ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 26 June 2019

Addressee: [redacted]

Decision number: TPE-D-2114475936-31-01/F
Substance name: Silver
EC number: 231-131-3
CAS number: 7440-22-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 05/04/2018
Registered tonnage band: Over 1000

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

Your testing proposal is accepted and you are requested to carry out:

Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: OECD TG 443) in rats, oral route with the analogue substance silver acetate (EC number 209-254-9; CAS number 563-63-3) specified as follows:

- **Ten weeks premating exposure duration for the parental (P0) generation;**
- **Dose level setting shall aim to induce systemic toxicity at the highest dose level;**
- **Cohort 1A (Reproductive toxicity);**
- **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**
- **Cohorts 2A and 2B (Developmental neurotoxicity); and**
- **Cohort 3 (Developmental immunotoxicity).**

You have to submit the requested information in an updated registration dossier by **3 January 2022**. You also have to update the chemical safety report, where relevant.

The reasons for this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3. More specifically, the test material shall be reported in accordance with the prescriptions set out in point 3 of Appendix 3.

On 3 December 2018, the European Commission adopted Commission Regulation (EU) 2018/1881 amending of the REACH Regulation by introducing notably new information requirements specific to nanomaterials in Annexes VI to XI.[1] The revised requirements will enter into mandatory application on 1 January 2020 for all registrations. After that date,

[1] *OJ L 308, 4.12.2018, p. 1–20.*

ECHA may examine your registration in accordance with Article 41(1) of the REACH Regulation, in order to verify that the information in your dossier complies with the requirements set out in the revised annexes. The present decision is without prejudice to future requests to submit any information needed to bring the registration into compliance with the revised information requirements.

The Substance is subject to regulatory processes under the REACH Regulation, the Classification, Labelling and Packaging Regulation (Regulation (EC) No 1272/2008) and the Biocidal Products Regulation (Regulation (EU) 528/2012). The present decision is without prejudice to the proceedings relating to other regulatory processes.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Authorised[2] by Ofelia Bercaru, Head of Unit, Hazard Assessment

[2] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposal submitted by you.

Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

The basic test design of an extended one-generation reproductive toxicity study (Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of Annex X , Section 8.7.3. of the REACH Regulation. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in in ECHA *Guidance on information requirements and chemical safety assessment,* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for an extended one-generation reproductive toxicity study according to OECD TG 443, conducted by the oral route in rats using the analogue substance silver acetate (EC number 209-254-9; CAS number 563-63-3). The study design you proposed is without the extension of cohort 1B to include the F2 generation, without cohort 2A/2B but you propose to include cohort 3.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Reproductive toxicity (extended one-generation reproductive toxicity study). ECHA notes that you provided your considerations and you applied read-across to fulfil the respective information requirement, and no other alternative methods were available. ECHA has taken these considerations into account.

A. The substance proposed for testing

ECHA has evaluated your proposal to perform the test with the analogue substance silver acetate (EC number 209-254-9; CAS number 563-63-3). Article 13(1) of the REACH Regulation requires information on intrinsic properties of substances on human toxicity to be generated whenever possible by means other than vertebrate animal tests, including from information from structurally related substances (grouping or read-across), "*provided that the conditions set out in Annex XI are met*".

According to Annex XI, 1.5 there needs to be structural similarity among the substances within a group or a category and furthermore, it is required that the relevant properties of a substance within the group can be predicted from the data for reference substance(s) by interpolation, and the data should be adequate for the purpose of classification and labelling and/or risk assessment.

1) Description of the grouping and read-across approach proposed by you

You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group: the testing outcome from this study with the analogue substance silver acetate (EC number 209-254-9) is "*anticipated to be fully applicable to ionic silver (Ag^+) irrespective of the donor silver substance forming this ion, i.e. to freely soluble salts such as silver nitrate, sparingly soluble compounds including disilver(I) oxide, and metallic silver including nanosilver forms*." ECHA understands that by using the wording 'donor silver substance' you refer to any other silver substance for which the potential toxicity would be driven by the properties and presence of the free ion.

You provide the following reasons: "*Silver acetate is a silver(1+) salt, which is soluble in water – it possesses a solubility value of approximately 10 g/L at 20C (Merck, 2013). It has been commonly utilised in a variety of published toxicology studies as a soluble silver(1+) reference compound, and is so referenced for read-across purposes in the dossier. It is known to exhibit satisfactory bioavailability via the oral route*".

This analogue substance is being proposed "*instead of silver nitrate because the latter compound is highly irritant to mammalian tissues due to the properties of the anion.*"
As an integral part of this prediction, you propose that the source substance in solution is a suitable donor of silver ions.

ECHA considers that this information is your read-across hypothesis.

ECHA notes that in your registration dossier you have registered several forms/compositions of your substance:
- *Silver >= 99.9% Ag in massive form (>1 mm) with no classified impurities – not classified,*
- *Silver < 99.9% Ag in massive form (>1 mm) with no classified impurities – not classified,*
- *Silver >= 99.9% Ag in powder form (<1 mm but which does not fulfil the EU definition of nanomaterial) with no classified impurities - classified for environment),*
- *Silver < 99.9% Ag in powder form (<1 mm but which does not fulfil the EU definition of nanomaterial) with no classified impurities - classified for environment),*
- *Silver >= 99.9% Ag in nano form (<100 nm) with no classified impurities – classified for environment, and*
- *Silver < 99.9% Ag in nano form (<100 nm) with no classified impurities – classified for environment,*

Moreover, most of the human health studies available in the dossier are performed using a nanoform, including the studies for the reproductive toxicity endpoint.

2) ECHA analysis of the grouping approach and read-across hypothesis in light of the requirements of Annex XI, 1.5.

ECHA observes that you have balanced the considerations regarding the most appropriate Ag^+ donor and considers that the proposed analogue may be suitable as a test material to address potential toxicity due to free silver ions. However, ECHA notes that you have not considered other possible factors influencing toxicity such as translocation, and subsequent release of free ions and toxicity due to formation of reactive oxygen species following oxidation of silver particles.
ECHA furthermore understands that you consider that the metal ion is responsible for the common property or effect and that there is no toxicity of the counter ion.

ECHA considers the hypothesis as plausible to address toxicity where it is due to free silver ions which will be expected from all donors of silver ions.

Finally, ECHA agrees that the use of silver acetate is preferred compared to silver nitrate due to the irritant properties of the nitrate anion.

3) Conclusion on the read-across approach

For the reasons as set out above, ECHA considers that this grouping and read-across approach may provide a reliable basis whereby the human health effects of the registered substance, where it is due to the free ions, may be predicted from data from a reference substance.
Hence, this approach is considered plausible for the purpose of the testing proposal evaluation. As noted above, there may be a need to consider other possible factors influencing the prediction of toxicity for the registered substance.

ECHA emphasises that any final determination on the validity of the read-across approach proposed by you, would be premature at this point in time. The eventual validity of the read-across hypothesis and grouping approach will be reassessed once the requested information is submitted.

B. Specifications of the study design

You propose to conduct the extended one-generation reproductive toxicity study without the extension of Cohort 1B to include the F2 generation, without Cohorts 2A/2B but you propose to include Cohort 3. ECHA considers that, based on the currently available information, the proposed study design needs further specification to fulfil the information requirement of Annex X, Section 8.7.3. of the REACH Regulation.

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement. Thus, an extended one-generation reproductive toxicity study according to columns 1 and 2 of Section 8.7.3., Annex X is required. The following refers to the specifications of this required study.

1) Premating exposure duration and dose-level setting

You propose "*a default pre-mating treatment period of 10 weeks*". ECHA agrees with your proposal.

ECHA notes that to ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).
As regards the dose-level selection you indicate that it will be determined "*based on ancillary / dose-range finder studies, also incorporating toxicokinetic endpoints*".

ECHA reminds you that the highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

2) Extension of Cohort 1B

If the column 2 conditions of 8.7.3., Annex X are met, Cohort 1B must be extended, which means that the F2 generation is produced by mating the Cohort 1B animals. This extension provides information also on the sexual function and fertility of the F1 animals.

You proposed not to include an extension of Cohort 1B and provided justifications taking into account the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Therefore, ECHA agrees that the criteria to extend the Cohort 1B are not met, despite widespread use, and concludes that Cohort 1B must not be extended to include mating of the animals and production of the F2 generation.

3) Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity as described in column 2 of Section 8.7.3., Annex X. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

You proposed not to include Cohorts 2A and 2B and provided justifications following the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Subsequent to a Proposal for Amendment (PfA) submitted by one of the Member States Competent Authorities (MSCAs), ECHA notes existing information on the substances structurally analogous to the registered substance which you referred to in your read across hypothesis. This concerns the information available from the *in vivo* studies Charehsaz *et al.* DARU Journal of Pharmaceutical Sciences (2016) 24:24 (DOI 10.1186/s40199-016-0162-9) and Hadrup 2012 [redacted] which show evidence of particular concern for neurotoxicity. Specifically, Charehsaz *et al.* dosed rat dams with $AgNO_3$ (20 mg Ag/kg/day) from Gestation Day 7 to 20, and there was a high incidence of mild to moderate hippocampal pyramidal neuronal loss and mild gliosis in the brain of treated rats. ECHA considers that these histopathological findings in brain are clear evidence of an abnormality observed in the central nervous system, and evidence of neurotoxicity. As supporting evidence, the study of Hadrup dosed rats with silver acetate (9 mg Ag/kg/day) for 28 days, and reported statistically-significant increased levels of dopamine and noradrenaline in the brain, although the size of the increase was modest. ECHA considers that the perturbation of neurotransmitter levels in brain is evidence of a specific mode of action closely linked to neurotoxicity.

Therefore, the developmental neurotoxicity cohorts 2A and 2B need to be conducted

because there is a particular concern on (developmental) neurotoxicity based on the results from the above-identified *in vivo* studies on substances structurally analogous to the registered substance.

In your comments to the PfA, you raised a number of detailed technical concerns about the studies which give rise to concern. After setting out these concerns, you stated "*Setting divergences in high-level findings aside, it is acknowledged that several of the publications ostensibly provide limited evidence of CNS neuropathological findings following silver exposure (in adults and developing animals), mainly in respect of possible hippocampal region changes; for instance, the reports by Rungby et al., 1987; Liu et al., 2012; Wu et al., 2015; Skalska et al., 2015; Charehsaz et al., 2016.*" You concluded that "*Based on weight-of-evidence considerations, it has not been demonstrated that ionic Ag or elemental Ag forms (also encompassing AgNP), meet the evidential triggers for DNT concerns (or alerting adult neurotoxicity), i.e. involving findings from scientifically robust studies which also meet the thresholds for severity (adversity). However, on a precautionary basis it could be argued that limited indications of neurotoxicity have now been described in several investigational studies. The EPMF is willing to include the DNT cohorts 2A and 2B in the EOGRTS test design in case this is deemed justified by the European regulators.*" Based on this, ECHA notes that you agree that Charehsaz *et al.* 2016 provides limited evidence of CNS neuropathological findings following silver exposure, and that you are willing to include the DNT cohorts 2A and 2B in the EOGRTS test design. ECHA notes that you consider the Hadrup *et al.* study (2012) to be of Klimisch reliability 2. In respect of the Charehsaz *et al.*, 2016 study, ECHA notes that the points you make do not invalidate the study, and that the results seen in this study are sufficient to give rise to a particular concern.

With reference to the list of points provided in your comments on the PfA, ECHA accepts that (i) the study was not performed according to guideline or GLP (ii) the study does not report all information that would be reported in a GLP, guidline study (iii) there is only a single treatment dose of $AgNO_3$, which impedes assessment of the dose-response pattern (iv) there is not a comprehensive reporting of histopathology findings for all regions of the brain. ECHA considers that your points (v, vii, viii and ix) are not relevant for the $AgNO_3$ group in the Charehsaz paper, and that your point (vi) has no validity, since the Ag content in total brain would not necessarily be expected to correlate with specific damage to a small region of the brain (i.e. the hippocampus). Nonetheless, the study is well reported, and provides evidence of an adverse effect in hippocampus at one specific dose in adult animals, linked to evidence of higher levels of silver in total brain. ECHA considers this is clear evidence of neurotoxicity, albeit limited. In performing a weight of evidence assessment for repeated dose toxicity studies, ECHA notes that Hadrup *et al.* (Archives of Toxicology, Volume 86, Issue 4, pp 543–551 2012) did not examine brain histopathology, and Kim *et al.* (2010) and Garcia *et al.* (2016) did not study ionic silver. Thus these studies do not contribute significantly to the weight of evidence. Boudreau *et al.* (2016) is a reliable study performed with a different silver salt (silver acetate), and it did not find evidence of adverse effects in brain, but ECHA does not consider that this study extinguishes the particular concern from the Charehsaz study, which was performed with a different silver salt.

In addition, as explained in page 2 of the Decision, Commission Regulation (EU) 2018/1881 amending the REACH Regulation imposes registrants to submit by 01 January 2020 information specific to nanomaterials under Annexes VI to XI requirements. In that respect, ECHA brings your attention on the particular concerns identified by the PfA resulting from studies on silver nanoparticles. In the prospect of ensuring the compliance of your dossier concerning silver nanoparticles by 01 January 2020, we encourage you to take account of the concerns raised by the PfA and to consider the need to propose any further testing.

4) Cohort 3

The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity as described in column 2 of 8.7.3., Annex X.

You proposed to include Cohort 3 and provided justifications following the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Therefore, ECHA agrees that the criteria to include Cohort 3 are met.

5) Species and route selection

You proposed testing in rats. According to the test method OECD TG 443, the rat is the preferred species. On the basis of this default consideration, ECHA considers that testing should be performed in rats.

You proposed testing by the oral route. You also indicate that the "*oral exposure via dietary administration is the most appropriate route to model human exposure. Exposure via incorporation in the drinking water is a possible alternative but is less preferred due to the potential for both silver adsorption on to container surfaces, and also due to experience that silver can affect the drinking water intake of experimental animals.*" ECHA agrees that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route. Moreover, ECHA also considers that the test substance should be administered through the diet.

C. Required test

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposedstudy with the analogue substance silver acetate (EC number 209-254-9; CAS number 563-63-3), following the conditions described below:

Extended one-generation reproductive toxicity study (test method OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;
- Cohorts 2A and 2B (Developmental neurotoxicity); and
- Cohort 3 (Developmental immunotoxicity).

While the specifications for the study design are given above, you shall also submit with the new endpoint study record a scientific justification on each of the following aspects: 1) length of the premating exposure duration and dose level selection, 2) reasons for why or why not Cohort 1B was extended, 3) termination time for F2 generation, and 4) reasons for why or why not Cohorts 2A/2B and/or Cohort 3 were included.

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. However, you may expand the study by including the extension of Cohort 1B if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the available information, together with the new information, shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented.

Deadline to submit the requested information in this decision

In the initial draft decision communicated to you, the deadline to provide the requested information was 24 months from the date of adoption of the decision. In your comments on the draft decision, you requested an extension of the deadline to 36 months. To justify this request you referred to the planning, preparatory efforts and the general complexity of the EOGRTS. You also indicated that you would need to perform additional studies prior to commencement of the EOGRT study, such as toxicokinetic studies, palatability and dose-range finding studies. In your justification you also refer to the limited capacity in testing laboratories and have provided documentary evidence of such from three laboratories. ECHA has reviewed your justifications and has extended the deadline to 30 months.

Appendix 2: Procedural history

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 5 July 2017.

ECHA held a third party consultation for the testing proposals from 27 September 2017 until 13 November 2017. ECHA did not receive information from third parties.

You updated your registration on 5 April 2018 (submission number [redacted]). ECHA took the information in the updated registration into account. The updated information is reflected in the Reasons (Appendix 1).

This decision does not take into account any updates after **11 March 2019**, 30 calendar days after the end of the commenting period.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and amended the deadline.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-65 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different compositions, the sample used for the new tests must be suitable to assess these compositions. Finally there must be adequate information on substance identity for the sample tested and the compositions registered to enable the relevance of the tests to be assessed. For each study record reported, adequate information on the test material used to generate the data needs to be documented in the test material record linked to the EndPoint Study Record. The test material record must document as a minimum the constituent concentration values and any other parameter that is relevant (for instance the size or the shape of the particles).

4. If the required tests are conducted with an analogue substance in the context of a read-across approach, the identity of the test material used to perform the test should be specified in line with the ECHA's Practical Guide on "How to use alternatives to animal testing to fulfil your information requirements" (chapter 4.4). This is required to show that the test material is representative of the analogue substance identified in the read-across approach and used to predict the properties of the registered substance.