EPMF comments on the JRC document ‘Silver_draft factsheet_v1’

EXECUTIVE SUMMARY

- We would like to refer to the silver REACH registration dossier as a reliable source for information on silver, which is continually being updated to account for relevant new scientific information.
- We consider the UK EQS for silver and the REACH freshwater PNEC more reliable than the DK EQS used in the JRC monitoring report as it takes into account more recent toxicity data which fulfil the ECHA requirements regarding the taxonomic group coverage for SSD derivation. Furthermore, it is derived using an assessment factor of 3 which is considered more appropriate than the assessment factor used for the DK EQS.
- The analytical techniques used to determine the silver concentrations for the dataset are not sufficiently sensitive (they do not have a sufficiently low detection limit to allow a proper comparison with the proposed PNEC) and the resulting dataset is not sufficiently reliable (dataset highly skewed towards higher concentrations, inclusion of possible false positives, too much uncertainty because of high number of non-quantified values and high LOD/Qs, extremely high dissolved concentrations) to select silver as a candidate for EQS derivation.
- While substances were compared and pre-shortlisted on the basis of their STE score for scenario Sc2-PNECQC, several statements are made for silver (impact of PNEC change, impact of dissolved versus whole water concentrations) on the basis of scenario Sc2, which leads to artificially high STE scores (because of the high LOD/Qs in comparison with the PNEC).
- **We strongly believe that there is a need for reliable monitoring data using more sensitive analytical techniques before making conclusive statements about silver exposures or potential aquatic silver risks.**
- Silver is currently under REACH Substance Evaluation. The prioritisation of silver under the WFD would be adding another suggested route as part of a much broader regulatory review. In view of all efforts made by the European Commission towards a better regulatory agenda, we wonder if it would not make sense to wait until the results of the Substance Evaluation become available before taking further regulatory actions.

1. SOURCES FOR INFORMATION ON SILVER

Without going into full detail on all statements made on silver in the factsheet, we notice that the information is taken from several non-scientific sources (e.g. Wikipedia) and that for some statements, no clear references are provided. We would like to refer to the **silver REACH registration dossier** as a reliable source of information, which is available on the ECHA dissemination website. It was originally submitted in 2010, and is continually being updated to account for relevant new information (last updated in November 2015; next update foreseen when the results from the silver Substance Evaluation become available in 2017-2019, depending on the extent of the testing).

Furthermore, we would like to point out that **silver is currently under Substance Evaluation**, focusing on the environmental compartment. Under the Substance Evaluation process, ECHA has issued the final decision in July 2016 (which should become publicly available soon). In order to check the registrants' hypothesis that the driver for silver toxicity for all nanoforms registered is the silver ion and that read-across use of toxicity values from ionic to nanosilver is thus a 'worst case' approach, the decision requests ecotoxicity testing (on algae, long term toxicity on aquatic invertebrates and on soil microorganisms) on the smallest nanoform with the highest specific surface area that is covered by the REACH registration dossier. Only in case any of the ecotoxicity tests show higher toxicity for nanosilver as compared to ionic silver, further fate testing will have to be undertaken. Furthermore, information on the uses for each individual nanoform is requested. Since the substance evaluation of silver is still ongoing, conclusions can only be made when the outcome of the evaluation would become available. The prioritisation of silver under the WFD would be adding another suggested route as part of a much broader regulatory review. **In view of all efforts made by the European Commission towards a better regulatory agenda, we wonder if it would not make sense to wait until the results of the Substance Evaluation become available before taking further regulatory actions.**

2. PROPOSED QUALITY STANDARDS (QS)

The PNEC selected for the STE run was a value of 0.017 µg/L, reported as an EQS for Denmark. EPMF considers the UK EQS and the REACH freshwater PNEC of 0.04 µg/L more reliable. Here below, an overview of the major differences between the chronic toxicity datasets as prepared by EPMF and submitted in the REACH dossier (ECHA, 2013) and the Danish Environmental Protection Agency (DK, 2009) are discussed. In addition, the considerations used to select an appropriate assessment factor are critically assessed.

a. **Chronic toxicity database:** Considerable differences in retained chronic toxicity data for freshwater organisms are observed between both datasets, i.e. DK (2009) and ECHA (2013) (see Annex 1):

- The EQS derivation as elaborated by the Danish Environmental Protection Agency (DK, 2009) uses only 'old' chronic toxicity data. Indeed, the most recent toxicity data in the DK (2009) report are dated from the year 1997 and more recent published toxicity data have not been considered. On the contrary the **REACH dossier considers also more recent data**.

- Several **discrepancies** in extracted EC10/NOEC data were observed between both datasets. E.g. for the species *Stenomena modestum* a chronic toxicity value of 3.7 µg/l was reported by DK (2009), while in the ECHA (2013) file a value of 1.0 µg/l was retained for PNEC derivation. The details of the studies where discrepancies were observed, including the criteria used to select chronic toxicity data, should therefore be re-assessed.

- For EQS derivation the requirements of the REACH guidance with respect to the number of taxa and species to be included in the dataset (ECHA, 2008) should be followed. ECHA guidance (ECHA, 2008) requires an ecotoxicological dataset to be comprised of at least 10 (preferably 15) long-term NOEC or EC10 values across a minimum of eight taxonomic groups before an SSD approach to PNEC derivation can be used (see Annex 2). The DK (2009) document reports chronic toxicity data for 14 different freshwater species. It is noted that toxicity tests using bacteria (*Pseudomonas putida*) and protozoans (*Chilomonas* *paramaecium, Entosiphon sulcatum & Uronema parduczi*) are included in the assessment performed by DK (2009). However, such species are typically used in the derivation of the PNEC for the STP and should therefore not be used for the derivation of the EQS for the pelagic aquatic community. In case these species are excluded from the DK (2009) dataset, the number of freshwater species is lowered to 10 species covering 6 taxonomic groups, while the ECHA (2013) database incorporates 17 freshwater species covering the required 8 different taxonomic groups. Therefore, **the DK (2009) dataset does not fulfil the ECHA requirements regarding the taxonomic group coverage for SSD derivation** (lack of chronic toxicity data for higher plants and for 'a family in any order of insect or any phylum not already represented').

b. Selection of Assessment Factor: According to ECHA (ECHA, 2008), an additional safety factor of between 1 and 5 should be applied to the HC5 derived from a SSD in order to derive a final PNEC/EQS value. Deviation from an assessment factor of five must be fully justified and is dependent on the overall quality and diversity of the ecotoxicological dataset and the level of uncertainty at the HC5. **In the ECHA (2013) file an assessment factor of 3 is selected while the DK (2009) report uses a factor of 4, which in our view is overly conservative**. The decision in relation to the selection of an appropriate assessment factor to the HC5 should reflect the residual uncertainties. In order to structure this assessment, key considerations when using the ECHA (2013) dataset are:

1) <u>the overall quality of the database and the endpoints covered, e.g., if all the data are generated from "true" chronic studies (e.g., covering all sensitive life stages)</u>: All the toxicity data in the ECHA (2013) file can be considered to have been generated from chronic studies and cover sensitive life stages. Indeed, where more than one endpoint from a test has been generated the most sensitive has been selected for inclusion in the SSD Summary of silver PNECs. *Conclusion*: no residual uncertainty related to this criterion.

2) <u>the diversity and representativity of the taxonomic groups covered by the database, and the extent to which differences in the life forms, feeding strategies and trophic levels of the organisms are represented</u>: The diversity of the aquatic silver dataset covers key taxonomic groups (including insects and mollusks and cyanobacteria) and also sensitive life forms and feeding strategies. *Conclusion*: no residual uncertainty related to this criterion.

3) <u>knowledge on presumed mode of action of the chemical (covering also long-term exposure)</u>: There is limited knowledge of the mode of action for long-term silver toxicity in the aquatic environment, but the tests do cover long-term exposures. In some cases – exposures are >200 days in duration (long-term data for fish). However, according to Wood *et al.* (2008) one mechanism of chronic toxicity appears to be similar to that of acute toxicity, i.e. interference with Na+ en Cl- regulation. Accompanying physiological disturbances include reductions in Na+ uptake, Na/K-ATPase activity, Na+ content, Cl- content and increases in whole-body cortisol and ammonia levels. *Conclusion*: no residual uncertainty related to this criterion.

4) <u>statistical uncertainties around the 5th percentile estimate, e.g., reflected in the goodness of fit or the size of confidence interval around the 5th percentile, and consideration of</u>

<u>different levels of confidence (e.g. by a comparison between the 5% of the SSD (50%) with the 5% of the SSD (95%))</u>; The log-normal SSD, applied to the toxicity data from the ECHA (2013) file, passes goodness of fit tests ($p>0.05$) at both the body and tail of the distribution. The use of the log-normal distribution resulted in a HC5-50 of 0.12 (0.12-0.27) µg/l.

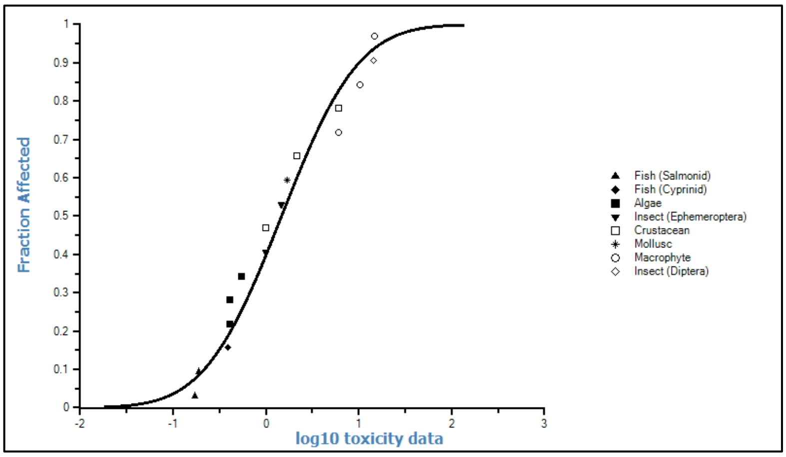

Figure 1: Log-normal fitting distribution to the chronic silver toxicity data (ECHA, 2013)

The log-normal distribution is a pragmatic choice from the possible families of distributions because of the available description of its mathematical properties (methods exist that allow for most in depth analyses of various uncertainties). However, according to the ECHA guidance document the Anderson–Darling (A-D) goodness of fit test can be used, as a criterion for the choice of a parametric distribution for comprehensive data sets, because it gives more weight to the tails of the distribution. The A-D goodness-of-fit statistics of the log-normal distribution function is 0.39 while applying best fitting techniques to the ECHA (2013) toxicity database revealed lower A-D goodness-of-fit statistics of 0.27 (and thus a better data fitting at the lower tail of the SSD). Selection of the best fitting SSD (Figure 2) revealed much higher HC5-50 values, i.e. 1.5 (0.45-8.58) µg/l using the Raleigh distribution.

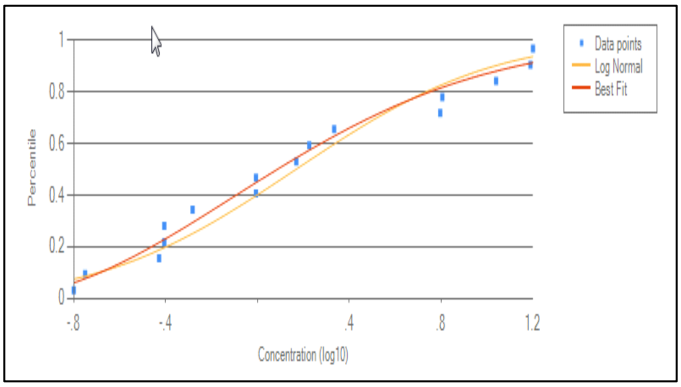

Figure 2: Best fitting distribution to the chronic silver toxicity data (ECHA, 2013)

Conclusion: no residual uncertainty related to this criterion.

5) <u>comparisons between field and mesocosm studies, where available, and the 5th percentile and mesocosm/field studies to evaluate the laboratory to field extrapolation</u>: *Conclusion*: No relevant data on field to mesocosm studies are available, and therefore the uncertainty remains.

In addition, it must be emphasized that the assessment for EQS derivation is overly conservative for the following reasons:

- Conservative in **data selection**: the EQS/PNEC derivation is based on the lowest available chronic toxicity values for the freshwater organisms. Indeed, in case several toxicity data are available for the same species and endpoint, only the lowest NOEC/EC10 was selected.
- Conservative in **bioavailability**: the selected chronic toxicity data typically reflects conditions of low bioavailability. Indeed, the concentrations of variables known to alter the bioavailability and toxicity of silver, e.g. the chromium reducible sulphides (CRS) or dissolved organic carbon (DOC), are much lower in standard test media compared to environmentally realistic levels.
- Conservative in **SSD choice**: the log-normal SSD is used instead of the best fitting distribution. As demonstrated here above, the use of the log-normal SSD resulted in more conservative HC5-50 values.

Furthermore, the Environment Agency of England and Wales, after an independent peer review procedure, applied an assessment factor of 3 to the HC5 in order to derive an EQS / PNECfreshwater. This was based on the recognition of the diversity of the available information and, particularly, the availability of data for blue-green algae (cyanobacteria), which were considered to be particularly at risk from silver exposures in the environment (due to the anti-microbial properties of silver ions).

***Conclusion*: an assessment factor of 3 to the HC5-50 for derivation of the PNEC is proposed** because:

- the only major remaining uncertainty is related to the absence of field/mesocosm data while all other criteria as specified in the ECHA guidance document are fulfilled,
- the conservatism, related to data selection/bioavailability/SSD choice, used in the EQS derivation.

3. MAJOR USES

This section seems mainly focused on the antimicrobial activity of (nano)silver but silver has many other applications. While nanosilver is mainly used for its antimicrobial properties, silver is also used in silverware, jewellery, bank investments, coinage (in which silver has been used globally for thousands of years), electronics, catalysts, photovoltaics and brazing alloys.

In this section, reference is made to the total aggregated tonnage of silver registered under REACH (100 000 - 1 000 000 t/a). It should be noted that only about 3 t/a of the total aggregated tonnage registered under REACH relates to nanosilver.

4. ENVIRONMENTAL BEHAVIOUR AND EFFECTS

Without going into full detail on all statements made on silver under this section, we notice that a lot of emphasis is put on nanosilver. In this respect, we would like to refer to a recent publication by Li et al. (2016) showing that, on the basis of field analysis of representative wastewater treatment plants in Germany, more than 96.4% of silver-based nanoparticles from wastewater influent are removed through wastewater treatment plants. Furthermore, please note our comments made earlier on the fate and behaviour of silver (which are attached to the silver factsheet).

5. MEASURED ENVIRONMENTAL CONCENTRATIONS (2006-2014)

We would like to reiterate that, based on the limited summary statistics provided in the factsheet, it is difficult to fully assess the dataset and its interpretation. **We would like to request again that these data are shared to support a transparent and evidence-based process.** We understand the difficulty in making the fully disaggregated monitoring data available due to confidentiality agreements between JRC and some data providers, but it may be possible that information on statistics per country, per year and LOD/Q are made available without compromising confidentiality. The provision of such data would greatly improve the common understanding of the monitoring data.

Based on the JRC report 'Second Review of the Priority Substances list under the Water Framework Directive: Monitoring-based Exercise' and the summary statistics provided in the factsheet, and in addition to our previous comments on the use of 'whole water' concentrations and the fact that these should not be compared with a PNEC derived using measured dissolved silver concentrations, we would however already like to add the following issues/observations:

a. **The used dataset is highly skewed towards the highest concentrations, leading to an overestimation of risk levels and a high STE score for silver.** This is due to the fact that:

   1) In Sc2 and Sc2-PNECQC, non-quantified records for which no LOD/Q is available are excluded but quantified records for which no LOD/Q is available are kept (as described in the criteria for concentrations and measurement limits on page 21 of the JRC report on the monitoring-based exercise), and

   2) In Sc2-PNECQC, non-quantified records for which 1/2 LOD/Q > PNEC are furthermore excluded.

   We do not know how many samples have been excluded from the dataset based on the first criterion, but comparing the total number of samples in Sc2 and Sc2-PNECQC shows that **89%** of the Sc2 records have been excluded for Sc2-PNECQC because of the second criterion (i.e. they are non-quantified and have an LOD/Q > 34 ng/L (2 x PNEC used)). Because of the high LOD/Qs in comparison to the used PNEC, a large number of non-quantified records (which are likely to be below the PNEC given the log normal shape that environmental concentration curves typically have) have therefore been excluded from the dataset, and mainly the quantified records (which are almost automatically above the PNEC given the high LOD/Qs) are kept.

b. In Sc2-PNECQC, we note that both the P25 and the median are 0.1 µg/L, meaning that at least 25% of the concentrations are exactly 0.1 µg/L. We further note the JRC remark "some of the reported 0.1 µg/L concentrations might be "false positive" non-quantified measurements". **If 25% or more of the records in the dataset might be false positives, the dataset is not reliable.**

c. Using the summary statistics provided for Sc2 and Sc2-PNECQC, we have summarised in below table the number of quantified/non-quantified samples and LOD/Qs:

	Samples	**% in Sc2**	**% in Sc2-PNECQC**
Non-quantified records with LOD/Q > 0.034 µg/L	11772	89.2%	Excluded
Non-quantified records with LOD/Q = 0.025 µg/L	259	2.0%	18.2%
Quantified records with LOD/Q not available	988	7.5%	69.4%
Quantified records with LOD/Q = 0.1 µg/L	175	1.3%	12.3%
Quantified records with LOD/Q = 0.8 µg/L	2	0.0%	0.1%
Total	**13196**		

Looking at this table, the following observations can be made:

1) For almost 70% of the samples in Sc2-PNECQC, no LOD/Q was available. This makes us question the reliability of these data. Also, as already stated under point a) above, since the criteria for concentrations and measurement limits state that non-quantified records for which no LOD/Q is available are excluded but quantified records for which no LOD/Q is available are kept, we wonder how many records have been excluded from Sc2.

2) It is remarkable that all the samples which have been measured with the most sensitive monitoring technique (i.e. with the lowest LOQ) are all non-quantified.

3) It appears that for 98% of all samples in Sc2 and for 82% of all samples in Sc2-PNECQC, the LOD/Q is greater than 0.034 µg/L or not available. For the remainder of the samples, the LOD/Q is 0.025 µg/L. This means that **for all samples, the LOD/Q appears to be above the PNEC used in the monitoring-based exercise (0.017 g/L)** and thus that methods of analytical determination are not compliant with the technical specification for chemical analysis set out by the European Commission for water quality monitoring (EC, 2009), which requires a method to have an LOQ of equal to or below a value of 30% of the relevant environmental quality standard. **As stated in our previous comments, the analytical techniques used to determine the silver concentrations for the dataset are not sufficiently sensitive to make conclusive statements about silver exposures.**

d. **PNEC changes** are assessed on the STE score for Sc2 rather than for Sc2-PNECQC. However, given the fact that in Sc2 non-quantified records are set to 1/2 LOD/Q, and the LOD/Qs are high compared to the PNEC (even if the PNEC of 0.04 µg/L is used), 89% of potentially artificial PNEC exceedances are introduced in this scenario (see also point a) above). Therefore, in the case of silver, **STE scores based on Sc2 will be artificially high, regardless of the PNEC used.**

e. A limited amount of summary statistics is also provided for the **dissolved fraction**. It is remarkable that the mean and max concentrations in the dissolved fraction are higher than in the whole water. **Silver monitoring data available in literature are significantly lower than the values reported here.** Indeed, the mean dissolved silver concentration was 5.935 µg/L (Sc2). In a UK monitoring campaign (425 samples from 84 sites), the mean of the maximum dissolved silver concentration at each site was just 0.006 µg/L (Peters et al. 2011). The maximum dissolved concentration recorded from the JRC data set was 210 µg/L. However, the worst case predicted concentration of high effluent discharge and low flow scenario by Johnson et al. (2014) is only

0.135 µg/L. The factsheet also states that silver concentrations in polluted water are in the range of 0.01-0.1 µg/L. The extremely high dissolved silver concentrations reported by JRC make us wonder if perhaps the wrong units have been used when reporting the sample concentrations.

Furthermore, we notice Sc2 was used to derive an STE score for the dissolved fraction. We would like to state again that because of the high LOD/Qs for silver as compared to the PNEC and because of the high amount of non-quantified values, Sc2 introduces a lot of potentially artificial PNEC exceedances and leads to an artificially high STE score.

References:

EC 2009. Laying down, pursuant to Directive 2000/60/EEC of the European Parliament and the council, technical specifications for chemical analysis and monitoring of water status. Off J Eur Union L210:36–38.

ECHA, 2008. Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose[concentration]-response for environment. European Chemicals Agency.

ECHA, 2013. Annex 4 of the Chemical Safety Report of the silver REACH Registration Dossier ‘Summary of the derivation of predicted no effect concentrations (PNECs) for silver in the environment’. Report from WCA to PMC.

Johnson A.C., Jürgens M.D., Lawlor A.J., Cisowska I., Williams R.J., 2014. Particulate and colloidal silver in sewage effluent and sludge discharged from British wastewater treatment plants. Chemosphere, 112: 49–55.

Li L., Stoiber M., Wimmer A., Xu Z., Lindenblatt C., Helmreich B., Schuster M., 2016. To What Extent Can Full-Scale Wastewater Treatment Plant Effluent Influence the Occurrence of Silver-Based Nanoparticles in Surface Waters? Environ. Sci. Technol. 50 (12), 6327-6333.

Peters, A., Simpson, P., Merrington, G., Rothenbacher, K., Sturdy, L., 2011. Occurrence and Concentration of Dissolved Silver in Rivers in England and Wales. Bulletin of Environmental Contamination and Toxicology 86, 637-641.

Annex 1: Retained chronic toxicity data for EQS derivation of silver

Species	DK, 2009	ECHA, 2013	Comments
Bacteria			
Pseudomonas putida	6.0 µg/l - Bringmann & Kuhn 1978	/	Data not considered in ECHA, 2013
Protozoa			
Chilomonas paramaecium	2.6 µg/l - Bringmann & Kuhn 1978	/	Data not considered in ECHA, 2013
Entosiphon sulcatum	580 µg/l - Bringmann 1980	/	Data not considered in ECHA, 2013
Uronema parduczi	100 µg/l - Bringmann & Kuhn 1978	/	Data not considered in ECHA, 2013
Algae			
Chlamydomonas reinhardtii	/	0.54 µg/l – Hiriart-Baer et al 2006	Data not considered in DK, 2009
Snedesmus quadricauda	9.5 µg/l - Bringmann & Kuhn 1978	/	Data not considered in ECHA, 2013
Pseudokirchneriella subcapitata	/	0.41 µg/l – Hiriart-Baer et al 2006	Data not considered in DK, 2009
Microcystis putida	0.7 µg/l – Bringmann & Kuhn 1978	/	Data not considered in ECHA, 2013
Nostoc muscorum	/	0.0.16 – 0.66 µg/l – Rai & Raizada, 1987	Data not considered in DK, 2009
Higher plants			
Salvinbia natans	/	14.8 µg/l – Hutchinson & Czsyrska, 1975	Data not considered in DK, 2009
Lemna paucicostata	/	6.4 – 16.7 µg/l – Nasu & Kugimoto, 1981	Data not considered in DK, 2009
Lemna minor	/	6.0 µg/l - Hutchinson & Czsyrska, 1975	Data not considered in DK, 2009
Invertebrates			
Ceriodaphnia dubia	0.53 µg/l – Rodgers et al. 1997	0.53 µg/l – Rodgers et al 1997	/
Ceriodaphnia dubia	/	2.48 µg/l – Kolts et al 2009	Data not considered in DK, 2009
Ceriodaphnia dubia	/	6.5 – 10.1 µg/l – Naddy et al 2007	Data not considered in DK, 2009
Daphnia magna	1.6 – 8.8 µg/l – Nebeker et al. 1983	2.2 – 9.1 µg/l – Nebeker et al 1983	Discrepancies between datasets
Daphnia magna	/	2.14 – 3.49 µg/l – Bianchini & Wood 2008	Data not considered in DK, 2009
Daphnia magna	/	3.27 µg/l – Naddy et al 2007	Data not considered in DK, 2009
Hyalella Azteca	0.9 µg/l – Diamond et al. 1990	0.95 µg/l – Diamond et al 1990	/
Isonychia bicolor	3.1 µg/l – Diamond et al. 1990	1.48 µg/l – Diamond et al 1990	Discrepancies between datasets
Stenomena modestum	3.7 µg/l – Diamond et al. 1992	1.0 µg/l – Diamond et al 1992	Discrepancies between datasets
Ceriodaphnia reticulate	/	1.0 µg/l – Elnabarawy & Welter 1986	Data not considered in DK, 2009
Chirnonomus tentans	/	14.4 µg/l – Call et al 1999	Data not considered in DK, 2009
Corbicula fluminea	2.6 – 7.8 µg/l – Diamond et al. 1990	1.68 µg/l – Diamond et al 1990	Discrepancies between datasets
Fish			
Salmo trutta	/	0.19 µg/l – Davies et al 1998	Data not considered in DK, 2009

Species	DK, 2009	ECHA, 2013	Comments
Oncorhynchus mykiss	0.09 – 0.17 µg/l – Davies et al., 1978	0.18 – 0.34 µg/l – Davies et al 1978	Discrepancies between datasets
Oncorhynchus mykiss	0.1 - 0.36 µg/l – Nebeker et al., 1983	0.39 - 0.72 µg/l – Nebeker et al 1983	Discrepancies between datasets
Oncorhynchus mykiss	/	0.17 – 0.63 µg/l - Davies et al 1998	Data not considered in DK, 2009
Oncorhynchus mykiss	/	0.37 µg/l – Dethloff et al 2007	Data not considered in DK, 2009
Oncorhynchus mykiss	/	0.79 – 1.21 µg/l – Morgan et al 2005	Data not considered in DK, 2009
Oncorhynchus mykiss	/	1.2 µg/l – Guadagnolo et al 2001	Data not considered in DK, 2009
Pimephales promelas	0.37 – 1.07 – Holcombe et al., 1983	0.37 – 1.07 µg/l – Holcombe et al 1983	/
Pimephales promelas	/	0.35 – 0.44 µg/l – Naddy et al 2007	Data not considered in DK, 2009
Pimephales promelas	/	0.729 µg/l – Norberg-King 1989	Data not considered in DK, 2009

Annex 2: Taxonomic group requirements according to ECHA (2008)

Taxonomic groups	Species (DK, 2009)	Species (ECHA, 2010)
Fish (usually tested species like salmons, bluegill, channel catfish, etc.)	*Oncorhynchus mykiss*	*Salmo trutta* *Oncorhynchus mykiss*
A second family in the phylum Chordata (fish, amphibian, etc.)	*Pimephales promelas*	*Pimephales promelas*
A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)	*Daphnia magna* *Ceriodaphnia dubia* *Hyallela azteca*	*Daphnia magna* *Ceriodaphnia dubia* *Ceriodaphnia reticulate* *Hyallela azteca*
An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)	*Isonychia bicolour** *Stenonema modestum**	*Isonychia bicolour** *Stenonema modestum**
A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.)	*Corbicula fluminea*	*Corbicula fluminea*
A family in any order of insect or any phylum not already represented	/	*Chironomus tentans*
Algae	*Microcystis putida* *Scenedesmus quadricauda*	*Pseudokirchneriella subcapitata* *Chlamydomonas reinhardtii* *Nostoc muscorum*
Higher plants	/	*Lemna minor* *Lemna paucicostata* *Salvinia natans*

*: Belonging to the same Order of the Ephemeroptera