lastly, endocardial hyperplasia of Schwann cells in the heart was observed in males at all dose levels (1/50, 2/50 and 2/50 at the low, mid and top doses compared to 0/50 in controls) and in females at the mid and top doses (2/50 and 1/49, respectively, compared to 0/49 in the control). In one mid-dose female, this finding was observed in early death.

TABLE 10: Relevant non-neoplastic incidences highlighted in RAC's assessment. Numbers of tissues examined presented next to each organ (adapted from pages 58-62 of the RAC opinion, ECHA, 2025).

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Kidney	50	50	50	50	50	50	50	50
Calculus (pelvis)	3	6	14	43	12	14	19	33
Early death	*1*	*1*		*13*	*2*	*3*	*5*	*8*
Minimal (uni-/bilateral)	*-/2*	*3/-*	*7/1*	*2/8*	*6/5*	*8/1*	*6/7*	*5/*
Mild (uni-/bilateral)	*-/1*	*1/-*	*5/1*	*4/2*	*-/1*	*4/1*	*4/2*	
Moderate (uni-/bilateral)	*-/-*	*1/-*	*-/-*		*-/-*	*-/-*	*-/-*	*7/*
Marked (uni-/bilateral)	*-/-*	*-/1*	*-/-*	*-/6*	*-/-*	*-/-*	*-/-*	*2/*
				-/-				*-/-*
Urothelial hyperplasia	5	7	9	36	9	7	4	14
Early death	*1*			*9*	*1*	*1*	*1*	*1*
Minimal (uni-/bilateral)	*4/-*	*1/-*	*5/-*	*6/1*	*5/3*	*4/1*	*2/1*	*1/-*
Mild (uni-/bilateral)	*1/-*	*3/2*	*3/1*	*4/1*	*-/1*	*2/-*	*1/-*	
Moderate (uni-/bilateral)	*-/-*	*-/1*	*-/-*		*-/-*	*-/-*	*-/-*	*2/*
				-/1				*-/2*
Angiectasis (papilla)	0	0	0	6	0	2	1	10
Hyaline casts	0	1	0	4	2	1	2	40
Early death		*1*			*1*		*1*	*4*
Tubular basophilia	1	0	1	29	2	1	2	40

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Early death	*1*		*1*	*7*			*1*	*9*
Tubular dilatation	0	0	0	35	0	0	0	44
Early death				*9*				*11*
Tubular inflammation	0	1	1	24	0	0	0	29
Early death								*8*
Tubular hypertrophy	0	1	0	10	1	1	4	8
Mononuclear interstitial cell infiltration	1	0	1	1	0	0	1	17
Liver	50	50	50	50	50	50	50	50
Basophilic foci	4	9	7	19	9	10	12	21
Early death	*1*	*1*		*2*	*3*		*1*	*1*
Minimal	*1*	*7*	*5*	*14*	*5*	*8*	*7*	*6*
Mild	*2*	*2*	*1*	*5*	*4*	*2*	*4*	*12*
Moderate	*1*	*-*	*1*	*-*	*-*	*-*	*1*	*2*
Marked	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*1*
Eosinophilic foci	1	0	3	16	2	3	10	17
Minimal	*1*	*-*	*-*	*13*	*2*	*2*	*8*	*10*
Mild	*-*	*-*	*3*	*3*	*-*	*1*	*2*	*7*
Hepatocellular focal hyperplasia (non-regenerative)	1	1	4	1	1	2	5 *1*	5

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Early death								
Ovary	-	-	-	-	48	50	50	49
Interstitial cell hypertrophy/hyperplasia	-	-	-	-	3	4	7	29
Early death					*1*	*2*	*3*	*8*
Uterus/cervix	-	-	-	-	49	50	50	50
Endometrial stromal polyps	-	-	-	-	9*	2	10	12
					2	*1*	*2*	*3*
Cystic endometrial hyperplasia	-	-	-	-	14	22	21	30
Early death					*4*	*3*	*6*	*4*
Mesenteric lymph nodes	49	50	50	50	48	-	50	49
Angiomatous hyperplasia	1	0	1	16	0	0	0	2
Early death				*1*				
Mild	-	-	1	3	-	-	-	1
Moderate	1	-	-	4	-	-	-	1
Marked	-	-	-	2	-	-	-	-
Severe	-	-	-	7	-	-	-	-
Focal hyperplasia	2	3	3	2	0	0	2	3
Early death		*1*						
Parathyroid gland	46	48	46	48	49	48	43	46
Focal hyperplasia	11	7	8	29	4	2	5	17

	Dose (mg/kg bw/d)							
	Males				Females			
	0	20	80	240	0	20	80	320
Early death	*3*		*1*	*4*	*1*	*1*		*3*
Minimal	*4*	4	*2*	*5*	*1*	*1*	*3*	*7*
Mild	*6*	*2*	*5*	*14*	*2*	*0*	*2*	*8*
Moderate	*1*	1	*1*	*6*	*1*	*1*	*-*	*2*
Marked	*-*	*-*	*-*	*4*	*-*	*-*	*-*	*-*
Stomach	50	50	50	50	50	50	50	50
Vascular/perivascular inflammation	0	2	0	0	0	0	0	5
Early death								*2*
Glandular focal hyperplasia	4	7	10	17	2	0	1	2
Early death	*1*			*2*				
Minimal	*3*	*4*	*3*	*10*	-	-	-	-
Mild	*1*	*2*	*5*	*5*	-	-	-	-
Moderate	*-*	*1*	*2*	*2*	-	-	-	-
Schwann cells (heart)	50	50	50	50	49	50	50	49
Endocardial hyperplasia	0	1	2	2	0	0	2	1
Early death							*1*	

*Asterix on control = statistically significant Peto's trend test
- Not applicable

Other non-neoplastic findings were acknowledged by RAC but considered to be more relevant to other hazard classes. These included findings in the testis, which consisted of

interstitial oedema, degeneration/atrophy of seminiferous tubules, and decreased sperm, and are discussed further under reproductive toxicity. The following effects were considered more relevant for discussion under STOT RE: degeneration of the caudate nucleus, putamen and thalamus in the brain in mid/top-dose males and top-dose females; slightly increased incidences of nerve fibre degeneration in the sciatic nerve and spinal cord in mid and top-dose males; cardiac effects including cardiomegaly, myocardial hypertrophy/degeneration and progressive myopathy; increased bone; increased incidence of spleen extramedullary haematopoiesis and mineralisation of Zymbal's gland blood vessels.

RAC conclusion on classification

RAC considered the adenomas and carcinoma observed in the kidneys to be relevant for classification. They noted that at the top dose, the 4/50 adenomas, 1/50 carcinoma in males and 3/50 adenomas in females were associated with significant toxicity in the form of ~30% reductions in body weight. However, they noted that kidney adenomas were observed at lower doses in both sexes (1/50 in mid dose males and 1/50 in low dose females), with a statistically significant positive result obtained in a Peto's trend test. Renal changes including tubular dilatation were observed at lower doses in the carcinogenicity and chronic phases, where body weight changes were less pronounced; as discussed above, RAC could not exclude that these findings were pre-neoplastic changes. Overall, they concluded that the kidney tumours were likely to have human relevance.

RAC also considered the endometrial adenocarcinomas in their conclusion, noting that whilst the incidence of this finding was particularly high at the top dose, there was no clear dose-response. However, a Peto's trend test gave a positive result. Mesothelioma in the epididymis was also detected at the top dose in the presence of extreme body weight loss.

The following tumours were considered by RAC to be supportive of classification: schwannomas, severe tumours which appeared to be rare when compared to the concurrent control, but which did not show dose-dependence or statistical significance; histiocytic sarcomas, which appeared to occur earlier after treatment with silver acetate; parathyroid gland adenomas and liver adenomas, both of which were rare according to concurrent controls but were benign and occurred without dose-dependency. RAC additionally noted the reduced latency of liver adenomas in females, including at the lowest dose.

Overall, RAC acknowledged that there were uncertainties in the data owing to a lack of malignant or dose-dependent findings at dose levels below the top dose where high levels of toxicity occurred. They concluded that the study provided 'limited evidence of carcinogenicity', therefore supporting classification in Category 2. Based on read-across from silver acetate, RAC concluded that silver nitrate should be classified as **Carc. 2; H351 (Suspected of causing cancer)**.

Classification proposed by the Agency:

The Agency agrees with RAC's conclusion on classification. Silver nitrate meets the classification criteria for **Carc. 2; H351 (Suspected of causing cancer)**.

Reproductive toxicity

Classification agreed by RAC:

For sexual function and fertility and developmental toxicity, there were no studies using silver nitrate available to RAC. Therefore, the assessment relied upon read-across of data from studies performed on silver acetate, silver nanoparticles and silver chloride. The read-across argument is further discussed above. In contrast to the DS, RAC did not support read-across from complex substances such as silver zeolites and silver sodium hydrogenophosphate.

Sexual function and fertility

A summary table of the studies and effects considered most relevant by RAC is provided below. This has been adapted from the RAC opinion, with additional information added where necessary for clarity:

Table 11: RAC summary of studies relevant for the assessment of sexual function and fertility (Taken from pages 71-75 of the RAC opinion (ECHA, 2025))

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
Rat studies		
AgAc 0, 0.4, 4.0 and 40.0 mg/kg bw/d Administered via drinking water One gen study Not GLP Sprando *et al*., 2016	Parental toxicity ↓ Stomach weight (dose dependent; -40% (stat sign) Sexual Function and Fertility ↓ implantation sites at top dose (-22%; p<0.05) ↓ fertility index at top dose (-10%) No effect on testes or gestation length	RAC considered reliable Missing investigations (i.e. sperm parameters) Reduction in implantation sites considered as **some evidence** of an adverse effect on fertility. Not considered a secondary effect of systemic toxicity.

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
AgAc F0 gen: 0, 4, 40, 80, 160 and 320 mg/kg bw/d (12 rats/sex) F1 gen: 0, 4 and 40 mg/kg bw/d (10 rats/sex) Dietary administration EOGRTS preliminary study	<u>General toxicity</u> All parental females were sacrificed at 160 and 320 mg/kg bw/d either for total litter loss (4/12 at 320 mg/kg bw/d and 2/12 at 160 mg/kg bw/d) or welfare reasons (between GD 20 and LD4) Inactive mammary glands in those females with total litter loss ↓bw in males (pre-pairing) at 320 mg/kg bw/d (-20% - statistically significant) at study termination <u>Sexual Function and Fertility</u> ↑ testis relative weight in top dose F0 males (+24%; p<0.01) (dose-dependent) ↑ gestation length in F0 females. Statistically significant at 40 mg/kg bw/d (p<0.05) and higher (p<0.01)	RAC considered reliable **No sperm parameter investigations** RAC noted comparison (From PND 28 in females and PND 38 in males) to concurrent control only possible for the doses at 4 and 40 mg/kg bw/d. **Further discussion in main text below.** RAC concluded that longer gestation length and dystocia were clear evidence of effect on sexual function/fertility. Testis effects were considered supportive.
AgAc F0/F1: 0, 40, 80 and 120 mg/kg bw/d Dietary administration OECD TG 443 and GLP compliant Main EOGRTS	<u>F0 General toxicity</u> 1 male death at 80 and 2 male deaths at 120 mg/kg bw/d (unknown causes) 1 female died at 120 mg/kg bw/d unrelated to treatment (mammary lesion). Males showed haematology and immune cell changes	RAC considered results reliable. They did not consider general toxicity seen to affect the assessment of effects on sexual function and fertility. Overall RAC considered the effects on sperm parameters and longer gestation length as clear

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
	↑ spleen extramedullary haematopoiesis (males) Epithelial degeneration of the glandular mucosa (females) <u>F1 General toxicity</u> All animals at 120 mg/kg bw/d killed at 10 weeks mainly due to brain lesions. Slight reduction in bw at 80 mg/kg bw/d (-11%). <u>Sexual function and fertility</u> ↑ gestation length in F0 females at 120 mg/kg bw/d ($p<0.05$) Delay in mean age of vaginal opening at 120 mg/kg bw/d (F1 females). Increased by 1.2 days. Statistically significant decrease in testis spermatids of F0 males at 120 mg/kg bw/d. ↓ in spermatids (total counts and total million) and cauda epididymis sperm total million (F1 males except 120 mg/kg bw/d group who were sacrificed prematurely) ↓ testis and epididymis weights (F1) from 80 mg/kg bw/d	evidence of an effect on sexual function and fertility.

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
AgAc OECD TG 453 and GLP compliant study 0, 20, 80 and 240(m)/320(f) mg/kg bw/d 52 weeks chronic and 104 weeks carcinogenicity study Dietary exposure Anonymous., 2024	<u>Males (chronic/carc)</u> ↑ testis size. Dose dependent and statistically significant. <u>Males (Chronic)</u> ↑ prostate, seminal vesicle and testis weight/bw (statistically significant at 80/240 mg/kg bw/d). ↑ absolute testis weight at 240 mg/kg bw/d <u>Males (Carc)</u> At 20 mg/kg bw/d, interstitial oedema, Degeneration/atrophy of the seminiferous tubules, decreased sperm and haemorrhage	RAC noted strong decrease in body weight at 240/320 mg/kg bw/d (exceeding MTD). RAC considered effects on reproductive system at 80 mg/kg bw/d relevant for classification.
AgNPs (70nm) 0, 25, 50, 100 and 500 ‘mg/kg concentration’, oral gavage for 48 days (Miresmaeili., 2013) 0, 25, 50, 100 and 500 mg/kg bw/d, oral gavage for 45 days (Baki *et al.,* 2014) Published literature (Non-GLP) Both studies in collaboration with each other	<u>Miresmaeili., 2013</u> Statistically significant difference in acrosome reaction in all dose groups compared to control. ↓ primary spermatocytes (p=0.012) and ↓ spermatids and spermatozoa (p=0.03) from 50 mg/kg bw/d <u>Baki *et al*., 2014</u> ↓ sperm progressive motility, sperms with normal morphology and Leydig cell number from 25 mg/kg bw/d	RAC notes lack of information on general toxicity Effects on sperm were considered supportive since they start at the lowest doses.

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
	↑ number of immobile sperm and spermatozoa with progressive motility from 25 mg/kg bw/d ↓ testosterone blood serum concentration and ↑ luteinising hormone blood serum concentration (from 50 mg/kg bw/d)	
AgNPs (60nm) 0, 15 and 30µg/kg bw/d 35 days oral gavage using young rats (PND 23-58) Mathias *et al.,* 2015 Published literature (Non-GLP)	At the lowest dose: A statistically significant ↓ in acrosome and plasma membrane integrity ↓ mitochondrial activity A statistically significant ↑ in number of abnormal sperm Authors refer to a previous set of results (same concentration of AgNPs) which showed a ↓ in sperm reserves at PND53/90 and in sperm transit time through the epididymis (PND 53)	RAC noted no signs of systemic toxicity. Sperm effects occurring at very low doses in young rats. Data were considered supportive and relevant.
Citrate-capped AgNPs (5-20nm) 0 and 20 µg/kg bw/d 90 days oral gavage Thakur *et al.* (2014) Published literature (Non-GLP)	Germ cells severely impaired and apoptotic Severe cellular changes in the cytoplasm of spermatogonia, primary and secondary spermatocytes. Round and elongating spermatids and Sertoli cells	RAC notes no signs of systemic toxicity. Sperm effects occurring at very low doses in young rats. Data were considered supportive and relevant

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
PVP-capped AgNPs (20-30nm) 0, 50, 100 and 200 mg/kg bw/d 90 days oral gavage Lafuente *et al*., 2016 Published literature (Non-GLP)	Non-statistically significant changes in number of epididymal sperm, sperm viability or sperm motility were found. An increased number of epididymal sperm morphological abnormalities was seen at 100 mg/kg bw/d but not at 200 mg/kg bw/d. Spermatocytes were not counted.	No general toxicity reported RAC considered the results as equivocal and not supportive
AgNPs (15nm) 0, 30 and 300 mg/kg bw/d 1-4 weeks oral Amr El-Nouri *et al.,* 2013 Published literature (Non-GLP)	Study performed in female rats In ovary, mononuclear cell infiltration, congestion in the stroma with extravasations of blood (dose- and duration-dependent), excess depositions of collagen fibers (indicating fibrosis) and positive reactions for apoptosis gene expression. A relative increase in atretic and degenerated follicles were reported.	RAC noted that these effects were not consistent with the lack of effects seen in the EOGRTS performed with AgAc. RAC concluded that these findings were to be treated with caution.
AgNPs (unknown size) 25, 50, 100 and 200 mg/kg bw/d 28 days oral Rezaei-Zarchi et al. (2012)	↓ testosterone concentration at 200 mg/kg bw/d ($p<0.05$)	Published literature in Arabic. Abstract is in English. RAC gave lower weight to this study but considered it supportive.

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
Published literature (Non-GLP)		
AgNP (unknown size) 25, 50, 100 and 200 mg/kg bw/d 35 days (1 dose/12 hrs) of oral gavage Amraie *et al.,* (2013) Published literature, non-GLP	↓ sperm mobility ($p<0.05$; dose dependent) with ↑ immobile sperm ↓ normal sperm (morphology) ($p<0.05$; dose-dependent)	No information on general toxicity but effects were considered consistent with those seen in the EOGRTS. RAC considered the data supportive.
AgNPs (20 and 200nm) 0, 5 and/or 10 mg/kg bw One single intravenous dose Gromadzka-Ostrowska *et al., (*2012). Published literature (Non-GLP)	↓ gonadosomatic index ($p<0.05$) ↓ epididymal sperm count ($p<0.002$). AgNP size/dose dependent ↑ abnormal spermatozoa ($p<0.05$) Histological findings were identified in the testes from rats exposed 200nm AgNPs	No general toxicity and effects were consistent with those seen in the EOGRTS study. RAC considered study relevant but gave lower weight due to the exposure route.
Rabbit Study		
AgNP (45 nm) 0 and 0.6 mg/kg bw One single intravenous dose Castellini *et al.,* 2014 Published literature (Non-GLP)	↓ Sperm count ($p<0.05$) ↑ Seminal Reactive Oxygen Species ($p<0.05$) ↑ less motile sperm ($p<0.05$) Lower curvilinear velocity and oxygen consumption ($p<0.05$) AgNP were visible in ejaculated sperm. Within	No general toxicity was noted. The effects were consistent with the effects on sperm in the EOGRTS study

Substance/dose/reference	Most relevant effects according to RAC	Final weight provided by RAC
	cytoplasmic residues and section of the nucleus, acrosome and axoneme.	Increased concern since this was within a second species RAC considered relevant but lower weight provided due to the exposure route.

In addition to those studies noted in the table above, RAC also referred to Boudreau *et al*., 2016, which was discussed under germ cell mutagenicity. This GLP and OECD-compliant study exposed rats for 13 weeks, via oral gavage, to concentrations of AgAc of 0, 100, 200 and 400 mg/kg bw/d and citrate coated AgNPs (10, 75 and 110nm) of 9, 18 and 36 mg/kg bw/d. Ten animals/sex/dose were used, which RAC highlighted was fewer than other studies on AgAc, such as Sprando *et al*., 2016 (i.e., 20/sex/dose). Due to poor survivability at 400 mg/kg bw/d AgAc, no reproductive parameters in this group were measured. There was no biologically significant body weight effect in the AgNP groups but significantly lower bw was reported in groups exposed to AgAc (more severe in males). A non-significant but dose-dependent increase in relative testis weight was reported in AgAc-exposed groups. There were no effects on sperm or gestation length irrespective of treatment type.

RAC further referred to the toxicokinetic studies performed by Van der Zande *et al*. (2012) and Lee *et al*. (2013). These studies highlighted that silver ions accumulate within the testes/brain in animals exposed to silver nitrate and AgNP. The elimination of these ions is very slow.

RAC conclusion

Overall, RAC considered all of the information presented above. The lack of study data on silver nitrate was not considered to affect the conclusion since read-across from AgAc, primarily, and AgNPs, as supportive information, was sufficient.

RAC noted that a statistically significant increase in the gestation length was reported in both the preliminary and main EOGRTS, in the absence of systemic toxicity at lower doses. In the preliminary study, they considered the prolonged gestation to occur alongside dystocia at the higher doses. Furthermore, effects on the spermatids and their parameters were also observed in the main EOGRTS. RAC noted that these were seen without marked systemic toxicity, and severity was increased in the F1 generation.

In contrast to the EOGRTS, RAC highlighted that the repeated dose study by Boudreau *et al.,* 2016 showed no significant differences in sperm/sperm parameters between controls and treatment groups. However, they noted that CLP guidance places more weight on reproductive toxicity studies compared to repeated dose studies. Hence, more weight was provided to the EOGRTS where sperm effects were noted. Furthermore, RAC highlighted that effects on sperm appeared more sensitive in the F1 generation, which the repeated dose toxicity study did not address.

With regards to the study by Sprando *et al*. (2017), RAC noted that although the study was robust, the results were inconsistent with the rest of the data set; reduced implantations and fertility index. They considered that the route of exposure could have had an impact (i.e. drinking water vs diet). RAC concluded that more weight would be provided to the EOGRTS.

Additionally, a range of studies from the published literature (non-GLP) were available on AgNPs, with effects in sperm reported in many of them. RAC considered these as supporting evidence only, noting that data on silver salts should take priority. RAC highlighted that negative studies in AgNPs do not remove the concern from silver nitrate studies.

RAC concluded that the effects observed in sperm/spermatids and longer gestation length (with presumed dystocia at higher doses), were clear evidence of adversity on sexual function and fertility, occurring in the absence of other toxic effects. These were considered relevant to humans. Hence RAC classified silver nitrate as **Repr. 1B; H360F (May damage fertility).**

Developmental toxicity

A summary table of the studies and effects considered most relevant by RAC is provided below. This has been adapted from the RAC opinion, with additional information added where necessary for clarity:

Table 12: RAC summary of studies relevant for the assessment of developmental toxicity (Taken from pages 78-81 in the RAC opinion (ECHA, 2025))

Substance/dose/reference	Effects seen	Final weight provided by RAC
Rat studies		
AgAc 0, 0.4, 4.0 and 40.0 mg/kg bw/d	Maternal toxicity Dose dependent decrease in stomach weight which was statistically significant at top dose (-40%). Effect also seen	RAC considered reliable. Maternal toxicity considered to have a limited impact on developmental toxicity.

Substance/dose/reference	Effects seen	Final weight provided by RAC
Administered via drinking water One gen study Not GLP Sprando *et al*., 2016	in pups at top dose (-34/26%, in m/f respectively). <u>Development (40 mg/kg bw/d)</u> 16/20 dams had a litter vs 20/20 in other litter groups. Two dams without a litter had a total resorption of implantation. Litter viability: 95%, 95%, 90% and 78% at 0, 0.4, 4 and 40 mg/kg bw/d, respectively. <u>Development (4 mg/kg bw/d)</u> Increased number of runts/litter.	Development effects were considered treatment-related. Increased mortality and number of runts was considered clear evidence of developmental toxicity
AgAc F0 gen: 0, 4, 40, 80, 160 and 320 mg/kg bw/d (12 rats/sex) F1 gen: 0, 4 and 40 mg/kg bw/d (10 rats/sex) Dietary administration GLP EOGRTS preliminary study	<u>Maternal toxicity (F0 females)</u> All parental females were sacrificed at 160 and 320 mg/kg bw/d either for total litter loss (4/12 at 320 mg/kg bw/d and 2/12 at 160 mg/kg bw/d) or welfare reasons (between GD 20 and LD4) Inactive mammary glands in those females with total litter loss ↓ bw and food consumption in top dose associated with decreased number of pups <u>Developmental toxicity (F1 Gen)</u> At two highest doses, incidences of dead pups at	RAC considered results reliable The 80 mg/kg bw/d had no concurrent control so comparison was not accepted by RAC. RAC considered pup mortality as treatment-related and adverse. No milk in stomachs and dams had pale and inactive mammary gland. RAC said it was difficult to discriminate between dev tox and sexual function/fertility.

Substance/dose/reference	Effects seen	Final weight provided by RAC
	top two doses. At lower doses, no specific clinical signs or toxicity were reported Litter size statistically significantly reduced at top dose (7.8 vs 15.3). ↓ pup bw in top two doses (day 1) Litter loss was 2/12 and 4/12 at 160 and 320 mg/kg bw/d No neurohistopathology findings at PND21.	Lower pup bw considered as a developmental effect.
AgAc F0/F1: 0, 40, 80 and 120 mg/kg bw/d Dietary administration OECD TG 443 and GLP compliant Main EOGRTS	<u>Maternal toxicity (F0)</u> No marked maternal toxicity related to treatment was seen <u>F1 general toxicity</u> High dose animals killed at 10 weeks due to welfare (brain lesions in accordance with neuropathological findings identified in these cohorts). <u>Developmental toxicity (120 mg/kg bw/d)</u> ↓ live births (89% vs 97% in controls) ↓ viability index (90% vs 99% in controls) ↓ bw from day 1 until weaning (both sexes). Also seen at **80 mg/kg bw/d** at days 14 and 21	RAC considered results reliable Maternal toxicity identified in F0 dams is considered low Neurotoxicity and increased mortality are considered as clear evidence of developmental toxicity

Substance/dose/reference	Effects seen	Final weight provided by RAC
	↑ incidence of severe/dose-dependent neuro-histopathological findings identified in both sexes (seen throughout cohorts). ↑ incidence of neurological/neurobehavioral effects.	
AgAc 10, 30 or 100 mg/kg bw/d via oral gavage Dosed through Gestation Days 6-19 Price and George., 2002 Published literature (Non-GLP)	No maternal toxicity <u>100 mg/kg bw/d</u> ↑ late foetal deaths (statistically significant) ↓ mean foetal bw per litter	RAC considered increased foetal deaths and reduced bw to be consistent with EOGRTS. This was considered relevant and supportive for classification.
Silver chloride 250 mg/kg bw/d in diet Exposed during gestation days 1-20 or 7-15 Shavlovski *et al.,* (1994) Published literature (non-GLP)	No maternal toxicity GD 1-20 ↑ implantation loss (36% vs 9.6% in controls) ↑ incidence of hydronephrosis and cryptorchidism ↓ lower body average mass GD 7-15 No embryotoxicity. Authors noted that this could be explained by the gradual decrease of ceruloplasmin	RAC considered the post implantation loss and decreased fetal bw as consistent with the EOGRTS This was considered relevant and supportive for classification.

Substance/dose/reference	Effects seen	Final weight provided by RAC
	from the blood and short exposure period.	
AgNPs (55mm) 0, 0.2, 2 or 20 mg/kg bw/d AgAc 20 mg/kg bw/d Oral gavage Gestation Days 7-20 Charehsaz *et al.,* 2016 Published literature	No maternal systemic toxicity but hippocampal pyramidal neuronal loss and mild gliosis were seen following exposure to both AgNPs and AgAc Offspring showed no histopathological findings in the brain, heart, liver, kidney or lung tissue. A dose-dependent increase in silver concentration in the brain was reported, however.	RAC noted that the increase in silver content of pup brains was concerning taking into account the low clearance from this organ. The lack of histopathological findings in the pups could be due to the short exposure time Overall, RAC considered the data do not contradict EOGRTS findings.
AgNPs (7.5 nm) 0, 100, 300 and 1000 mg/kg bw/d via oral gavage GD 6-20 Yu *et al*., 2013 Published literature (non-GLP)	No significant maternal toxicity No developmental toxicity ↑ pre-implantation loss was reported. Statistically significant at higher dose but also increased at low dose.	Overall RAC considered the data do not contradict the EOGRTS findings.
AgNPs (20nm) 25 mg/kg bw/d via oral gavage Gestation day 9 till parturition.	No maternal toxicity ↓ bw and brain weight/bw ratio in exposed pups	Overall, RAC considered the data to be consistent with EOGRTS findings. The data were relevant and supportive for classification

Substance/dose/reference	Effects seen	Final weight provided by RAC
Fatemi *et al.,* 2013 Published literature (non-GLP)	↑ silver concentrations and incidence of microvacuolar structures in the brain ↓ antioxidant activity in brain ↑ peroxidation in brain	
AgNPs (20nm) 0.1, 0.2, 0.5 ad 1 mg/kg bw/d Intranasal instillation for 14 weeks Yin *et al.,* 2015.	In neonatal rats: Cerebellar ataxia-like symptoms (Dysfunction of motor coordination and impairment of locomotor activity) with some statistically significant at the lowest dose tested Top dose showed distortions in both Purkinje layer and granular layer.	RAC considered that findings from direct neonatal exposure should be taken into account for classification for developmental toxicity.
Mice		
AgNPs (20nm) 0, 10, 100 or 1000 mg/kg One dose gavage (gestation day 9) Philbrook *et al.,* 2011	No maternal toxicity ↑ incidence of non-viable foetuses at GD19.	RAC notes that the effect on foetus viability was not dose-dependent. However they noted that the finding was consistent with the whole dataset. It was suggested that agglomeration of AgNPs at higher doses may reduce toxicity and clearance. Data considered supportive for classification

Substance/dose/reference	Effects seen	Final weight provided by RAC
AgNPs (20nm) 0, 0.2 and 2 mg/kg bw administered subcutaneously once per 3 days. From GD3 to parturition Ghaderi *et al.,* 2015 Published literature (non-GLP).	No maternal systemic toxicity was described. In adult offspring, cognitive behaviour was significantly impaired. Adult offspring also showed increased number of defecations/leanings in the open field assay and increased passages in light/dark test.	No information on general toxicity, which RAC considered to reduce relevance of results. However, they noted the neurobehavioral effects were consistent with the EOGRTS. RAC considered the route of exposure to lower the weight this study has on classification
Rabbit		
AgNPs (2-10nm) 1% in drinking water Before mating until weaning Hang *et al.* (2013) Published literature (non-GLP)	No maternal toxicity reported No statistically significant changes were seen in litter size and weight at birth. Survival until weaning was increased following treatment.	No information on general toxicity, which decreased the relevance of the results. No effects seen. RAC noted this could indicate the rabbit being less sensitive to silver ion developmental toxicity.

Through toxicokinetic data (Lee *et al.,* 2013 and Van der Zande *et al*., 2012), it is established that silver ions are able to accumulate in organs such as the brain and testis, with a very slow rate of elimination. RAC noted that this fact increases the concern of observed developmental neurotoxicity from the studies presented above. They also noted that the EOGRTS (preliminary and main) on silver acetate showed that silver can be transferred to foetuses *in utero* or via lactation, which was also observed in studies with AgNPs.

RAC considered a potential mechanism for developmental toxicity; silver ion-induced loss of ceruloplasmin, leading to perturbation of Cu homeostasis and thus reduced serum copper levels. In a study by Shavloski *et al*. (1995), it was noted that exposure to silver chloride reduced, not depleted, Cu tissue levels but that Cu levels in the serum, placenta

and embryonic tissues dropped to almost zero. The study authors hypothesised that duration of exposure would lead to a gradual decrease of ceruloplasmin activity until the induction of developmental defects. A study by Pribyl *et al*. (1989) was considered by RAC to confirm this hypothesis. Similar effects on Cu have been seen in studies using other forms of silver (prelim/main EOGRTS), including silver nitrate (Zatulovskiy *et al*., 2012). Overall, RAC explains that ceruloplasmin has the same function in humans so this potential mechanism cannot be considered irrelevant.

There was no available information in the CLH report on potential links between AgNPs and ceruloplasmin and/or Cu homeostasis. However, RAC highlighted a peer reviewed study by Skomorokhova *et al*. (2020), where AgNPs (10, 20 and 75nm) were injected intraperitoneally into C57Bl/6 mice. The results highlighted that AgNPs could reduce Cu plasma concentration and ceruloplasmin activity (dependent on nanoparticle size) but that Cu levels in the organs remained unaffected. RAC concluded that although this study used a non-standard route of exposure, it did show similar effects on Cu/ceruloplasmin as seen in studies with other forms of silver. With data on toxicokinetics and the consistency of effects, RAC considered it to be scientifically justified to consider the toxicity detected after AgNP exposure to evaluate the developmental toxicity of silver nitrate.

RAC conclusion

Overall, in the absence of data on silver nitrate, a read-across from silver salts (silver acetate and silver chloride) was relied upon by RAC. Data on AgNPs were supportive but RAC considered that the data on silver salts were sufficiently reliable to make a conclusion by itself.

The findings on offspring mortality (increased post implantation loss and early deaths), reduced pup bw, neurobehavioral impairments and associated histopathological findings seen in studies with silver salts were considered clear evidence of developmental toxicity. RAC considered these effects to be independent of maternal toxicity. Furthermore, mechanistic information did not raise doubt on the relevance of these findings in humans. The effects seen in studies on AgNPs were considered to be in alignment with the EOGRTS on silver acetate. Therefore, RAC concluded that silver nitrate meets the criteria for classification as **Repr. 1B; H360D (May damage the unborn child)**.

<u>Lactation</u>

In the EOGRTS preliminary toxicity study, a dose-dependent increase in the incidence of inactive and pale mammary gland was seen in dams exposed to silver acetate, which RAC noted could fulfil the classification criteria. This finding was seen in the top two doses of the preliminary study (160 and 320 mg/kg bw/d) but not in the main study, which had a top dose of 120 mg/kg bw/d. RAC noted that this effect could have been a secondary effect to dystocia which was also reported at similar doses.

In addition, RAC also noted that silver is able to enter the milk. In the study by Charehsaz *et al*. (2016), silver was reported in the milk following exposure to 20 mg/kg bw/d of silver nitrate or AgNPs. Furthermore, the preliminary EOGRTS showed that the highest concentration of silver was seen in the milk pellets obtained from offspring on day 4 for those exposed to 80 mg/kg bw/d (See table 13 below). The same table also shows that silver is able to bioaccumulate in the offspring brain throughout the lactation period, so a question was raised as to whether the threshold for toxicity could be reached in the pups. However, no neurohistopathology findings were identified in the preliminary study. RAC therefore concluded that the current dataset do not allow the determination of a threshold for toxicity in the pups. Thus, RAC considered that the available evidence was not robust enough to classify for lactation, despite the likeliness of possible toxicity effects via lactation.

Table 13: Mean concentrations of silver in the pup brains and milk pellets (EOGRTS preliminary study)

Post-natal day and Tissue examined	**Control group (silver concentration in ng/g)**	**4 mg/kg bw/d group (silver concentration in ng/g)**	**40 mg/kg bw/d group (silver concentration in ng/g)**	**80 mg/kg bw/d group (silver concentration in ng/g)**
PND 4 Female brain	<LOQ	73.1	285	437
PND 21 Female brain	<LOQ	385	2123	2926
PND 21 Male brain	<LOQ	369	2141	2936
PND 4 Female milk pellet	60.7	327	301	19163

Classification proposed by the Agency:

The Agency notes the absence of information on silver nitrate to assess reproductive toxicity but agrees with RAC that data on silver acetate and silver chloride (for developmental toxicity) can be read-across. It is also agreed that available data on AgNPs

can be used to support the assessment. Please refer to read-across discussion at the beginning of the report.

Sexual function and fertility

The Agency has considered the available dataset and provided a summary of the key studies and findings below.

EOGRTS in silver acetate (preliminary and main)

RAC has provided most weight to the results of the preliminary and main EOGRTS on silver acetate. The key findings from this study, as highlighted in their opinion, were increased gestation length, reduced testicular and epididymis spermatid counts/totals and reduced testis/epididymis absolute weights. RAC noted these to be clear signs of an adverse effect on sexual function and fertility.

Gestation length

The increased gestation length seen in the preliminary and main EOGRTS, whilst statistically significant at higher doses, is only lengthened by a maximum of 0.5 days in the treatment groups, when compared to the controls. Whilst a treatment-related effect cannot be ruled out, the increase in gestation length is minor and not considered to be adverse.

Table 14: Gestation length in the EOGRTS preliminary and main studies.

Dose group (mg/kg bw/d)	**Number of pregnant animals / gestation index**	**Gestation Length (Days)**					**Mean Length (days)**
		21	22	22.5	23	23.5	
Preliminary study (Doses of 160 and 320 mg/kg bw/d terminated early so not included here)							
Control	12 / 92%	0	0	4	5	2	22.41
4	11 / 100%	1	0	0	6	4	22.5
40 ($p<0.05$)	11 / 100%	0	0	0	3	8	22.86

80 (Not treated concurrently with controls)	11 / 100%	0	0	0	2	9	22.9
Main study							
Control	25 / 100%	0	7	8	8	2	22.6
40	25 / 100%	0	2	5	17	1	22.84
80	24 / 100%	0	0	9	15	0	22.81
120 ($p<0.05$)	24/ 100%	0	0	6	18	0	22.87

Effects on male reproduction

Only the main EOGRTS study gave a thorough assessment of male reproduction since sperm parameters were not measured in the preliminary study. At doses of 80 and 120 mg/kg bw/d, the F0 generation showed reduced testis weight of around 6-7% ($p<0.05$) compared to controls. Testis sperm total was also reduced. However, these were not dose-dependent and considered by the Agency not to be clearly related to treatment (i.e., 166, 141, 163 and 141 million sperm at 0, 40, 80 and 120 mg/kg bw/d, respectively). For the F1 generation the absolute weights of the testis and epididymis were reduced by 11-15% and 10-37%, respectively. Additionally, the total spermatids (millions) were statistically significantly ($p<0.01$) reduced, compared to controls, in the testis but this was not dose-dependent (-23.6%, -37.1% and -18.3% at 40, 80 and 120 mg/kg bw/d, respectively). Spermatid counts (millions/g) in the testis were also statistically significantly ($p<0.01$) reduced at 40 and 80 mg/kg bw/d. In the epididymis, a statistically significant ($p<0.01$) reduction in total sperm (millions) was reported at all dose groups, compared to controls (-19.9%, -19.2% and -44.5% at 40, 80 and 120 mg/kg bw/d, respectively). A reduction in epididymis sperm counts (millions/g) was also observed at all dose groups but this was not statistically significant and without a clear dose-response. A summary of these findings are presented in table 15 below.

Table 15: Male reproductive parameters in the main EOGRTS (PO and F1 generations)

Dose (mg/kg bw/d)	Control	40	80	120
P0				
Testis Weight (g)	1.96	1.89 (-3.6%)	1.83* (-6.6%)	**1.85* (-5.6%)**
Testis Spermatid Count (Millions/g)	85	75 (-11.8%)	89 (+4.7%)	76 (-10.6%)
Testis Spermatid Total (Million)	166	141 (-15.1%)	163 (-1.8%)	**141* (-15.1%)**
Epididymis Weight (g)	0.299	0.291 (-2.7%)	0.285 (-4.7%)	0.293 (-2.0%)
Epididymis Sperm Count (Millions/g)	455	470 (+3.3%)	532 (+16.9%)	419 (-7.9%)
Epididymis Sperm Total (Million)	136	136 (0%)	152 (+11.8%)	123 (-9.6%)
F1				
Testis Weight (g)	1.86	1.76 (-5.4%)	1.66** (-10.8%)	**1.59** (-14.5%)**
Testis Spermatid Count (Millions/g)	101	82** (-18.8%)	70** (-30.7%)	92 (-8.9%)
Testis Spermatid Total (Million)	186	142** (-23.7%)	117** (-37.1%)	**152** (-18.3%)**
Epididymis Weight (g)	0.244	0.236 (-3.3%)	0.220** (-9.8%)	**0.153** (-37.3%)**
Epididymis Sperm Count (Millions/g)	600	499 (-16.8%)	537 (-10.5%)	521 (-13.2%)
Epididymis Sperm Total (Million)	146	117** (-19.9%)	118** (-19.2%	**81** (-44.5%)**

*p≤0.05; **p≤0.01

Grey area denotes sacrifice at 10 weeks (prior to scheduled at 13-14 weeks) so RAC considered this not comparable.

The Agency considers that the male reproductive effects show an adverse effect on sexual function and fertility in the F1 generation. In the P0 generation, there is no clear treatment-related effect in either the testicular spermatids or the epididymis sperm. Statistically significant findings here appear to be spontaneous rather than an actual treatment-related effect, since no clear dose-response is observed. The F1 generation shows a clearer

treatment-related effect. The testis weight and spermatid number is reduced at all treatment groups, compared with the control. However, reductions in spermatid number (total millions) lacks a dose-response, as the reduction is less pronounced at the top dose. However, as noted by RAC, the top dose was sacrificed early and was considered not be directly comparable with the controls. If this group are removed from the dataset, a clear dose response is observed. The effect on epididymis weight and epididymis sperm number (total millions) clearly correlates with increased dose. All in all, there is evidence of an effect on male reproduction in the main EOGRTS. It could be argued that no actual effect on fertility has been seen and hence a clear effect on sexual function and fertility has not been demonstrated. However, an important consideration is that the effects on sperm were more pronounced in the F1 generation but this generation has not been mated. Therefore, the effect on fertility would not have been measured.

One generation toxicity study in silver acetate (Sprando et al., 2016)

RAC considered this study reliable, despite not being conducted according to GLP, but noted that it showed contrasting effects to the EOGRTS. Unfortunately, the study did not include some investigations, such as those related to sperm, and individual animal data were not available. The study reported a statistically significant decrease in the number of implantation sites (-22%) and a reduction in fertility index (-10%) at the top dose of 40 mg/kg bw/d. Some general toxicity in the dams was reported but this was limited to a statistically significant reduction in stomach weight (-40%) at the top dose. Overall, the Agency considers this to be limited evidence of an adverse effect on sexual function and fertility. The lack of reporting and contrasting effects seen compared to the more reliable EOGRTS, makes the reliability of the study questionable.

Chronic and Carcinogenicity study in silver acetate (Anon., 2024)

RAC summarised a GLP/OECD TG 453 compliant chronic/carcinogenicity study (Anon., 2024), not included in the CLH report, where silver acetate was administered to rats for two years in their diet at doses of 0, 20, 80 and 240/320 mg/kg bw/d in males/females, respectively. Due to strong decreases in bodyweight, the dose level of 240/320 mg/kg bw/d was considered to be above the maximum tolerated dose (MTD). However, effects relevant for reproductive toxicity were still observed at doses of 80 mg/kg bw/d and less.

In the testis, the incidence and severity of interstitial oedema increased in a dose-dependent manner. Additionally, degeneration/atrophy of the seminiferous tubules (reduced sperm), haemorrhage, pigmented macrophages and fibroplasia were also reported and the incidences of these were increased dose-dependently. The authors of the study further evaluated the testes and were able to exclude an obstructive and/or vascular pathogenesis for the observed effects. These effects were seen in old age animals from the carcinogenicity phase of the study, which reduces their reliability compared with the EOGRTS. A summary of these effects is presented in table 16 below.

At doses below 320 mg/kg bw/d, the main finding in female reproductive organs was ovary interstitial cells hypertrophy/hyperplasia. The Agency does not consider this to be a relevant effect for classification for reproductive toxicity, since it is not associated with any adverse impact on female fertility.

Table 16: Summary of testicular effects in the chronic/carc study (Anon., 2024)

Dose (mg/kg bw/d)	**0**	**20**	**80**	**240T**
Number of animals	50	50	50	50
Interstitial Oedema	6 (12%)	24 (48%)	41 (82%)	40 (80%)
Degeneration/atrophy of seminiferous tubules	6 (12%)	10 (20%)	22 (44%)	34 (68%)
Haemorrhage	0 (0%)	0 (0%)	3 (6%)	6 (12%)
Pigmented macrophages	0 (0%)	4 (8%)	7 (14%)	13 (26%)
Fibroplasia	0 (0%)	2 (4%)	3 (6%)	6 (12%)
Cellularity, decreased sperm (epididymis)	7 (14%)	9 (18%)	12 (24%)	28 (56%)
Depletion, germ cell; spermatid (testis)	1 (2%)	0 (0%)	0 (0%)	3 (6%)

T = Exceedance of MTD

Overall, the Agency concludes that the effects seen in male reproductive organs at doses below the MTD are treatment-related and adverse. However, since this is a chronic/carcinogenicity study, the effect on sexual function and fertility has not been measured and the effects were only seen in old age animals. It is therefore used alongside other studies in a WoE approach.

Available information on AgNPs

In agreement with RAC, the published literature studies on AgNPs can be used as supportive evidence but studies on silver acetate and other simple silver salts are most representative of silver nitrate. Please see the read-across section for further information.

The general outline of effects from AgNP's, as summarised in the study table above, is a consistent pattern of effects on sperm parameters including number, morphology, motility and/or acrosomal reaction seen in two different species (rats and rabbits). The impacts on sperm number are consistent with that seen in the silver acetate EOGRTS but there is no clear impact on other sperm parameters. Therefore, this could point to an alternative mechanism for AgNPs. Nonetheless, the data on AgNPs does not contradict the spermatogenic findings from the more reliable AgAC EOGRTS. Hence, this increases the overall confidence in the classification prediction of silver nitrate.

Overall conclusion on sexual function and fertility

The Agency considers that the most relevant effect seen across studies on silver acetate and AgNPs are the effects on sperm and associated reproductive organs (epididymis and testis). The general pattern of effects clearly shows diminished sperm counts and organ weights amongst treatment groups. The available literature on AgNPs further supports this pattern of effects. Hence, it can be assumed that silver nitrate would also effect male reproductive organs and sperm parameters.

The Agency does not agree with RAC that the effects on gestation warrant classification, due to it being minimal and not adverse.

For a substance to be classified in Category 1B for sexual function and fertility, a clear adverse effect on sexual function and fertility must be observed in test animals. The Agency notes that whilst there are effects seen, the available data do not allow unequivocally demonstrate an effect on male fertility. Nonetheless, in the EOGRTS, the male reproductive parameters were more significantly affected in the F1 generation for which a conclusion on fertility cannot be drawn (as this generation was not mated). Additionally, the evidence from other studies including the body of evidence on silver nanoparticles further supports an effect on male reproduction. Therefore, in this situation, the Agency supports the RAC opinion that Category 1B is most appropriate but considers it to be a borderline case between 1B and category 2.

Overall, the Agency considers that silver nitrate meets the criteria for classification as **Repr. 1B; H361F (May damage fertility).**

<u>Developmental toxicity</u>

The Agency has considered the available dataset and provided a summary of the key studies and findings below

EOGRTS in silver acetate (preliminary and main)

As for sexual function and fertility, the preliminary and main EOGRTS on silver acetate was considered by RAC to be the most reliable studies. For the preliminary study, the key

effects highlighted in the RAC opinion were increased pup mortality and lower pup bw from PND1. In the main study, pup mortality at birth was increased at the highest dose whilst the remaining pups were sacrificed early due to welfare issues, where brain lesions were cited as a contributing factor. Furthermore, all dose groups showed adverse neurological and neurobehavioral effects.

Pup mortality

For the preliminary study, some P0 females at the two highest dose groups (160 and 320 mg/kg bw/d) were terminated prematurely due to total litter loss (2 and 4 at each dose, respectively). The Agency notes that it is challenging to distinguish whether this is an effect on development or sexual function and fertility (i.e., dystocia). The remaining females were also terminated prematurely due to welfare reasons suggesting these two doses were in exceedance of the MTD. No treatment-related mortality was seen at the lower doses.

In the main study, live birth and viability indices (during PND 1-4) were reduced at 120 mg/kg bw/d. This was observed in the absence of any marked maternal toxicity and thus the Agency considers this to be a clear treatmentrelated developmental effect. Numerical data are presented in Table 17.

Table 17: Perinatal survival in the main EOGRTS

Dose group (mg/kg bw/d)	**0**	**40**	**80**	**120**
Number of pups (Total) mean	14.9	15.1	14.4	14.7
Number of alive pups (Day 1) mean	14.5	14.4	13.9	13.0
Number of alive pups (Day 4) mean	14.4	14.2	13.6	11.7**
Live birth index (%)	97.3	94.7	96.6	89.1*
Viability index (Day 4) (%)	99.1	99.0	97.9	90.2**

* <0.05; **p<0.01

Between days 19 and 47 post-weaning, nine males and two females of the F1 generation died. Microscopic examinations revealed that a major factor contributing to mortality were brain lesions. The remainder of this group were sacrificed at 10 weeks of age.

Overall, the Agency considers that increased mortality of pups during the perinatal period and post weaning, in the main EOGRTS, represents clear and adverse developmental toxicity from exposure to the test substance. The lack of significant maternal toxicity highlights that developing offspring (F1 adults) are more susceptible to silver acetate induced toxicity.

Pup body weight

In the preliminary study, RAC noted that at the two highest doses (160 and 320 mg/kg bw/d) pup body weight was decreased from PND1. Unfortunately, the numerical data for these two dose groups was not included in the CLH report or RAC opinion.

Reduced pup bw was also observed in the main study for which numerical data were provided. This was statistically significant at all doses and timepoints post weaning in males and was statistically significant up to day 29 post weaning at 40 mg/kg bw/d, up to day 36 post weaning at 80 and 120 mg/kg bw/d. The numerical data is presented in Table 18 and 19 below:

Table 18: Body weight – group mean values (g) for F1 males (Main EOGRTS)

		Day 1 Post weaning	Day 8 Post weaning	Day 15 Post weaning	Day 22 Post weaning	Day 29 Post weaning	Day 36 Post weaning	Day 43 Post weaning	Day 50 Post weaning	Day 57 Post weaning	Day 64 Post weaning	Day 71 Post weaning
Group/Sex (target dose mg/kg bw/day												
1M (0)	Mean	94	152	217	272	335	379	420	455	484	508	527
	SD	11.0	16.3	20.3	25.1	27.7	30.7	36.9	41.2	44.2	48.0	49.4
	N	60	60	60	60	60	60	50	40	40	40	20
2M (40 mg/kg bw day)	Mean	86** (↓9%)	138** (↓9%)	196** (↓10%)	247** (↓9%)	307** (↓8%)	352** (↓7%)	391** (↓7%)	421** (↓7%)	448** (↓7%)	471** (↓7%)	492** (↓7%)
	SD	12.2	17.8	22.1	25.4	30.7	34.8	40.7	45.1	48.0	52.8	45.0
	N	60	60	60	60	59	59	49	39	39	39	20
3M (80 mg/kg bw day)	Mean	82** (↓13%)	131** (↓14%)	189** (↓13%)	239** (↓12%)	297** (↓11%)	340** (↓10%)	376** (↓10%)	405** (↓11%)	429** (↓11%)	451** (↓11%)	457** (↓13%)
	SD	9.5	14.2	18.4	21.6	24.5	26.1	30.2	28.9	30.5	32.7	38.4

	N	60	60	60	60	59	59	49	39	39	39	19
4M (120 mg/kg bw day)	Mean	82** (↓13%)	131** (↓14%)	187* (↓14%)	238** (↓13%)	294** (↓12%)	334** (↓12%)	361** (↓14%)	-	-	-	-
	SD	10.1	15.0	19.5	22.2	26.7	33.5	47.7	-	-	-	-
	N	60	60	60	58	56	53	44	-	-	-	-

* p<0.05
** p<0.01

Table 19: Body weight – Group mean values (g) for F1 females (main EOGRTS)

		Day 1 Post weaning	Day 8 Post weaning	Day 15 Post weaning	Day 22 Post weaning	Day 29 Post weaning	Day 36 Post weaning	Day 43 Post weaning	Day 50 Post weaning	Day 57 Post weaning	Day 64 Post weaning	Day 71 Post weaning
Group/Sex (target dose mg/kg bw/day)												
1F (0)	Mean	86	129	163	183	206	220	233	244	253	263	263
	SD	9.0	11.0	11.7	14.6	16.0	17.3	18.5	20.5	20.6	22.0	23.8

	N	60	60	60	60	60	60	50	40	40	40	30
2F (40 mg/kg bw day)	Mean	80** (↓7%)	119** (↓8%)	151** (↓7%)	173** (↓5%)	198** (↓4%)	216 (↓2%)	229 (↓2%)	242 (↓1%)	252 (↓0.4%)	259 (↓2%)	264 (↑0.4%)
	SD	11.1	14.2	14.3	14.6	16.6	16.8	18.9	18.3	20.8	21.4	21.0
	N	60	60	60	60	60	60	50	50	40	40	30
3F (80 mg/kg bw day)	Mean	77** (↓10%)	114** (↓12%)	146** (↓10%)	169** (↓8%)	194** (↓6%)	211* (↓4%)	227 (↓3%)	238 (↓2%)	247 (↓2%)	258 (↓2%)	261 (↓1%)
	SD	8.9	11.3	14.2	15.2	17.8	17.7	18.4	18.4	20.2	19.8	22.1
	N	60	60	60	60	60	60	50	50	40	40	30
4F (120 mg/kg bw	Mean	74** (↓14%)	111** (↓14%)	144* (↓12%)	167** (↓9%)	193** (↓6%)	211** (↓12%)	225 (↓4%)	240 (↓2%)	-	-	-

day)											
	SD	9.2	12.3	14.8	16.9	21.0	21.6	25.0	23.7	-	-	-
	N	60	60	60	60	60	58	48	18	-	-	-

* p<0.05
** p<0.01

The Agency considers the reduction in pup bw to be treatment-related and adverse where reductions exceed 10%.

Neurotoxicity

In the preliminary study, only the pups from the 4 or 40 mg/kg bw/d groups were analysed macroscopically for brain lesions. The only finding was a lower absolute brain weight in females treated with 40 mg/kg bw/d. No other abnormalities were reported.

In the main study, as noted above, some animals from the 120 mg/kg bw/d group were found dead before study termination and the cause of death was associated with brain lesions. For the F1 generation (cohort 1A), the incidence of brain related microscopic findings were reported in the CLH report (Table 20).

Table 20: Incidence and severity of silver acetate-related microscopic findings in F1 cohort 1A.

Dose group (mg/kg bw/d)	**0M**	**40M**	**80M**	**120M**	**0F**	**40F**	**80F**	**120F**
Number examined	20	19	20	20	20	20	20	20
Edema, Intramyelinic	0	0	2 (minimal) 1 (slight)	6 (minimal) 2 (slight)	0	0	0	3 (minimal)
Necrosis, Neuron, Hippocampus (minimal)	0	0	8	11	0	0	3	9
Necrosis, Neuron/Glial Cell, Thalamus	0	0	3 (minimal)	3 (minimal) 1 (slight) 1 (moderate)	0	0	2 (minimal)	3 (minimal) 1 (slight)
Pigment extracellular (minimal)	0	11	8	5	0	2	9	6

Cohort 2A underwent neurobehavioral testing and a microscopic evaluation of the brain was performed at termination (day 75). The study authors noted abnormal motor movements (chewing mouth movements, licking around mouth) in two males at both 80 and 120 mg/kg bw/d. Two females at 120 mg/kg bw/d were observed with their eyelids completely closed, one recorded as asleep and the other as awake. Three males at 120 mg/kg bw/d were noted as slightly awkward to handle. Other relevant effects included reduced activity and rearing counts in treatment groups, a possible effect on startle latency to peak response and habituation at 120 mg/kg bw/d, increased group mean landing foot splay values in the top two doses in males and reduced forelimb and hindlimb grip strength at the top two dose (both sexes). Landing foot splay and grip strength values are presented in table 21 below. Furthermore, the microscopic findings in the brain were presented for cohort 2A (table 22) and confirm the findings also seen in Cohort 1A. In addition to these microscopic findings, brain morphometry evaluations revealed a dose dependent reduction in mean hippocampus size (2.1, 2.1, 1.97 and 1.91mm at 0, 40, 80 and 120 mg/kg bw/d, respectively) in males. This was statistically significant at 80 and 120 mg/kg bw/d.

Table 21: Mean landing foot splay (mm), forelimb grip strength (kg) and hindlimb grip strength (kg) in cohort 2A males/females.

Parameter	0M	40M	80M	120M	0F	40F	80F	120F
Body temperature (°C)	37.7	37.8	37.9	37.7	39.0	38.6	38.5**	38.4**
SD	0.3	0.3	0.6	0.5	0.2	0.4	0.5	0.4
Body weight (g)	412.5	394.4	368.3*	355.1**	225.6	233.3	229.0	216.4
SD	39.2	37.0	35.9	41.7	17.7	22.6	15.2	28.6
Landing footsplay (mm)	127	115	134	146*	111	106	120	101
SD	20	19	17	15	17	21	14	22
Forelimb grip strength (kg)	1.13	1.17	1.15	1.05	1.01	0.99	1.01	0.86**
SD	0.09	0.11	0.09	0.12	0.12	0.10	0.08	0.14

Hindlimb grip strength (kg)	0.60	0.56	0.53**	0.51**	0.49	0.44	0.46	0.40**
SD	0.07	0.04	0.04	0.06	0.04	0.04	0.06	0.06

*p≤0.05; **p≤0.01

Table 22: Incidence and severity of silver acetate-related microscopic findings in F1 cohort 2A.

Dose group (mg/kg bw/d)	**0M**	**40M**	**80M**	**120M**	**0F**	**40F**	**80F**	**120F**
Number examined	10	10	10	7	10	10	10	10
Brain Cerebrum								
Edema, Intramyelinic	0	0	0	1 (minimal) 1 (slight) 1 (moderate)	0	0	0	3 (minimal) 1 (slight) 1 (moderate)
Necrosis, Neuron, Hippocampus (minimal)	0	0	5	5	0	0	3	7
Necrosis, Neuron/Glial Cell, Thalamus	0	0	0	2 (minimal) 1 (slight)	0	0	0	4 (minimal) 1 (slight)
Brain, Forebrain								
Edema, intramyelinic (minimal)	0	0	0	2	0	0	0	2
Brain, Medulla Oblongata								

Pigment, Extracellular (minimal)	0	6	3	3	0	2	1	0

Overall, the microscopic examinations in the brain and neurobehavioral assessments show a clear treatment related effect on the developing brain. These are both relevant for classification.

One generation toxicity study using silver acetate (Sprando et al., 2016)

In this study, some maternal toxicity was reported at the top dose (40 mg/kg bw/d) in the form of reduced stomach weight (-40%), which was also noted in the pups. The impact of this finding on pup development is unclear since there was no other maternal toxicity (e.g., bw reductions). Regarding developmental toxicity, 4 dams at 40 mg/kg bw/d did not produce a litter although all were sperm positive. Two of these dams had no implantation sites whereas the other two reabsorbed their litters. A further two pups were lost during lactation. This resulted in viable litters remaining at PND21 of 19, 19, 18 and 14 at 0, 0.4, 4 and 40 mg/kg bw/d, respectively.

Additionally, an increased number of runts (with increased reduced pup bw) were seen at 4 mg/kg bw/d but not to the same extent at 40 mg/kg bw/d. According to the authors and RAC, the reduced amount of runts at the top dose is likely due to the increased pup mortality at this dose. The Agency agrees.

Overall, the Agency concludes that these effects are relevant for classification and support those already discussed in the EOGRTS.

Price and George., 2002

The study was GLP compliant and broadly followed OECD TG 414. Unfortunately, only the abstract was available. Doses of 0, 10, 30 or 100 mg/kg bw/d silver acetate were administered to female CD albino rats, via oral gavage, during GD 6-19. The main developmental toxicity findings were foetal deaths in the 100 mg/kg bw/d group (10% mortality vs 0% in all other groups) and a dose-dependent reduction in the average foetal body weight per litter (combined sexes). There was no significant maternal toxicity.

Therefore, the Agency agrees with RAC that this finding is supportive of similar effects seen in the EOGRTS.

***Shavlovski et al*., 1994**

The study was non-GLP and non-guideline. Silver chloride was administered to 20 females at a dose of ~250mg/kg bw/d through gestation days 1-20 and a further 5 females were

exposed to the same dose only during days 7-15. No significant maternal toxicity was reported and no embryotoxic effects were reported in the group exposed during GD 7-15. However, in the group exposed throughout GD 1-20, post-implantation loss was statistically significantly increased compared to the controls. Furthermore, all newborn animals died within 24 hours, an increased incidence of hydronephrosis and cryptorchidism was reported and the average body mass of embryos was lower. Available numerical data is presented in Table 23 below.

Table 23: Key findings from Shavlovski *et al*., 1994

Parameter	**Control Group**	**250 mg/kg bw/d (GD 1-20)**	**Historic control (Number/dates unknown)**
Post-implantation loss (%)	9.6	36	8.7
Average mass of the foetus (g)	2.24	1.75	2.26
Incidence of Hydronephrosis (%)	30.6	5.6	1.2
Incidence of cryptorchism (%)	34.7	1.3	0.8

An additional analysis was provided which aimed to elucidate a possible mechanism involving copper/ceruloplasmin. In this study, 4 groups were formed (control group, silver chloride exposed group, silver chloride + ceruloplasmin supplementation on GD 2-14 and silver chloride + ceruloplasmin supplementation on GD 8-21). It was shown that intraperitoneal injections of human ceruloplasmin improved the survival of newborns and that this was dependent on the timing during gestation. Percentage of newborns that died were 100%, 34.5%, 5.9% and 0% in the silver chloride, ceruloplasmin GD2-14 group, ceruloplasmin GD8-21 group and controls, respectively. It was additionally noted that incidences of hydronephrosis drops to 3.6% and incidence of cryptorchism were 0% in the GD 2-14 group. Furthermore, another analysis reported that silver chloride developmental toxicity was potentiated using sub-embryotoxic doses of penicillamine and bipyridyl (chelators of copper and iron, respectively).

Overall, although this is a non-guideline and non-GLP study, it does support the results of the EOGRTS and provides further insight into the possible mechanism of action through

which silver ion developmental toxicity is potentially mediated. Hence the Agency considers this supportive of classification.

Studies on AgNPs

In agreement with RAC, the Agency considers that available data on AgNPs can be used as supportive evidence but that studies on silver salts are most representative of silver nitrate. Please see read-across section.

There were a number of non-GLP studies referenced from the published literature and these are summarised in the table above. Only those considered most robust by RAC have been discussed.

Two studies performed on rats (Charehsaz *et al*. (2016) and Yu *et al*. (2013)) reported no specific developmental toxicity effects, but as noted by RAC the exposure times were short (13-14 days). Two further studies on rats did report some developmental toxicity from AgNPs (Fatemi *et al*., 2013 and Yin *et al*., 2015). The study by Fatemi dosed dams from GD9 until parturition, and effects in the pups included reduced bw and brain/bw ratio plus neuronal defects. The study by Yin dosed neonatal rats via intranasal instillation for 14-weeks. Effects included cerebellar-ataxia like symptoms from the lowest dose of 0.1 mg/kg bw/d and distortions in the Purkinje and granular layer of the cerebellar cortex at the top dose of 1 mg/kg bw/d. Although intranasal instillation is not a standard route of exposure, the developmental effects seen are consistent with those seen in the more reliable EOGRTS.

There were two AgNP studies in mice (Philbrook *et al*., 2011 and Ghaderi *et al*., 2015). In the study by Philbrook, one gavage dose was administered to dams at 0, 10, 100 and 1000 mg/kg bw. In the absence of maternal toxicity, the only developmental effect was a possible increase in the incidence of non-viable foetuses in treatment groups. However, this lacked a dose-response. RAC postulated that the absence of a doseresponse could be due to the agglomeration of silver nanoparticles. Ghaderi used subcutaneous injections (one injection every 3 days) in dams of 0, 0.2 and 2 mg/kg bw from GD3 to parturition. Neurobehavioral effects were reported in the adult offspring. Both of these studies are of questionable relevance due to limitations in observed effects and routes of exposure. However, effects seen are consistent with those from the EOGRTS.

Finally, one study was presented in rabbits (Hang *et al*., 2013). Dams were exposed to AgNPs in water (1%) *ad libitum* prior to mating until weaning of pups. Overall, no maternal toxicity of development toxicity was reported. The lack of effects seen here could be due to a number of reasons such as using drinking water as the vehicle or because rabbits are less sensitive than rats and mice to AgNP effects.

Overall, the developmental effects from AgNPs are mostly consistent to those seen in studies performed with silver acetate (particularly neurodevelopmental). Therefore, the

Agency agrees with RAC that these studies provide supportive evidence of an effect on development.

Mechanism of action

The proposed mechanism for the observed developmental toxicity is discussed in the RAC summary above. Overall, the mechanism is well described, supported by data and relevant to humans. Therefore, the Agency considers it to support classification.

Overall conclusion on developmental toxicity

The Agency considers that the available data on silver salts is sufficient to conclude on classification of silver nitrate. Key developmental findings from these studies were offspring mortality, decreased pup bw and neurobehavioral impairments with associated histopathological changes in the brain. These effects were seen in the absence of significant maternal toxicity and a potential mechanism of action is clearly defined and relevant to humans.

In accordance with the guidance on the application of the CLP criteria (ECHA, Version 5.0, 2024b), the Agency concludes that these are clear adverse effects on development, in the absence of other toxic effects, based on animal studies. **Therefore, classification as Repr. 1B., H360D (May damage the unborn child) is proposed.**

Lactation

The Agency agrees with RAC that the available data are not sufficient to classify for effects on lactation.

Aspiration hazard

Not assessed in the CLH report or RAC opinion.

Environmental hazards

Hazardous to the aquatic environment

The classification strategy for metal compounds (Annex IV.5 of ECHA, 2024c) was followed with silver nitrate being considered a readily soluble metal compound because its water solubility (approximately 2340 g/L at 25°C) exceeded the acute ecotoxicity reference values (ERVs) of the dissolved metal ion. M-factors for readily soluble metal compounds are applied in the same way as for organic substances.

Classification agreed by RAC:

Rapid transformation of inorganic substances:

RAC considered the rate silver nitrate dissolves and transforms. RAC agreed that silver nitrate was **not rapidly transformed to non-bioavailable forms** for the purpose of hazard classification based on the following information presented in the CLH report (CLH, 2023) and the RAC Opinion (ECHA, 2025):

- Although silver nitrate does not have any chemical bonds prone to hydrolysis, it readily dissolves in water and dissociates into its constituent ions.

- No quantitative data on the effects of photolysis on silver in water are available although the CLH report noted that photo-reduction and photo-oxidation may affect the rate at which silver ions are released from silver nitrate and the speciation of these ions in the water compartment.

- Sulphide is normally present at low concentrations in natural waters and forms a strong complex with silver ions. A study submitted by industry during the public consultation on the CLH proposal calculated that at the environmental average concentration of dissolved organic matter, the concentration of chromium reducible sulphides (CRS) is between 10 and 50 nM. At these levels of CRS, full precipitation of silver as Ag_2S was modelled. However, RAC noted that CRS is below 1 nM in pristine waters and calculations at this concentration do not support silver precipitation. RAC also considered that potential reverse change cannot be ruled out.

- Depending on the levels of sulphide and silver ions present in water, other speciation reactions with varying binding constants may occur, such as binding with chloride and NOM. With increasing salinity, concentrations of silver-chloro complexes increase while sorption to suspended solids and phytoplankton decreases.

- Complexing ligands plus competing cations decrease the toxicity by reducing the bioavailability of silver, although there is also information suggesting that chloride as a ligand to silver does not protect against silver toxicity to fish.

- Two OECD GD 29 T/D tests with silver powder (CIMM, 2009; ECTX, 2010, in CLH, 2023) are available. These T/D tests at a loading of 1 mg/L at pH 6 and 8 demonstrate an increase in dissolved metal concentrations with time up to day 28, despite the medium containing a high concentration of chloride which is expected to remove some of the silver due to formation of insoluble silver chloride.

- During the public consultation on the CLH report, industry proposed that an extended T/D study supports the concept of rapid removal of silver from the water column and should be used in preference to the OECD GD 29 T/D tests. This extended T/D test contained a low binding sediment substrate. Within 96 hours, 89% of dissolved silver was removed from the water column due to binding with sulphide substances in the substrate and industry considered the potential for re-solubilisation was negligible. However, RAC noted that the concentration of silver used was almost 5 orders of magnitude higher than environmentally relevant concentrations which RAC considered could explain the study results. The equilibrium concentration of silver after 96 hours of settling in the presence of the substrate was also considerably above toxic levels. RAC concluded the extended T/D study was not relevant to the hazard classification of silver.

Overall, RAC agreed that the information on dissolution, speciation and sorption did not provide clear evidence of rapid and irreversible environmental transformation of silver from soluble to insoluble forms.

Bioaccumulation:

RAC agreed that silver nitrate was **not bioaccumulative** for the purpose of hazard classification based on the following information presented in the CLH report (CLH, 2023) and the RAC Opinion (ECHA, 2025):

- Silver is a non-essential element. The ability of silver to accumulate varies widely between species depending on silver chemical species and external conditions.
- Fish BCF values for silver ranged from 0.4 to 327 L/kg ww indicating fish have physiological mechanisms to keep silver levels low.
- Invertebrate BCF values for silver ranged from 2.5 to 27500 L/kg ww. Toxic effects were not observed even at the highest tissue concentrations due to efficient sequestration mechanisms, whereby silver is stored in metabolically unavailable forms.

In conclusion, RAC considered that aquatic organisms have evolved mechanisms to regulate, store, detoxify or remove silver. Based on valid experimental results for fish supported by invertebrates information, RAC agreed that silver nitrate does not meet the classification criteria for bioaccumulation under CLP.

Aquatic Toxicity:

Silver nitrate is readily soluble with 100% expected to be dissolved in water. Released silver ions are considered the environmentally relevant species that cause observed toxicity in ecotoxicity tests. On this basis, nitrate was not considered further. Only the presence of dissolved silver (<0.45 µm filter) in ecotoxicity tests was taken into account in

the CLH assessment and ecotoxicity results were recalculated based on the content of silver in silver nitrate using the molecular mass (64%).

RAC acknowledged that extensive acute and chronic aquatic toxicity data for silver were available for all three trophic levels. Full details are presented in the CLH report (CLH, 2023) and summarised in the RAC Opinion (ECHA, 2025).

In accordance with guidance on CLP and SSDs (ECHA, 2008; 2024c), RAC considered the probabilistic approach for the acute aquatic toxicity was not applicable due to a lack of data for major taxonomic groups such as molluscs, amphibians and insects. RAC also noted that different experimental set-ups affecting the bioavailability of silver would not allow consistent comparison of the sensitivity between species.

RAC considered that the lowest reliable acute ERVs for the dissolved silver ion for each trophic group were the:

- *Pimephales promelas* 96-hour LC_{50} of 0.0031 mg/L (Van Genderen *et al.*, 2003, in CLH, 2023);
- *Daphnia magna* 48-hour LC_{50} of 0.00022 mg/L (Bianchini *et al.*, 2002, in CLH, 2023); and
- *Pseudokirchneriella subcapitata* 72-hour E_rC_{50} of 0.00096 mg/L (Schlich *et al.*, 2017, in CLH, 2023).

On this basis, RAC agreed that the overall lowest dissolved silver acute ERV was the *Daphnia magna* 48-hour LC_{50} of 0.00022 mg/L which corresponds to a silver nitrate acute ERV of 0.00034 mg/L.

As the silver nitrate acute ERV falls within the 0.0001 mg/L < acute ERV ≤ 0.001 mg/L range, RAC agreed that silver nitrate should be **classified as Aquatic Acute 1 with an Acute M-factor of 1000.**

RAC considered that the lowest reliable chronic ERVs for the dissolved silver ion for each trophic group were the:

- *Oncorhynchus mykiss* 73-77-day NOEC $_{growth}$ of 0.00021 mg/L (Dethloff *et al.*, 2007, in CLH, 2023);
- *Daphnia magna* 21-day $EC_{10\ growth}$ of 0.00214 mg/L (Bianchini and Wood, 2008, in CLH, 2023); and
- *Pseudokirchneriella subcapitata* 72-hour E_rC_{10} of 0.00010 mg/L (Schlich *et al.*, 2017, in CLH, 2023).

On this basis, RAC agreed that the overall lowest dissolved silver chronic ERV was the *Pseudokirchneriella subcapitata* 72-hour E_rC_{10} of 0.00010 mg/L which corresponds to a silver nitrate chronic ERV of 0.00016 mg/L.

Given RAC considered silver nitrate is not rapidly transformed, and since this endpoint for silver nitrate falls within the 0.0001 mg/L < chronic ERV ≤ 0.001 mg/L range, RAC agreed that silver nitrate should be **classified as Aquatic Chronic 1 with a Chronic M-factor of 100.**

RAC considered that this deterministic approach for the aquatic chronic hazard classification was supported by the probabilistic approach using results from the draft dossier on the Environmental Quality Standard (EQS) for silver and a study (Arijs *et al*., 2021, in ECHA, 2025) submitted by industry. RAC noted the inclusion of a study by Diamond *et al.* (1990, in ECHA, 2025) increased the number of taxonomic groups and robust data points in the SSDs from both of these sources. Although differences between the datasets were noted, assuming a normal distribution the HC_5 values were very similar at 0.000084 mg/L for the EQS SSD and 0.000088 mg/L for Arijs *et al.* (2021). These values correspond to 0.00013 mg/L and 0.00014 mg/L when recalculated to silver nitrate and are also in the hazard classification range 0.0001 mg/L to 0.001 mg/L range.

RAC Opinion:

RAC agreed to classify silver nitrate as:

- **Aquatic Acute 1 (H400) with an Acute M-factor of 1000** based on the dissolved silver *Daphnia magna* 48-hour LC_{50} of 0.00022 mg/L equating to a silver nitrate acute ERV of 0.00034 mg/L.

- **Aquatic Chronic 1 (H410) with a Chronic M-factor of 100** based on the dissolved silver *Pseudokirchneriella subcapitata* 72-hour E_rC_{10} of 0.00010 mg/L equating to a silver nitrate chronic ERV of 0.00016 mg/L and a not rapidly transformed substance.

Classification proposed by the Agency:

The Agency agrees that silver nitrate should be classified following the classification strategy for metal compounds set out in Annex IV.5 of the ECHA (2024c) Guidance on CLP.

The Agency agrees that silver nitrate is not rapidly transformed to non-bioavailable forms based on the fate data. Silver nitrate dissociates to silver and nitrate ions in water. In standard T/D tests with silver powder, the concentration of dissolved silver continued to increase throughout the 28-day study periods. Although silver forms complexes with various ligands in the natural aquatic environment, such as sulphides, chlorides and NOM, these processes depend on the abundance of these ligands, the concentration of silver and the water salinity. Under typical environmental conditions, complete precipitation of silver complexes is not expected meaning silver ions are anticipated to remain the aqueous phase and potentially be bioavailable to aquatic life. Additionally, changes could be reversed.

The Agency agrees that silver nitrate is not bioaccumulative for the purpose of hazard classification because aquatic organisms have evolved mechanisms to regulate silver, as demonstrated by experimental data for fish and invertebrates. Fish BCF values were below the hazard classification criterion of ≥500 L/kg and while certain BCF values for invertebrates exceeded ≥500 L/kg, no toxic effects were observed due to the sequestration of silver in non-bioavailable forms. The Agency concluded that silver had a low bioaccumulation potential based on the same information in the GB MCL Technical Report for silver (HSE, 2023).

The Agency agrees that silver nitrate is a readily soluble metal compound and that the classification should, i) reflect ERVs for the metal compound, and ii) M-factors should be applied to the compound ERVs in the same way as for organic substances. The reliability and relevance of the acute and chronic ecotoxicity studies and SSDs discussed below was considered by the Agency in the GB MCL Technical Report for silver (HSE, 2023).

The acute and chronic ERVs for dissolved silver presented in the RAC Opinion for silver nitrate (ECHA, 2025) are the same as those used in the RAC Opinion on silver (ECHA, 2022), except that the lowest acute endpoint for algae was given as the E_yC_{50} from the study by Schlich *et al.* (2017). Growth rate endpoints are preferred for algae and aquatic plants because growth rate is not dependent on test design (ECHA, 2023). Therefore, the Agency agrees that the growth rate endpoint from this study is the most reliable and relevant acute endpoint for algae and aquatic plants for hazard classification.

For the acute fish toxicity, the CLH (2023) proposal for silver nitrate included a more sensitive 96-hour LC_{50} of 0.0023 mg/L for *Pimephales promelas* based on dissolved silver (Nebeker, 1983). This endpoint is in the same concentration range as the Van Genderen *et al.* (2003) *P. promelas* 96-hour LC_{50} of 0.0031 mg/L for dissolved silver.

Overall, the Agency agrees that the lowest relevant and reliable acute ERV is the *Daphnia magna* 48-hour LC_{50} of 0.00022 mg/L for dissolved silver, which corresponds to a silver nitrate acute ERV of 0.00034 mg/L. As this endpoint for silver nitrate is in the 0.0001 mg/L < acute ERV ≤ 0.001 mg/L range, the Agency agrees with RAC that silver nitrate meets the classification criteria as **Aquatic Acute 1 (H400) with an Acute M-factor of 1000**.

The Agency agrees that the lack of relevant and reliable acute toxicity data for certain taxonomic groups prevents the use of the probabilistic approach for the aquatic acute classification because the minimum species requirements to use an SSD (ECHA, 2008) is not met.

In relation to the aquatic chronic toxicity, the RAC Opinion on silver (ECHA, 2022) highlighted an additional *Oncorhynchus mykiss* 196-day LC_{10} of 0.00017 mg/L (Davis *et al.*, 1998). However, it was considered this study could be R2 (reliable with restrictions), or R4 (not assignable) and the endpoint is in the same hazard classification range as the Dethloff *et al.* (2007) *O. mykiss* NOEC above.

For invertebrates, the CLH (2023) proposal for silver nitrate included a more sensitive *Daphnia magna* 21-day NOEC of 0.0007 mg/L based on dissolved silver (Nebeker, 1983). This more sensitive invertebrate endpoint does not affect the classification because algae were the most chronically sensitive trophic group.

The Agency agrees that the overall lowest relevant and reliable chronic ERV is the *Pseudokirchneriella subcapitata* 72-hour E_rC_{10} of 0.00010 mg/L for dissolved silver which corresponds to a silver nitrate chronic ERV of 0.00016 mg/L. As this endpoint for silver nitrate falls within the 0.0001 mg/L < chronic ERV ≤ 0.001 mg/L range, and considering that silver nitrate is not rapidly transformed, the Agency agrees that silver nitrate meets criteria for classification as **Aquatic Chronic 1 (H410) with a Chronic M-factor of 100**.

The Agency agrees that the silver nitrate HC_5 values of 0.00013 mg/L and 0.00014 mg/L calculated from the SSD for the silver EQS and the SSD by Arijs *et al.* (2021), respectively are in the same 0.0001 to 0.001 mg/L hazard classification range and provide supporting information for this chronic classification and M-factor.

Other hazards

Hazardous to the ozone layer

Not assessed in the CLH report or RAC opinion.

Overall conclusion

The Agency has evaluated the RAC Opinion, its rationale and any additional scientific evidence that may have been made available to HSE against the criteria for classification and labelling in the GB CLP Regulation and technical guidance.

The Agency technical report **agrees** with the classification proposed by RAC for the following hazards:

Ox. Sol. 1; H271 (May cause fire or explosion; strong oxidiser)

Met. Corr. 1; H290 (May be corrosive to metals)

Repr. 1B; H360FD (May damage fertility. May damage the unborn child)

Acute Tox. 2; H300 (Fatal if swallowed), with an ATE of 50 mg/kg bw

Skin Corr. 1A; H314 (Causes severe skin burns and eye damage)

Eye Dam. 1; H318 (Causes serious eye damage)

STOT RE 1; H372 (Causes damage to the nervous system through prolonged or repeated exposure)

Carc. 2; H351 (Suspected of causing cancer)

Aquatic Acute 1; H400 (Very toxic to aquatic life), with an M-factor of 1000

Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects), with an M-factor of 100

Supplemental hazard statement: **EUH071** (Corrosive to the respiratory tract)

The Agency technical report **disagrees** with the classification proposed by RAC for the following hazards:

NOT CLASSIFIED for skin sensitisation

Germ cell mutagenicity: the Agency is aware of new information on silver nitrate for the assessment of germ cell mutagenicity so has not proposed classification for this hazard class in this technical report.

Overall, the conclusion is to **disagree** with the RAC opinion.

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Documents published as part of the EU CLH process: Source: European Chemicals Agency, http://echa.europa.eu/

Glossary of terms used in Agency technical reports

Agency, the	HSE, acting in its capacity as the GB CLP Agency
AgNPs	Silver nanoparticles
AR	Applied radioactivity
ATE	Acute toxicity estimate
BCF	Bioconcentration factor
BOD	Biological Oxygen Demand
bw	Body weight
CAR	Competent Authority Report
CAS	Chemical Abstracts Service
CI	Confidence interval
CL	Confidence limits
CLH	Harmonised Classification and Labelling
CLP	Classification, labelling and packaging (of substances and mixtures)
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
d	Day
DAR	Draft Assessment Report
DOC	Dissolved Organic Carbon
DS	Dossier Submitter
DT	Dissipation time OR degradation time (also DissT or DegT where apparent)
DT_{50}	Dissipation half-life OR degradation half-life (hours or days), see also above
dw	Dry weight
ECHA	European Chemicals Agency
ECx	x% effect concentration
EFSA	European Food Safety Authority
EOGRTS	Extended One-Generation Reproductive Study
E_rCx	x% effect concentration based on growth rate
EU	European Union
GLP	Good Laboratory Practice
GVs	Guidance values
h	Hours
K_{OC}	Organic carbon-water partition coefficient
K_{OW}	Octanol-water partition coefficient
LC_x	x% lethal effect concentration

MCL	Mandatory Classification and Labelling
M-factor	Multiplying factor
MW	Molecular weight
NOEC	No-observed effect concentration
OECD	Organisation for Economic Co-operation and Development
QSAR	Quantitative structure-activity relationship
RAC	Risk Assessment Committee
RAR	Renewal Assessment Report
RCOM	Response to comments document
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals regulation
SCAS	Silver-containing active substances
STOT-RE	Specific target organ toxicity – repeated exposure
STOT-SE	Specific target organ toxicity – single exposure
TG	Test Guideline
UNTGs	UN Model Regulations on the Transport of Dangerous Goods
US EPA	United States Environmental Protection Agency
wt	Weight
wwt	Wet weight

Further information

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This publication is available on the HSE website.

Published by the Health and Safety Executive