Ag effects assessment

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1 AIM OF THE PROJECT

The present research project proposes to develop an acute & chronic aquatic ecotoxicity database for Ag and Ag-compounds which could be used for both classification and risk assessment purposes. The initial classification/effects assessment report includes a detailed overview of the acute & chronic effects thresholds and the bioavailability factors which may affect the toxicity of Ag & Ag-compounds in the freshwater environment. The latter is particularly important for the implementation of bioavailability models in the derivation of ecotoxicity threshold concentrations (PNEC). Consequently, based on this initial analysis, a data gap analysis was performed and a prioritization and ranking of the follow-up research programs to fill the data gaps is proposed.

2 SPECIATION

Toxicity data extracted from literature are often reported as total or dissolved Ag concentrations. However, literature clearly demonstrates that neither total nor dissolved metal is a good predictor of metal, of Ag, toxicity to aquatic organisms across a wide range of water quality conditions. Therefore, for silver as well as for other metals, it would be important to define the actual or bioavailable concentration, which is important for toxicity, both in the laboratory tests and in the real environment.

3 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

3.1 GENERAL

The aim is to extract high quality acute and chronic toxicity data which could be further used for the derivation of both the reference (acute & chronic) and PNEC values.

Based on the experience of data evaluation in the EU risk assessment process of metals, the data quality and relevance of all data points obtained were thoroughly assessed and ranked. The assignment to the different quality classes were conducted according to the guidelines set out by the MERAG project. In this effects assessment report the 'total risk approach' has been used for the derivation of the PNEC value for the freshwater environment, suggesting that both the background and the anthropogenic Ag could contribute to the observed effects. Depending on the data richness, this safe threshold value will be derived from toxicity data (either NOEC values or LC_{10} and EC_{10} values from laboratory tests), using either assessment factors or the statistical extrapolation approach.

3.1.1 Sources and selection of ecotoxicological data

3.1.1.1 Sources of ecotoxicological data

The ecotoxicological data in this report are derived from original papers on the subject, published in international journals and from research projects. Databases searched for literature were Web of Science and Elsevier ScienceDirect. The Web of Science, available through the library of Ghent University, provides Web-based access to the following ISI citation databases: Science Citation Index, Expanded Social Sciences Citation Index and Arts & Humanities Citation Index. The Science Citation Index, covering approximately 4600 journals, provides complete bibliographic data plus citations to world-wide literature across a wide range of scientific and technological disciplines. Data are available from 1972 onward.

The Elsevier ScienceDirect database contains citations and abstracts from 3000 journals in fields such as the life sciences, physical sciences, social sciences and technology. ScienceDirect also provides access to full text articles to the following: the Elsevier Science Journals to which the University Library Ghent subscribes, and over 200 Academic Press journals previously available on IDEAL bookmark.

Review articles covering silver in the environment were also searched for data sources. However, only original literature data should be used in the silver effects assessment.

3.1.1.2 Selection of ecotoxicological data

Specific selection criteria have been developed for aquatic effects data that pay attention to both the reliability and relevancy aspect of the toxicity data. In this report the results of the aquatic toxicity studies are expressed as either the actual (measured) concentration or as the nominal (added) concentration. The actual concentrations include the background concentration of silver. The total risk approach is based on nominal concentrations to which background concentrations were added or on actual concentrations (i.e. NOEC). If the background concentration is reported as below detection limit (DL), the background concentration is estimated as DL/2.

The assessment of data adequacy involves a review of individual data elements with respect to how the study is conducted and how the results are interpreted in order to accept (or reject) a study in accordance with the purpose of the assessment.

The term adequacy covers both the reliability of the available data and the relevance of the data for environmental hazard and risk assessment. These two basic elements have been defined by the TGD as follows:

- Reliability: covering the inherent quality of a test relating to test methodology and the way that the performance and results of a test are described.
- Relevance: covering the extent to which a test is appropriate for a particular risk assessment.

Only ecotoxicity data that comply with all of the above-mentioned criteria can be considered valid and should be used in the risk assessment.

In this section of the report the adequacy of all chronic toxicity data that were generated using silver as toxicant is discussed. Based on this evaluation, the data were classified into three different categories which are presented in section 3.1.1.3.

Relevance

Biological relevancy

The toxicity data on algae, higher plants, invertebrates, amphibians and fish are from single-species tests that study relevant ecotoxicological parameters such as survival, growth, reproduction and developmental malformations.

Relevancy of the test substance

Ag-only exposures are considered relevant for the effects assessment. Studies should be rejected if indications exist that impurities or other substances might have an effect on the toxic properties of the substance under investigation.

Ecological relevancy of data

The assessment of the ecological relevancy of a particular study requires that certain basic ecological considerations be taken into account (e.g.; test organisms should be representative for the environment). This is especially true when non-ecological relevant species are the driver for the PNEC.

Relevancy of the test medium

The range of the physico-chemistry of the test media should be within the range of ecologically relevant conditions that occur in the European environment. Information on key physico-chemical parameters of the test medium that may alter the bioavailability & toxicity of Ag should be reported, i.e., pH, hardness and dissolved organic matter. The latter parameter is rarely reported, but in most cases a reliable estimate of the DOC-content can be made based on the type of test medium that is used (well water, artificial water, natural water, tap water, etc.). If not reported, assumptions should be based upon knowledge of the composition of the toxicity test source water (e.g. Lake Superior water). If toxicity tests were conducted in reconstituted laboratory water, where deionized or Milli-Q water was used as the water source, DOC are assumed to equal 0.5 mg/L. If toxicity test dilution water was prepared with laboratory water, other than deionized or Milli-Q, DOC is assumed to be 1 mg/L. If toxicity tests are conducted with actual surface water, DOC is assumed to be 2 mg/L.

The establishment of the physico-chemical borders of these parameters in EU surface waters is based on the analysis of recent monitoring data that were collected from various environmental agencies and governmental institutes. An overview of these data is given in section 4.

Test duration

According to the Zn risk assessment, chronic exposure was defined as > 4 days for all invertebrates and fish. With respect to the chronic effect values it is noted that the fact whether or not a NOEC value is considered chronic is not determined exclusively by the above exposure limit of 4 days. For unicellular algae, other micro-organisms (bacteria, protozoa) and even invertebrates (e.g. rotifers), an exposure time of 4 days or less already covers one or more generations, thus for these organisms chronic NOEC values may be derived from experiments taking less than 4 days. However, chronic exposure depends upon the exposure duration and is also a function of the life cycle of the test organisms. Therefore, *a priori* fixed exposure durations are therefore not relevant and should instead be related to the species, their typical life cycle and to the recommended exposure duration as described in standard ecotoxicity protocols (e.g. 7 days for Ceriodaphnids (ASTM, 2005), 21 days for daphnids (OECD, 1998), 30 days for fish (OECD, 1992).

Reliability

Standardised tests, as prescribed by organisations such as OECD and USEPA, are used as a reference when test methodology, performance and data treatment/reporting are considered. Indeed, the thorough description of key requirements guarantees the (high) reliability of the reported ecotoxicity data. Non-standardised test data, however, may also have a high reliability, but require a more thorough check on their compliance with reliability criteria before being used for risk assessment purposes. The specific items considered in this study for data selection are the following:

Type of test

Both standard OECD approved tests and non standardised tests have been considered as suitable. Ideally, tests should be performed according to international accepted standard operational procedures and/or guidelines (e.g., OECD Guidelines, ISO-Guidelines, EPA-Guidelines,…). Preference is given to data extracted from peer reviewed publications, but data from national environment agencies (US EPA, RIVM,…) are also retained. GLP and non-GLP tests can be used provided that the latter fulfil the stipulated requirements. For risk assessment purposes preference is given to the use of chronic tests.

Description of test material and methods

A detailed description of methods employed in the study should be provided. Including, but not limited to preparation of the test solutions (environment), timing of administration and observations recorded, etc.

Test substance

Toxicity data in this report cover the different silver compounds found in literature which is mainly silver nitrate. There is at present not justifiable reason to differentiate between the different silver compounds, as no indication is given that silver toxicity in the environment would depend on the salt that was administered (as aqueous solution). Moreover, at the moment, there are too few data enabling an effect assessment for each silver compound. Finally, it is doubtful whether discrimination between silver compounds would have an ecological relevance, as the compound will change with time and under various environmental conditions. Hence, it can be concluded that the silver compounds can be pooled with regard to toxicity.

Chemical analysis

In general metal effects assessment should only be based on NOEC values which were derived from actual (measured) effect concentrations. This is especially relevant for those metals where effects concentrations are close to the background concentrations. For those metals where the background level is negligible compared to the observed NOEC, effects levels based on nominal values can be considered if there is clear indication that actual values are within 10% of nominal values.

Test acceptability

Minimal requirements for endpoints such as mortality, growth, reproduction (e.g. control mortality for chronic exposure < 20 %) are often given in standard procedures. Therefore chronic data were rejected if evidence is provided that these minimal requirements were not met in the control. For algae, control division rate was checked for conformity with OECD (1983) and ASTM (2005) guidelines. These guidelines suggested a cell division rate of 1.33 for the OECD guideline (i.e. cell concentration in the control cultures should have increased by a factor of at least 16 within 3 days) and 1.0 for the ASTM (2005) guideline (i.e. cell concentration in the control cultures should have increased by a factor of at least 16 within 4 days).

Concentration-effect relationships

Clear dose-response should be observed. Because effect concentrations are statistically derived values, information concerning the statistics should be used as a criterion for data selection. In that respect $L(E)C_{10}$ values are considered as equivalent to NOEC. If no methodology is reported or if values are 'visually' derived, the data were considered unreliable. Effect levels derived from toxicity tests using only 1 test concentration always results in unbounded and therefore unreliable data. Therefore, only the results from toxicity tests using 1 control and at least 2 silver concentrations should be retained. Tests that do not comply with the above-mentioned stipulations are rated as not reliable and are not recommended for use in the risk assessment exercise. However, the use of unbounded NOEC/LOEC values could be justified on a case by case basis, e.g. when no other toxicity values are available for a particular species or when scientific evidence exist that the true toxicity towards a specific organism would be biased if such data were not taken into account.

3.1.1.3 Classification of toxicological studies (publications, reports) for risk assessment (RA) purposes

In theory, for risk assessment (RA) purposes preference is given on the use of *high quality chronic data*. In practice, however, this may result in a very limited amount of studies and toxicity data, especially for compounds like silver for which the number of toxicological studies is rather limited compared to some other metals or other chemical compounds. It was therefore decided to classify the evaluated studies into three categories:

Category 1; High quality data (Q1-data for RA-purposes): Relevant studies that comply with all criteria described in section 3.1.1.2 (full description of test method and conditions, based on measured concentrations, clear effect-concentration relationship that allows the derivation of a reliable LC_{10} or NOEC).

Category 2: Satisfactory quality data (Q2-data for RA-purposes): Relevant studies for which no or insufficient information is given on one of the reliability criteria described in section 3.1.1.2:

- effect concentrations are based on nominal values;

or

- test method and conditions are insufficiently described;

or

- unbounded NOEC/LOEC values for a particular species for which no other data are available or when scientific evidence exist that the true toxicity towards a specific organism would be biased if such data were not taken into account

If evidence is given that the criterion for which information is lacking, does not meet the quality standards set in section 3.1.1.2, (e.g., nominal values are given, but precipitation of silver is reported), this study should be categorised as Q3 (Low quality data)

Category 3: Low quality data (Q3-Data for RA purposes): Irrelevant studies or relevant studies for which no or insufficient information is given on more than one of the reliability criteria described in section 3.1.1.2.

3.1.2 Derivation of NOEC values (methods)

The toxicological variables are estimated based on NOEC (No Observed Effect Concentration) or $L(E)C_{10}$ values. The methods that have been used for the derivation of NOEC values, being "real" NOEC values or

NOEC values derived from effect concentrations, are based on the recommendations outlined in the revised TGD (2003).

If $L(E)C_{10}$ data are reported or if both NOEC and $L(E)C_{10}$ data are available (as is often the case in research projects), the $L(E)C_{10}$ values were used for the effects assessment. It is recommended that the $L(E)C_{10}$ value should not be extrapolated below the lowest applied (non-zero) concentration. According to the draft ISO document estimation of $L(E)C_{10}$ values outside the concentration range tested introduces a great deal of uncertainty. Furthermore for metals/metal compounds it is imperative that this value should fall within the range of tested concentrations to avoid extrapolating $L(E)C_{10}$ values below the natural background.

If no reliable $L(E)C_{10}$ values are available, "real" NOEC values should be derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test and should be derived using appropriate statistics (significance level usually: $p = 0.05$ (optional: the $p = 0.01$ level if reported instead of the $p = 0.05$ level)).

The use of LOEC/MATC or unbounded NOEC/LOEC values could also be considered in specific cases, e.g. if other toxicity values are not available for a particular species. For example, > NOEC can be used as a conservative estimate for the "real" NOEC.

In addition, to avoid over-representation of ecotoxicological data from one particular species and as used in the above mentioned EU risk assessment, the chronic silver toxicity data values used here were treated as following:

- If for one species several chronic NOEC values based on the same toxicological endpoint are available, these values are averaged by calculating the geometric mean, resulting in the "species mean" NOEC. With respect to this it is noted that the NOEC values should be from equivalent tests, for example from tests with similar exposure times. However, NOEC values derived from tests with a relatively short exposure time may be used together with NOEC values derived from tests with a longer exposure time if the data indicate that a sensitive life stage was tested in the former tests.
- If for one species several chronic NOEC values based on different toxicological endpoints are available, the lowest value is selected. The lowest value is determined on the basis of the geometric mean if more than one value for the same endpoint is available (see above).
- In some cases, NOEC values for different life stages of a specific organism are available in a specific publication. If from these data it becomes evident that a distinct life stage is more sensitive, the result for the most sensitive life stage is selected. The life stage of the organisms is indicated in the tables as the life stage at start of the test (e.g. fish: yearlings) or as the life stage(s) during the test (e.g. eggs → larvae, which is a test including the egg and larval stage).
- If species data were dependent of each other, e.g., toxicity data for more species were obtained in a multispecies system, then the data were not used in the SSD approach as they were not independent as required for the this approach.

3.1.3 Derivation of PNEC using assessment factors

When only a limited number of data are available (data poor substances), PNEC setting is based on the use of assessment factors reflecting the degree of uncertainty in extrapolating from laboratory toxicity test data for a limited number of species.

The PNEC values were derived from the ecotoxicity data (either NOEC values or EC_{10} values from laboratory tests), using assessment factors, which is described in the TGD 2003).

The proposed assessment factors are presented in Table 1.

When only short-term toxicity data are available, an assessment factor of 1000 will be applied on the lowest L(E)C_{50} of the relevant available toxicity data, irrespective of whether or not the species tested is a standard test organism. A lower assessment factor will be applied on the lowest NOEC derived in long-term tests with a relevant test organism.

For some compounds, a large number of validated short-term L(E)C_{50} values may be available. Therefore, it is proposed to calculate the geometric mean if more than one L(E)C_{50} value is available for the same species and endpoint. Prior to calculating the geometric mean an analysis of test conditions must be carried out in order to find out why differences in response were present.

The algal growth inhibition test of the base-set is, in principle, a multigeneration test. However, for the purposes of applying the appropriate assessment factors, the EC_{50} is treated as a short-term toxicity value. The NOEC from this test may be used as an additional NOEC when other long-term data are available. In general, an algal NOEC should not be used unsupported by long-term NOECs of species of other trophic levels. However, if the short-term algal toxicity test is the most sensitive of the short-term tests, the NOEC from this test should be supported by the result of a test on a second species of algae.

Micro-organisms representing a further trophic level may only be used if non-adapted pure cultures were tested. The investigations with bacteria (e.g. growth tests) are regarded as short-term tests. Additionally, blue-green algae should be counted among the primary producers due to their autotrophic nutrition.

The assessment factors presented in Table 1 below should be considered as general factors that under certain circumstances may be changed. In general, justification for changing the assessment factor could include one or more of the following:

• Evidence from structurally similar compounds (Evidence from a closely related compound may demonstrate that a higher or lower factor may be appropriate);

• Knowledge of the mode of action including endocrine disrupting effects (Some substances, by virtue of their structure, may be known to act in a non-specific manner);

• The availability of test data from a wide selection of species covering additional taxonomic groups other than those represented by the base-set species;

• The availability of test data from a variety of species covering the taxonomic groups of the base-set species across at least three trophic levels. In such a case the assessment factors may only be lowered if these multiple data points are available for the most sensitive taxonomic group.

Table 1: Assessment factors to derive a $PNEC_{aquatic}$

Available data	**Assessment factor**
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, Daphnia and algae	1000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1 (case by case)
Field data or model ecosystems	Reviewed on a case by case basis

3.1.4 Derivation of PNEC using statistical extrapolation

When a large data set for different taxonomic groups is available, the PNEC can be calculated using the statistical extrapolation method in which the susceptibility of a set of species for a given toxicant can be described by some statistical distribution (i.e. Species Sensitivity distribution or SSD). A SSD can be visualized as a cumulative distribution function (

Figure 1). The cumulative distribution function curve follows the distribution of the sensitivity data obtained from ecotoxicological testing, plotting effect concentrations derived from acute or chronic toxicity tests, for example LC_{50} values and No Observed Effect Concentrations (NOECs), respectively.

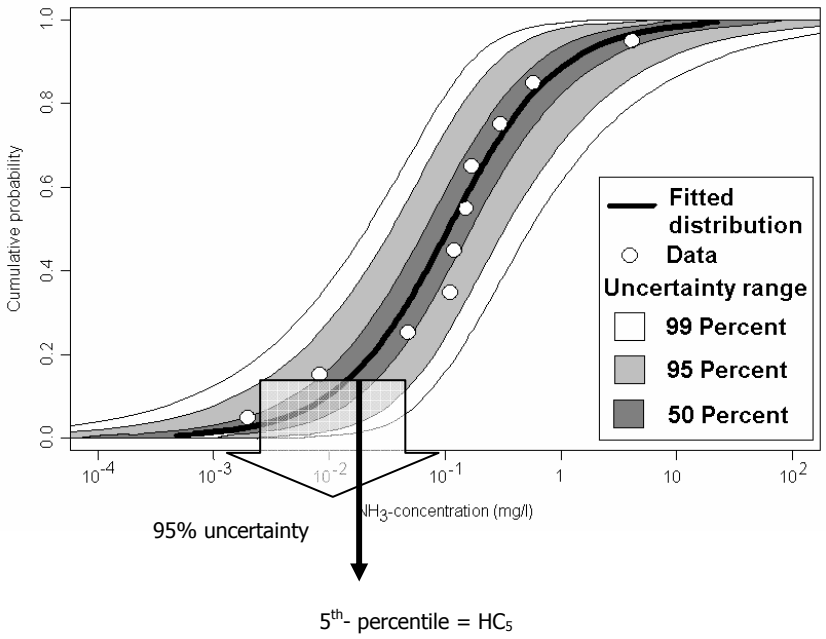

Figure 1: Example of a SSD (Species Sensitivity Distribution - loglogistic distribution) with uncertainty band and its HC5 (Hazardous Concentration at 5%)

The lowest AF that could be used when using the statistical extrapolation approach = 1. But usually it varies between 2 and 5. The exact number is usually decided on a case by case basis. This factor should be applied on the 5th percentile of the SSD derived from chronic toxicity testing with at least 10 species representing 8 different taxonomic groups.

3.2 TOXICITY TO AQUATIC ORGANISMS

In this section the effects assessment report an overview and evaluation of different ecotoxicological studies that have been performed in the aquatic environment using silver as toxicant. Sections 3.2.1 to 3.2.3 present the different studies that are evaluated according to different trophic levels: fish and amphibians (section 3.2.1), invertebrates (section 3.2.2), and algae and higher plants (section 3.2.3).

3.2.1 Toxicity to freshwater fish and amphibians

Data on single-species toxicity tests resulting in NOEC/LOEC values for freshwater fish and amphibians are given in Table 2. More detailed information on the test method of the individual studies is given in the Annexes. For PNEC derivation either NOEC or $L(E)C_{10}$ are used. Most chronic tests report NOECs and nearly all of these data are of relatively high quality (Q1 and Q2). These studies are discussed below. Note that all chronic fish tests were performed with rainbow trout *Oncorhynchus mykiss*.

Brauner and Wood (2002) (1) exposed rainbow trout *Oncorhynchus mykiss* from fertilisation to one week post-hatch (~ 30d) to the following nominal silver concentrations: 0, 0.1, 1 µg/L. Measured total silver concentrations were <0.05, 0.098, 0.85 µg/L, respectively. Test medium was dechlorinated Hamilton hard water with flow rates of 100 mL/min. Mortalities were recorded. NOEC was 0.098 and LOEC 0.85 µg/L. This study was considered as highly reliable (Q1).

Davies et al. (1978) exposed eyed eggs of *Oncorhynchus mykiss* for 18 months to the following nominal silver concentrations: 0, 0.06, 0.12, 0.25, 0.5, and 1 µg Ag/L. The test was conducted at the ambient temperature of dechlorinated Fort Collins city water (tap water) which ranged from 3°C in winter to a summer high of 17°C. Mean temperature was 11.1 °C. For the endpoint mortality, a NOEC of 0.09 µg/L was reported. The study was assigned as highly reliable (Q1).

Galvez and Wood (2002) studied the effect of silver on the growth rate of juvenile *Oncorhynchus mykiss*. Experiment 1 tested the nominal total Ag concentrations of 0.1 and 1 µg/L. Total Ag concentrations were measured daily. The measured concentrations were control (<0.05 µg/L), low silver exposure: 0.2 +/- 0.05 µg/L, high silver exposure: 1.04 +/-0.43 µg/L. Experiment 2 tested the nominal Ag concentrations of 3 and 5 µg/L. For this chronic exposure period the test was repeated as no effects were observed at the low test concentrations of the first test. The measured concentrations were control (<0.05 µg/L), low silver exposure: 3.02 +/-0.1 µg/L and high silver exposure: 4.81 +/-0.28 µg/L. The NOEC for growth rate was 3.02 and the LOEC 4.81 µg/L. This study was considered reliable with restrictions (Q2) since all fish were marked before the start of the test.

Morgan et al. (2005) exposed eggs of *Oncorhynchus mykiss* for 64 days until the swim-up stage. Eggs were exposed in three different water hardnesses and to three different silver concentrations (nominal concentrations of 0, 0.1, and 1 µg/L). The measured total silver concentrations in the water column were generally within 20% of the nominal concentrations. NOEC for mortality was 0.1 and LOEC 1 µg/L for the three different hardness levels tested. The study was assigned as highly reliable (Q1).

Brauner and Wood (2002) (2) reported the results obtained in a flow-through test design in which embryos of *Oncorhynchus mykiss* were exposed for 51 days to three silver concentrations (nominal levels of 0, 0.1, and 10 µg/L total silver; measured levels of <0.05, 0.14, 10.7 µg/L total silver) in dechlorinated Hamilton hard water. The NOEC for mortality was 0.14 and the LOEC 10.7 µg/L. This study was considered as highly reliable (Q1).

Guadagnolo et al. (2001) exposed embryos of *Oncorhynchus mykiss* for 32 days to 0, 0.1, 10 µg/L total silver (nominal values) plus a control (<0.05 µg/L). Measured values were 0.117, 1.22, and 13.51 µg/L. The NOEC for mortality was 1.2 and the LOEC 13.5 µg/L. This study was considered as highly reliable. Control mortality in embryos was 33%. According to the OECD guidelines (OECD 210) overall survival of fertilized eggs in the control or hatching success should be > 66%.

Galvez et al. (1998) exposed *Oncorhynchus mykiss* for 28 days to the nominal total silver concentrations 0.5 and 2.0 µg/L. Measured Ag concentrations were 0.7 +/-0.2 and 1.7 +/-0.4 µg/L, respectively. The NOEC for growth rate was 0.7 and the LOEC 1.7 µg/L. This study was considered reliable with restrictions (Q2) since the test method is not fully described. The age and/or size from the test organism and the pH of the test medium are not reported.

Table 2: Overview of chronic endpoints for fish and amphibians

Sub-stance	Species	Age and/or size of test organism	Test duration	Effect parameter	End-point	Value (µg/l)	Analysis of concentr-ations	Dose – response	Admini-stration of test substance	Temp (°C)	pH	Hardness (mg/l)	DOC (mg/l)	Test water	Ag-back-ground	Quality of the study	Reference
$AgNO_3$	*Oncorhynchus mykiss*	embryos	+/-30d fertilization to 1 week post-hatch	Mortality	NOEC LOEC	0.098 0.85	Measured	Yes	Flow-through	13.7	7.5	120	1.3	Tap water (dechlor-inated Hamilton hard water)	<0.05	Q1	Brauner and Wood, 2002 (1) Ag-3
$AgNO_3$	*Oncorhynchus mykiss*	Eyed eggs	18m	Mortality	NOEC	0.09	Measured	yes	Flow-through	11.1 (3-17)	7	27.5	n.r. 1*	Tap water	<0.05	Q1	Davies et al., 1978 Ag-6
$AgNO_3$	*Oncorhynchus mykiss*	Juvenile	23d	Growth rate	NOEC LOEC	3.02 4.81	Measured	Yes	Flow-through	15	8	120	1.3	Tap water	<0.05	Q2	Galvez and Wood, 2002 Ag-12
$AgNO_3$	*Oncorhynchus mykiss*	Eggs	64d	Mortality	NOEC LOEC	0.1 1	Measured	Yes	Flow-through	11	7	2, 150, 400	0.5	Tap water	<0.05	Q1	Morgan et al., 2005 Ag-14
$AgNO_3$	*Oncorhynchus mykiss*	Embryos	51d	Mortality	NOEC LOEC	0.14 10.7	Measured	Yes	Flow-through	12	7.5-8	120	2.86	Tap water	<0.5	Q1	Brauner and Wood, 2002 (2) Ag-51
$AgNO_3$	*Oncorhynchus mykiss*	Embryos	32d	Mortality	NOEC LOEC	1.2 13.5	Measured	Yes	Flow-through	12.2	7.5	120	1.3 (DOM)	Tap water	<0.05	Q1	Guadagnolo et al., 2001 Ag-70

Sub-stance	Species	Age and/or size of test organism	Test duration	Effect parameter	End-point	Value (µg/l)	Analysis of concentr-ations	Dose – response	Admini-stration of test substance	Temp (°C)	pH	Hardness (mg/l)	DOC (mg/l)	Test water	Ag-back-ground	Quality of the study	Reference
$AgNO_3$	*Oncorhynchus mykiss*	n.r.	28d	Growth rate	NOEC LOEC	0.7 1.7	Measured	Yes	Flow-through	15.5-17.5	n.r	140	n.r. 1*	Tap water	n.r.	Q2	Galvez et al., 1998 Ag-93

*estimated DOC

3.2.2 Toxicity to freshwater invertebrates

Few chronic toxicity studies are performed with invertebrates exposed to Ag. Data on single-species toxicity tests for invertebrates are given in

Table 3. More detailed information on the test method of the individual studies is given in the Annexes. For PNEC derivation either NOEC or L(E)C_{10} are used.

The only reliable chronic study of silver exposed to invertebrates is reported by Bielmyer et al. (2002). Bioassays with neonates (<24h) lasted until 60% of surviving control organisms had three broods of offspring (~ 8d). Nominal concentrations were 0.001, 0.01, 0.1, 1, and 10 µg/L. Control medium was US EPA moderately hard water. The NOEC for mortality was 0.1 µg/L. This study was considered reliable with restrictions (Q2) since 0.1 µg/L was undetectable on the atomic absorption (detection limit 2 µg/L) and the value is derived from a figure. The NOEC for reproduction was 0.001 and the LOEC 0.01 µg/L. This endpoint was not considered reliable (Q3) as the lowest test concentration 0.001 µg/L is a factor 1000 under the detection limit and no information is given about dose-response relationship or control reproduction.

Table 3: Overview of chronic endpoints for invertebrates

Substance	Species	Age and/or size of test organism	Test duration	Effect parameter	End-point	Value (µg/l)	Analysis of concen-trations	Dose – response	Admini-stration of test substance	Temp (°C)	pH	Hardness (mg/l)	DOC (mg/l)	Test water	Ag-back-ground	Quality of the study	Reference
$AgNO_3$	*Daphnia magna*	Neonates	21d	Reproduction	LOEC	≤ 5	Measured	No	Static renewal	20	7.5	115	n.r. 1*	Reconstituted water	n.r.	Q3	Bianchini and Wood, 2002 Ag-52
$AgNO_3$	*Ceriodaphnia dubia*	<24h	8d	Mortality Reproduction	NOEC NOEC LOEC	0.1 0.001 0.01	Nominal (under detection limit)	Yes	Static renewal	25	7.4-7.8	80-100	n.r. 1*	EPA moderately hard water	n.r.	Q2 Q3	Bielmyer et al., 2002 Ag-64

*estimated DOC

3.2.3 Toxicity to freshwater algae and higher plants

No chronic experiments (NOEC or L(E)C_{10}) are reported with algae and higher plants exposed to silver. Only acute toxicity tests (EC_{50} and IC_{50}) with algae are available (see classification).

3.3 PNEC DERIVATION

Table 4 summarizes all chronic toxicity data that originate from Q1/Q2 studies and that can be used for the derivation of the PNEC.

Table 4: Overview of Q1/Q2 chronic Ag-toxicity data in the aquatic environment for HC_5 and PNEC-derivation

Substance	Species	Endpoint	Value (µg/l)	Chemical analysis*	pH	Quality of the study	Reference
$AgNO_3$	*Oncorhynchus mykiss*	+/-30d-NOEC mortality	0.098	Measured	7.5	Q1	Brauner and Wood, 2002 (1)
$AgNO_3$	*Oncorhynchus mykiss*	18m-NOEC mortality	0.09	Measured	7	Q1/Q2	Davies et al., 1978
$AgNO_3$	*Oncorhynchus mykiss*	23d-NOEC growth rate	3.02	Measured	8	Q2	Galvez and Wood, 2002
$AgNO_3$	*Oncorhynchus mykiss*	64d-NOEC mortality	0.1	Measured	7	Q1	Morgan et al., 2005
$AgNO_3$	*Oncorhynchus mykiss*	51d-NOEC mortality	0.14	Measured	7.5-8	Q1	Brauner and Wood, 2002 (2)
$AgNO_3$	*Oncorhynchus mykiss*	23d-NOEC mortality	1.2	Measured	7.5	Q1	Guadagnolo et al., 2001
$AgNO_3$	*Oncorhynchus mykiss*	28d-NOEC growth rate	0.7	Measured	n.r.	Q2	Galvez et al., 1998
$AgNO_3$	*Ceriodaphnia dubia*	8d-NOEC mortality	0.1	Nominal (under detection limit)	7.4-7.8	Q2	Bielmeyer et al., 2002

*when no indication is given if measured concentrations are dissolved or total it is assumed to be total silver concentrations

Section 3.1.3 describes the derivation of the PNEC using assessment factors, which is reported in the TGD (2003). For silver, only two reliable long-term NOECs from species representing two trophic levels (fish and Ceriodaphnia) are available. Therefore, an assessment factor of 50 is used.

The lowest value is used to determine the PNEC. If for one species several chronic NOEC values based on the same toxicological endpoint are available, these values are averaged by calculating the geometric mean, resulting in the "species mean" NOEC. Mortality is a more sensitive endpoint than growth rate for *Oncorhynchus mykiss*. The geometric mean from the different NOECs with as endpoint mortality is 0.17 µg silver/L. The lowest value to determine the PNEC is the 8d-NOEC value of 0.1 µg/L for *Ceriodaphnia dubia* (Bielmeyer et al., 2002).

PNEC= 0.1 ug/L (lowest NOEC)/50 (assessment factor) = 0.002 µg/L or 2 ng/L.

4 WATER CHARACTERISTICS OF EUROPEAN FRESHWATER BODIES

4.1 SOURCES OF EXPOSURE DATA

Physicochemical properties of surface waters that were monitored in various surveys throughout Europe were gathered and used for the construction of frequency distributions. The key water characteristics that were taken into account were pH, dissolved organic carbon and hardness (Figure 2 to Figure 4). Based on these distributions relevant ranges in EU-surface waters for each physicochemical characteristic were derived and compared with the physicochemical properties of the test media in which reliable Ag-effects data were generated. Ranges were selected in such a way that they covered 90% of all data available for a specific parameter (i.e., based on the 5th and 95th percentile).

Data on the EU freshwater characteristics were obtained for the following regions and sources: Belgium, Germany, The Netherlands, Sweden, United Kingdom and Spain. An additional source is the FOREGS-database, which is the outcome of an international monitoring survey for the determination of baseline levels of numerous elements and parameters in water, sediment and soil.

Belgium

The monitoring data from the Flemish surface waters were obtained from the Flemish Environment Agency (VMM, Vlaamse MilieuMaatschappij). The database contains information on more than 1000 stations spread over 6 different river systems in Flanders. Data were collected between 1989 and 2003. The monitoring data for the Walloon region were generated and reported by the Scientific Institute for Public Services (ISSeP). The database includes 62 stations representing 42 rivers and 4 different water catchments areas (Scheldt, Meuse, Rhine and Seine). Each station was sampled 6 to 12 times in 2000. From the Belgian databases 12,884 individual data points on pH, 1931 on DOC and 1944 on hardness were collected.

Germany

The German monitoring data included in this report, originate from the Wassergütestelle Elbe (Hamburg) and are only representative for the river Elbe. Eleven locations were monitored monthly or bimonthly in 1996 and 2000. From this German database 362 individual data points on pH, 325 on DOC and 263 on hardness were collected.

The Netherlands

The monitoring data from the Netherlands were gathered by the Rijkswaterstaat (RWS; executive organisation of the Dutch Ministry of Transport, Public Works and Water Management), and represent 25 different rivers and stations throughout the Netherlands. Data are available for the period 1990-2000. From this Dutch database 1510 individual data points on pH and 821 on DOC were collected.

Sweden

The Swedish monitoring data in SWAD were gathered from the Swedish University of Agricultural Science. Various national, regional and reference surface waters (incl. the four large Swedish lakes) were monitored between 1995 and 2001. From this Swedish database 3428 individual data points on pH, 2952 on DOC and 3192 on hardness were collected.

United Kingdom

The data for the United Kingdom were collected in the framework of "The Harmonised Monitoring Scheme" a monitoring campaign that is conducted since 1974 and which covers the whole of England

and Wales. The database contains the measurements of 143,079 samplings obtained at 275 different locations and 195 rivers. Up to 116 different parameters were determined, including physico-chemical parameters. From this UK database 3583 individual data points on pH, 1146 on DOC and 3075 on hardness were collected.

Spain

The COMMPS database reports data on the physico-chemical characteristics of Spanish freshwaters in 1997. From this Spanish database 5901 individual data points on pH were collected.

4.2 FREQUENCY DISTRIBUTIONS FOR KEY WATER CHARACTERISTICS

A summary of the calculated percentiles for the main parameters is presented in Table 5. A distinction between FOREGS and the other data sets has been made, as the latter data sets are relevant for ambient levels, whereas FOREGS aimed at baseline levels. Based on the values shown in Table 5, however, can be concluded that little difference exists between both data sets. Discussion of the different parameters is based on the ambient levels.

All relevant data gathered from the different databases were incorporated in the frequency distributions that were constructed for typical key water characteristics: pH, dissolved organic carbon and hardness (Figure 2, Figure 3 and Figure 4, respectively). These distributions were then used for the estimation of relevant ranges in EU-surface waters for each physicochemical characteristic. Ranges were selected in such a way that they covered 90% of all data available for a specific parameter (i.e., based on the 5th and 95th percentile).

- Hardness

The hardness content of most EU freshwaters (90%) ranges between 37.4 and 323.3 mg/l $CaCO_3$ (range as 10th and 90th percentile, see (Table 5) as shown in Figure 2. A typical hardness (50th percentile) of 99.4 mg/l $CaCO_3$ was estimated in EU freshwaters. Hardness levels in the test media that are described in Tables 2 and 3 are situated between 2 and 400 mg/L as $CaCO_3$. For fish, only the test media reported by Davies et al (1978) (hardness of 27.5 mg/L as $CaCO_3$) and by Morgan et al (2005) (hardness of 2 and 400 mg/L as $CaCO_3$ are beyond the 90% range for EU surface waters. All other test media (fish and invertebrates) had a hardness that was close to the EU-median of 99.4 mg/L as $CaCO_3$ (80-150 mg/L as $CaCO_3$). The hardness of most the test media that are associated with the toxicity data included in the Ag-effects database are therefore relevant for EU-surface waters

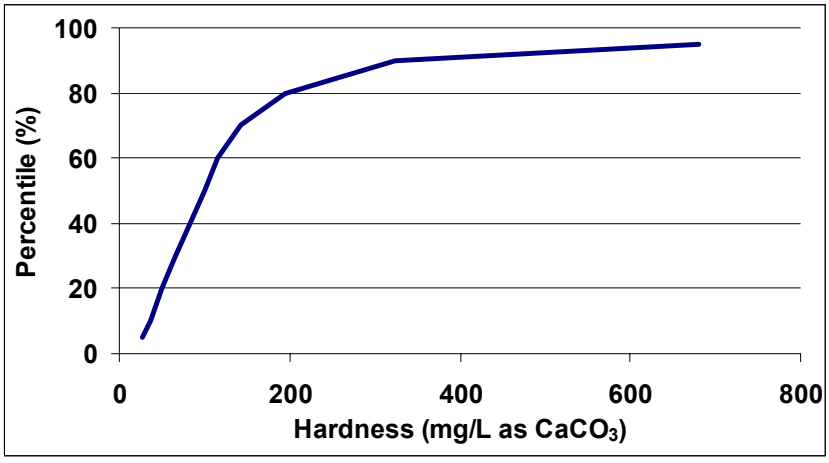

Figure 2: Frequency distribution of the observed hardness in EU freshwaters.

- pH

The pH of 90% of EU freshwaters ranges between 6.2 and 8.3 (range as 5th and 95th percentiles, see (Table 5) as shown in Figure 3. A typical pH value (50th percentile) of 7.5 has been derived for EU freshwaters. pH-levels of the test media were situated between 7 and 8, so it can be concluded that the collected effects data were generated in test media with pH-levels that are relevant for EU-surface waters.

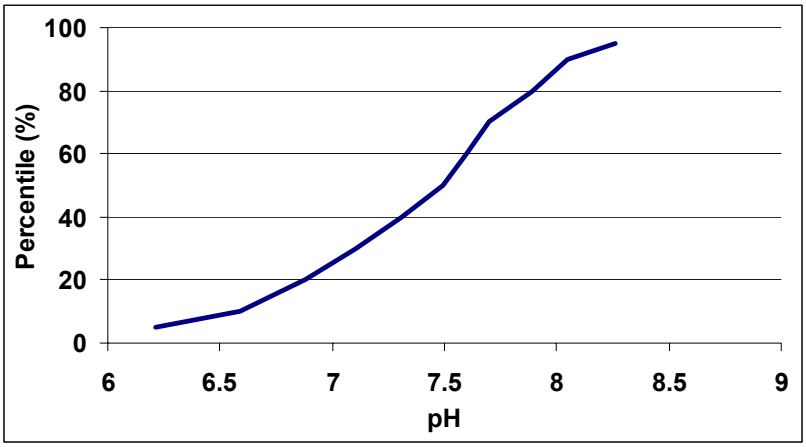

Figure 3: Distribution of the observed pH in EU freshwaters.

Table 5: Range of physico-chemical parameters in European freshwaters.

Parameter	5th percentile	10th percentile	50th percentile	90th percentile	95th percentile
EU-monitoring data sets					
pH	6.2	6.6	7.5	8.1	8.3
DOC (mg/L)	2.1	2.6	6.4	12.4	15.2
Hardness (mg/L)	27.0	37.4	99.4	323.3	680
FOREGS-data set					
pH	6.10	6.40	7.70	8.30	8.50
DOC (mg/L) [(1)]	--	--	4.99	17.0	--
Ca (mg/L)	1.7	2.8	40.2	118.5	146.6
Mg (mg/L)	0.5	0.7	6.0	27.1	37.9
Hardness (mg/L)	6.1	9.9	125	406	521.8

[(1)]: data taken from FOREGS-website

- Dissolved organic carbon

The dissolved organic carbon range that covers 90% of EU-surface waters is situated between 2.1 and 15.2 mg/L (range based on 5th and 95th percentile, (see Table 5) as shown in Figure 4. A typical DOC concentration in EU freshwaters (50th percentile) of 6.4 mg/L was estimated. DOC-levels were not always reported in the original publications, but for those test media where DOC was measured, values ranged between 0.5 and 2.86 mg/L. These values are situated at the lower end of the distribution, and under the

conditions the reported NOECs represent worst case Ag-toxicity in EU-surface waters (no toxicity reduction resulting from Ag-DOC complexation).

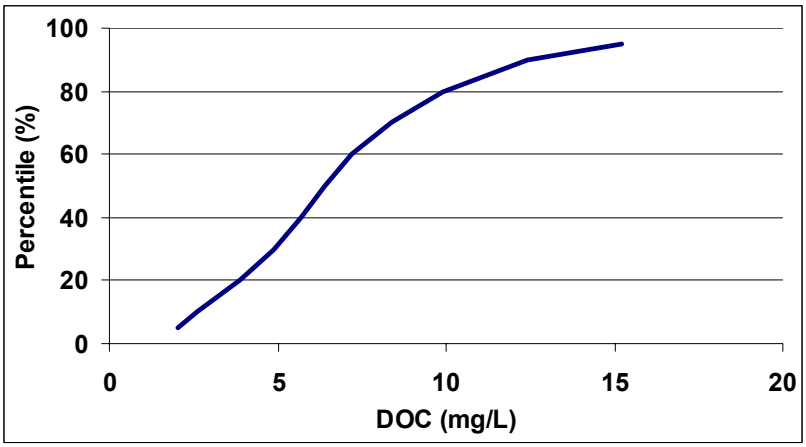

Figure 4: Distribution of the observed DOC content in EU freshwaters.

5 DATA GAPS AND RECOMMENDATIONS

It is proposed to refine the effects assessment in a tiered approach, as suggested in Figure 5. Tier 1 of this approach is to perform toxicity testing in order to fulfil REACH requirements. Silver will have to fulfil the requirements for more than 1000 tonnes/y. For risk assessment purposes this can be translated to the calculation of a PNEC according to the Assessment Factor (AF) methodology. In case the use of this approach leads to the identification of a potential risk, additional testing can be performed, leading to the development of a Species Sensitivity Distribution (SSD). From this SSD it is possible to determine the $HC_{5,50\%}$ with 5%-95% confidence interval (i.e., 5^{th} percentile, based on Monte-Carlo analysis), and this value should be used for PNEC-derivation (i.e., PNEC = HC5,50% / Assessment Factor). The applied AF-value will depend on the uncertainty that is associated with the derived $HC_{5,50\%}$.

If the Tier 2 approach still leads to the identification of potential risks, it can be considered to develop bioavailability models (e.g., Biotic Ligand Models) which allow a) the incorporation of bioavailability into the effects assessment and b) the derivation of site/region specific PNEC values (Tier 3). When possible risks are identified in the previous tier, it is recommended to develop alternative approaches (e.g. field studies) or consider risk management measures (Tier 4).

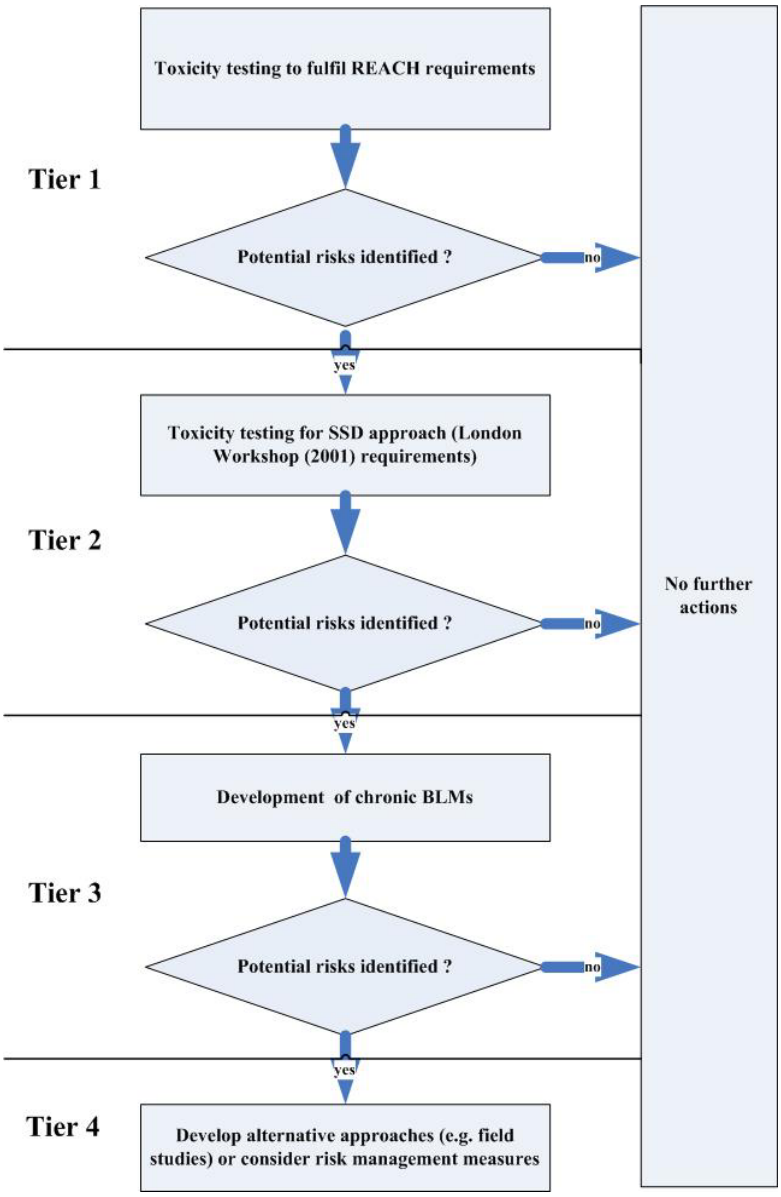

Figure 5: Tiered approach gathering toxicity data within the effects assessment framework**.**

Table 6 reports the different toxicity testing necessary to fulfil REACH requirements (Tier 1). This reasoning only considers testing prescribed for >1000 tonnes/y chemicals (REACH annexes VI-X) and does not take into account possible exemptions for testing (e.g. exposure-based waiving) or intelligent testing strategies (read-across, 'upper threshold concentration step-down approach' to limit fish tests, qsars…).

Table 6: REACH requirements (> 1000 tonnes/y)

REACH requirements	**Ag-database**
short-term toxicity testing on invertebrates (preferred species *Daphnia*) (> 1 tonne/y)	Fulfilled, see classification report
growth inhibition study aquatic plants (algae preferred) (> 1 tonne/y)	No reliable data available in the Ag-database
short-term testing on fish (> 10 tonne/y)	Fulfilled, see classification report
long-term toxicity testing on invertebrates (preferred species *Daphnia*) (>100 tonnes/y)	Fulfilled, (*Ceriodaphnia* sp.)
long-term toxicity testing on fish (fish early-life stage (FELS) toxicity test; fish short-term toxicity test on embryo and sac-fry stages; fish juvenile growth test) (> 100 tonnes/y)	Fulfilled, (*Oncorhynchus mykiss*)

The requirements for REACH are fulfilled, except for the algae growth inhibition test. In order to fulfil all REACH requirements, data has to be gathered for algae growth inhibition study.

Algal toxicity data are lacking, resulting in an assessment factor (AF) of 50 on the lowest NOEC (LC_{10}) for invertebrates/fish for $PNEC_{aquatic}$ derivation. An algal NOEC needs to be generated in order to reduce the AF to 10.

Please note that both long-term toxicity tests with fish and invertebrate not report dissolved silver concentrations. The invertebrate test only relies on nominal values and the fish test on measured total silver concentrations.

Initially, all REACH requirements should be performed. Further, when a potential risk is identified in the Tier 1 approach, it is recommended that a SSD is developed. With regard to the minimum species requirements when using the SSD approach for the aquatic compartment the London workshop (2001) formulated some recommendations. The SSD should cover at least 8 taxonomic groups containing at least 10 NOECs (preferably more than 15) for different species (**From** the extracted data, it is clear that the Ag-database does not fulfil the requirement of 10-15 different NOEC values. Only two different species NOEC values were compiled from the Q1/Q2 database. The London Workshop (2001) defined 8 different taxonomic groups that should be included in the effects database (Fehler! **Ungültiger Eigenverweis** auf Textmarke.). However, the current database includes organisms for only two of these categories: a fish and a crustacean.

Table 7). In reality for some metal/metal compounds it will be difficult to obtain 10 NOEC data. In those cases an SSD could still be constructed as long as the associated sampling uncertainty with the HC_5 estimate is properly quantified.

From the extracted data, it is clear that the Ag-database does not fulfil the requirement of 10-15 different NOEC values. Only two different species NOEC values were compiled from the Q1/Q2 database. The London Workshop (2001) defined 8 different taxonomic groups that should be included in the effects database (**Fehler! Ungültiger Eigenverweis auf Textmarke.**). However, the current database includes organisms for only two of these categories: a fish and a crustacean.

Table 7: Minimum taxonomic group requirements for the derivation of the PNEC water (freshwater) using the statistical extrapolation technique

	Taxonomic groups	**Ag-database**
1	Fish (usually tested species like salmons, bluegill, channel catfish, etc.)	Fulfilled, (*Oncorhynchus mykiss*)
2	A second family in the phylum Chordata (fish, amphibian, etc.)	Not included in the Ag-database
3	A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)	Fulfilled, (*Ceriodaphnia* sp)
4	*An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)*	Not included in the Ag-database
5	A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.)	Not included in the Ag-database
6	A family in any order of insect or any phylum not already represented	Not included in the Ag-database
7	Algae	Not included in the Ag-database
8	Higher plants	Not included in the Ag-database

The Tier 3 approach should be performed when a risk is characterized in Tier 2. This approach includes the development of a chronic BLM. When possible risks are identified in the previous tier, it is recommended to develop alternative approaches (e.g. field studies) or consider risk management measures (Tier 4).

In conclusion, research should be focused first on the development of all the requirements of REACH for toxicity testing. This will include the development of reliable toxicity data for an algae growth inhibition test.

6 REFERENCES

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7 ANNEXES

Reference:	Bianchini and Wood, 2002
Type of test:	Chronic invertebrate test/static renewal
Species	*Daphnia magna*
Exposure period:	21d
Unit:	µg/L
NOEC:	LOEC <5 (reproduction)
E(L)C50:	
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	ASTM, 1997
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Adult gravid *Daphnia magna* were obtained from Aquatic Research Organisms (ARO, Hampton, NH, USA). Neonate daphnids were chronically exposed to a single concentration (total measured= 5.0 +/-0.04 µg Ag/L) of silver for 21 days. Test procedures followed the ASTM standard guide for conducting *Daphnia magna* life-cycle toxicity tests (ASTM, 1997). 10 neonates were individually exposed in 50 mL of synthetic water (20°C).
Result:	Q3: unbounded value
pH	7.5
DOC (mg/L)	NR
Ca (mg/L)	40
Mg (mg/L)	3.6
Na (mg/L)	13.8
Total Hardness (mg/L)	115

Reference:	Bielmyer et al., 2002
Type of test:	Acute and chronic invertebrate test/static renewal
Species	*Ceriodaphnia dubia*
Exposure period:	96h, 8d
Unit:	µg/L
NOEC:	8d-NOEC= 0.1 (endpoint=mortality) 8d-NOEC= 0.001; 8d-LOEC= 0.01 (endpoint=reproduction)
E(L)C50:	96h-LC50= 0.5; 8d-LC50= 0.32
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Bioassay procedures followed US EPA short-term methods. Each test was initiated with <24h-old neonates randomly placed in 30 mL cups filled with 15 mL of test solution. Control medium was US EPA moderately hard water (MHW) (25°C). Nominal concentrations were 0.001, 0.01, 0.1, 1, and 10 µg/L. Media were changed at approximately the same time each day. Following renewal, mortality and fecundity were recorded. Each treatment was replicated 10 times. Bioassays lasted until 60% of surviving control organisms had three broods of offspring (~ 8d). LC50 values were determined using trimmed Spearman-Karber analysis. Reproduction data were also analysed, generating NOEC and LOEC values, using an analysis of variance (ANOVA) followed by Dunnett's test.
Result:	Q2 for chronic mortality test since value is derived from figure and 0.1 µg/L is under the detection limit. Q3 for chronic reproduction test since lowest test concentration is 0.001 µg/L and this concentration is undetectable on the atomic absorption (detection limit 2 µg/L) and no information is given on dose-response relationship and control mortality.
pH	7.4-7.8
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR

Na (mg/L)	NR
Total Hardness (mg/L)	80-100

Reference:	Brauner and Wood, 2002 (1)
Type of test:	Chronic fish test/flow-through
Species	Rainbow trout *Oncorhynchus mykiss*
Exposure period:	Fertilization to 1 week post-hatch (+/- 30d)
Unit:	µg/L
NOEC:	NOEC 0.1 LOEC 1 Endpoint is mortality
E(L)C50:	
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Freshly fertilized rainbow trout eggs were obtained from Rainbow Trout Hatchery. Three hours following fertilization, embryos were randomly distributed to one of three flow-through silver exposure conditions, nominally of 0 (below the detection limit of 0.05 µg/L), 0.1 µg/L (measured= 0.098 µg/L) and 1 µg/L (measured= 0.85) total silver, as $AgNO_3$ with flow rates of 100 ml/min (in dechlorinated Hamilton hard water, 13.7°C). Embryos were exposed to all silver concentrations in triplicate, with 300 embryos in two of the three replicates and 600 in the third replicate which was the source for embryos sampled throughout the experiment. Mortalities were recorded. Early in the development, the criterion for mortality was opaqueness of embryos while later in development it was cessation of heartbeat. ANOVA was used for testing of statistical significance.
Result:	

pH	7.5
DOC (mg/L)	1.3
Ca (mg/L)	40
Mg (mg/L)	4.86
Na (mg/L)	13.8
Total Hardness (mg/L)	120

Reference:	Brauner and Wood, 2002 (2)
Type of test:	Chronic fish test/flow-through
Species	*Oncorhynchus mykiss*
Exposure period:	51d
Unit:	µg/L
NOEC:	NOEC= 0.1 LOEC= 10 Endpoint= mortality
E(L)C50:	
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$

Test method:	Freshly fertilized rainbow trout embryos were purchased from Rainbow Springs Trout Hatchery. Within 3 h following fertilization, embryos were randomly distributed to one of three flow-through silver exposure concentrations (nominal of 0, 0.1, and 10 µg/L total silver; measured of <0.05, 0.14, 10.7 µg/L total silver) in dechlorinated Hamilton hard water (12°C). Embryos were exposed in duplicate, with 200 embryos in the first replicate (0.5 l chambers) and 700 embryos in the second replicate (2 l chambers), the latter of which was the source for embryos and larvae terminally sampled throughout the experiment. Embryos were kept in dark during development. Flow-through of 100 mL/ min. Mortalities, %hatch, %swim-up were calculated. Differences in mortality were tested using a one-way ANOVA on the number of mortalities per day (i.e. on non-cumulative mortality).
Result:	Chronic exposure to 10 µg/L total silver (nominal), but not to 0.1 µg/L total silver, greatly increased mortalities relative to controls.
pH	7.5-8
DOC (mg/L)	2.86
Ca (mg/L)	40
Mg (mg/L)	4.86
Na (mg/L)	13.8
Total Hardness (mg/L)	120

Reference:	Davies et al., 1978
Type of test:	Acute and chronic fish test/flow-through
Species	*Salmo gairdneri* (*Oncorhynchus mykiss*, rainbow trout)
Exposure period:	96h, 18months
Unit:	µg/L
NOEC:	18m-NOEC= 0.09 (endpoint is egg survival/mortality)
E(L)C50:	96h-LC50= 6.5 (soft water); 13 (hard water)
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR

GLP:	NR
Test substance:	$AgNO_3$
Test method:	Acute test: Four different 96h- flow-through toxicity tests were performed. Three soft-water experiments (mean hardness: 26 mg/L $CaCO_3$) and one hard-water experiment (hardness- 350 mg/L $CaCO_3$). Each experiment tested five different silver concentrations and a control with a replicate of each. Nominal silver concentrations for the soft-water experiments were: 0, 2.5, 5, 10, 20 µg Ag/L. Nominal silver concentrations for the hard-water experiments were: 0, 5, 10, 20, 40, 80 µg Ag/L. Each aquarium contained 10 rainbow trout of the same size and age which were hatched from the same lot of eggs. The mean size of fish for the four experiments were 69, 146, 173, and 167 mm, respectively. LC50's were determined by log-probit analysis. Mean temperature for soft-water tests was 11.5°C and for hard-water test 15.5°C. Chronic test: the toxicity tests were initiated with 300 eyed eggs per concentration. Nominal concentrations for the first experiment were 0, 0.6, 1.2, 2.5, 5, 10 µg Ag/L. This experiment was terminated after 10 weeks because of excessive mortalities in the three highest concentrations. A second experiment was initiated to more closely define the "no effect" range for silver. Nominal concentrations were 0, 0.06, 0.12, 0.25, 0.5, 1 µg Ag/L. Endpoints were egg survival, hatching success, and growth. The test was conducted at the ambient temperature of dechlorinated Fort Collins city water (tap water) which ranged from 3°C in winter to a summer high of 17°C. Mean temperature was 11.1 °C.
Result:	Chronic experiment was conducted from 3 to 17°C. This looks like a mesocosm study which reflects the environment more then a laboratory test. This study is assigned as highly reliable (Q1).
pH	Acute soft water experiment: 6.7 Acute hard water experiment: 8 Long term experiment: 7
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	Acute soft water experiment: 26 Acute hard water experiment: 350 Long term experiment: 27.5

Reference:	Galvez and Wood, 2002
Type of test:	Acute and chronic fish test/flow-through
Species	Juvenile *Oncorhynchus mykiss* (rainbow trout)
Exposure period:	96h, 7d, 23d
Unit:	µg/L
NOEC:	23d-NOEC= 3 (endpoint specific growth rate) 23d-LOEC= 5 (endpoint specific growth rate)
E(L)C50:	96h-LC50= 7.6-15.1 7d-LC50= 9.9-22.4
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Juvenile rainbow trout were purchased from Humber Springs Trout Hatchery (Orangeville, Canada). All fish were marked 2 weeks before the start of the experimental exposures. Chronic test: Experiment 1 tested the nominal total Ag concentrations of 0.1 and 1 µg/L. Total Ag concentrations were measured daily. The measured concentrations were control (<0.05 µg/L), low silver exposure: 0.2 +/-0.05 µg/L (n=24), high silver exposure: 1.04 +/-0.43 µg/L (n=24). Experiment 2 tested the nominal Ag concentrations of 3 and 5 µg/L. The measured concentrations were control (<0.05 µg/L), low silver exposure: 3.02 +/-0.1 µg/L (n=24) and high silver exposure: 4.81 +/-0.28 µg/L (n=24). Mean fish weights were calculated on days 0, 5, 10, 15, and 23. Specific growth rates (SGR) in percentages per day were calculated for each treatment from the slope of the least-squares regression through the natural logarithm (ln) transformed weight versus time data (SPSS). Toxicity test: five Ag concentrations in dechlorinated Hamilton tap water (15°C) plus a simultaneous control was used. 60 fish were randomly distributed into the six test containers. The nominal Ag concentrations used for the lethality tests included: 10, 18, 32, 56, and either 5.6 or 100 µg/L (nominal values). The 96h and 168h LC50 were calculated by log probit analysis using measured total aqueous Ag concentrations.

Result:	Since all fish were marked before the start of the test (Q2). For the chronic test 2 tests were performed since no effects were observed in the first test.
pH	8
DOC (mg/L)	1.3
Ca (mg/L)	40
Mg (mg/L)	NR
Na (mg/L)	14.4
Total Hardness (mg/L)	120

Reference:	Galvez et al., 1998
Type of test:	Chronic fish test/flow-through
Species	*Oncorhynchus mykiss*
Exposure period:	28d
Unit:	µg/L
NOEC:	NOEC= 0.5 LOEC= 2 Endpoint= growth rate
E(L)C50:	
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$

Test method:	Juvenile rainbow trout (3.01 +/- 0.27 mg?) were purchased from Rainbow Springs Hatchery (Thamesford, Ontario). Nominal total silver concentrations tested were 0.5 and 2.0 µg/L. Measured Ag concentrations were 0.7 +/-0.2 and 1.7 +/-0.4 µg/L, respectively. For the 0.5 µg/L Ag exposure, 450 fish were randomly selected from the original stock and divided into two equal groups of 225. For the 2.0 µg/L exposure, 300 fish were divided into two equal groups of 150. In all cases, fish were placed in 400 L tanks, each supplied with 1000 mL/min of dechlorinated tap water (15.5-17.5°C). Fish weights obtained at each sampling period (n=30) were used as an index of fish growth over the course of the experiment. Growth was expressed as average weight per fish. Mean values of both Ag-exposed groups were statistically compared to their simultaneous controls using Student's two-tailed t-test.
Result:	
pH	8
DOC (mg/L)	NR
Ca (mg/L)	40
Mg (mg/L)	4.86
Na (mg/L)	13.8
Total Hardness (mg/L)	140

Reference:	Guadagnolo et al., 2001
Type of test:	Chronic fish test/flow-through
Species	*Oncorhynchus mykiss*
Exposure period:	32d
Unit:	µg/L
NOEC:	NOEC= 1.2 LOEC= 13.5 Endpoint= mortality
E(L)C50:	
Limit test:	No

Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Freshly fertilized rainbow trout eggs were obtained from Rainbow Springs Trout farm (Thamesford, ON, Canada). Because embryos are photosensitive early in development, they were kept in opaque chambers. Over a period of 32d, from 2 to 3h postfertilization to hatch, 1,000 embryos were exposed to 0, 0.1, 10 µg/L total silver (nominal values) plus a control (<0.05 µg/L). Test medium was dechlorinated Hamilton tap water (12.2°C) and flow rate was 140 mL/min. Criteria for embryo death was cessation of heartbeat. Mortality between treatments was tested for statistical significance using a one-way analysis of variance on the number of mortalities per day (i.e. on noncumulative mortality).
Result:	Q2: 33% mortality in control embryos. OECD 210 overall survival of fertilized eggs in the control: hatching success > 66%.
pH	7.5
DOC (mg/L)	1.3 (DOM)
Ca (mg/L)	40
Mg (mg/L)	4.8
Na (mg/L)	13.8
Total Hardness (mg/L)	120

Reference:	Morgan et al., 2005
Type of test:	Chronic fish test/flow-through
Species	*Oncorhynchus mykiss* (rainbow trout)
Exposure period:	64d (fertilization to swim-up)
Unit:	µg/L
NOEC:	NOEC= 0.1; LOEC=1 (for hardness: 2, 150, and 400 mg/L) Endpoint= daily % mortality, following 50% hatch

E(L)C50:	
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Approximately 15,000 freshly fertilized rainbow trout eggs were obtained from the Rainbow Springs Trout Hatchery (Thamesford, Canada). Eggs were distributed to exposure containers and exposed in three different water hardnesses and to three different silver concentrations (nominal concentrations of 0, 0.1, and 1 µg/L). Measured total silver concentrations are reported for the three different silver concentrations at the three different hardnesses tested in the original paper in Table 1. The measured water total silver concentrations were generally within 20% of the nominal concentrations. For each treatment, two exposure containers were used with tap water of 11°C. The first exposure container contained 500 eggs (in 4L water) and the second contained 500 eggs (in 2L water). The containers were covered. Mortality, percent hatch, and percent swim-up were determined daily. Statistics: one-way analysis of variance on the number of mortalities per day, followed by a Turkey's post hoc test.
Result:	
pH	7
DOC (mg/L)	0.5
Ca (mg/L)	0.8
Mg (mg/L)	0
Na (mg/L)	1.2
Total Hardness (mg/L)	Tested concentrations: 2, 150, and 400