Additional information report

For a Substance under Harmonised Classification and Labelling Process

Substance Name: Silver

EC Number: 231-131-3

CAS Number: 7440-22-4

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1 INTRODUCTION

During the CLH process of silver, ECHA received a copy of the following newly performed studies:

- The final Audited Study report of the 90-Day Study of Silver Acetate by Dietary Administration in Wistar Han Rats (Lourens et al., 2022)

To assure an efficient and transparent assessment of the new information provided by the data submitter and giving parties concerned an opportunity to comment, it is the responsibility of the data submitter to elaborate non-confidential detailed study summaries and explain the relevance of the provided information for the classification at issue. To facilitate the task of the data submitter, ECHA has compiled a format for Additional information report.

Every study should be presented individually. The relevance of each study for the classification at issue should be clearly presented. A non-confidential detailed study summary and the results of the study should be reported transparently and objectively as in the original data source without any interpretations by the data submitter. A detailed study summary includes test type (e.g. test guideline followed and deviations from the guideline, the GLP and quality assurance status of the study, identity of the test substance (including degree of purity), information about the test subjects, route of administration, mode of exposure and numerical results. If read-across to structurally or mechanistically similar substances is used a justification for using data from a different substance should be provided and, if known, calculations to convert dose/concentration levels from the test substance to the substance for which CLH is proposed should be presented.

2 REPEATED DOSE TOXICITY

Study reference: Lourens N.J.J. et al. (2022) A 90-Day Study of Silver Acetate by Dietary Administration in Wistar Han Rats (Test Facility Study No. 20274170)

2.1 Justification for using data from different substance (Silver Acetate)

Silver acetate was proposed because it is a silver (1+) salt, which is soluble in water – it possesses a solubility value of approximately 10 g/L at 20°C (Merck, 2013). It has been commonly utilised in a variety of published toxicology studies as a soluble silver (1+) reference compound. Ag acetate is known to exhibit satisfactory bioavailability via the oral route. Testing outcomes from a study utilising silver acetate are anticipated to be fully applicable for read-across to ionic silver (Ag+) donor compounds such as silver nitrate and disilver(I) oxide but not to silver metal (powder and massive). For Ag metal (massive and powder), a weight of evidence is supported based on *in-vivo* comparative toxicokinetic data in the dossier.

2.2 Relevance of the study:

This study assesses the potential toxicity of silver acetate (AgAc), when given via diet for 90 days to the Wistar Han rats. This study is shared in the context of the STOT RE discussion by providing additional evidence regarding repeated dose exposure to silver ions.

The repeated dose toxicity assessment of silver metal (massive and powder) (AgMP) is based on a 90-day study performed with silver acetate (AgAc). However, a correction of the findings in the study with AgAc is required based on a demonstrated lower bioavailability of Ag metal (massive and powder) vs soluble Ag salts (like AgAc).
It is assumed that systemic toxicity of silver substances is driven by Ag^+ (silver ions) as the primary species for systemic circulation and relevant for tissue exposure, and hence hazard assessment. Thus, a very low bioavailability leads to a very low internal concentration of Ag^+ (toxicophore), to a lack of biological interaction and hence the absence of effects at similar dosing levels (expressed as Ag concentration) in comparison with highly bioavailable silver compounds.
The bioavailability of AgAc via oral route is consistent between independent estimates presented in the scientific literature (Barraclough and Cotton 2017, Boudreau et al. 2012, Park et al. 2011 and Van der Zande et al. 2012) and the comparative *in-vivo* TK study – in the order of 3-5%. In comparative terms, silver metal powder (AgMP) is substantially less absorbed. Based on matched dose assessments performed in the *in-vivo* TK study (Melvin et al., 2021 and Charlton et al., 2021, the extent of systemic exposure was about 10 to 30-fold lower in the case of AgMP vs AgAc. Unlike the situation of AgAc, the degree of uptake of AgMP is not linear as the amount of administered of AgMP was increased up to a limit dose, but instead there was evidence of absorption plateauing (**figure 1**). As a

generic observation, the following trend in bioavailability is being observed: AgAc / $AgNO_3$ >> AgNP >>> AgMP.

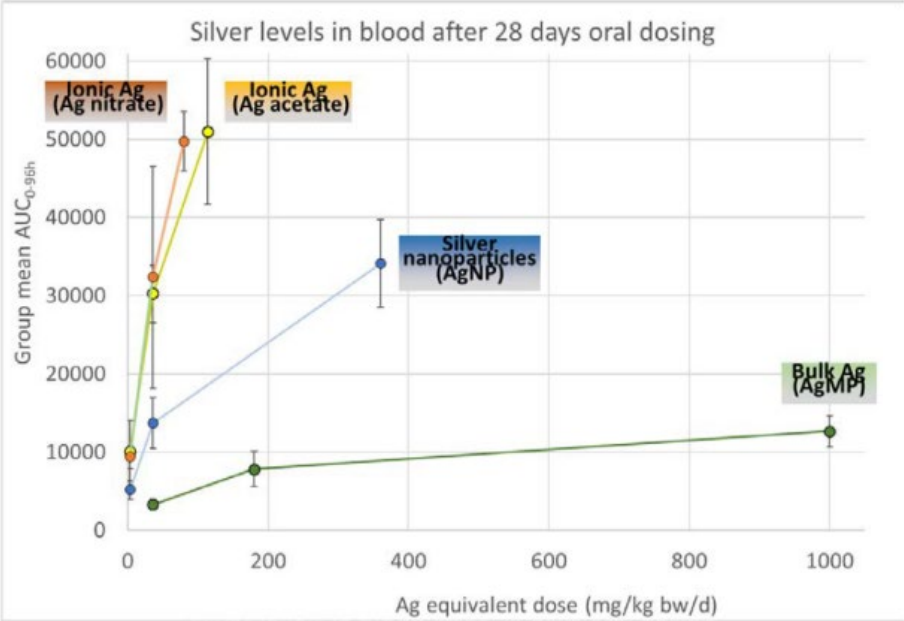

Figure 1. Marked differences exist in the extent of absorption observed for ionic Ag (tested as Ag acetate and Ag nitrate) versus that of bulk Ag (sub-micron Ag metal powder, 'AgMP').

Results are for female animals receiving the test items (very similar findings were also obtained in the case of males).

Data are expressed as Ag equivalent dose. Standard deviations are added.

Therefore, the toxicokinetic findings strongly suggest that the direct read-across of mammalian toxicity data with soluble silver salts (like silver acetate and silver nitrate) and nanosilver to silver metal (powder and massive) is <u>not</u> justified (based on their respective toxicokinetic profiles, presented in **figure 1).** This new evidence demonstrates that oral intake of AgMP results in markedly lower absorption, distribution and systemic tissue/organ exposure to silver than more bioavailable forms like AgAc.

Therefore, the Toxicokinetic / bioavailability / Ag^+ release parameter (Melvin et al., 2021; Charlton et al., 2021) is considered as key piece of evidence in the weight of evidence reasoning for the repeated dose toxicity of silver metal (massive and powder) by oral route.

2.3 <u>Detailed study summary and results:</u>

The objective of this study was to determine the potential toxicity of silver acetate, when given via diet for 90 days to the Wistar Han rat. In addition, a No Observed Adverse Effect Level (NOAEL) was evaluated. The toxicokinetic characteristics of silver acetate (via the analytical determination of silver) were also determined.

The design of this study is based on the following study guidelines:

- EC No 440/2008, B.26 Repeated Dose (90 days) Toxicity (oral), 2008.
- OECD 408, Repeated Dose 90-day Oral Toxicity Study in Rodents, 2018.
- OPPTS 870.3100, EPA 712-C-98-199, 90-Day Oral Toxicity in Rodents, 1998.
- Guideline on Bioanalytical Method Validation, European Medicines Agency (EMA), EMEA/CHMP / EWP/192217/2009, 21 July 2011.

- Guidance for industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), May 2018.
- OECD Guideline 417. Toxicokinetics.

Information substance Identity :

Identification: silver acetate
CAS number: 563-63-3
Molecular weight: 166.92
Expiry date: 11 October 2021
Physical Description: beige solid
Purity/composition: See Certificate of analysis
Silver content: 64.58%
Purity: >99.5%
Storage condition: in refrigerator protected from light

The study design was as follows:

Text Table 1
Experimental Design

Group No.	Test Item Id.	Target dose level (mg/kg (body weight)/day)	Subsets	Number of Animals	
				Males	Females
1	Control	0	Main TK	10 3	10 3
2	Silver acetate	40	Main TK	10 3	10 3
3		120	Main TK	10 3	10 3
4		320	Main TK	10 3	10 3

Id. = Identification; TK = Toxicokinetic animals

Text Table 2
Test Article Intake

Group	Target Dose Level (mg test item/kg body weight/day	Actual Mean over Means Intake [mg test item/kg body weight/day] (mean range indicated within brackets)			
		Males		Females	
2	40	41	(33-48)	42	(37-48)
3	120	122	(108-141)	126	(109-142)
4	320	287	(270-328)	319	(299-354)

The target dose level was reached for Group 2 and 3 animals and Group 4 females. Group 4 males had an actual mean dose level of 287 mg/kg body weight/day as a consequence of a dose related reduction in food consumption/palatability for Group 4 males. For the identification of Group 4 males in this report the target dose level (320 mg/kg body weight/day) is used.

Chemical analyses of dietary preparations were conducted in Weeks 1, 6 and 13 to assess accuracy and homogeneity. Dietary analyses confirmed that formulations of test item in diets were prepared accurately and homogenously.

The following parameters and endpoints were evaluated in this study: mortality, clinical signs, functional observation tests, body weights, food consumption, ophthalmology, oestrus stage determination, clinical pathology parameters (hematology, coagulation, clinical chemistry, thyroid hormones and immunophenotyping), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations.

Results

At 40 mg/kg body weight/day, a non-adverse test item-related increase in alkaline phosphatase activity (ALP) in females and HDL cholesterol concentration in males was noted.

At 120 mg/kg body weight/day, non-adverse test item-related changes in clinical pathology included increased eosinophil count in females, increased red blood cell distribution width in males, along with a decreased mean corpuscular volume and mean corpuscular haemoglobin in in both sexes. In addition, mean corpuscular haemoglobin concentration was decreased in males only. Moreover, slightly shortened prothrombin time (PT) was observed in males.
Furthermore, increased ALP activity in females and increased (HDL) cholesterol and calcium concentrations in males and females were noted.
At necropsy, a test item-related higher relative liver weight was observed in males, which was without microscopic correlate and therefore considered to be non-adverse.
At 320 mg/kg body weight/day, a test item-related lower body weight and body weight gain as seen in males throughout the administration period. In females, a slightly lower body weight and/or body weight gain was seen starting at Day 50 onwards. The food consumption in males was decreased throughout the administration period, while in females it was decreased during six out of thirteen weeks only. The effects on food consumption at 320 mg/kg body weight/day and consequently on body weight are at the severity observed considered to be adverse for males and not adverse for females.
At clinical pathology, non-adverse changes included increased eosinophils (females), increased red blood cell distribution width (both sexes) and decreased mean corpuscular volume (both sexes) and mean corpuscular haemoglobin (both sexes) and mean corpuscular haemoglobin concentration (males) and hemoglobulin concentration (males). In addition, a slightly shortened PT was observed in males. Clinical chemistry analysis revealed increased alanine aminotransferase activity and urea concentration in males, ALP activity in females and (HDL) cholesterol, triglyceride and calcium concentrations in males and females.
Test item-related non-adverse organ weight differences consisted of slightly higher liver weight in males and females and slightly lower pituitary gland and epididymis weight in males, which were without a microscopic correlate.
Microscopic evaluation showed a slightly higher incidence and severity of extramedullary hematopoiesis in the spleen in females compared to the control females. At the severity observed, this finding was not adverse.
At necropsy, test item-related non-adverse macroscopic grey discoloration was present in all animals at all dose levels. Affected organs included: kidneys, harderian gland, clitoral or preputial gland, thymus, urinary bladder, liver, thyroid gland, stomach, small and large

intestines, mesenteric and mandibular lymph nodes, mandibular salivary gland, skin, lacrimal gland, pituitary gland, uterus, and prostate gland. Test item-related microscopic black-brown pigment (ranging from minimal to moderate degree) was present in tissues from all animals at all dose levels. Affected organs included the brain (area postrema and subfornical organ), kidney, liver, lymph nodes (mesenteric, mandibular), pancreas, skin, stomach, small and large intestines, thymus, urinary bladder, harderian gland, preputial/clitoral gland, and lacrimal gland. In the spleen at 320 mg/kg body weight/day increased pigment was noted in males.

The presence of the pigment microscopically was caused by the silver accumulation and was not associated with any other tissue alterations and was therefore considered to be a non- adverse change.

No toxicologically significant changes were noted in any of the remaining parameters investigated in this study (i.e. clinical signs, functional observation, ophthalmoscopy, thyroid hormones and immunophenotype parameters).

There was no evidence of exposure to silver in control animals. All silver acetate-treated animals were exposed to Silver confirming administration and absorption. Exposure to Silver, expressed as AUC/Dose, increased less than dose proportionally over the used target dose range of 40 to 320 mg/kg body weight/day on Day 1 and Week 13 in both sexes. Slight accumulation of Silver seemed to occur after administration with Silver Acetate. This effect was most pronounced at a target dose level of 320 mg/kg body weight/day. On Day 1, a slight trend towards higher exposure levels in females compared to males was observed, whereas in Week 13, no clear sex differences in exposure were observed during the study. No clear sex differences in exposure were observed during the study.

Conclusion

In conclusion, administration of silver acetate by dietary inclusion was well tolerated in Wistar Han rats at target dose levels up to 320 mg/kg body weight/day. Non-adverse test item-related macroscopic grey discoloration and microscopic pigment were observed in several tissues and organs of all animals starting at 40 mg/kg body weight/day, which was regarded to be due to accumulation of silver. The only other histopathology finding was a non-adverse minor increase in incidence and severity of extramedullary hematopoiesis was noted in the spleen of females at 320 mg/kg body weight/day. Finally, minimal and non-adverse changes in clinical pathology parameters were noted mainly at 120 and 320 mg/kg body weight/day.

At 320 mg/kg body weight/day, lower body weight gain and lower food consumption were observed in males and females, which were adverse in males only.

Based on these results, the No-Observed-Adverse-Effect level (NOAEL) was considered to be at a target dose level of 120 mg/kg body weight/day for males and a target dose level of at least 320 mg/kg body weight/day for females.

3 ANNEXES

Deviations:

All deviations that occurred during the study have been authorized/acknowledged by the Study Director, assessed for impact, and documented in the study records.

Formulations and Dosing		
Study Plan Section No.	**Deviation**	**Impact**
6.1	For the diet preparation of Group 1 for Week 8 an insufficient amount of water was added to the diet before pellet procedure (11.7% was added instead of approximately 15%).	As the pellets had a good consistency after pelletizing and drying, this deviation had no impact on the study integrity.
6.1	The weighing of the test item for the diet preparation was done under yellow light. The actual preparation of the test item diet batches was prepared under normal laboratory light conditions instead of under yellow light because it was practically not possible to prepare diet batches under yellow light.	All diet analysis results were within the acceptable range and the discoloration of the diet batches (silver acetate reaction to light) was also limited after preparation. Furthermore, as the diet was stored in dark bags prior to use, this short duration of exposure to light during the preparation is considered not to have an effect on the test item. Therefore, it can be concluded that this deviation has no effect on the study integrity.