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DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For Silver, CAS No 7440-22-4 (EC No 231-131-3)

Addressees: Registrant(s)[1] of Silver

This decision is addressed to the Registrant(s) of the above substance with active registrations pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided as an Annex to this decision.

The scope of this substance evaluation is limited to the properties of and information on nanoforms of Silver.

Consequently, only those Registrants whose registration covers nanoforms[2] of Silver shall provide the information requested in this decision.

Concerning the coverage of nanoforms of Silver in registration dossiers of the Registrants, in absence of explicit and suitable information in all available individual registration dossiers that they do/do not cover nanoforms of Silver, ECHA is not in a position to determine whether and which individual registration dossier actually covers any specific nanoform of the substance.

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[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

[2] For the purpose of this substance evaluation, the evaluating MSCA distinguishes two broad (groups of) forms (which may be divided in subgroups): the nanoforms, and the bulk forms (*i.e.* larger than nanoform). A nanoform of a substance is a form that would fulfil the definition in the Commission Recommendation (2011/696/EU). Different nanoforms of Silver are further characterised by a specific size, shape and surface chemistry. The specific surface chemistry can be due to deliberate modification (e.g. surface treatment or coating), or the absence of surface modification (no surface treatment). As indicated on the first page of this document, the scope of this substance evaluation is limited to the properties of and information on nanoforms of Silver.

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Only each respective Registrant of Silver knows the relevant forms in which the substance is registered. Only the Registrant is therefore able to determine the particle size distribution or primary particles and to report sufficient information on the respective forms registered.

In case where a Registrant actually manufactures or imports nanoforms of Silver as defined for this substance evaluation (see footnote 2), failure to report sufficient information on each form of a substance in the registration dossier, including nanoforms, may result in these forms not being covered by this registration. All Registrants of Silver shall therefore determine whether their individual registration dossier cover nanoforms of Silver in order to establish certainty as to which manufacturer or importer will have to provide the information requested in this decision.

Based on an evaluation by Bureau REACH on behalf of the Ministry of Infrastructure and the Environment as the Competent Authority of the Netherlands (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 1 November 2015 *i.e.* the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

I. PROCEDURE

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of the Netherlands has initiated substance evaluation for Silver, CAS No 7440-22-4 (EC No 231-131-3) based on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Nanoparticles/Ecotoxicity of different forms of the substance; Environmental fate; Exposure/Wide dispersive use; aggregated tonnage, Silver was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The updated CoRAP was published on the ECHA website on 26 March 2014. The Competent Authority of the Netherlands was appointed to carry out the evaluation.

This evaluation does not include a full evaluation of all elements of the registration dossiers but is targeted to the characterisation of the substance, environmental fate properties, environmental hazard assessment and exposure assessment of the nanoforms of Silver that are covered by the REACH registration dossier(s) submitted for Silver.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 23 March 2015.

On 7 May 2015 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant(s) commenting phase

By 15 June 2015 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay.

By the end of October 2015 the Registrant(s) provided an update of the Registration Dossier. In the Joint Chemical Safety Report (J-CSR) the main updates are summarised as *"inclusion of data relevant for the Substance Evaluation by RIVM (new information found in literature and included in comments on Substance Evaluation draft decision; Navarro et al. 2014 and Notter et al. 2014), improvement of the composition section to mark generic compositions, inclusion of testing proposal for reproductive toxicity endpoint (EOGRTS)."*

The evaluating MSCA considered the comments received from the Registrant(s) and the dossier updates. Where relevant, the information contained therein is reflected in the Statement of Reasons (Section III) whereas minor amendments to the Information Required (Section II) were made.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 21 January 2016 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of the draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Following this invitation, on 22 February 2016, ECHA submitted 9 proposals for amendment to the draft decision, of which the Registrant(s) was notified on 26 February 2016 with an invitation for comments on those proposals for amendment within 30 days of the receipt of the notification pursuant to Articles 52(2) and 51(5) of the REACH Regulation. No proposals for amendments were received from other Member States.

Referral to Member State Committee

On 7 March 2016 ECHA referred the draft decision to the Member State Committee.

By 29 March 2016 the Registrant(s) provided comments on the proposals for amendment, in accordance to Article 51(5) and on the draft decision. The Member State Committee took the comments on the proposal(s) for amendment of the Registrant(s) into account.

The evaluating MSCA considered the proposals for amendment as well as the comments received from the Registrant(s) on these. Where relevant, the Statement of Reasons (Section III) and the Information Required (Section II) were amended.

Based on the current information in the registration dossiers, the information as requested in section II is required. Evaluation of the information submitted in response to these requests might reveal that the safety of the nanoforms of Silver still cannot be adequately assessed and might lead to additional requests for information.

Considerations of proposals for amendment and Registrant(s)' comments on them.
One proposal for amendment regarded the definition of form(s), suggesting the following text: "*A nanoform of a substance is a composition that would fulfil the EU recommendation for the definition of a nanomaterial that has a specific shape and surface treatment*". The Registrant(s) emphasise the importance of a fixed definition for the course of the Silver substance evaluation (viz. regulatory certainty) and therefore suggest to apply the definition of a nanoform as proposed.

ECHA acknowledges the need for clear terminology, but proposes that a further distinction between different nanoforms should be made, based on shape, surface treatment, and on size, when differences in these parameters result in different toxicological properties. The footnote was amended accordingly.

After discussion in the Member State Committee meeting on 25 – 29 April 2016, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 27 April 2016. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

1 INFORMATION REQUIRED

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods/instructions (in accordance with Article 13(3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

1. Information on ecotoxicity on the smallest nanoform of Silver with the highest specific surface area that is covered by the REACH registration dossier(s) submitted for Silver, i.e. information on:
   - Toxicity to algae, according to OECD Test Guideline No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. The test medium to be used is AAP medium as described in OECD Test Guideline No. 201, with the following adaptations: to minimise complexation of silver ions, the amount of $Na_2EDTA\cdot 2(H_2O)$ shall be minimised (by balancing the molar concentrations of iron and $Na_2EDTA\cdot 2(H_2O)$) and chloride salts shall be replaced by nitrate salts. The pH shall be the pH at which the medium equilibrates with air (generally around 7.6) and temperature 20 °C.
   - Long-term toxicity to aquatic invertebrates, according to OECD Test Guideline No. 211: *Daphnia magna* Reproduction Test. The test medium to be used is Elendt M7 medium as described in OECD Test Guideline No. 211, with the following adaptations: to minimise complexation of silver ions, the amount of $Na_2EDTA\cdot 2(H_2O)$ shall be minimised (by balancing the molar concentrations of iron and $Na_2EDTA\cdot 2(H_2O)$) and chloride salts shall be replaced by nitrate salts. The pH shall be the pH at which the medium equilibrates with air (generally around 7.6) and temperature 20 °C.

- Toxicity to soil microorganisms, according to OECD Test Guideline No. 216: Soil Microorganisms, Nitrogen Transformation Test in three different soils. The three soils to be used are the same as the ones used for Request 2, i.e. they need to cover as wide a range as possible of soil properties expected to affect the fate of the nanoform of Silver in soil; especially pH, clay content and organic matter content are expected to be of major relevance in this respect. The selection of soils shall be justified and include LUFA 2.2 soil as this nowadays is a commonly used reference soil. Soils shall be sufficiently characterised: at least pH, clay content, organic matter content, and moisture content shall be reported.

While conducting these studies, the OECD Guidance on Sample Preparation and Dosimetry (ENV/JM/MONO(2012)40, in particular sections III, IV, and V-A through V-C) should be consulted. The composition of the test media shall be fully reported (including at least ionic strength, calcium concentration and hardness, pH, alkalinity, dissolved organic matter, and presence of dispersing agents). In addition, the following important conditions shall be taken into account:

Throughout the study, the ratio between particulate and ionic silver shall be monitored in samples from the test vessels, using analytical techniques that enable distinction between the concentrations of the nanoform of Silver and ionic silver.

The study setup shall include a control with exposure to silver nitrate to enable distinction between the toxicity of the nanoform of Silver and ionic silver.

The same analytical techniques shall be applied in the ionic silver control as are applied in the test with the nanoform of Silver to enable detection of the potential formation of nanoparticles in a solution with ionic silver only.

The nanoform of Silver that is tested shall be sufficiently characterised, by providing information on the following physico-chemical properties, using the indicated test method(s):

- The granulometry, which shall include primary particle size and shape, aggregate/agglomerate size and primary particle size distribution (number-based). Method: Method for powders is Transmission Electron Microscopy (TEM) combined with Energy Dispersive X-ray (EDX), and Laser Diffraction; method for suspensions is Transmission Electron Microscopy (TEM) combined with Dynamic Light Scattering;

- The specific surface area (by volume). Method: for powders BET (ISO 9277:2010); for suspensions calculation based on theoretical model;

- The surface treating agent(s), including chemical identity (IUPAC name and numerical identifiers (CAS and EC)), type of reaction with the silver surface, relative coverage of the silver surface (as this information is part of the substance identity, the information shall be added in IUCLID sections 1.2 and 1.4);

- The dissolution rate for the specific test media used in the tests. Method: OECD Guidance Document 29, taking note of the OECD Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (ENV/JM/MONO(2012)40, in particular sections III, IV, and V-A through V-C). The composition of the test medium shall be fully reported (including at least ionic strength, calcium concentration and hardness, pH, alkalinity, dissolved organic matter, and presence of dispersing agents);
- The density. Method: OECD 109 Density of Liquids and Solids;
- The point of zero charge. Method: micro-electrophoresis or electrophoretic light scattering to be performed at fixed low salt concentration and at fixed particle concentration, as described above for dissolution rate.

In case the information in Request 1 indicates a higher toxicity in one of the toxicity tests performed for the nanoform than for the ionic silver, information on request 2 shall be provided.

2. Quantitative information shall be provided on the fate of nanoform of Silver in soil pore water and the soil solid fraction, following their introduction in three different soil types. The nanoform of Silver and its transformation products shall be determined in pore water and in clay, organic matter and remaining inorganic solid fractions. A proper mass balance needs to be established and documented. Total test duration is 12 months and test temperature is 20 C.

In addition, silver nitrate shall be tested to enable a comparison with ionic silver under the same test conditions (as specified below).

The three soils to be selected need to cover – as wide as possible – a range of soil properties expected to affect the fate of the nanoform of Silver in soil; especially pH, clay content and organic matter content are expected to be of major relevance in this respect. The selection of soils shall be scientifically justified and shall include LUFA 2.2 soil as this nowadays is a commonly used reference soil. Soils shall be sufficiently characterised: at least pH, clay content, organic matter content, and moisture content shall be reported.

Test temperature shall be 20 °C. Test duration after introduction of (nanoforms of) silver in soil shall at least be 12 months, with a minimum of 3 time points for sampling, including start and finish of the test duration.

To minimize loss of particles during centrifugation, collection of pore water shall be done by centrifugation of a saturated and equilibrated fixed amount of soil at relatively low centrifugal forces (preferably < 2000 g) over a filter of 0.45 µm, followed by filtration over a 0.2 µm filter. To determine dissolved silver species in the pore water, ultracentrifugation or ultrafiltration (1 kDa filter) is required. A proper mass balance needs to be established.

Quantitative measurements shall focus on assessing the partitioning between the solid phase and the pore water, and on assessing particle transformation, including dissolution and formation of silver complexes, in particular silver sulphide particles. It shall be assured that no significant modification of the particles takes place during any of the steps to be taken during processing of soil and pore water samples.

3. Information on the uses of each individual nanoform of Silver that is registered; as specified in section III.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA an update of the registration dossier(s) containing either the information required by Requests 1 and 3 of this decision by **13 July 2017** or the information required by Requests of 1, 2, and 3 of this decision by **14 January 2019**[3], including robust study summaries and, where relevant, an update of the Chemical Safety Report.

2 STATEMENT OF REASONS

According to the current Commission Recommendation on the definition of nanomaterial, a nanomaterial is a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm (EU, 2011). The Commission Recommendation on the definition of nanomaterial also includes aggregates and agglomerates of nanoparticles. Agglomerated or aggregated particles may or may not exhibit the same properties as unbound particles. Moreover, there can be cases during the life cycle of a nanomaterial where the particles are released from weakly bound agglomerates or under certain conditions even from more strongly bound aggregates[4]. The Commission Recommendation on the definition of nanomaterial (EU, 2011) therefore includes particles in agglomerates or aggregates whenever the constituent particles are in the size range 1 nm - 100 nm. Where technically feasible and requested in specific legislation, compliance with the definition as mentioned above may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition as mentioned above where the specific surface area by volume of the material is greater than 60 m^2/cm^3 (EU, 2011). However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition even if the material has a specific surface area lower than 60 m^2/cm^3 (EU, 2011).

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[3] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

[4] http://europa.eu/rapid/press-release_MEMO-11-704_en.htm

Nanomaterials are being engineered for their specific physico-chemical and biological characteristics, meaning that their reactivity and/or behaviour (such as their interaction with their environment) will depend on these characteristics. Due to their specific physico-chemical properties, nanoscale particles can have specific characteristics that distinguish them from the non-nanoparticles and from other nanoparticles of the same material. Although the toxicological profile of the chemical components of a given nanomaterial may be well known, there may be cases where its specific properties raise concerns about the specific potential to harm humans and the environment (SCENIHR, 2010). It is further concluded in this SCENIHR report that the reaction rate of nanoparticles often relates to the available surface area. Consequently, chemical reactivity per mass dose increases with decreasing particle size. Partly based on these observations the Second Regulatory Review on Nanomaterials (EC, 2012) concludes that "*possible risks are related to specific nanomaterials and specific uses. Therefore, nanomaterials require a risk assessment, which should be performed on a case-by-case basis, using pertinent information."* In 2013 the General Report on REACH (EC, 2013) echoed these observations.

In addition, nanomaterials may change during their life cycle. Parameters such as size, aggregation states, surface charge, coatings and other properties may change in different solvents, test media, and biological environments (SCCS, 2012; EC, 2013).

To ensure a high level of protection of human health and the environment, the REACH Regulation imposes the determination of hazards, exposures and risk irrespective of the form of the substances concerned. This includes more specifically nanoforms of substances, which may trigger specific hazardous properties and risks, as already highlighted by various institutions, including the European Parliament[5].

From the registration dossier(s) it is not clear how much of the total silver is produced as nanoform(s) of silver, but based on the information available, the Lead Registrant estimates that the total production volume of nanoforms of silver is around [redacted] per annum[6].

Annex 7 to the Joint Chemical Safety Report (J-CSR) for Silver (EC No 231-131-3), titled "Nanosilver read-across justification for environmental information requirements", focusses on showing that nanoforms of Silver are less toxic than ionic silver and that read-across can be applied with ionic silver as a worst-case. Based on the existing information ECHA, however, is not convinced that nanoforms of silver are less toxic than ionic silver. Available studies show cases where nanoforms of silver induce higher toxicity than ionic silver (e.g.

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[5] Recital D of European Parliament Resolution of 24 April 2009 on Regulatory aspects of nanomaterials (2008/2208(INI)), pages 267-275 of the document available at http://www.europarl.europa.eu/RegData/seance_pleniere/textes_adoptes/provisoire/2009/04-24/P6_TA-PROV(2009)04-24_EN.pdf.

[6] In the J-CSR no estimation of the total production volume of nanoforms of silver is indicated. In the Registrant(s) comments to the Draft Decision [redacted] estimate of [redacted] tonnes per annum was indicated. This estimate of [redacted] tonnes per annum was reiterated in the Registrant(s) comments to the Proposals of Amendment. The estimation of [redacted] tonnes per annum was only indicated by the Registrant(s) at MSC-47.

Georgantzopoulou et al., 2012; Wang et al., 2012; Notter et al., 2014), which would then result in lower PNEC values than those provided for ionic silver. The Registrant(s) indicate in their exposure scenarios that risk management measures (RMMs) should be implemented that ensure that the environmental release will not result in exceedance of the PNEC value in the receiving waters. Consequently, when specific nanoforms are more toxic than currently assumed by the Registrant(s), more stringent operational and/or regulatory RMMs might be needed, such as improved waste treatment and more stringent classification, even at the tonnage level of nanomaterial currently produced as indicated by the Registrant(s). Furthermore, insufficient information is provided by the Registrant(s) to judge to what extent the information in Annex 7 to the J-CSR is representative for the nanoforms that are registered by the Registrant(s). There is, therefore, a concern on the safe use of nanoforms of Silver registered by the Registrant(s).

2.1 CONSIDERATIONS OF PROPOSALS FOR AMENDMENT AND REGISTRANT(S)' COMMENTS ON THEM

Related to the Registrant(s)' statement that *"less than 0.02% of the total silver volume registered under REACH is nanosilver"* one proposal for amendment suggested to insert text that clarifies that the tonnage by itself may not be a relevant consideration for risk assessment of nanomaterials. The Registrant(s) add that the 0.02% may be an overestimation and the tonnage appears to be as low as [redacted] per annum (see footnote 6). *"Due to the restricted number of nanoforms that lie in scope of the silver REACH registration (versus the number of nanoforms used for antibacterial and antifungal properties)"*, the Registrant(s) reiterated a concern about the proportionality of the decision.

While indeed the tonnage by itself may not be relevant, ECHA is of the opinion that the number of forms by itself may not be relevant, as the registration should identify the hazards, including those of nanoforms, irrespective of tonnage considerations or the number of nanoforms registered.

2.2 ECOTOXICITY

2.2.1 Establishing the concern

In their read-across justification document (Annex 7 to the J-CSR) the Registrant(s) argue that nanoforms of silver are less toxic than ionic silver and consequently the toxicity of ionic silver can be taken as a worst case for the hazard assessment of nanoforms of silver. The read-across document contains toxicity studies on acute and chronic effects of nanoforms of silver and ionic silver. Test organisms include aquatic invertebrates, algae, fish, aquatic and terrestrial plants, soil macro-organisms, and aquatic and terrestrial microorganisms. However, the available data on chronic toxicity of nanoforms of silver are very limited and also the Registrant(s) conclude "there is currently insufficient information available to make any conclusions regarding the influence of particle size, morphology or coating on the long-term toxicity of nanosilver" for aquatic invertebrates and fish. Furthermore, acute data show that nanoforms of silver can be more toxic than ionic silver (e.g. Georgantzopoulou et al., 2012; Wang et al., 2012; Notter et al., 2014), which raises questions on the validity of the read-across argumentation provided by the Registrant(s).

In October 2015 the Registrant(s) provided an update on the registration which included the following paragraph to further substantiate the read-across of toxicity of nanoforms of silver from the toxicity of free silver ions:

> *"Notter et al. (2014) presents a meta-analysis of published EC50 values for ionic silver and nanosilver. The authors demonstrate that almost 94% of acute toxicity values assessed for freshwater, seawater and terrestrial systems using algae, annelid, arthropoda, bacteria, crustacea, fish, nematoda, plant, protozoa and rotatoria show that the nanoform of silver is less toxic than the dissolved metal (when normalised for total metal concentration). Therefore, taking the full body of evidence into account, the read-across use of toxicity values from ionic to nanosilver as a 'worst case' approach is justified and scientifically defensible."*

This paragraph was added twice in the section on environmental hazard assessment (Chapter 7), both in the section on the aquatic compartment (section 7.1), and in the section on the terrestrial compartment (section 7.2).

Closer inspection of the data as outlined in the Figures 1 and 2 of the publication of Notter et al. (2014), however, reveals two important issues. First, the frequency of exceedance of 1 of the ratio of the dissolved metal toxicity value (LD, LC, IC, EC50) to the nanoform toxicity value (LD, LC, IC, EC50) (exceedance of 1 of this ratio is used by Notter et al. as the indicator that toxicity of nanoforms of silver is therefore an insufficient justification of read-across of silver toxicity data to toxicity of nanoforms of silver. In addition it should be noted that, when all other physico-chemical properties are kept the same, smaller particles are more toxic than larger ones (e.g. Delmaar et al., 2015). Second, for specific groups of test organisms (see the bottom row of Figure 1 of the publication of Notter et al.) the ratio of the dissolved metal toxicity to the nanoform toxicity exceeds 1 for a high percentage of the cases investigated. While acknowledging the relative low number of studies for some groups of test organisms, the ratio indicates that the toxicity of nanoforms of silver may exceed that of ionic silver in 10 – 33 % of cases for some groups of organisms (*e.g.* for annelids, algae and plants), which may have consequences for a species sensitivity distribution and consequently for hazard assessment.

Only few of the studies provided in the J-CSR enable a comprehensive and comparative evaluation of toxicity exerted by silver nanoparticles and ionic silver. Such an evaluation demands sufficient information on each of the essential elements, *i.e.* fate of the total amount of silver in the test, fate of the silver nanoparticles in the test, a control with ionic silver only, and sufficient identification and characterisation of the nanoparticle tested. Despite all the details provided, the available data cannot univocally support the Registrant(s) conclusion that nanoforms of silver are less toxic than ionic silver. This is further discussed below under 'justification why new information is needed'. Furthermore, although the tested (nano)forms of silver are different from the forms that are registered, the Registrant(s) do not justify why the tested forms are representative for the registered forms.

Further data on the different nanoforms of silver that are registered are therefore needed in order to either verify or reject the basic hypothesis of silver ions being fully responsible for adverse effects of exposure of biota to nanoforms of silver. This information is essential to verify the relevance of ecotoxicity data in the J-CSR for the registered forms.

In the CSR of the Registrant(s) no exact Risk Characterisation Ratio (RCR) for the aquatic environment has been calculated. The Registrant(s) indicate in their exposure scenarios that manufacturers could ensure safe use by implementing risk management measures (RMMs) that ensure that the environmental release will not result in an exceedance of the PNEC value in the receiving waters. Consequently, when specific nanoforms are more toxic then currently assumed by the Registrant(s), more stringent regulatory RMMs might be needed to ensure safe use of all nanoforms of silver, which may include more stringent classification and or restriction of certain uses.

2.2.2 Justification why new information is needed

As indicated above, acute data show that nanoforms of silver can be more toxic than ionic silver (e.g. Georgantzopoulou et al., 2012; Wang et al., 2012; Notter et al., 2014), which raises questions on the validity of the read-across argumentation provided by the Registrant(s). Furthermore, the bioavailability of the nanoforms of silver in toxicity tests is not always clear. Many of the available studies in the read-across document lack details to distinguish the toxicity of the nanoparticles from that of ionic silver, and to gain sufficient information on the bioavailability of the nanoforms of silver in the test. The following factors should be paid attention to within a study, both in characterisation and in concentrations of nanoforms of silver as well as ionic silver:

- *Fate of the total silver concentration.* The total silver concentration in the test medium could decrease rapidly, for example due to sorption of the ionic silver to the wall of the test vessel or organic matter in the medium. This is indicated by some of the studies discussed in the read-across justification (e.g. Griffitt et al., 2008; Bilberg et al., 2012; Wang et al., 2012) where recoveries of nanoforms of silver lower than 50% are reported, but effect concentrations are defined by nominal concentrations, which will lead to underestimation of toxic levels. Furthermore, the fate of the silver in the test medium could be different from that in the stock solution or that in a separate vessel without test organisms present. The test organism itself could influence the fate of the silver, *e.g.* due to sorption of the silver to the test organism or through excretion of faeces which could bind the silver. Therefore, the fate of the silver should preferably be determined in samples from the test vessels. The total concentration of the silver in the test medium should be measured at least at the start and the end of the test. Alternatively, the test medium could be renewed frequently, although also in this case the concentration of silver in the test medium should be monitored.
- *Fate of the silver nanoparticles.* The concentration of the nanoparticles could decrease due to precipitation, but also through dissolution of the silver from the particles. Therefore, the ratio between free ionic silver and the nanoparticles should also be determined at the start and at the end of the test. Determination of this ratio could be performed by measuring the free silver concentration with a special probe or by analysis of samples of the test medium after filtration over a filter with pore sizes smaller than the nanoparticles.

From the difference between the concentration of the free silver ions and the total silver concentration, the fraction of nanoparticles of silver can be estimated. In this estimation it is presumed that all undissolved silver is present as nanoform(s) of silver. Alternatively, a chelator could be added to the test solution that removes free silver ions from the solution, thus ensuring that the test organisms are only exposed to nanoparticles of silver.

- *A control with ionic silver only* (e.g. silver nitrate). To distinguish between the toxicity of the nanoparticles and that of free ionic silver it is important that the effect of free ionic silver is determined in the same test setup. Because of variation between different test laboratories, for example due to variation in the sensitivity between different strains of the same species or different exposure media, the use of test results from other studies for comparison is best avoided. Because of the potential formation of nanoparticles in a solution with only ionic silver, the same analytical techniques should be applied in this control as are applied in the test with the nanoform(s) of silver.
- *Sufficient details on the nanoparticle tested.* Particle size, coating and shape all may influence exposure and toxicity. Therefore, details on the nanoparticle properties should be provided in order to be able to compare results across studies and to distinguish whether the extent of observed effects are related to a specific parameter of the nanoform(s) of silver. The nanoform(s) of silver tested should be sufficiently characterised.

Many of the available studies in the read-across document do not address each of these essential factors. Therefore, from these studies it cannot be concluded that silver nanoparticles are less toxic than ionic silver (or vice versa). This hampers a proper risk assessment of the nanoform(s) of silver.

For example, Wang et al. (2012) tested the effect of a nanoform of silver on the aquatic species *Chydorus sphaericus, Raphidocelis subcapitata* and *Danio rerio* while the fate of the nanoform of silver was monitored during the test by measuring the total and free silver concentrations. With this approach they could distinguish between the toxicity of the total silver concentration and that of the silver particles. One of the main results is that the toxic effect of the nanoform of silver partly can be attributed to the silver ions released by the particles, but also to the particles themselves. Wang et al. (2012) also showed that, especially for *D. rario,* the fraction of the toxicity caused by the nanoparticles themselves can be higher than that for the silver ions released from the particles. This study further showed that the difference in behaviour between ionic silver and nanoparticles causes a different exposure in the toxicity studies. For example, filter feeders can expose themselves to higher levels of nanoforms of silver than when the silver was fully available as free ions. In the read-across justification by the Registrant(s), two further studies (Gao et al., 2009; McLaughlin and Bonzongo, 2012) are referenced, but these lack a control with ionic silver, which hampers a comparison between the toxicity of nanoform(s) of silver and that of ionic silver. However, in these studies acute LC50 values are reported for nanoform(s) of silver on the crustacean *Ceriodaphnia dubia* that are comparable to the LC50 value for ionic silver to *C. dubia* given in the CSR. Assuming that the nanoparticles of silver are not fully dissolved during the test, they should have an effect that is very similar to that of silver ions released.

Similar to the results for *Ceriodaphnia dubia* above, studies on wetland plants and wheat also suggest that certain types of silver nanoparticles may have greater toxicity to terrestrial plants than silver ions alone (Yin et al., 2012; Dimkpa et al., 2013), although both studies had some limitations regarding the measurements of ionic silver. Either the actual ion release of the silver nanoparticles in the soil was not measured (Yin et al., 2012), or the concentration of ionic silver was not measured in the treatment with silver nitrate (Dimkpa et al., 2013).

In conclusion, only a few studies give insight in the actual toxicity of the nanoforms of silver. Of those, in particular the studies in aquatic invertebrates and terrestrial plants indicate that the toxic effects observed after exposure to nanoforms of silver are unlikely to be caused by the release of silver ions alone, and suggest higher toxicity of the nanoparticles. It should also be considered that the characteristics of the nanoform(s) of Silver could give a different exposure for the invertebrates tested in comparison with exposure to silver ions resulting in differences in toxicokinetics that can be anticipated for nanoparticles. Based on the available data the read-across of toxicity of nanoforms of silver from the toxicity ionic silver is considered not sufficiently substantiated.

Different physico-chemical characteristics are expected to play a decisive role in toxicology of nanomaterials or specifically (nanoforms of) silver. In particular particle size and shape, particle size distribution, aggregation and agglomeration state, specific surface area, surface treating agent(s), water solubility and dissolution rate, density, and surface charge are considered key parameters (SCENIHR, 2010; ECHA, 2012). As such information on these parameters is essential to characterise the test material.

- Granulometry: Despite the importance of granulometry (*i.e.* particle size and particle size distribution) in identifying nanomaterials, the current registration dossier includes only limited information on this parameter and it is not clear whether that information represents all forms that are registered. The primary particle size could be of influence on characteristics of the agglomerate/aggregate states of nanoforms of silver. Information on the aggregate/agglomerate state and the size distribution of these particles are of influence on the dissolution rate. Furthermore, smaller particles appear to be more toxic than larger particles to such an extent that the toxicity of the nanoforms of silver exceeds that of the ionic silver (*e.g.* Notter et al., 2014). Therefore, information on the primary particle size and the particle size distribution of the registered substance is required to decide on the worst-case nanoform(s) of silver.
- Specific surface area: The specific surface area has impact on the solubility and reactivity of materials. Especially nanomaterials, including nanostructured materials, have an increased surface-to-volume ratio. This relatively high surface area may also lead to a higher toxicity. As an example, Georgantzopoulou et al. (2012) showed that a silver particle of 23 nm with a surface area of 8.2 m^2/g, was much more toxic to bacteria, algae and daphnids than two similar sized nanoforms of silver with a smaller surface area (20 and 27 nm, both with a surface area of ca 2.2. m^2/g). Acknowledging the increased surface-to-volume ratio of nanomaterials, the European Commission included in its recommendation that a material should be considered as falling under the definition when it has a large specific surface area by volume (*i.e.* $> 60\ m^2/cm^3$; EU, 2011).

For powders, the surface area can be determined by Brünauer, Emmett and Teller (BET) measurements on gas adsorption; for suspensions the surface area can be estimated based on particle size distribution and density.

- Dissolution rate: Dissolution of nanoforms of silver affects the bioavailable concentration in the environment. Where nanoparticles are taken up by an organism this may lead to elevated internal (ionic) concentrations that may lead to a higher toxicity that cannot be predicted from toxicity tests with ionic silver. Dissolution of (nanoforms of) silver is an oxidative process involving protons (H^+) and dissolved oxygen. This process is influenced by the type of medium. Thio et al. (2012) for instance, found differences in dissolution between freshwater and seawater, and Angel et al. (2013) showed the influence of humic acid and chloride in synthetic media. Furthermore, the role of H^+ in the dissolution process clearly indicates an influence of pH as well. To enable a comparison between different nanoforms the dissolution rate should therefore be determined in the same medium for all different forms, preferably the same as used for the tests on ecotoxicity and on the fate in soil. The different test protocols for the different tests on ecotoxicity and on the fate in soil, however, prescribe different test media. Apart from expressing the dissolution rate in dissolved concentration by loading, additional information may be provided by expressing dissolution rate per surface area. Information on the method used to calibrate release per surface area, as well as the underlying assumptions, however, will in this case be essential.
- Density: the density of nanoparticles of silver may affect the precipitation. The density is also required to determine the specific surface area for suspensions. Density may be influenced by surface modifications, in particular for smaller nanoparticles where the volume specific surface area (and relative contribution of surface modifications to overall density) will be high.
- Point of zero charge: the surface charge is a determining property for the tendency of a material to agglomerate or otherwise interact with its surroundings, and is therefore relevant both for the fate and hazard of nanoforms of silver. El Badawy et al. (2011) showed that surface charge strongly influenced the toxicity of nanoforms of silver for bacteria where positively charged nanoforms of silver were most toxic and toxicity decreased with increasing negative charge. Surface charge is not an intrinsic property as it depends on the testing conditions. Therefore, in order to compare different nanoforms they have to be tested at the same conditions. In order to compare the point of zero charge of different nanoforms of silver, this parameter shall be determined under the same standardised conditions, similar to those described above for dissolution rate.

The description of the tested forms should include information on the core of the particle (including impurities), as well as information on the surface characteristics (including surface modification).

2.2.3 What is the request

To ensure that all nanoforms of silver are safely produced and used, information is requested on the chronic ecotoxicity to algae, daphnia, and soil microorganisms for the smallest nanoform of silver with the highest specific surface area that is covered by the REACH registration dossier submitted for silver. In any case, the Registrant(s) need to justify and document in the dossier the selection of the form for testing.

In the tests requested sufficient monitoring should be performed to enable a distinction between the toxic contributions of the nanoforms of silver and ionic silver.

For interpretation of the results (and extrapolation of the data to other nanoforms), the nanoforms of silver should be sufficiently characterised. As environmental factors strongly influence physico-chemical parameters of nanoforms of silver and consequently its environmental behaviour, differences may occur in dissolution rate and/or reactivity of nanoforms of silver in the different environmental compartments.

Furthermore, to some extent the validity of using effects of silver ions as a worst-case may depend on the test species as well. As a consequence comparisons between effects of representative nanoforms of silver and ionic silver may potentially be necessary for all ecotoxicological endpoints that are required under REACH.

For the smallest nanoform of silver with the highest specific surface area that is covered by the REACH registration dossier submitted for silver both aquatic toxicity testing and soil toxicity testing is required in order to assess the toxicity profile of nanoforms of silver and to verify that in both compartments, the silver ion is more toxic than nanoforms of silver and safe use of all registered nanoforms of silver can be ensured.

2.2.4 Consideration of Registrant(s)' comments

In their comments, the Registrant(s) stated that *"the overall scope of the draft decision is extensive and aims to resolve complex research issues that academia have strived to resolve for more than 10 years".* While the overall scope of the draft decision may be interpreted as extensive by the Registrant(s), ECHA considers this scope justified and in line with the REACH objectives.

To ensure a high level of protection of human health and the environment, the REACH Regulation imposes the determination of hazards, exposures and risk irrespective of the form of the substances concerned. This includes more specifically nanoforms of substances, which may trigger specific hazardous properties and risks, as already highlighted by various institutions, including the European Parliament. The Registrant(s) argue that (toxicity of) ionic silver can be seen as a worst-case scenario to estimate the potential hazards of nanoforms of silver. ECHA has concerns about the justification of this statement as toxicity of some nanoforms of silver exceeds that of ionic silver. In addition, there are several shortcomings in the characterisation of the different forms of silver in the dossier that are registered. Therefore, ECHA is unable to verify the validity of the argumentation of the Registrant(s) based on the (environmental) toxicity tests provided. The requests in the decision comprise a minimal set of information to substantiate and justify the argumentation of the Registrant(s) and remove the concerns of ECHA.

The outcome of the toxicity data from Request 1 confirming a more toxic nanoform of silver may result in lower PNEC values than that of ionic silver, which would result in more stringent operational and/or regulatory RMMs might be needed, such as improved waste treatment and more stringent classification, even at the tonnage level of nanomaterial currently produced as indicated by the Registrant(s).

The Registrant(s) asked for further clarification on the "methodological limitations" of the references studies in the read-across justification document (Annex 7 to the J-CSR). This has been added in the establishment of the concern for the ecotoxicity.

To further support their read-across arguments, the Registrant(s) have recently added the meta-analysis of Notter et al. (2014) to the dossier(s). ECHA acknowledges the value of this paper, but cannot agree with the Registrant(s) that with the analysis of Notter et al. (2014) the use of ionic silver data as a worst-case approach is now sufficiently justified. This is further substantiated in the text above.

The Registrant(s) objected to the request for test media without $Na_2 \cdot EDTA \cdot 2(H_2O)$ in the toxicity studies on algae and *Daphnia magna*, which the evaluating MSCA deemed necessary to avoid complexation of EDTA with silver. The Registrant(s) argued that a minimal amount of $Na_2 \cdot EDTA \cdot 2(H_2O)$ is needed in order to obtain sufficient availability of iron to allow algal growth. ECHA acknowledges the arguments of the Registrant(s) and adapted the decision by allowing a minimised amount of $Na_2 \cdot EDTA \cdot 2(H_2O)$. As this appears common practice in testing metal toxicity (*e.g.* this approach is indicated in OECD Guideline 201; OECD, 2011), ECHA sees no need for the preliminary work that the Registrant(s) requested to test this approach. Similarly, ECHA sees no need in extensive preliminary work to test an approach where chloride salts are replaced by nitrate salts in the media, as this appears common practice in testing silver toxicity.

2.2.5 Considerations of proposals for amendment and Registrant(s)' comments on them

ECHA agrees with the evaluating MSCA that the Registrant(s)' hypothesis that the driver for silver toxicity for the compositions registered is the silver ion (i.e. the transformation product of elemental silver) is not sufficiently substantiated, but proposed to specify that the forms to be tested should represent the worst-case for physico-chemical parameters, and that the Registrant(s) will have to justify and document in the dossier the selection of the nanoform of sSilver for testing. This will have consequences for the forms to be tested for Request 2 as well. The Registrant(s) indicated that only two nanoforms of silver are registered and can be tested.

The evaluating MSCA agrees with ECHA that the forms to be tested should represent the worst case and adapted the draft decision accordingly. The evaluating MSCA agrees with the Registrant(s) that the number of forms to be tested should be limited by the number of forms that are registered.

2.2.6 Conclusion

Pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision:

Information on the ecotoxicity on the smallest nanoform of silver with the highest specific surface area that is covered by the REACH registration dossier(s) submitted for silver, i.e. information on:

- Toxicity to algae, according to OECD Test Guideline No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. The test medium to be used is AAP medium as described in OECD Test Guideline No. 201, with the following adaptations: to minimise complexation of silver ions, the amount of $Na_2EDTA \cdot 2(H_2O)$ shall be minimised (by balancing the molar concentrations of iron and $Na_2EDTA \cdot 2(H_2O)$) and chloride salts shall be replaced by nitrate salts. The pH shall be the pH at which the medium equilibrates with air (generally around 7.6) and temperature 20 °C.
- Long-term toxicity to aquatic invertebrates, according to OECD Test Guideline No. 211: *Daphnia magna* Reproduction Test. The test medium to be used is Elendt M7 medium as described in OECD Test Guideline No. 211, with the following adaptations: to minimise complexation of silver ions, the amount of $Na_2EDTA \cdot 2(H_2O)$ shall be minimised (by balancing the molar concentrations of iron and $Na_2EDTA \cdot 2(H_2O)$) and chloride salts shall be replaced by nitrate salts. The pH shall be the pH at which the medium equilibrates with air (generally around 7.6) and temperature 20 °C.
- Toxicity to soil microorganisms, according to OECD Test Guideline No. 216: Soil Microorganisms, Nitrogen Transformation Test in three different soils. The three soils to be used are the same as the ones used for Request 2, *i.e.* they need to cover as wide a range as possible of soil properties expected to affect the fate of nanoforms of silver in soil; especially pH, clay content and organic matter content are expected to be of major relevance in this respect. The selection of soils shall be justified and include LUFA 2.2 soil as this nowadays is a commonly used reference soil. Soils shall be sufficiently characterised: at least pH, clay content, organic matter content, and moisture content shall be reported.

While conducting these studies, the OECD Guidance on Sample Preparation and Dosimetry (ENV/JM/MONO(2012)40, in particular sections III, IV, and V-A through V-C) should be consulted. The composition of the test media shall be fully reported (including at least ionic strength, calcium concentration and hardness, pH, alkalinity, dissolved organic matter, and presence of dispersing agents). In addition, the following important conditions shall be taken into account:

Throughout the study, the ratio between particulate and ionic silver shall be monitored in samples from the test vessels, using analytical techniques that enable distinction between the concentrations of nanoform(s) of silver and ionic silver.

The study setup shall include a control with exposure to silver nitrate to enable distinction between the toxicity of nanoform(s) of silver and ionic silver.

The same analytical techniques shall be applied in the ionic silver control as are applied in the test with the nanoform of silver to enable detection of the potential formation of nanoparticles in a solution with ionic silver only.

The nanoform of silver that is tested shall be sufficiently characterised, by providing information on the following physico-chemical properties, using the indicated test method(s):

- The granulometry, which shall include primary particle size and shape, aggregate/agglomerate size and primary particle size distribution (number-based). Method: Method for powders is Transmission Electron Microscopy (TEM) combined with Energy Dispersive X-ray (EDX), and Laser Diffraction; method for suspensions is Transmission Electron Microscopy (TEM) combined with Dynamic Light Scattering;
- The specific surface area (by volume). Method: for powders BET (ISO 9277:2010); for suspensions calculation based on theoretical model;
- The surface treating agent(s), including chemical identity (IUPAC name and numerical identifiers (CAS and EC)), type of reaction with the silver surface, relative coverage of the silver surface (as this information is part of the substance identity, the information shall be added in IUCLID sections 1.2 and 1.4);
- The dissolution rate for the specific test media used in the tests. Method: OECD Guidance Document 29, taking note of the OECD Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (ENV/JM/MONO(2012)40, in particular sections III, IV, and V-A through V-C). The composition of the test medium shall be fully reported (including at least ionic strength, calcium concentration and hardness, pH, alkalinity, dissolved organic matter, and presence of dispersing agents);
- The density. Method: OECD 109 Density of Liquids and Solids;
- The point of zero charge. Method: micro-electrophoresis or electrophoretic light scattering to be performed at fixed low salt concentration and at fixed particle concentration, as described above for dissolution rate.

In case information on one (or more) of the requested physico-chemical properties is already available (taking the specific test conditions indicated above into account), this information may be provided. Only the Registrant(s) of the substance know the details of each of its forms necessary for their characterisation. Based on this knowledge, they may consider that a test method requested by ECHA is not suitable in order to characterise each form of the substance. Nevertheless, it is the exclusive responsibility of the Registrant(s) 1) to ensure that ECHA is in a position to identify precisely each form of the substance registered and 2) to verify the reasons for the use of another test method instead of a method explicitly required in the present decision.

The evaluating MSCA will examine the information provided by the Registrant(s). In case the concern is still not clarified or new information raises further concerns, additional testing may be requested in a new decision based on the information that is provided in response to the current decision.

2.2.7 Considerations of proposals for amendment and Registrant(s)' comments on them

A proposal of amendment suggested to better justify or, preferably, remove request (1) from the decision. On one hand it was argued that it was not clear how information will be used, while on the other hand it was not clarified why existing information on some of the parameters was insufficient. In case the request remains it was proposed to give the Registrant(s) the possibility to group the information. The Registrant(s) agreed with the proposal, although they indicated that grouping may not be necessary, because the Registrant(s) indicated that only two nanoforms of silver are registered.

ECHA agrees that the justification for physico-chemical properties could be improved, and chose to request the physico-chemical properties for the nanoform tested in the ecotoxicity tests. The decision was amended with an improved justification for the individual parameters.

2.3 FATE

2.3.1 Establishing the concern

In case the information in Request 1 indicates a higher toxicity in one of the toxicity tests performed for the nanoform than for the ionic silver, potential differences in fate of nanoforms of silver becomes relevant.

Fate of nanomaterials in the environment determines in which environmental compartment (soil, air, water, sediment) they (mainly) accumulate, and thus which biota are at risk. For environmental exposure the Registrant(s) state in the J-CSR (section 9.0.2.1, annex 1) that: *"As per the properties of other zerovalent forms of silver (e.g. massive and powder forms), the properties of nanosilver relevant to the development of environmental exposure scenarios (i.e. environmental fate characteristics and ecotoxicity) are based on "read-across" from the properties of ionic silver."*

In the read-across justification document (Annex 7 to the J-CSR), the Registrant(s) conclude that "*the behaviour of nanosilver in the environment appears to be largely controlled by physical processes, particularly the tendency for both homo- and hetero-aggregation of nanosilver particles which may cause them to settle out of suspension, and their tendency to be retained in soils due to either physical straining or sorption processes*". They further conclude that "*the retention of nanosilver in soils is broadly comparable to ionic silver (median* K_D *4023 L/kg). However, this may not result from the same chemical process*".

This statement is based on a limited number of studies and does not allow a conclusion on the fate in the different environmental compartments of all different nanoforms of silver, in particular of the most toxic forms.

In the aquatic environment, information on the fate of nanoforms of silver appears mainly related to dissolution rate (which is part of Request 1). Furthermore, some additional information is available from several studies (e.g. Johnson et al., 2011; Angel et al., 2013), indicating the influence of stabilising environmental factors, *e.g.* presence of natural organic matter.

In contrast, for the terrestrial environment information on the fate of nanoforms of silver is grossly lacking. The available data show that environmental fate of nanoforms of silver is different from that of ionic silver (e.g. Cornelis et al., 2010; Navarro et al., 2014). Furthermore, as the behaviour of metals depends on the composition of the environmental compartments, it is not valid to use a median K_D value to assess the fate of nanoforms of silver in different soils types in Europe. The fate assessment is further hampered by the lack of knowledge on the processes that are actually occurring after introduction of nanoforms of silver in soil, including speciation of nanoforms of silver in the soil solid phase and the pore water.

The available information indicates that high clay content and high pH enhance the retention of nanoforms of silver. A recent paper by Navarro et al. (2014) investigated the retention and release of nanoforms of silver in spiked soils, but it provides insufficient quantitative information on the fate of nanoparticles of silver following their introduction in soil. In this study two test soils were used that are in the low pH range (which stimulates the release of ions), and thus may not be representative for European soil types in general.

Overall, read-across for fate characteristics is therefore not adequately substantiated in the dossier and therefore not justified.

2.3.2 Justification why new information is needed

In the read-across justification document (Annex 7 to the J-CSR), the Registrant(s) conclude that "*the behaviour of nanosilver in the environment appears to be largely controlled by physical processes, particularly the tendency for both homo- and hetero-aggregation of nanosilver particles which may cause them to settle out of suspension, and their tendency to be retained in soils due to either physical straining or sorption processes*". They further conclude that "*the retention of nanosilver in soils is broadly comparable to ionic silver (median K_D 4023 L/kg). However, this may not result from the same chemical process*".

The general conclusion of the Registrant(s) that settling out of suspension, and retention in soils are important in the behaviour of nanoforms of silver in soil is supported. Nevertheless, as the retention of nanoforms of silver and hence partitioning of nanoforms of silver in soils is due to different processes than those for ionic silver, the available information is too limited to assess the behaviour of nanoforms of silver in different soil types. Additional studies are therefore needed to allow for quantifying the fate of nanoforms of silver in soil.

There is a need for studies on the overall stability and partitioning of nanoforms of silver between the soil solid phase and the pore water as this determines the exposure (route) for soil organisms and consequently the (potential) risk. These studies should especially take account of dissolution of the nanoparticles of silver, as this is influenced by the physico-chemical properties of the soil and the pore water (*e.g.* clay content, pH, organic matter content) in addition to the physico-chemical properties of the nanoparticles of silver themselves (*e.g.* surface modification may affect the release of ions, whereas surface charge influences interaction with soil particles).

For instance, positively charged particles are expected to have a stronger interaction with natural organic matter in soil, which generally show a negative surface charge, and less with clay particles that generally show a positive surface charge (*e.g.* Cornelis et al., 2012). Clearly, time plays an important role as well, as for instance shown by Waalewijn-Kool (2013). This author found a dynamic pattern of decreased, increased and then again decreased pore water concentrations of metal ions following incubation of nanoparticles. A study on fate of nanoforms of silver should therefore be long enough to capture such dynamics. In work by [redacted] ([redacted]., 2010) that is referenced in the J-CSR, insight was gained into the behaviour and fate of ionic silver. Based on this work it would seem necessary to age the soils with exposure to nanoforms of silver for at least 12 months, as was done for ionic silver. This is also in line with the work by Waalewijn-Kool (2013).

As indicated above, Navarro et al. (2014) investigated the retention and release of nanoforms of silver in spiked soils, but their test soils (in the low pH range, which stimulates the release of ions) may not be representative for European soil types in general. In addition, their observation that less than 25% of the silver released in the thiosulphate extracts was in the < 1 kDa size fraction does not automatically imply that less than 25% of the released silver was ionic silver. Silver ions and silver nanoparticles may also have been present in the larger size fractions prior to thiosulphate extraction. The results indicate that during 'chemical' extraction (using *e.g.* thiosulphate) conversion/dissolution of particles takes place, while during 'non-chemical' extraction (using *e.g.* KNO_3) silver is released in much lower quantities but in the nanoform of silver. The results, however, do not allow quantitative estimates of the proportion of silver that was released as either ionic silver or as the nanoform of silver, nor was aging taken into account. Thus, quantitative information on the distribution and speciation of nanoforms of silver in (bioavailable) soil fractions is still needed to determine the dominant exposure route for soil organisms. The Registrant(s) further point out that "*this study shows the importance of colloidal silver associations and highlights that while these nanosilver particles can be present in low concentrations in solutions they are not the original manufactured nanosilver particles.*" While this may be true, the nanoparticles of silver released as a result of the addition of manufactured nanoparticles of silver are still the responsibility of the Registrant(s).

2.3.3 What is the request

Quantitative information on the fate of nanoparticles of silver following their introduction in soil for the most toxic nanoform of silver that is registered, and for three different representative soils.

The nanoform of silver tested shall be the one tested in Request 1. Small particle sizes (*e.g.* Notter et al., 2014) and/or large (volume specific) surface areas (*e.g.* Georgantzopoulou et al., 2012) are expected to result in higher toxicity. In addition, a comparison with ionic silver (*e.g.* silver nitrate, which is commonly used in ecotoxicity studies with ionic silver) should be made in the same test set-up.

The three soils to be selected need to cover as wide a range as possible of the soil properties expected to affect the fate of nanoforms of silver in soil. Especially pH (generally ranging from 6 to 8), clay content (generally ranging from 5 to 20 %) and organic matter content (generally ranging from 1 to 10 %) are expected to be of major relevance in this respect. The choice for the soils should be scientifically justified.

No specific guidance is available on pore water collection. A combination of centrifugation and filtration should be used for obtaining pore water, assuring that modification of the particles is minimised during any of the steps to be taken during processing of soil and pore water samples. A proper mass balance needs to be established.

Fate assessment should focus on assessing the partitioning between the solid phase and the pore water, and on assessing particle transformation, including dissolution and formation of silver complexes, in particular silver sulphide particles. As indicated in Request 1, a link should be established between media used to determine dissolution rate in Request 1 and the test media used here for Request 2. Formation of silver sulphide is of interest in this respect, as this is an insoluble silver species that is generally less toxic than either nanoforms of silver or ionic silver. Clearly, time is an important factor to take into account as well.

2.3.4 Consideration of Registrant(s)' comments

The Registrant(s) have proposed to include an ageing process of 12 months in the environmental fate studies to increase the environmental relevance of the request. ECHA agrees with this proposal and adapted the request accordingly, including an extension of the deadline for submitting the required information to 30 months.

The original deadline to execute Request 1 (Eco-toxicity) and Request 3 (Uses) was set to 12 months, based on the length of the toxicity study of 9 months and the additional 3 months which is granted to allow Registrant(s) to decide who will perform the test and to share the test results. In the adapted decision Request 2 (Fate) is now only required in case the information in Request 1 indicates a higher toxicity for the nanoform in one of the toxicity tests performed in comparison with ionic silver. In case the fate study is performed the deadline is set to 30 months, which is considered to be sufficient for provision of the required information.

In October 2015 the Registrant(s) added a paper by Navarro et al. (2014) that furthers the discussions in relation to the fate of nanoforms of silver in soils. ECHA acknowledges the added value of this paper, but is of the opinion that the paper does not remove the concern, because the tested soils in this paper do not fully represent European soil types in general. Consequently, the request for quantitative information on the fate of nanoparticles of silver following their introduction in a range of representative soils is not changed.

The Registrant(s) commented that *"there is significant overlap between the information requirements on the fate of nanosilver in soil detailed in the draft decision ("Request 2") and Deliverables in the recently funded EU Nanomaterial FAte and Speciation in the Environment (Contract Agreement:[redacted])".* ECHA is aware of this project that aims to deliver an integrated Exposure Assessment Framework of models and characterisation protocols that will allow all stakeholders to assess the full diversity of industrial nano-enabled products to a standard that is acceptable in regulatory registrations and will underpin public and consumer confidence. However, the aims of this 48-month continuing project go far beyond Request 2 that aims to get insight in the fate of nanoforms of silver in soil and will also provide information about the behaviour of nanoforms of silver in a sewage treatment plant.

Provided that a sufficiently wide range of soils is selected, ECHA is quite convinced that the Registrant(s) can provide sufficient information to substantiate (or falsify) their hypothesis that ionic silver can be seen as a worst-case approach in the Chemical Safety Assessment of nanoforms of silver. Nevertheless, since this safety assessment depends on the outcome of the ecotoxicity tests as well, the study on the fate of nanoforms of silver in soil has been made conditional.

2.3.5 Considerations of proposals for amendment and Registrant(s)' comments on them

An amendment was proposed regarding the forms to be tested in Request 1, which has consequences for forms to be tested in Request 2 as well.

ECHA agrees on the forms to be tested in Request 1 (see above), and amended Requests 1 and 2 accordingly.

2.3.6 Conclusion

Pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision:

In case the information in Request 1 indicates a higher toxicity in one of the toxicity tests performed for the nanoform than for the ionic silver, information on request 2 shall be provided.

Quantitative information shall be provided on the fate of nanoparticles of silver in soil pore water and the soil solid fraction, following their introduction in three different soil types. The nanoforms of silver and its transformation products shall be determined in pore water and in clay, organic matter and remaining inorganic solid fractions. A proper mass balance needs to be established and documented. Total test duration is 12 months and test temperature is 20 C.

In addition, silver nitrate shall be tested to enable a comparison with ionic silver under the same test conditions (as specified below).

The three soils to be selected need to cover – as wide as possible – a range of soil properties expected to affect the fate of nanoforms of silver in soil; especially pH, clay content and organic matter content are expected to be of major relevance in this respect. The selection of soils shall be scientifically justified and shall include LUFA 2.2 soil as this nowadays is a commonly used reference soil. Soils shall be sufficiently characterised: at least pH, clay content, organic matter content, and moisture content shall be reported.

Test temperature shall be 20°C. Test duration after introduction of (nanoforms of) silver in soil shall at least be 12 months, with a minimum of 3 time points for sampling, including start and finish of the test duration.

To minimize loss of particles during centrifugation, collection of pore water shall be done by centrifugation of a saturated and equilibrated fixed amount of soil at relatively low centrifugal forces (preferably < 2000 g) over a filter of 0.45 µm, followed by filtration over a 0.2 µm filter. To determine dissolved silver species in the pore water, ultracentrifugation or ultrafiltration (1 kDa filter) is required. A proper mass balance needs to be established.

Quantitative measurements shall focus on assessing the partitioning between the solid phase and the pore water, and on assessing particle transformation, including dissolution and formation of silver complexes, in particular silver sulphide particles. It shall be assured that no significant modification of the particles takes place during any of the steps to be taken during processing of soil and pore water samples.

Equally suitable methods may be used to provide the requested information, provided that their use is scientifically justified.

2.4 USE AND EXPOSURE

2.4.1 Establishing the concern

For environmental exposure the Registrant(s) state in the J-CSR (section 9.0.2.1, annex 1) that: *"As per the properties of other zerovalent forms of silver (e.g. massive and powder forms), the properties of nanosilver relevant to the development of environmental exposure scenarios (i.e. environmental fate characteristics and ecotoxicity) are based on "read-across" from the properties of ionic silver."*

As argued above for Request 2, the environmental fate of nanoforms of silver and ionic silver may differ. Both fate and ion release strongly depend on physico-chemical properties (including surface modifications) of the different (nano)forms of silver, as well as on environmental factors (*e.g.* pH, soil properties). Therefore, environmental fate may also depend on the specific route of entry into the environment, and information on which form(s) enter the environment via which route is essential.

Furthermore, toxicity of (nanoforms of) silver is also influenced by environmental factors (*e.g.* pH, soil properties), further emphasising the need for information on the environmental compartment that is most impacted by release of (nanoforms of) silver. In addition, even within one compartment toxicity of nanoforms of silver differs from toxicity of ionic silver and also differences between different nanoforms of silver are observed. Differences in toxicity are further discussed under Requests 1 and 2 above, but they further support the need for information on which form(s) enter the environment via which route.

As the Registrant(s) do not specifically distinguish uses of nanoforms of silver from uses of other forms of silver, it is unclear what the main exposure route of nanoforms of silver to the environment is. In addition, in their comments the Registrant(s) indicate they "*believe that a number of companies purchase bulk silver and convert it into nanosilver*". This suggests that companies that produce bulk silver may not be aware of this use, which raises questions on the extent to which these down-stream companies are covered by the current registration.

2.4.2 Justification why new information is needed

In May 2015, in addition to their company-specific composition, [redacted] had included in IUCLID section 1.2 the generic compositions of all forms covered by the joint registration leading to a lack of clarity about the number of individual Registrant(s) registering the nanoform of silver. The October 2015 registration update(s) clarified this issue to some extent and [redacted] Registrant(s) indicated a registration of the nanoform of Silver by indicating either group 5 or 6 in section 1.2.

In the registration dossiers a list of uses of silver by industrial workers, professional workers and consumers is included. In general the uses are applicable to silver as one substance. Where use of nanoforms of silver is mentioned, that specific use is indicated for other forms of silver as well. No information is provided on the uses of each individual nanoform of silver.

This leads to several uncertainties in the uses of nanoforms of silver. As mentioned above the Registrant(s) indicate they "*believe that a number of companies purchase bulk silver and convert it into nanosilver*", which raises questions on the extent to which these downstream companies are covered by the current registration(s). The Registrant(s) further comment that "[redacted] *believes that the majority of nanosilver being placed on the market are actually within the scope of the EU Biocidal Product Regulation rather than REACH*", while the dossier(s) do not provide sufficient detail to verify this assumption.

Further details on uses of nanoforms of silver are therefore needed to identify the (dominant) route of environmental exposure and clarify the scope of the current registration under the provisions of the REACH Regulation.

2.4.3 What is the request

It is requested to provide more information on the uses of each individual (nano)form of silver that allows a clear distinction between uses that fall within the scope of REACH and other uses, *e.g.* those that fall within the scope of the EU Biocidal Product Regulation. For uses that fall within the scope of REACH, a selection of relevant exposure scenarios and relevant (surface modified) nanoforms of silver can be made. To ensure safe use of nanoforms of silver, it may be necessary to provide specific exposure estimations in a follow up of the present decision.

2.4.4 Consideration of Registrant(s)' comments

The Registrant(s) indicate that "[redacted] *believes that the majority of nanoforms of silver being placed on the market are actually within the scope of the EU Biocidal Product Regulation rather than REACH*". The information provided by the Registrant(s), however, is not sufficient to verify this assumption. In fact, no distinction is made between uses of nanoforms of silver and other forms of silver, nor is the nanoform of silver used in biocidal products only. Furthermore, the production of nanoforms of silver will still be within the scope of REACH. The scope of the REACH Registration for nanoforms of silver is thus broader than biocidal products alone. Therefore, there is no need to change the original request.

The Registrant(s) further indicate that "*We believe that a number of companies purchase bulk silver and convert it into nanosilver. These companies would be considered downstream users under REACH and would not be required to register silver even if their output is more than 1 tonne per annum. We consider it unfair that these companies will not contribute to the testing required in the draft decision but will benefit from the efforts of a small number of* [redacted] *companies.*" A check on 9 February 2016 indicates no downstream user reports being submitted for Silver. Therefore, according to REACH the downstream uses should be covered in the Registration Dossiers or the Registrant(s) should explicitly not cover these uses in their registration dossier and/or advise against this specific use.

2.4.5 Considerations of proposals for amendment and Registrant(s)' comments on them

It was proposed to clarify the aspect on the Registrant(s) indication of the biocidal use of nanoforms of silver being outside the scope of REACH. The Registrant(s) further substantiate this comment by referring to reports by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).

In response to the Registrant(s)' comments that downstream users may convert bulk silver into nanoforms of silver, ECHA checked but found no indications of downstream user reports being submitted for silver, indicating that such downstream uses should be covered in the registration dossier(s).

Further clarification on the biocidal aspect and downstream uses has been added in the dossier(s). The references to reports by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) are acknowledged. ECHA, however, cannot find a direct link between the very general statements in these reports that nanoforms of silver are mainly/widely used for their antibacterial/antifungal properties, and the nanoforms in the REACH Registration for silver. Based on these reports, ECHA saw no need to amend the decision.

2.4.6 Conclusion

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit for the registered substance information on the uses of each individual nanoform of slver that is registered; as specified above.

3 AVOIDANCE OF UNNECESSARY TESTING BY DATA- AND COST-SHARING

In relation to the experimental studies the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) whose registration covers nanoforms of Silver and that have to provide the information requested in this decision are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at: https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at http://echa.europa.eu/regulations/reach/registration/data-sharing.

If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the studies on behalf of all of them.

4 INFORMATION ON RIGHT TO APPEAL

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[13] by Leena Ylä-Mononen, Director of Evaluation

Annex: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

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

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