Substance Evaluation for Silver
Precious Metals & Rhenium Consortium (PMC) comments to draft decision

The European Precious Metals Federation (www.epmf.be) represents the interests of its member companies and national associations vis-à-vis the European authorities on several topics of relevance for the precious metals sector such as the safety of chemicals and waste management; access to raw materials, recycling and sustainability, including responsible sourcing; energy and climate change; trade, customs, tax and competitiveness; etc. EPMF is hosting the Precious Metals & Rhenium Consortium (PMC - http://www.epmf.be/index.php/menuepmf/reachconsortium) aiming to comply with the REACH and CLP regulations. The main objective of the PMC is to steer collective response of entities mining, refining, recycling, manufacturing, importing, trading, banking Precious Metals and Rhenium to the Registration, Classification, Evaluation, Restriction and Authorisation requirements laid down in the REACH and CLP regulations.
Heraeus Precious Metals GmbH & Co. KG will act as the silver Registrants Contact Point and represents the PMC members, and more specifically all the addressees of the draft decision.

Executive summary

The addressees of the draft decision would like to summarize here below the comments and concerns raised in this document. The comments are organized in 4 main sections: general comments, comments on the requirements on the physicochemical properties, comments on the requirements on fate in soil and comments on the requirements on ecotoxicity testing.

First of all, the general comments can be summarized as follows:
- We are concerned by the proportionality and the hypothesis-driven research nature of certain requests in the draft decision (Comments 1 and 2).
- We would like to request that “grade” is replaced by “form”. Additionally, the term “form” should be clearly defined. (Comment 3)
- We would like to request an extension of the indicated timeframe to comply with the decision (Comment 4).
- We have been unable to identify all nanosilver Registrants despite extensive effort and we would like ECHA to consider mediating in the identification of these nanosilver Registrants. Additionally, we would like the final decision to more accurately reflect the number of nanosilver Registrants (Comment 5).
- We would like to request the eMSCA to re-assess the weight-of-evidence issue with regard to the read-across of the environmental effects of nanosilver from those of ionic silver (Comments 6 and 17).

Secondly, our comments on the requirements on physicochemical properties are related to the methodology proposed in the draft decision, while we agree with the overall requests on physicochemical properties and related nanoform characterisation (Comments 7-11).

Thirdly, regarding the requirements on fate in soil:
- We suggest that, in addition to the LUFA 2.2 soil, two soils are selected from those that were used for the previous soil testing in the CSR (Comment 12). This comment is also applicable to the soil micro-organism testing (Comment 18).
- It would be very helpful to obtain further clarifications or advice from the eMSCA during the course of the test protocol development, to ensure that the selected experimental design adequately addresses the concerns raised in the draft decision (Comment 13).
- There are some recent quantitative data published on the remobilisation of silver from silver nanoparticles and also silver sulphide nanoparticles that further the discussions in relation to the fate of nanosilver in soils and which are not considered in the draft decision (Comment 14).
- We suggest an aging process of 12 months for the soils (Comment 15).

Fourthly, to increase the transparency in the draft decision’s critique of some aspects of the ecotoxicity profile of silver, and hence our ability to respond to any perceived methodological gaps, access to the draft Substance Evaluation Report is requested (Comment 16). Content wise, the main comments / suggestions on the ecotoxicity testing are related to the media and soils to be used (Comment 18).

General comments

1. We are concerned by the proportionality of certain requests in the draft decision for the following reasons:

    a. On page 10 of the draft decision, last paragraph, it is stated that "it is unclear how representative the products in Table 1 are for all nanosilver products put on the market. Consequently, based on the information provided, safe use cannot be verified for all nanosilver forms that are manufactured, imported and/or placed on the market by the Registrant(s)." PMC believes that the majority of nanosilver forms being placed on the market are actually within the scope of the EU Biocidal Product Regulation rather than REACH. Furthermore, on page 11-12 of the draft decision it is stated that "In order to either verify or reject the basic hypothesis of silver ions being fully responsible for adverse effects of exposure of biota to silver nanoparticles, data are needed on particles that are expected to represent worst case conditions with regard to the inherent toxicity of the particles, and particles that are expected not to release silver ions." A central contradiction exists between this stated objective and reality due to the restricted number of nanoforms that lie in scope of the silver REACH registration (versus the totality of nanoforms when biocides regime nanosilver is taken into account).

       This will also have an impact on the selection of the nanosilver forms to be tested in "Request 2" (fate in soil) and "Request 3" (ecotoxicity testing). The draft decision requests three representative nanosilver forms to be selected with differing surface characteristics (draft decision page 4, third paragraph). While deviation from the requested forms is allowed, we anticipate further dialogue with the eMSCA will be necessary on the allowable deviation from what they would ideally like to be covered.

    b. According to the information currently available to PMC, less than 0.02% of the total silver volume registered under REACH is nanosilver[1].

    c. 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. In effect, the draft decision requests a small number of Registrants (see also Comment 5) to address these complex issues within a very limited timeframe given the attendant experimental complexities of the program.

2. Some of the information requirements in the draft decision are clearly research focused and there is no certainty that they will generate results that will definitively support the Chemical Safety Assessment. For example, 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: SEP-210187494). For instance, in respect of dissolution and formation of silver complexes (in particular silver sulphide particles; draft decision page 17, second paragraph). The overarching objective of this multi-million euro project – with 34 research partners – is 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. This project will start at the end of the summer of 2015, prior to the Substance Evaluation decision being issued, with its delivery anticipated only in 2019.

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[1] The aggregated tonnage mentioned on the ECHA dissemination website for silver is 100,000 – 1,000,000 tonnes per annum, whereas according to the information available to PMC, less than 20 tonnes per annum of this registered volume is nanosilver.

Furthermore, we believe it would be useful for the final decision to take into account the forthcoming nanosilver data of the Testing Programme of Manufactured Nanomaterials launched by OECD's Working Party on Manufactured Nanomaterials (WPMN).

3. On page 2 of the draft decision, the term "grade" is used several times, whereas throughout the rest of the draft decision, the term "form" is used when referring to nanosilver. For consistency, we would like to request that one term is used. In the metals sector, "grade" usually refers to (im)purity and can e.g. refer to LME standard grades, which can be misleading. In addition, the REACH regulation is substance focused, not product focused as the use of the term "grade" might implicitly suggest. Therefore, we would like to request that "grade" is replaced by "form". Furthermore, we would like to request that the decision includes a definition of the term "form".

4. On page 6 of the draft decision, under "Deadline for submitting the required information" it is stated that "Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by [exact date – 12 months from the date of the decision] an update of the registration(s) containing the information required by this decision, including robust study summaries and, where relevant, an update of the Chemical Safety Report." We recommend an extension of the 12 months to at least 24 months, and preferably 30 months given the hypothesis-driven research nature of the requests. Specific justification for this request is as follows (see also timeline in Annex 1):

    a. Information on the physicochemical properties ("Request 1") is required for each individual form of nanosilver (draft decision page 3, fourth paragraph). One of these physicochemical properties is the dissolution rate, to be determined according to OECD Guidance Document 29, but with one of the ecotoxicity test media to be used in place of the standard medium in the test protocol. As pilot studies will first be required to establish the workability of the suggested ecotoxicity test media (see also Comment 18), this will impact the timing for the physicochemical testing. Furthermore, as the physicochemical parameters ultimately guide selection of further testing for "Request 2" (fate in soil) and "Request 3" (ecotoxicity testing), the physicochemical properties will need to be fully determined before the main testing required in request 2 and 3 can be initiated.

    b. Since the information requirements on the behaviour and fate of nanosilver in soils ("Request 2") are clearly research focused, the organisations able to undertake this work are limited, and the experimental timeframes will be considerable. Significant time will be needed for study preparation and method development and, again, given the basic research nature of this study, i.e. lack of test guidelines or specific guidance, we would request the decision builds in a reasonable time contingency to allow for unexpected results and/or repeat testing. Overall, these constraints represent a considerable project management challenge.

    c. Some suggestions for determining the ratio of dissolved silver and nanosilver in ecotoxicity test media are made in the draft decision (i.e. filtration and use of a chelator) (draft decision page 19). Defining the appropriate separation / ultrafiltration technique will be extremely challenging, not least because of the low concentrations of silver being tested. Such approaches will need to be investigated and validated prior to implementation in the ecotoxicity tests. It is anticipated that this will take a minimum of 4-6 weeks which will need to be added to the time required to fulfil the ecotoxicity information requirements.

    d. We consider the aging of silver in soils an important process if the findings of the information Request 2 in the Draft Decision are to have environmental relevance (see also Comment 15). An aging process of 12 months is proposed as realistic, which needs to be added to the total time to submit the required information.

    e. We suggest that, in addition to the LUFA 2.2 soil, two soils are selected from those that were used for the previous soil testing in the CSR (see also Comment 12). Based on our experience in similar work, the gathering, characterisation and preparation of the soil types takes on its own 2-3 months.

5. On page 9 of the draft decision, at the start of the last paragraph, it is stated that “From the 56 individual Registrants only 10 indicated that they register the nanoform of silver by indicating either group 5 or 6 in section 1.2 of IUCLID.” We have the following comments on this assertion:

    a. In order to adequately address the information requests in the draft decision, and then update the REACH registration(s), whilst ensuring a fair sharing of costs, it is imperative that all co-registrants impacted by the decision and all nanoforms of silver covered by the registration(s) are identified. However, despite extensive effort and communication within the SIEF, PMC has so far only been able to identify two of the 10 individual Registrants (both PMC members) who have registered the nanoform of silver. Given the lack of success in these good-faith efforts, we request that ECHA mediate in the identification of the remaining Registrants.

    b. 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 there output is more that 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 PMC companies.

    c. We believe that several Registrants have been erroneously identified as nanosilver Registrants in the draft decision as they have not only included their company-specific composition in section 1.2 of IUCLID, but also the generic compositions of all forms covered by the joint registration. We intend to discuss the possibility of a registration update for these Registrants with the eMSCA, in order to more accurately reflect the number of nanosilver Registrants in the final decision.

6. The strategy for the read-across of the environmental effects of nanosilver from those of ionic silver that is presented as a supporting document to the silver CSR concludes that, ‘the available reliable data on ecotoxicity of nanosilver outlined in this report has been compared to the available key data for ionic silver [and] the properties of ionic silver are significantly more hazardous than any available data on nanosilver, irrespective of any data available describing morphology, size, size distribution or coating’. This conclusion is based on a weight-of-evidence approach, taking into account all of the available reliable and relevant studies on ionic silver and nanosilver. While it is accepted that there are some individual studies that are equivocal in this regard, or that show that the toxicity of some forms of nanosilver are potentially of equivalent toxicity to that of ionic silver, the overwhelming body of evidence is that the primary mechanism of toxicity of nanosilver is caused by dissolution of ions from the nanosilver particles, and that soluble silver salts possess greater toxicity than nanosilver particles over comparable time periods. Indeed, a recent paper by Notter et al. (2014), which presents a meta-analysis of published EC_{50} values for ionic silver and nanosilver, demonstrates that almost 94% of (acute) toxicity values assessed 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 remains justified and scientifically defensible. We would like to request that the eMSCA re-assess this important weight-of-evidence point. We intend to discuss the possibility of a registration update with the eMSCA to include the recent Notter et al. (2014) study.

Comments on the requirements on physicochemical properties

7. The draft decision states that the determination of the dissolution rate of each individual form of nanosilver should be done according to OECD Guidance Document 29 (draft decision page 3, point ‘d’) but instead of the standard transformation / dissolution medium (ISO 6341), the Elendt M7 medium as described in OECD Test Guideline 211 (*Daphnia magna* Reproduction Test) should be used, with the following adaptations: “to minimise complexation of silver ions (which enhances dissolution rates), $Na_2EDTA \cdot 2(H_2O)$ should not be added and chloride salts should be replaced by nitrate salts (chloride will react with silver to form insoluble silver chloride complexes).” While we agree that

using one of the ecotoxicity test media for the dissolution rate testing is a pragmatic and cost-efficient proposal, we would like to raise the following comments:

a. Before using the suggested medium for the dissolution rate testing, pilot studies will first be required to establish the workability of the suggested ecotoxicity test media (see also Comment 18).

b. The OECD Guidance Document 29 was developed for hazard classification purposes, and therefore any changes in the medium that may affect the exposure of the compound will make the results not directly useable for hazard classification.

8. The draft decision states that the determination of the dissolution rate of each individual form of nanosilver should be done according to OECD Guidance Document 29 (draft decision page 3, point ‘d’) but the pH should be 7.5. The pH for testing should in this case be the pH at which the medium equilibrates with air, which is usually slightly above pH 7.5 and in line with what would happen in an ecotoxicity test.

9. While recognising that according to the OECD Guidance Document 29, transformation / dissolution endpoints should be determined after 24 hours, and thereafter at 7 days and 28 days, we believe that we should first investigate the kinetics of nanosilver dissolution to check if an equilibrium is reached sooner. This may dismiss the need for a 28 days extension (or even a 7 days extension if dissolution is rapid) and it may save time.

10. In connection to the above, it may be worthwhile to reflect here on the philosophy we think applicable to follow within the research program. We note the following key points should be evaluated:

    a. In first instance, any difference in dissolution kinetics and equilibrium between nanosilver and ionic silver;

    b. Secondly, the relative ecotoxicity of nanosilver and ionic silver;

    c. Finally, the establishment of a relationship between ecotoxicity and dissolution kinetics / equilibrium as determined by step ‘a’ and ‘b’ above, in order to definitively determine if there is a discernible nano-specific effect, or not.

    Given the uncertainties at the present time on what represents the most appropriate protocols (e.g. the optimally relevant testing medium etc.), we would respectfully suggest that the draft decision only describes these 3 key objectives rather than attempting at this early stage to provide details on how to reach them. In turn we would offer the opportunity for an ongoing dialogue with the eMSCA as the research program progresses in order to discuss and refine the experimental design.

11. As dissolution kinetics involving particles need to take into account differences in surface area, we propose to express the dissolution rate both in dissolved concentration by loading and in release per surface area. The release per surface area will allow comparison with dissolution rates of other physical forms of silver.

Comments on the requirements on fate in soil

12. On page 4 of the draft decision, fourth paragraph (and further on pages 16 and 17) it is stated that “The three soils to be selected need to cover – as wide as possible – a range of soil properties expected to affect the fate of nanosilver in soil; especially pH, clay content and organic matter content are expected to be of major relevance in this respect.” We suggest that, in addition to the LUFA 2.2 soil, two soils are selected from those that were used for the previous “Evaluation of toxicity and bioavailability of silver in soils” undertaken by CSIRO and given in the CSR. This would mean that full characterisation of the soils has already been undertaken and that practical

experimental knowledge, such as the behaviour of silver salts post spiking and dosing concentrations, is already known saving a great deal of time and allowing linking/comparing the new program outcome with the existing data sets of the silver registration file. For further information on the selection of soils covering the required wide range of physicochemical properties, please see Annex 2.

13. There is a major challenge associated with the determination of silver and characterisation of possible silver particles in soil solutions around the detection of very low levels of silver (draft decision page 4, sixth paragraph). Several methods can be used including isotope dilution techniques. However, as this is not routine testing it would be very helpful to obtain further clarifications or advice from the eMSCA during the course of the test protocol development, to ensure that the selected experimental design is appropriate, i.e. that it adequately addresses the concerns raised in the decision and that the mass balance is deemed “proper” (draft decision page 4, second paragraph).

14. The draft decision outlines the importance of understanding the behaviour and fate of nanosilver particles, beyond dissolution chemistry with speciation and partitioning between solid phases and solution (page 5, first paragraph; page 9, second paragraph; page 10, fourth paragraph; page 16, second paragraph). There are some recent quantitative data published on the remobilisation of silver from silver nanoparticles and also silver sulphide nanoparticles that further the discussions in relation to the fate of nanosilver in soils (Navarro et al. 2014). Specifically, these authors investigated the retention and release of nanoparticulate silver in spiked soils as citrate, PVP, humic acid coated forms and silver sulphide nanoparticle cysteine suspension. These forms were compared to silver nitrate and bulk silver sulphide (> 200 nm) spikes. Batch release experiments were performed on the spiked soils, in potassium nitrate solution and potassium nitrate solutions plus an environmentally/agriculturally relevant ligand (humic acids, citrate, thiosulphate, cysteine, mercaptopropionic acid). The measure of release of silver in soils was shown to vary between the two soils tested but to be less than 25% of that retained in the soils accept for the thiosulphate treatment. It is important to note that of the silver that was released in the thiosulphate extracts less than 25% was present in the < 1KDa size fraction. 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. This study also suggests that more is now known than indicated in the draft decision in regard to formation of silver complexes and solution nanoparticle behaviour (draft decision page 5, first paragraph). We intend to discuss the possibility of a registration update with the eMSCA to include the recent Navarro et al. (2014) study.

15. On page 17 of the draft decision, at the end of the second paragraph it is stated that “........time is an important factor to take into account as well”, in regard to further clarifying the fate of nanosilver in soils. From the previous experimental work undertaken by CSIRO it was shown that as for most metals, aging of silver in soils had a considerable influence upon behaviour, fate and ecotoxicity of the added silver salt. The aging timeframe for the CSIRO work, like that for other trace element bioavailability programs (e.g. copper and nickel) was 12 months. The aging process is an important one if the findings of the information requested in the draft decision are to have environmental relevance (Pellston Workshop on Hazard Assessment of Soils, 1999). For further information and argumentation on the importance of aging of silver in soils, please see Annex 3. PMC recommends an aging process of 12 months is applied, as was conducted for ionic silver. As previously stated, this would influence the timelines given for the experimentation and data submission. In respect of this proposed revision, a minimum of 18 months would be required to undertake the terrestrial component of the research experimentation alone.

Comments on the requirements on Ecotoxicity Testing

16. The draft decision references the read-across justification document (Annex 7 to the J-CSR) which focuses on showing that nanosilver is less toxic than ionic silver (draft decision pages 18-20), and

suggests that many of the studies referenced in the read-across justification "suffer from methodological limitations that are hampering a comprehensive and comparative evaluation of toxicity exerted by silver nanoparticles and ionic silver". Thus the conclusion of the eMSCA is that no firm conclusions on the toxicity of silver nanoparticles versus ionic silver can be drawn on the basis of those studies. However, without provision of a more detailed rationale for that conclusion, including scientific counter-points, this statement is not sufficiently transparent or verifiable. In order to prevent potentially similar methodological limitations occurring in our follow-up work, we respectfully request access to the draft Substance Evaluation Report.

17. A number of ecotoxicity studies are referenced in the draft decision (draft decision page 20) to support the hypothesis that at least part of the toxicity inferred by silver nanoparticles is due to the effects of the particles themselves, rather than simply the release of silver ions from the particles.

    a. Wang et al. (2012a) – it is stated in the draft decision that this study shows that the toxic effect of nanosilver can be attributed, partly, to the particles themselves, and that the fraction of toxicity caused by the particles can be higher than that caused by silver ions released from the particles. However, what is apparent from this study is that in a direct comparison of the toxicity of three differently coated silver nanomaterials with silver nitrate, the EC_{50} for silver nitrate to *Chydorus sphaericus* was lower than for all the silver nanomaterials tested (confidence intervals do not overlap), and thus that silver nitrate was (marginally) more acutely toxic to *Chydorus sphaericus* than the silver nanomaterials. The difference in effect between silver nitrate and silver nanomaterials was more pronounced in the other two species tested in this study.

    b. Gao et al. (2009), McLaughlin and Bonzongo (2012) – the rationale given in the draft decision with regard to these two studies appears to be that a similar level of acute effect in *Ceriodaphnia dubia* was derived from exposure to ionic silver and nanosilver, and therefore, assuming that the nanosilver particles are not fully dissolved, the effects from nanosilver cannot be derived solely from silver ions released from the particles. It is not disputed that there may be some effect of particles which acts in tandem with the released silver ions on organisms exposed to nanosilver, and in particular that such an effect may be more pronounced in organisms which sequester particles (for example by filter feeding). However, our contention is that the focus should be on the actual hazard presented, including consideration of toxicity equivalence, and not on inferences around the mechanism(s) of toxicity *per se*. There are many other substances that cause toxicity to exposed organisms by a number of different mechanisms based on their chemical activity and their toxicokinetics following uptake. The overall toxicity of the silver nanoparticles is similar (actually rather less) than that of ionic silver in these studies. In this context, we conclude that a read-across strategy predicated on the toxicity characteristic of ionic silver would hence protect against the overall effects of silver nanoparticles.

    c. Griffitt et al. (2008), Bilberg et al. (2012), Farmen et al. (2012), Hoheisel et al. (2012), Wang et al. (2012b) – These fish studies all indicate that ionic silver is of greater toxicity, or at worst of equivalent toxicity, to fish than nanosilver particles. Indeed, the Hoheisel et al. study concluded that 'regulatory approaches based on the toxicity of ionic silver to aquatic life would not be underprotective for environmental releases of nanosilver'.

The conclusion made by the eMSCA based on these (and presumably other) studies is that "the toxic effects observed after exposure to nanosilver are unlikely to be caused by the release of silver ions alone". We suggest that, based on the considerable weight-of-evidence, the release of silver ions from silver nanoparticles is likely to be the most significant mechanism of toxicity, but accept that there remains currently undefined potential for some forms of nanosilver particles to infer particle-specific effects in certain organisms. However, the read-across strategy proposed is not based on the mechanisms of toxicity of nanosilver, but on the overwhelming evidence that silver ion is more toxic to exposed organisms (or at worst, in some cases of equivalent toxicity) than the overall toxicity of nanosilver particles (regardless of the mechanisms of such nanosilver toxicity), and that hazard assessments based on ionic silver will protectively encompass any nano-specific effects. This basis for a read-across strategy is supported by a recent draft ECHA / JRC / RIVM guidance document on the criteria for read-across between nanoforms which states that the hypothesis building for read-across may imply developing a rationale to assure that the target nanoform is equally or less hazardous than the source material (hazard argument for following a worst case approach). Again, we would like to request the eMSCA to reconsider the weight-of-evidence arguments.

18. With respect to the specific test conditions required to support the ecotoxicity testing we have the following responses:

    a. The draft decision requests that $Na_2EDTA \cdot 2(H_2O)$ is not added in the media used for algal and *Daphnia* tests (page 21). It is well known that excess $Na_2EDTA \cdot 2(H_2O)$ can chelate metals in solution and therefore make them unavailable for toxic challenge to organisms exposed in toxicity tests, thus increasing the toxicity values derived in such tests. However, the lack of $Na_2EDTA \cdot 2(H_2O)$ in test media can also have a detrimental secondary effect on the biology of the test organisms, particularly algae. Most algal species require sufficient amounts of iron to grow to sufficient density to be used in ecotoxicity tests. This iron is taken up by the algal cells in chelated form, and lack of a suitable chelator in culture media prevents sufficient uptake of iron, and then severely limits the growth of the algae. While some iron can be taken up directly from the media, and minimal growth can be achieved, the use of media in toxicity tests that do not include an iron chelator can lead to erroneous results - the control's growth can be highly sub-optimal, and the test article treated exposures made more sensitive to the toxicants added. The low rate of algal growth over the duration of such tests often means that study validity criteria are not achieved, or that comparisons between control and treatments are not possible in any statistically reliable manner.

       The issue of potential chelation of the metal under investigation can be addressed more successfully by balancing the molar concentrations of iron and $Na_2EDTA \cdot 2(H_2O)$, thereby ensuring that all (or most) of the $Na_2EDTA \cdot 2(H_2O)$ present in the algal media is complexed with iron. This provides sufficient complexed iron for optimal algal growth while also minimising the concentration of excess uncomplexed $Na_2EDTA \cdot 2(H_2O)$ which may bind with the metal added to the test system (in this case, silver). There are a number of standard methodologies which highlight the use of media in which the molar concentrations of iron and EDTA are balanced (e.g. USEPA short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms (2002), Environment Agency/ Standing Committee of Analysts guideline on the direct toxicity assessment of aqueous environmental samples using the *Pseudokirchneriella subcapitata* freshwater algal growth inhibition test (2008)), however, even the OECD method for assessing inhibition of algal growth (OECD 201 (2011)), provides options with regard to the use of different growth media. With specific reference to AAP medium requested to be used for algal testing in the draft decision, the OECD guideline states that 'the molar ratio of EDTA to iron slightly exceed unity. This prevents iron precipitation and at the same time, chelation of metal ions is minimised'.

       The effect of eliminating $Na_2EDTA \cdot 2(H_2O)$ from the media used in *Daphnia* tests is likely to be less pronounced, since the *Daphnia* uptake trace metals from the algae on which they feed as well as from the surrounding media. Nevertheless, in preliminary proving work, it will be necessary to establish *Daphnia magna* cultures in media without $Na_2EDTA \cdot 2(H_2O)$ and to assess their performance with reference toxicants in terms of survival, growth and reproduction before undertaking such tests, to ensure that the toxicity values derived are representative and that validity criteria for the tests can be achieved.

    b. It is also requested that chloride salts are replaced by nitrate salts in algal and *Daphnia* media (draft decision page 20). The effects, if any, of using nitrate salts to replace chloride salts in algal and *Daphnia* media (owing to the potential for chloride to promote the precipitation of silver salts) are unknown. It will also be necessary to establish algal and *Daphnia magna* cultures in media which replace chloride salts with nitrate salts to assess their performance with reference toxicants in terms of survival, growth and reproduction before undertaking the main work, to ensure that the toxicity values derived are representative and that validity criteria for the tests can be achieved.

c. Considering the above comments on medium alterations, the ecotoxicity program will need to be conducted in a staged manner with pilot studies being undertaken to establish the workability of any medium alterations ahead of the main testing. This would in turn provide an answer (in a staged manner) as to which medium should be used for the determination of the dissolution rate, as the draft decision suggests using one of the ecotoxicity test media for the initial comparison of the dissolution rates of the different nanosilver forms. This pilot work obviously impacts on the timing required to complete the overall program.

d. On page 5 of the draft decision, third bullet (and further on page 21) it is stated that "The three soils to be selected need to cover – as wide as possible – a range of soil properties expected to affect the fate of nanosilver in soil; especially pH, clay content and organic matter content are expected to be of major relevance in this respect." For the soil micro-organism testing, we also suggest that, in addition to the LUFA 2.2 soil, two soils are selected from those that were used for the previous "Evaluation of toxicity and bioavailability of silver in soils" undertaken by CSIRO detailed in the CSR (see also Comment 12 and Annex 2).

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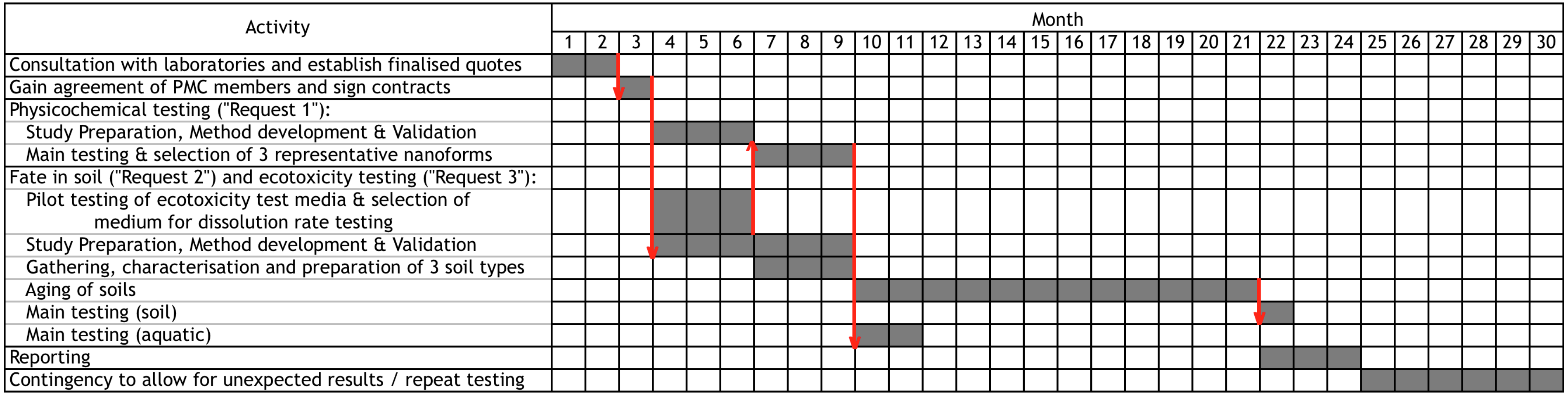
Annex 1: Time required to fulfil the requests of the draft decision

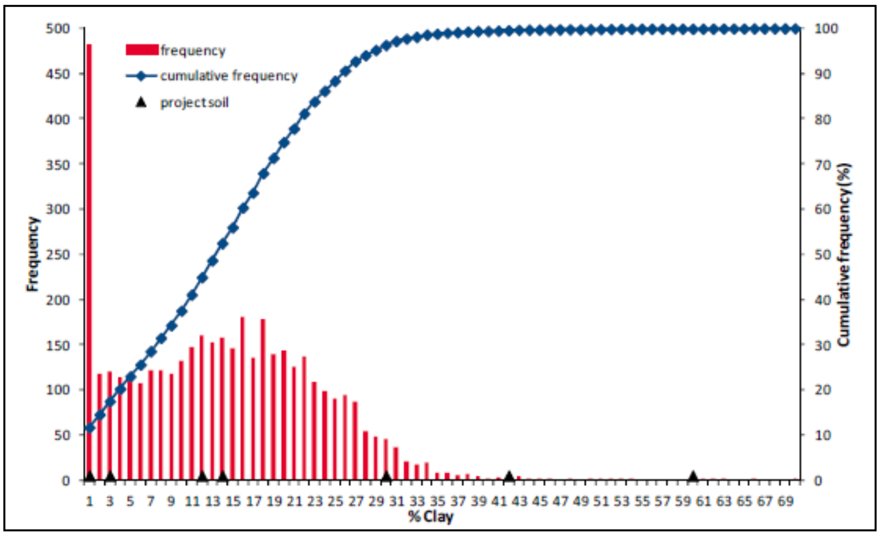


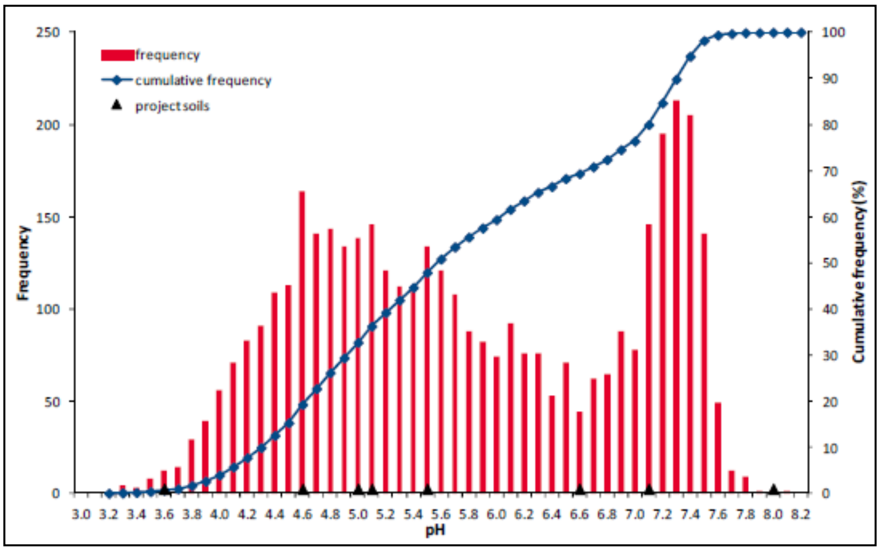
Activity	Month																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Consultation with laboratories and establish finalised quotes	▨	▨																												
Gain agreement of PMC members and sign contracts			▨																											
Physicochemical testing ("Request 1"):																														
Study Preparation, Method development & Validation				▨	▨	▨																								
Main testing & selection of 3 representative nanoforms							▨	▨	▨																					
Fate in soil ("Request 2") and ecotoxicity testing ("Request 3"):																														
Pilot testing of ecotoxicity test media & selection of medium for dissolution rate testing				▨	▨	▨																								
Study Preparation, Method development & Validation				▨	▨	▨	▨	▨	▨																					
Gathering, characterisation and preparation of 3 soil types							▨	▨	▨																					
Aging of soils										▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨									
Main testing (soil)																						▨								
Main testing (aquatic)										▨	▨																			
Reporting																						▨	▨	▨						
Contingency to allow for unexpected results / repeat testing																									▨	▨	▨	▨	▨	▨

The red arrows connect the steps that can only be started after another one has been completed.

Annex 2: Soil selection for testing

Frequency distributions of selected physicochemical properties of European soils, as taken from the GEMAS soils database (n = 5000) are shown in Figure 1. GEMAS was developed to map and define the European background of long series of metals, physicochemical properties of European soils as well as parameters that influence the bioavailability. It forms thereby the best available reference to define and select such soil types/conditions. The soils used in the CSIRO project are marked as black triangles on the x-axis and cover a wide range of the salient properties likely to influence the behaviour of silver. We recommend that two soils are selected from these, as shown in Table 1, for example Millicent and Balaklava as they, in addition to the LUFA 2.2 soil, cover the required wide range of physicochemical properties, specifically pH, clay content and organic matter content.





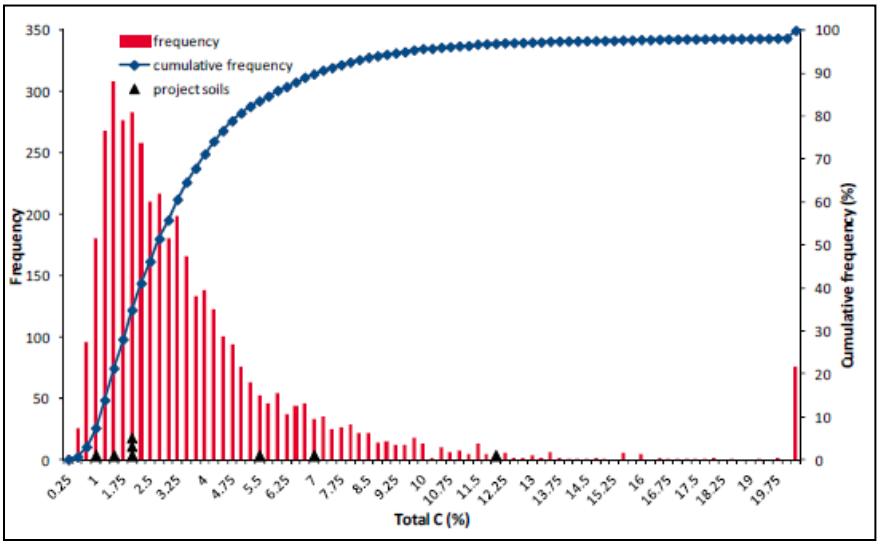
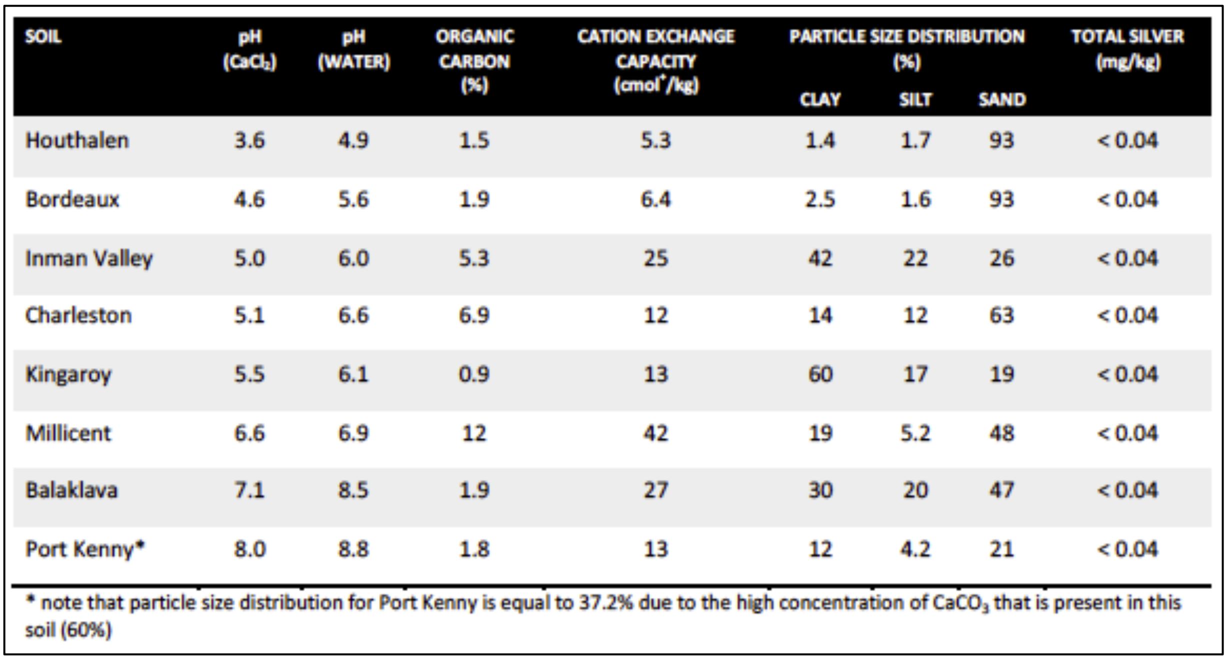


Figure 1. Frequency distributions of European soil properties, as given in the GEMAS soils database and the soils used in the CSIRO project.

Table 1. The physicochemical properties of the soils used for the CSIRO Project detailed in the CSR



SOIL	pH ($CaCl_2$)	pH (WATER)	ORGANIC CARBON (%)	CATION EXCHANGE CAPACITY (cmol$^+$/kg)	PARTICLE SIZE DISTRIBUTION (%)			TOTAL SILVER (mg/kg)
					CLAY	SILT	SAND	
Houthalen	3.6	4.9	1.5	5.3	1.4	1.7	93	< 0.04
Bordeaux	4.6	5.6	1.9	6.4	2.5	1.6	93	< 0.04
Inman Valley	5.0	6.0	5.3	25	42	22	26	< 0.04
Charleston	5.1	6.6	6.9	12	14	12	63	< 0.04
Kingaroy	5.5	6.1	0.9	13	60	17	19	< 0.04
Millicent	6.6	6.9	12	42	19	5.2	48	< 0.04
Balaklava	7.1	8.5	1.9	27	30	20	47	< 0.04
Port Kenny*	8.0	8.8	1.8	13	12	4.2	21	< 0.04

* note that particle size distribution for Port Kenny is equal to 37.2% due to the high concentration of $CaCO_3$ that is present in this soil (60%)

Annex 3: Aging of silver in soils

When soil ecotoxicity testing for metals is undertaken, soluble metal salts are used to spike a soil and the test then undertaken after perhaps 1-2 days equilibration. However, this type of metal exposure is unlikely to happen in the field and thus researchers have assessed the importance of aging (and leaching) in soils post spiking to more reasonably reflect what will happen in the field.

The process of aging the soils results in a decrease in the labile pool of metal ions as they become incorporated into the solid phase of the soils. An aging period for experimental soils is important as it allows the metal ions to equilibrate in the soil to reach a point that is more representative of field soils. This has been demonstrated as a difference between spiked soils in pots tested after one month (often using plants) and then retested in the same soils after 12 months. The difference between the ECx values for a metal for the same species are generally always more than a factor of 1 indicating that the toxicity has decreased over the aging period.

For silver the ECx values for plant growth tests in soils spiked with silver nitrate after 1 month and then again in the same soils after 12 months were different by factors of between 1.3 and 22. Therefore, after aging the silver was less toxic than before the soil was aged. The ECx values before aging were on average 2.4 times lower than those after aging. It is important to provide enough time to account for the aging process of silver in the soils, i.e. 12 months.

Furthermore, the extent of aging is dependent upon, in-part, soil physicochemical characteristics. The CSIRO project on the Evaluation of toxicity and bioavailability of silver in soils given in the CSR provides a relationship that was developed using multiple linear regression to estimate the aging factor if soil properties are know:

$$log_{10}AF = 0.78 + (0.46 \times log_{10}CEC) + (-0.50 \times log_{10}OC) + (-0.92 \times log_{10}pH)(R^2 = 0.92)$$

Where:
CEC = effective cation exchange capacity
OC = organic carbon
pH = pH in water.

This relationship could reasonably be considered to mean that the soil specific aging factors could robustly be predicted for soils without the need to incubate soils for 12 months. Yet, this relationship is developed for silver nitrate and not nanosilver particles. While it could be assumed to hold for nanosilver particles, one of the key areas identified in the draft decision requiring greater information is in relation to potential behaviour and fate differences between ionic silver soil exposures and nanosilver particle exposures.

Therefore, if an insight is to be gained into the behaviour, fate and ecotoxicity of nanosilver particles, it is necessary to not assume that the aging relationship for ionic silver potentially holds for nanosilver too. As such it would seem necessary to age the soils with nanosilver exposure for at least 12 months, as was done for ionic silver.