Ag classification report

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1 CLASSIFICATION

The existing classification schemes for substances can be used in a straightforward manner for soluble metal compounds by applying the current classification criteria as described in the Globally Harmonized system of Classification and Labelling of Chemicals (GHS, 2003). In this scheme, the evaluation of both short term and long term aquatic hazard potentials of the metal under investigation are achieved using appropriate standard ecotoxicity data as determined with the soluble metal salt (acute and chronic values) and comparison with the classification cut-off values (1-100 mg/L). The ecotoxicity of soluble inorganic silver compounds is dependent on the physico-chemistry of the medium, irrespective of the original metal species released in the environment. Reading across inorganic silver compounds can therefore be done by comparison of the soluble metal ion concentration (mg Ag/L) causing the appropriate standard ecotoxicity effect (acute, chronic) and translation of the results towards the compound under investigation using the molecular weight ratio (MW substance/MW metal ion) (GHS, 2003).

Classification and labelling of chemical compounds within REACH is intended to be in line with the Global Harmonized System (GSH) (UN, 2003). The GSH is similar to the current EU system (EC, 1996, 2003) as it A) provides one single system for hazard classification and labelling; B) covers approximately the same hazards; C) often uses similar or equal classification criteria; D) sets up an equivalent system of hazard communication. Differences between the GHS and the current EU-system, however, do exist, e.g., it defines further hazard classes and categories and categorizes some hazards in more than one class.

The solubility and bioavailability differs for silver compounds, whereas silver metal is not soluble and needs to be transformed to become bioavailable. Therefore, soluble silver compounds and silver metal should be evaluated in a different way, i.e., in accordance to the EU hazard classification system:

- Soluble silver species: ecotoxicity data obtained from tests carried out with soluble silver species are directly used for classification
- Silver powders and silver massives: ecotoxicity data obtained from tests carried out with soluble silver species are compared to the outcome of transformation/dissolution (T/D) tests and the need for classification is evaluated.

It should be noted that the followed methodology in this section is in line with the guidance given the Fact Sheets of the MERAG-document (Metals Environmental Risk Assessment Guidance, 2007), and this methodology may deviate from the OECD Guidance in some points: the latter guidance is focussed on the assessment of (organic) chemical compounds in general, and some of the concepts are not relevant for metals due to their specific properties, chemical behaviour and their role in the environment.

In this study only toxicity data generated with soluble silver salts as added toxicant are considered. It is assumed that all these salts are readily soluble and that the observed toxicity is only caused by the dissolved silver ion.

1.1 SELECTION OF ECOTOXICOLOGICAL DATA FOR HAZARD CLASSIFICATION

The classification of silver is based on acute and chronic toxicity data for fish, crustacea and algae/aquatic plants, i.e., taxa generally accepted as representative for aquatic fauna and flora for hazard classification. These data can be drawn from data required for regulatory purposes (e.g., IUCLID) as well as the relevant literature and/or internationally recognised toxicity databases.
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.

1.1.1 Selection of ecotoxicological data for hazard assessment purposes

The quality of the data and extracted information from scientific literature may vary considerably between individual source documents. It is therefore essential that the compiled ecotoxicity data is evaluated with regard to the adequacy for classification purposes. In general this evaluation involves a review of individual data 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 collected data and the relevance of the data for environmental classification purposes.
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. These two basic elements have been defined by the Technical Guidance Document (TGD) (EC, 1996, 2003) 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 to be used for the derivation of an ecotoxicity reference value for classification purposes.

Only those ecotoxicity data that comply with both criteria can be considered valid, should be retained for the assessment; and should be used for ecotoxicological benchmarking. General guidance on how to screen and select the most appropriate data is outlined hereunder. A detailed description of all criteria is provided in the MERAG-document, Fact Sheet 8 "Classification" (MERAG, 2007).

Reliability criteria

- Soluble silver compounds have been used as test compound;
- Standard OECD approved tests are preferred but non standardized tests can also be considered as suitable when described and executed in a proper way;
- Tests should be performed according standard operational procedures, and a detailed description of the test method that was employed in the study should be provided (detailed information on test-set up, test material, test organisms, test design);
- A clear concentration-effects relationship should be observed, control response (mortality, reproduction, growth) should be in line with test criteria and an NOEC (chronic toxicity) or $L(E)C_{50}$ (acute toxicity) should be derived according to appropriate statistical methods;
- Only effect levels based on actual (i.e., measured) concentrations have been found reliable;
- If different $NOEC/L(E)C_{50}$ values were reported, measured dissolved values were used as first priority, measured total values were used as second priority.

Relevancy criteria

- Only data obtained for the standard OECD test species were selected as relevant to hazard classification;
- Only standardized OECD endpoints and exposure duration were selected:
  Fish: LC_{50} values from 96h exposure duration as acute endpoints and NOECs for mortality, growth and reproduction for >4 days exposure periods as chronic endpoints;

Invertebrates: LC_{50}-values from 48 hours exposure duration as acute endpoints and NOECs for mortality, growth and reproduction for >4 days exposure periods as chronic endpoints;
Algae: EC_{50} (biomass and growth rate) values (acute) and NOECs (chronic) from exposure duration of 72 hours. If both biomass and growth rates were reported, growh rate values were used for further evaluation;

- 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;
- Only relevant test media were considered: tests carried out in test media with pH range between 5.5 and 8.5 were selected for both the acute and chronic endpoints. No selection was based on hardness values;
- Culture conditions were not used as a selection criterium.

Acute aquatic toxicity would normally be determined using a fish 96 hour LC_{50} (OECD Test Guideline 203 or equivalent), a crustacea species 48 hour EC_{50} (OECD Test Guideline 202 or equivalent) and/or an algal species 72 or 96 hour EC_{50} (OECD Test Guideline 201 or equivalent). These three types or organisms are considered as surrogate for all aquatic organisms. Data on other species as the higher plant *Lemna* (duckweed) may also be considered if the test methodology is suitable.
Chronic toxicity data are in general less available than acute data and the range of testing procedures is less standardized. Data generated according to the OECD Test Guidelines 210 (Fish Early Life Stage), 211 (*Daphnia* reproduction) and 201 (Algal Growth Inhibition) can be accepted. Other validated and internationally accepted tests could also be used. The chronic parameter that should be used is the NOEC or L(E)Cx (e.g., EC_{10}).

1.1.2 Classification methodology

CLASSIFICATION OF TOXICOLOGICAL STUDIES (PUBLICATIONS, REPORTS) FOR HAZARD CLASSIFICATION (HC) PURPOSES

In general, it has been agreed that both freshwater and marine species toxicity data can be considered as equivalent data and are preferably to be derived using OECD Test Guidelines or equivalent according to the principles of GLP. If this type of information is not available, classification should be based on the best available data. Indeed, the use of toxicity data generated according to OECD Test Guideline only may result in a very limited amount of available 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 HC-purposes): Relevant studies, executed according to OECD Test Guidelines, and that comply with all criteria described in section 1.1.1: thorough description of standard test method and conditions using standard test species (algae, invertebrates, fish), results based on measured concentrations, and clear effect-concentration relationship that allows the derivation of a reliable LC_{50} or NOEC.

Category 2: Satisfactory quality data (Q2-data for HC-purposes): Relevant studies with standard for which no or insufficient information is given on one of the reliability criteria described in section 1.1.1:

- Toxicity test method not according to OECD Test Guidelines, but the study complies with all other criteria given in section 1.1.1;

or

- effect concentrations are based on nominal values;

or

- test conditions are not well documented but indication is given that conditions are relevant for the EU-freshwater environment

If evidence is given that the criterion for which information is lacking, does not meet the quality standards set in section 1.1.1 (e.g., nominal values are given, but precipitation of Ag is reported), this study should be categorised as Q3 (Low quality data)

Category 3: Low quality data (Q3-Data for HC 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 1.1.1. Finally, studies that could be categorised as Q2, but that only report toxicity data for an organism not belonging to the standard species, are also categorized as Q3-data. Information on toxicity data for non-standard test species can only be considered for classification if no adequate data for standard test species are available (e.g., the use of plant data if no algal data are available).

CLASSIFICATION CATEGORIES AND CRITERIA

As reported in MERAG (2007), the harmonized classification system for substances (GHS, 2003) consists of three acute classification categories and four chronic classification categories (see Figure 1). The acute and the chronic classification categories are applied independently. The criteria for classification of a substance in acute categories I to III are defined on the basis of the acute toxicity data only (EC_{50} or LC_{50}). The criteria for classification of substances into chronic categories combine two types of information, i.e. acute toxicity data and environmental fate data (degradability and bioaccumulation data).
According to the "Harmonized integrated classification system for human health and environmental hazards of chemical substances and mixtures" (OECD, 2001), substances that are classified under the following criteria will be categorized as 'hazardous to the aquatic environment'.

In case the solubility is limited (metals/alloys and sparingly soluble metal compounds (SSMC)) the classification strategy is based on the comparison of the T/D data with the selected ecotoxicity reference value. The crucial question that has to be answered is: at which loading rate is the concentration of the dissolved metal ion greater or equal to the derived ecotoxicity reference value (L(E)C_{50}-NOEC), based on the soluble metal ion concentration, adjusted for molecular weight as needed. In order to allow reading across physical metal forms, the Critical Surface Area concept can be used to enable self classification of powders and massives. For appropriate classification of massives/powders, further refinement consists of the comparison of the calculated critical particle size with normal handling and use. The presented scheme, taken from Fact Sheet 8 of the MERAG-document (MERAG, 2007), is built around the EU classification strategy for metals and metal compounds (67/548/EEC, annex 6 w L225/263) but GHS terminology has already been introduced to broaden the scope and to facilitate the comparison with the GHS scheme. It should be noted that due to the unclarity of the GHS classification text with regard to the implementation there are some items with option for discussion. This is in particularly so with the removal of the R53. At the moment the methodology as is used in the EU has been adapted in this section of the report.

A more in-depth discussion of the methodology in provided in Fact Sheet 8 ("Classification") of the MERAG-document (MERAG, 2007).

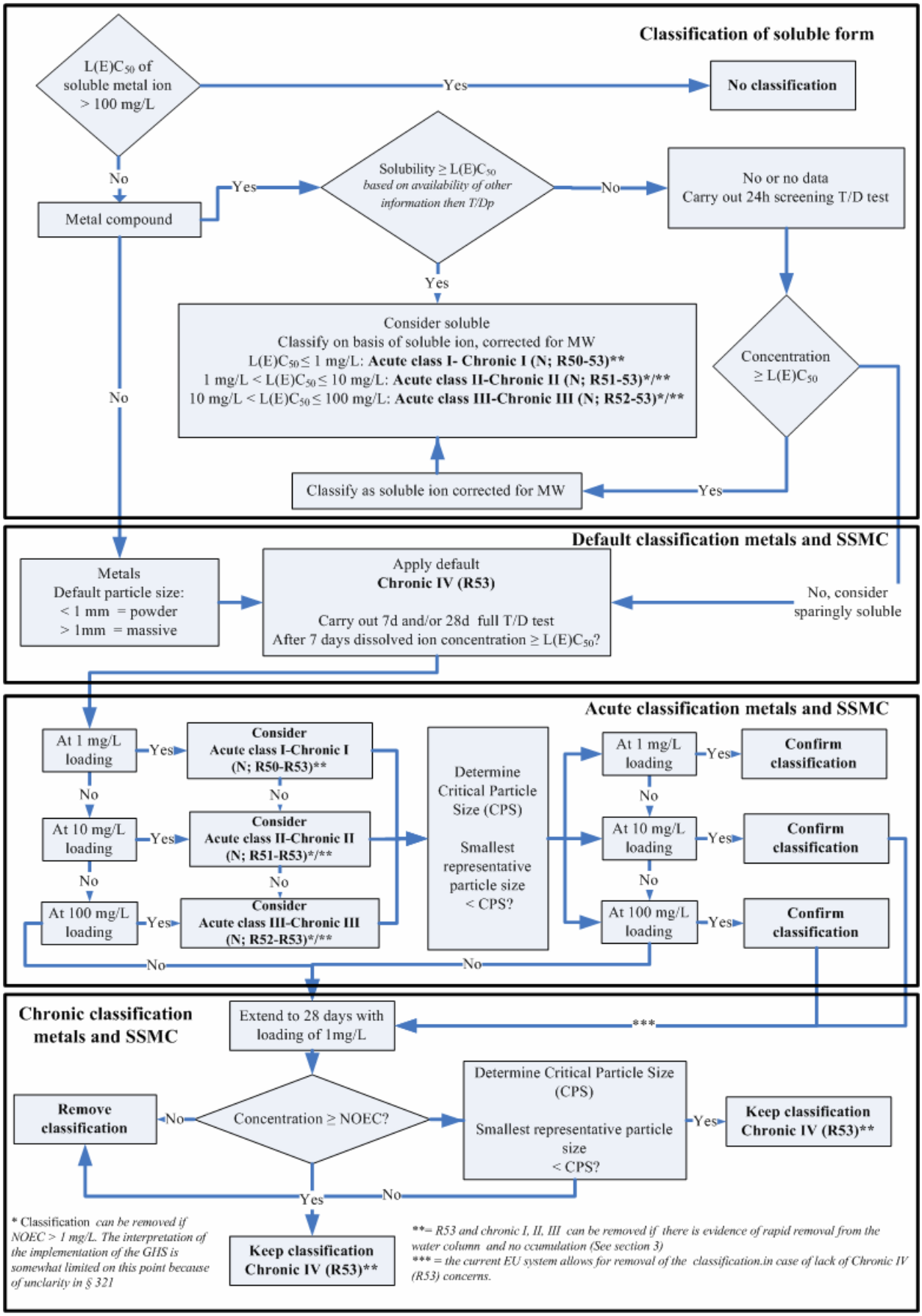

Figure 1: Classification strategy for metals and metal compounds

AGGREGATION OF L(E)C_{50}/NOEC DATA

In general, the following grouping rules can be applied:

- If for one species more than one L(E)Cx/chronic NOEC values based on the same toxicological endpoint are available for a give species, these values are averaged by calculating the geometric mean, resulting in the "species mean" NOEC/L(E)Cx. In case of a flawed dataset, e.g., only two data points are available and one represents a very low value and another a high value, it is recommended to repeat testing and take the geometric mean of all data. The Global Harmonized System (GHS) the use of a geometric mean is recommended for larger data sets (4 or more values);
- If for one species several acute L(E)Cx/chronic NOEC values based on different toxicological endpoints are available, the lowest value is selected. The lowest value is again determined on the basis of the geometric mean if more than one value for the same endpoint is available;
- In some cases, L(E)Cx/NOEC values for different life stages of a specific organism are reported in the same study. 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 to be indicated in the tables as the life stage at the 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 both the egg and larval stages);
- In case geometric means of different endpoints are given it is recommended to use the most sensitive endpoint;
- If acclimatization/adaptation is important test results should be grouped on the basis of the similarity of the background in the culture medium with the background found typically in the environment;
- When it is apparent from the data that the observed intra- species variability in toxicity test results can be assigned to differences in bioavailability and no bioavailability model is available to normalize the data, the effect data should be grouped by similar ranges of abiotic factors that control the bioavailability of metals. For example if the pH is driving the assessment toxicity data should be grouped in pH ranges around 6 (5.5-6.5) and 8 (7.5-8.5) relevant for pH 6 and 8 (8.5) used in the T/D for acute and chronic hazard classification respectively.

For each of the three test species, all adequate acute or chronic toxicity data are collected and a reference value is determined for different pH-classes.
The pH determines the silver speciation in solution. As not all silver species may be equally toxic or bioavailable, variation of L(E)C_{50}/NOEC values may occur as a function of changing pH-levels. It was therefore decided to categorise – where possible – the adequate toxicity data into three different pH-classes: 5.5-6.5, >6.5-7.5, and >7.5-8.5.

Depending on the amount of available data for a specific organism and exposure period (acute or chronic) within a pH-class, the reference value is determined as follows:

- **<4 data points available**: The lowest is selected as species-specific reference value for the pH-class and exposure period under consideration;
- **4 or more data points available**: The geometric mean is derived and considered as species-specific reference value for the pH-class and exposure period under consideration.

1.2 EVALUATION OF ACUTE ECOTOXICOLOGICAL STUDIES WITH SILVER

In this section of the Hazard classification reported an overview and evaluation of different acute toxicological studies that have been performed in the aquatic environment using silver as toxicant. Sections 1.2.1 to 1.2.3 present the different studies that are evaluated according to different trophic levels: fish (Section 1.2.1), invertebrates and insects (section 1.2.2), and algae and plants (section 1.2.3). Chronic data have been evaluated and reported in the effects assessment section of this report and the selection of adequate chronic toxicity data for hazard classification is based on the outcome of that evaluation.

1.2.1 Acute toxicity to freshwater fish

Acute data on single-species toxicity tests resulting in L(E)C_{50} values for freshwater fish are given in Table 1. More detailed information on the test method of the individual studies is given in the Annexes. The individual studies with reliable data quality (Q1 and Q2) are discussed below.

Table 1 reports whether silver concentrations are measured in the different studies evaluated. Reported concentrations are assumed to represent the total fraction when it is not specified whether the reported values reflect total or dissolved silver levels.

Lima et al. (1982) studied the acute effect of $AgNO_3$ on the flagfish *Jordanella floridae* and the fathead minnow *Pimephales promelas*. Fifteen 30 days old flagfish (0.044g) were used per replicate and for fathead minnows twenty 30 days old fish (0.079g) were used. Mean silver concentrations were 3.4, 8.4, 13.7, 29.6, and 67 µg/L and <0.2 µg/L in the control. Test concentrations were measured. The 96h-LC50 was 9.2 and 10.7 µg/L for *J. floridae* and *P. promelas*, respectively. This study was assigned as highly reliable (Q1).

Davies et al. (1978) performed acute toxicity tests with rainbow trout *Oncorhynchus mykiss* at different hardnesses. Nominal silver concentrations for the soft-water experiments (hardness 26 mg/L $CaCO_3$) were: 0, 2.5, 5, 10, 20 µg Ag/L. Nominal silver concentrations for the hard-water experiments (hardness 350 mg/L $CaCO_3$) were: 0, 5, 10, 20, 40, 80 µg Ag/L. Test concentrations were measured. Each aquarium contained 10 rainbow trout of the same size and age which were hatched from the same lot of eggs. The 96h-LC_{50} was 6.5 and 13 µg/L for soft and hard water, respectively (not specified if the values are based on nominal or measured concentrations). This study was assigned as highly reliable (Q1).

Galvez and Wood (2002) performed an acute toxicity test with juvenile *Oncorhynchus mykiss*. The nominal Ag concentrations that were used for the lethality tests were 10, 18, 32, 56, and either 5.6 or 100 µg/L (nominal values). Test concentrations were measured and ten fish were used per concentration. The 96h-LC_{50} was between 7.6 and 15.1 µg/L and the 168h-LC_{50} between 9.9 and 22.4. Fish were marked two weeks before the experiment. This study was assigned as highly reliable (Q1).

Morgan and Wood (2004) studied the acute effect of $AgNO_3$ on the rainbow trout *Oncorhynchus mykiss*. Fish were exposed to nominal concentrations of 0, 5, 20, 25, 30, and 40 µg of total silver/L. Test concentrations were measured. Exposure tanks contained 32 fish in 20 L synthetic soft water. The 96h-LC_{50} was 13.3 µg Ag_{tot}/L or 3.3 µg Ag_{dis}/L. This study was considered reliable with restrictions (Q2) as no replicates are reported.

Van Genderen et al. (2003) tested the acute effect of silver nitrate to fathead minnow, *Pimephales promelas*. Triplicate beakers were prepared for each of five Ag concentrations plus a control with 10

larvae per beaker. The Ag-concentrations in the different test media were determined. The 96h-LC50 was 6.2 µg Ag_{tot}/L or 4.5 µg Ag_{dis}/L. This study was considered as highly reliable (Q1).

Grosell et al. (2000) studied the acute effect of silver nitrate to the trout *Oncorhynchus mykiss* and the eel *Anguilla anguilla*. A total of eight fish (eel or trout) were transferred to each of five 25 L glass tanks supplied with a flow-through of 0.1 L/min 'low chloride' softwater. For both species, one tank served as control. Test concentrations were measured. The 96h-LC_{50} was 10.2 and 34.4 µg/L for *O. mykiss* and *A. anguilla*, respectively. This study was considered reliable with restrictions (Q2) since the hardness of the test medium is not reported.

Bury et al. (1999) performed acute toxicity tests with *Pimephales promelas* and *Oncorhynchus mykiss* at different calcium, chloride and DOC concentrations. Tests were performed according to ASTM guidelines. For each test, six to nine concentrations over the range of 3.2 to 40 µg $AgNO_3$/L were used. Ten rainbow trout and ten fathead minnows were used per replicate. The 96h-LC_{50} for *P. promelas* was 6.7, 13.3, 18.0 µg/L with DOC concentrations of 0.3, 1.6, 5.8 mg/L, respectively. The 96h-LC_{50} for *O. mykiss* was 7.5, 18.4, 27.7 µg/L with DOC concentrations of 0.3, 1.6, 5.8 mg/L, respectively. This study was considered reliable with restrictions (Q2) since the hardness is not reported.

Karen et al. (1999) performed acute toxicity tests with *Pimephales promelas* and *Oncorhynchus mykiss* at different hardness, chloride and DOC concentrations. Tests with *P. promelas* were blocked around chloride levels (3, 20, 40, and 60 mg/L), hardness (as $CaCO_3$; 50, 100, and 200 mg/L) and humic acid (0, 5, and 10 mg/L) were used in conjunction with six concentrations of silver (0, 2, 5, 10, 20, and 40 µg/L) in a complete factorial design, totalling 54 treatments per blocked test. For *O. mykiss*, chloride (0, 3, 20, and 40 mg/L) and humic acid (0, 2.5, and 5 mg/L) were used in conjunction with six silver nitrate concentrations (0, 2, 5, 10, 20, and 40 µg/L). Test concentrations are measured. Each treatment had three replicates containing 10 fish. For *P. promelas*, the 96h-LC_{50} ranges between 2.0 and 13.4 µg/L. For *O. mykiss*, the 96h-LC_{50} ranges between 1.5 and 28.4 µg/L. Details about the results are found in the article. Here, only the ranges are given of 36 different LC_{50}s. This study was assigned as highly reliable (Q1).

Erickson et al. (1998) tested the acute effect of silver nitrate on the test organism *Pimephales promelas*. Juvenile (30d) fish were exposed in duplicate chambers at each of five to seven silver concentrations (0.5 dilution factor) with 10 fish per chamber. Test concentrations were measured. The 96h-LC_{50} was 10.4 µg/L. This study was assigned as highly reliable (Q1).

Table 1: Overview of acute toxicity values for fish

Sub-stance	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
$AgNO_3$	*Jordanella floridae* *Pimephales promelas*	30d-old	96h	Mortality	LC_{50}	9.2 10.7	Measured	Yes	Static	25	7.4	44	n.r. 1*	Lake Superior water	<0.2	Q1	Lima et al., 1982 Ag-5
$AgNO_3$	*Oncorhynchus mykiss*	Mean 129mm Mean 167mm	96h	Mortality	LC_{50}	6.5 13	Measured	yes	Flow-through	11.5 15.5	6.7 8	26 350	n.r. 1*	Tap water	<0.05	Q1	Davies et al., 1978 Ag-6
$AgNO_3$	*Oncorhynchus mykiss*	Juvenile	96h 168d	Mortality	LC_{50}	7.6-15.1 9.9-22.4	Measured	Yes	Flow-through	15	8	120	1.3	Tap water	<0.05	Q1	Galvez and Wood, 2002 Ag-12
$AgNO_3$	*Oncorhynchus mykiss*	Mean weight 6.3 g	96h	Mortality	LC_{50}	13.3 Agtot 3.3 Agdis	Measured	Yes	Flow-through	10	7	10	0.7	Synthetic soft water	n.r.	Q2	Morgan and Wood, 2004 Ag-35
$AgNO_3$	*Oncorhynchus mykiss*	Mean weight 30.8 mg/L	96h	Mortality	LC_{50}	15 Agtot 13.2 Agdis	Measured	Yes	Static renewal	14	n.r.	n.r.	n.r. 1*	Tap water	n.r.	Q3	Mann et al., 2004 Ag-36
$AgNO_3$	*Pimephales promelas*	Larvae <24h	96h	Mortality	LC_{50}	6.2 Agtot 4.5 Agdis	Measured	Yes	Static renewal	25	8.2	79.2	4.4	Recon-stituted hard water	n.r.	Q1	VanGenderen et al., 2003 Ag-40
$AgNO_3$	*Anguilla anguilla*	Mean weight 60g 25g	96h	Mortality	LC_{50}	34.4	Measured	Yes	Flow-through	14	n.r.	n.r.	1.3	'Low chloride' softwater	n.r.	Q2	Grosell et al., 2000 Ag-82

Sub-stance	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
	Oncorhynchus mykiss					10.2											
$AgNO_3$	*Pimephales promelas*	ASTM guideline	96h	Mortality	LC_{50}	6.7 13.3 18.0	Measured	Yes	Static renewal	17	6.2 - 6.7	n.r.	0.3 1.6 5.8	Reverse osmosis tap water	n.r.	Q2	Bury et al., 1999 Ag-96
$AgNO_3$	*Oncorhynchus mykiss*	ASTM guideline	96h	Mortality	LC_{50}	7.5 18.4 27.7	Measured	Yes	Static renewal	17	6.2 - 6.7	n.r.	0.3 1.6 5.8	Reverse osmosis tap water	n.r.	Q2	Bury et al., 1999 Ag-96
$AgNO_3$	*Pimephales promelas*	Larvae <24h	96h	Mortality	LC_{50}	Range 2.0-13.4	Measured	Yes	Static	22	8.3	50, 100, 200	0, 5, 10	Recon-stituted water	n.r.	Q1	Karen et al., 1999 Ag-97
$AgNO_3$	*Oncorhynchus mykiss*	20 +/-3d	96h	Mortality	LC_{50}	Range 1.5-28.4	Measured	Yes	Flow-through	10-12	9	60	0, 2.5, 5	Recon-stituted water	n.r.	Q1	Karen et al., 1999 Ag-97
$AgNO_3$	*Pimephales promelas*	30d	96h	Mortality	LC_{50}	10.4	Measured	Yes	Static renewal	25	8	48	1.5	Laboratory water	n.r.	Q1	Erickson et al., 1998 Ag-109
$AgNO_3$ $Ag(S_2O_3)_n$ $AgNO_3$ $Ag(S_2O_3)_n$	*Oncorhynchus mykiss*	Juvenile	96h 96h 7d	Mortality	LC_{50}	11.8 161,000 9.1	Measured	Yes	Static renewal	15	7.9 - 8.2	n.r.	n.r. 1*	Tap water	n.r.	Q3	Hogstrand et al., 1996 Ag-112

Sub-stance	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
$AgCl_n$			7d			137,000 no mortality up to 100,000											
$AgNO_3$	*Pimephales promelas*	1-7d	48h	Mortality	LC_{50}	5.7	Measured	Yes	Static renewal	20	n.r.	70-90	<1	Recon-stituted water	n.r.	Q3	Diamond et al., 1997 Ag-113

*DOC estimated
**When measured silver concentrations are not specified to be total or dissolved it is assumed to be total silver levels

1.2.2 Acute toxicity to freshwater invertebrates

Acute data on single-species toxicity tests resulting in L(E)C_{50} values for freshwater invertebrates are given in Table 2. More detailed information on the test method of the individual studies is given in the Annexes. The individual studies with reliable data quality (Q1 and Q2) are discussed below.

Table 2 reports if the silver concentrations are measured in the different studies evaluated. Reported concentrations are assumed to represent the total fraction when it is not specified whether the reported values reflect total or dissolved silver levels.

Lima et al. (1982) studied the acute effect of silver nitrate to the scud *Gammarus pseudolimnaeus* and the midge *Tanytarsus dissimilis*. Mean silver concentrations for the test with scud were 0.80, 2.15, 4.9, 15.3, 35.6 µg/L and <0.2 µg/L in the control. For the midges, the silver concentrations were 371, 842, 1870, 3350, and 7190 µg/L and <0.2 µg/L in the control. The test concentrations were measured. The 96h-LC_{50} for *G. pseudolimnaeus* was 4.5 µg/L (48h-LC_{50} was 4.7 µg/L) and the 48h-LC_{50} for *T. dissimilis* was 3,160 µg/L. This study was considered as highly reliable (Q1).

Glover et al. (2005) performed an acute toxicity test with *Daphnia magna*. Ten daphnid neonates (age <24h) were added to dark chambers. Test medium was renewed every 24 hours and samples were taken to determine both total and dissolved Ag-concentrations. The 48h-LC_{50} was 0.23 µg/L (based on total silver concentrations). This study was considered as highly reliable (Q1).

Bury et al. (2002) studied the acute effect of silver nitrate to the three invertebrates *Daphnia magna*, *Daphnia pulex*, and *Gammarus pulex*. Toxicity tests with *D. magna* and *D. pulex* were conducted according to USEPA methods given in USEPA, 1993. All reported silver levels are nominal concentrations. The 48h-LC_{50} was 0.47, 0.65, 2.1 for *D. magna*, *D. pulex* and *G. pulex*, respectively. This study was considered reliable with restrictions (Q2) as it was not reported whether test concentrations were measured or not.

Bianchini et al. (2002) (1) assessed the acute effect of silver nitrate to *Daphnia magna* and the crayfish *Cambarus diogenes diogenes*. For crayfish toxicity test, mean measured concentrations tested were: 0, 2.23, 3.21, 6.0, 6.23, 13.07, 27.54, 37.14, 39.52, 43.28, 108.71, 125.24 and 128.46 µg of total Ag/L. The mean measured concentrations to which *D. magna* was exposed, were 0, 0.038, 0.083, 0.14, 0.323, 0.625, 1.294 µg of total Ag/L. The 48h-LC_{50} for *D. magna* was 0.26 µg Ag_{tot}/L, and the 96h-LC_{50} for *C. diogenes diogenes* was 65.85 µg Ag_{tot}/L. This study was considered reliable with restrictions (Q2) as the test method was not described into detail. Number of test species per replicate, size/age of test organisms used was not reported.

Bianchini et al. (2002) (2) studied short-term toxicity of silver nitrate to *Daphnia magna*. Nominal test concentrations were 0, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/L. Test concentrations were measured. The 48h-LC_{50} was 0.22 Ag_{dis}/L. This study was assigned as highly reliable (Q1).

Bielmeyer et al. (2002) performed an acute toxicity test with *Ceriodaphnia dubia*. Bioassay procedures followed US EPA short-term methods. Nominal concentrations were 0.001, 0.01, 0.1, 1, and 10 µg/L. Test concentrations were measured. This study was considered reliable with restrictions (Q2) as only the highest test concentrations were measured (detection limit was 2 µg/L).

Karen et al. (1999) performed acute toxicity tests with *Daphnia magna* at different hardness, chloride and DOC concentrations. Six tested silver concentrations were 0, 0.5, 1.0, 2, 3.5, and 5 µg/L. Test concentrations were measured. Tests were blocked around chloride levels (3, 10, 40, and 60 mg/L), hardness (as CaCO3/L; 100 and 200 mg/L) and humic acid (DOC; 0, 2, 5, and 10 mg/L) were used

together with six silver concentrations in a complete factorial design totalling 48 treatments per blocked test. The 48h-LC_{50} range was between 0.58 and 3.8 µg/L. Details about the results are found in the article. Here, only the ranges are given of 32 different LC_{50}s. This study was considered as highly reliable (Q1).

Erickson et al. (1998) studied the acute toxicity of silver nitrate to *Daphnia magna*. Each test consisted of duplicated control chambers and duplicated chambers at each of five silver concentrations (0.5 dilution factor) with 10 organisms per chamber. Test concentrations were measured. The 48h-LC_{50} was 0.58 µg/L. This study was considered as highly reliable (Q1).

Diamond et al. (1997) performed an acute toxicity test with *Ceriodaphnia dubia*. At least six (including control) metal concentrations were tested. Test concentrations were measured. The 48h-LC_{50} was 0.79 µg/L. This study was considered as highly reliable (Q1).

Table 2: Overview of acute toxicity values for invertebrates

Sub-stance	Species	Age and/or size of test organism	Test duration	Effect para-meter	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
Ag^+	*Aphelenchoides ritzemabosi* (nematode)	/	24h	Mortality	NOEC LOEC	10 100	n.r.	yes	static	5	n.r.	n.r.	n.r. 1*	Glass-distilled water	n.r.	Q3	Pitcher and McNamara, 1972 Ag-4
$AgNO_3$	*Gammarus pseudolimnaeus*	0.67+/-0.24 cm	48h 96h	Mortality	LC_{50}	4.7 4.5	Measured	Yes	Static	20	7.4	44	n.r. 1*	Lake Superior water	<0.2	Q1	Lima et al., 1982 Ag-5
$AgNO_3$	*Tanytarsus dissimilis*	3rd instar midge larvae (2.5-2.75 mm)	48h	Mortality	LC_{50}	3,160	Measured	Yes	Static	20	7.4	44	n.r. 1*	Lake Superior water	<0.2	Q1	Lima et al., 1982 Ag-5
$AgNO_3$	*Daphnia magna*	neonates <24h	48h	Mortality	LC_{50}	0.23 Agtot	Measured	Yes	Static renewal	20-22	8	100	n.r. 1*	Recon-stituted water	n.r.	Q1	Glover et al., 2005 Ag-20
$AgNO_3$	*Daphnia magna*	n.r.	48h	Immobility	EC_{84}	0.15 0.06	n.r.	yes	Static	22	8-8.2 7.2-7.6	250 45	n.r. 1*	Recon-stituted water	n.r.	Q3	Fjällborg et al., 2006 Ag-21
$AgNO_3$	*Daphnia magna* *Daphnia pulex* *Gammarus pulex*	<24h <24h n.r.	48h	Mortality	LC_{50}	0.47 0.65 2.1	n.r.	Yes	Static renewal	20 20 10	7.6 7.6 6.8	92 85 6	n.r. 1*	Artificial freshwater Tap water	n.r.	Q2	Bury et al., 2002 Ag-48

Sub-stance	Species	Age and/or size of test organism	Test duration	Effect para-meter	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*	n.r.	48h	Mortality	LC_{50}	0.26 Agtot	Measured	Yes	Static renewal	20	8.2	120	<0.1	Recon-stituted water	n.r.	Q2	Bianchini et al., 2002 (1) Ag-58
$AgNO_3$	*Cambarus diogenes diogenes*	adult	96h	Mortality	LC_{50}	65.85 Agtot	Measured	Yes	Flow-through	15	8.2	120	1.3	Tap water	n.r.	Q2	Bianchini et al., 2002 (1) Ag-58
$AgNO_3$	*Daphnia magna*	Neonates	48h	Mortality	LC_{50}	0.22 Agdis	Measured	Yes	Static renewal	20	8.2	115	n.r. 1*	Recon-stituted water	n.r.	Q1	Bianchini et al., 2002 (2) Ag-60
$AgNO_3$	*Ceriodaphnia dubia*	<24h	48h 96h	Mortality	LC_{50}	0.5 0.5	Highest concen-trations measured	Yes	Static renewal	25	7.4-7.8	80-100	n.r. 1*	EPA moderately hard water	n.r.	Q2	Bielmyer et al., 2002 Ag-64
Ag	*Simocephalus sp. and Ceriodaphnia dubia*	ASTM protocol	48h	Mortality	LC_{50}	27	n.r.	Yes	Static	20	7	16	0.2	Deionized water	0.01	Q3	Hook and Fisher, 2001 Ag-69
$AgNO_3$	*Daphnia magna*	<24h	48h	Mortality	LC_{50}	Range 0.58-3.8	Measured	Yes	Static renewal	22	8.3	100, 200	0, 2, 5, 10	Recon-stituted water	n.r.	Q1	Karen et al., 1999 Ag-97
$AgNO_3$	*Daphnia magna*	<24h	48h	Mortality	LC_{50}	0.58	Measured	Yes	Static renewal	20	8	48	1.5	Laboratory water	n.r.	Q1	Erickson et al., 1998 Ag-109

Sub-stance	Species	Age and/or size of test organism	Test duration	Effect para-meter	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
$AgNO_3$	*Ceriodaphnia dubia*	<24h	48h	Mortality	LC_{50}	0.79	Measured	Yes	Static renewal	20	n.r.	70-90	<1	Recon-stituted water	n.r.	Q1	Diamond et al., 1997 Ag-113

* DOC estimated
** When measured silver concentrations are not specified to be total or dissolved it is assumed to be total silver levels

1.2.3 Acute toxicity to freshwater algae

Table 3 gives an overview of the acute toxicity data for freshwater algae. The 72h algal growth inhibition test is a chronic test but the EC_{50} is treated as an acute value for classification purposes. More detailed information on the test method of the individual studies is given in the Annexes. All studies reported in Table 3 are of low quality (Q3) and can not be used for classification.

Table 3: Overview of acute toxicity values for algae

Sub-stance	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
Ag^+	*Pseudokirchneriella subcapitata*	Exponential phase	6h	Algal growth	EC_{50}	2.8	Measured	yes	static	20	7	n.r.	n.r. 0.5*	Ultrapure water	0	Q3	Lee et al., 2005 Ag-18
$AgNO_3$	*Lactuca sativa*	Seeds	96h	Root elongation	EC_{50}	1,000	n.r.	yes	Static	22	n.r.	n.r.	n.r. 0.5*	Pure Milli Q water	n.r.	Q3	Fjällborg et al., 2006 Ag-21
Ag^+(?)	*Chlamydomonas reinhardtii* *Pseudokirchneriella subcapitata*	Exponential phase	96h	Growth inhibition	EC_{50} EC_{50}	1.6 2.4	Measured	Yes	Static	20	7	15	n.r. 1*	Modified high salt medium (MHSM)	n.r.	Q3	Hiriart-Baer et al., 2006 Ag-24
Ag^+	*Chlamydomonas reinhardtii*	Exponential phase	96h	Growth inhibition	IC_{50}	2.3	Measured (?)	Yes	Static	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	Q3	Campbell et al., 2002 Ag-49

* DOC estimated
** When measured silver concentrations are not specified to be total or dissolved it is assumed to be total silver levels

1.3 SELECTED ACUTE TOXICITY DATA FOR CLASSIFICATION

An overview of reliable data for classification purposes is given below.

1.3.1 Fish

Table 4 presents reliable acute LC_{50} values for fish to different pH-classes. The lowest value in the 5.5-5.6; 6.5-7.5; 7.5-8.5 pH-classes are 6.7, 3.3 and 2.0 µg/L, respectively. These values are considered as reference values for the classification of silver at different pH-levels. Note that toxicity tests with measured dissolved silver concentrations are preferred. Studies with nominal or total silver concentrations can be used only when the silver speciation in the test media is well understood.
One study reports also a reliable LC_{50} (range 1.5-28.4 µg/L) which was however generated at pH 9 (Karen et al., 1999).

Table 4: Acute fish toxicity values (LC_{50} values in µg/L) for the hazard classification of silver

Substance	Species	Endpoint	Value (µg/l)	Ag_{dis} vs Ag_{tot} *	pH	Quality of the study	Reference
pH-class 5.5-6.5							
$AgNO_3$	*Pimephales promelas*	LC_{50}	6.7	Measured	6.2-6.7	Q2	Bury et al., 1999
$AgNO_3$	*Oncorhynchus mykiss*	LC_{50}	7.5	Measured	6.2-6.7	Q2	Bury et al., 1999
pH-class >6.5-7.5							
$AgNO_3$	*Jordanella floridae* *Pimephales promelas*	LC_{50}	9.2 10.7	Measured	7.4	Q1	Lima et al., 1982
$AgNO_3$	*Oncorhynchus mykiss*	LC_{50}	6.5	Measured	6.7	Q1	Davies et al., 1978
$AgNO_3$	*Oncorhynchus mykiss*	LC_{50}	3.3	Agdis	7	Q2	Morgan and Wood, 2004
pH-class >7.5-8.5							
$AgNO_3$	*Oncorhynchus mykiss*	LC_{50}	13	Measured	8	Q1	Davies et al., 1978
$AgNO_3$	*Oncorhynchus mykiss*	LC_{50}	7.6-15.1	Measured	8	Q1	Galvez and Wood, 2002
$AgNO_3$	*Pimephales promelas*	LC_{50}	4.5	Agdis	8.2	Q1	VanGenderen et al., 2003
$AgNO_3$	*Pimephales promelas*	LC_{50}	2.0-13.4	Measured	8.3	Q1	Karen et al., 1999
$AgNO_3$	*Pimephales promelas*	LC_{50}	10.4	Measured	8	Q1	Erickson et al., 1998

* if only measured is reported it is assumed to be total silver concentrations

1.3.2 Invertebrates

Table 5 presents reliable acute LC_{50} values for invertebrates to different pH-classes. The lowest value in the 6.5-7.5 and the 7.5-8.5 pH-classes are 2.1 and 0.22 µg/L, respectively. No reliable data are found for the 5.5-6.5 pH-range. Note that the toxicity tests with measured dissolved silver concentrations are

preferred. Studies with nominal or total silver concentrations can be used only if the silver speciation in the test media is well understood.

Table 5: Acute invertebrate toxicity values (LC_{50} values in µg/L) for the hazard classification of silver

Substance	Species	Endpoint	Value (µg/l)	Ag_{dis} vs Ag_{tot} *	pH	Quality of the study	Reference
pH-class 5.5-6.5: no reliable data available							
pH-class >6.5-7.5							
$AgNO_3$	*Gammarus pseudolimnaeus*	LC_{50}	4.7	Measured	7.4	Q1	Lima et al., 1982
$AgNO_3$	*Tanytarsus dissimilis*	LC_{50}	3,160	Measured	7.4	Q1	Lima et al., 1982
$AgNO_3$	*Gammarus pulex*	LC_{50}	2.1	n.r.	6.8	Q2	Bury et al., 2002
pH-class >7.5-8.5							
$AgNO_3$	*Daphnia magna*	LC_{50}	0.23	Measured	8	Q1	Glover et al., 2005
$AgNO_3$	*Daphnia magna*	LC_{50}	0.47	n.r.	7.6	Q2	Bury et al., 2002
$AgNO_3$	*Daphnia pulex*	LC_{50}	0.65	n.r.	7.6	Q2	Bury et al., 2002
$AgNO_3$	*Daphnia magna*	LC_{50}	0.26	Agtot	8.2	Q2	Bianchini et al., 2002 (1)
$AgNO_3$	*Cambarus diogenes diogenes*	LC_{50}	65.85	Agtot	8.2	Q2	Bianchini et al., 2002 (1)
$AgNO_3$	*Daphnia magna*	LC_{50}	0.22	Agdis	8.2	Q1	Bianchini et al., 2002 (2)
$AgNO_3$	*Ceriodaphnia dubia*	LC_{50}	0.5	Highest concentrations measured	7.4-7.8	Q2	Bielmyer et al., 2002
$AgNO_3$	*Daphnia magna*	LC_{50}	0.58-3.8	Measured	8.3	Q1	Karen et al., 1999
$AgNO_3$	*Daphnia magna*	LC_{50}	0.58	Measured	8	Q1	Erickson et al., 1998

*if only measured is reported it is assumed to be total silver concentrations

1.3.3 Algae

No adequate data on the acute toxicity of silver to freshwater algae were found in literature. Therefore, no reference value for hazard classification purposes could be determined for this taxonomical group.

1.4 SELECTED CHRONIC TOXICITY DATA FOR CLASSIFICATION

Chronic data have been evaluated and reported in the effects assessment section of this report and the selection of adequate chronic toxicity data for hazard classification is based on the outcome of that evaluation.

1.4.1 Fish

Table 6 presents reliable chronic toxicity values for fish to different pH-classes. The lowest value in the 6.5-7.5 and the 7.5-8.5 pH-classes are 0.09 and 0.14 µg/L, respectively. No reliable data are found for the 5.5-6.5 pH-range. Note that the toxicity tests with measured dissolved silver concentrations are preferred. Studies with nominal or total silver concentrations can be used only if the silver speciation in the test media is well understood.

Table 6: Chronic fish toxicity values (LC_{50} values in µg/L) for the hazard classification of silver

Substance	Species	Endpoint	Value (µg/l)	Ag_{dis} vs Ag_{tot} *	pH	Quality of the study	Reference
pH-class 5.5-6.5: no reliable data available							
pH-class >6.5-7.5							
$AgNO_3$	*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*	64d-NOEC mortality	0.1	Measured	7	Q1	Morgan et al., 2005
$AgNO_3$	*Oncorhynchus mykiss*	23d-NOEC mortality	1.2	Measured	7.5	Q2	Guadagnolo et al., 2001
pH-class >7.5-8.5							
$AgNO_3$	*Oncorhynchus mykiss*	23d-NOEC growth rate	3.02	Measured	8	Q2	Galvez and Wood, 2002
$AgNO_3$	*Oncorhynchus mykiss*	51d-NOEC mortality	0.14	Measured	7.5-8	Q1	Brauner and Wood, 2002 (2)

*if only measured is reported it is assumed to be total silver concentrations

1.4.2 Invertebrates

The only reliable chronic toxicity test with invertebrates is reported by Bielmeyer et al. (2002). The 8d-$NOEC_{mortality}$ was 0.1 µg/L at pH 7.4-7.8. Note that this concentration was under the detection limit.

1.4.3 Algae

No adequate data on the chronic toxicity of silver to freshwater algae were found in literature.

1.5 DATA GAP ANALYSIS

Acute and chronic toxicity data will serve for the classification of soluble, sparingly-soluble and insoluble inorganic silver and silver compounds. For the classification of sparingly soluble or insoluble inorganic silver and silver compounds, no transformation/dissolution (T/D) data have been generated yet. This type of information is necessary for the hazard classification of these inorganic silver compounds. As pH has a significant influence on T/D, all tests (ecotoxicity tests, T/D tests) should in principle be carried out at a pH that maximizes the concentration of the dissolved metal ions in solution. If no relevant literature data exist, a preliminary screening test may need to be carried out in order to ensure that the test is performed at a pH maximizing transformation/dissolution within the described pH ranges. With reference to the conditions generally found in the environment, a pH range of 6 to 8.5 must be used. So,

depending on the physicochemical properties of an insoluble or sparingly soluble inorganic silver compound, the pH at which the T/D is performed may differ.
With regard to silver/silver compounds and the evaluation of their acute aquatic toxicity, required toxicity data are:

- 96h-LC_{50} for fish (OECD Test Guideline 203 or equivalent);
- 48h-EC_{50} for crustacea (OECD Test Guideline 202 or equivalent);
- 72/96h-EC_{50} for algae (OECD Test Guideline 202 or equivalent); the 72h algal growth inhibition test is a chronic test but the EC_{50} is treated as an acute value for classification purposes.

These test species are considered as surrogate for all aquatic organisms and data on other species such as *Lemna* may also be considered if the test methodology is suitable. For the evaluation of the chronic toxicity of inorganic silver, accepted test procedures include the 7d-assay with Ceriodaphnids (ASTM, 2005), the 21d test for daphnids (OECD, 1998), the 30d test for fish (OECD, 1992), and the 72h algal growth inhibition test (endpoint: NOEC/EC_{10}).

Acute fish data is available for the three different pH-classes 5.5-6.5; >6.5-7.5; >7.5-8.5. For invertebrates, data is available for the two highest pH-classes but not for pH-range 5.5-6.5. No adequate data is available on the acute toxicity of silver to freshwater algae.

Note that the toxicity tests with measured dissolved silver concentrations are preferred. From all acute fish toxicity values reported, only two studies measured dissolved silver concentrations (Morgan and Wood, 2004 and Van Genderen et al., 2003). For invertebrates, only one study is based on dissolved silver concentrations (Bianchini et al., 2002 (2)). Studies with nominal or total silver concentrations can be used only if the silver speciation in the test media is well understood.

Chronic fish data is available for the two different pH-classes >6.5-7.5 and >7.5-8.5. For invertebrates, one reliable study was performed at pH 7.4-7.8 (Bielmeyer et al., 2002). No values are available for pH classes 5.5-6.5 for fish, 5.5-6.5 and >6.5-7.5 for invertebrates, and all classes 5.5-6.5; >6.5-7.5; and >7.5-8.5 for algae.

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3 ANNEXES

Reference:	Bianchini et al., 2002 (1)
Type of test:	Acute invertebrate tests/static renewal (*D. magna*); flow-through (*C. diogenes*)
Species	*Daphnia magna*; *Cambarus diogenes diogenes* (crayfish)
Exposure period:	48h, 96h
Unit:	µg/L
NOEC:	
E(L)C50:	48h-LC50= 0.26 Agtot (*D. magna*); 96h-LC50= 65.85 Agtot (*C. diogenes*)
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Adult crayfish *Cambarus diogenes diogenes* (18.57 +/-0.5 g; n=140) were obtained from Boreal Laboratory Supplies (St. Catharines, Ontario, Canada). Acclimated crayfish were exposed to silver in dechlorinated Hamilton city tap water (15°C). Mean measured concentrations tested were: 0, 2.23, 3.21, 6.0, 6.23, 13.07, 27.54, 37.14, 39.52, 43.28, 108.71, 125.24, 128.46 µg of total Ag/L. Several colonies of adult gravid *Daphnia magna* (ARO strain, Lot No. 090600 DM) were obtained from Aquatic Research Organisms (Hampton, NH). Reconstituted tap water was used as test solution (20°C). Mean measured concentrations tested were: 0, 0.038, 0.083, 0.14, 0.323, 0.625, 1.294 µg of total Ag/L. Three replicates were used. LC50 values are calculated using Probit analysis.
Result:	Q2: test method is not described in detail (number of test species per replicate, size/age of test organisms used).
pH	8.2
DOC (mg/L)	<0.1 (*D. magna*); 1.3 (*C. diogenes*)
Ca (mg/L)	40
Mg (mg/L)	4.8
Na (mg/L)	13.8
Total Hardness (mg/L)	120

Reference:	Bianchini et al., 2002 (2)
Type of test:	Acute invertebrate test/static renewal
Species	*Daphnia magna*
Exposure period:	48h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 0.22 Agdis
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Several colonies of adult gravid D. magna were obtained from Aquatic Research Organisms (ARO strain, lot 090600 DM, Hampton, NH, USA). Acute toxicity tests were performed in glass beakers containing 250 mL of synthetic water (20°C, designed to resemble Lake Ontario). Nominal test concentrations were: 0, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/L. 10 neonates were placed in each beaker. Tests were performed in triplicate. 48h-LC50 value was calculated using probit analysis.
Result:	
pH	8.2
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= 0.001; 8d-LOEC= 0.01 (endpoint=reproduction)

E(L)C50:	48h- or 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 test since lowest test concentration is 0.001 µg/L and this concentration is undetectable on the atomic absorption (detection limit 2 µg/L).
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:	Bury et al., 1999
Type of test:	Acute fish test/static renewal
Species	*Pimephales promelas* and *Oncorhynchus mykiss*
Exposure period:	96h
Unit:	µg/L
NOEC:	

E(L)C50:	Water chemistry Cl(µM):Ca(µM):DOC(mg/L) Chloride treatment: LC50 in µg Altot/L *P. promelas* *O. mykiss* 50:50:0.3 6.7 7.5 250:50:0.3 7.5 9.2 800:50:0.3 7.7 18.5 1500:50:0.3 8.8 25.6 calcium treatment: 50:500:0.3 7.8 9.9 50:2000:0.3 9.9 10.5 DOC treatment 50:50:1.6 13.3 18.4 50:50:5.8 18.0 27.7
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	ASTM guidelines
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Juvenile rainbow trout, weight 2.2 +/- 1.2 g, were obtained from Humber Spring Hatchery, Orangeville, Ontario, Canada and fathead minnows, weight 0.23 +/- 0.28 g, were obtained from Aquatic Biosystems, Fort Collins, Colorado, USA. All toxicity tests were performed in a static-renewal system and followed ASTM guidelines. For each test, at least six (max nine) concentrations over the range of 3.2 to 40 µg $AgNO_3$/L were used. Ten rainbow trout and ten fathead minnows were placed in separate chambers within the same tank with modified reverse osmosis tap water (17°C). Each day 80% of the medium from each tank was replaced. The LC50 values were calculated either by probit analysis or by the methods of Litchfield and Wilcoxon.
Result:	LC50 expressed as ionic silver concentration is also reported in the article.
pH	6.2-6.7
DOC (mg/L)	See E(L)C50
Ca (mg/L)	See E(L)C50
Mg (mg/L)	See table 1 in article for each treatment
Na (mg/L)	See table 1 in article for each treatment
Total Hardness (mg/L)	See table 1 in article for each treatment

Reference:	Bury et al., 2002
Type of test:	Acute invertebrate test/static renewal
Species	*Daphnia magna*, *Daphnia pulex*, *Gammarus pulex*

Exposure period:	48h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 0.47 (*D. magna*); 0.65 (*D. pulex*); 2.1 (*G. pulex*)
Limit test:	No
Analytical monitoring:	
Test procedure:	USEPA, 1993 (*D. magna* and *pulex*)
GLP:	NR
Test substance:	$AgNO_3$
Test method:	*D. magna* were obtained from Aquatic World, London, UK and *D. pulex* from Kellogg Biological Station, Michigan. USA and were kept in well-aerated artificial freshwater at 20°C. Toxicity tests were conducted according to USEPA methods given in USEPA, 1993. Ten daphnid neonates (<24h) were added to 200 mL of well-aerated medium, and all experiments were performed in triplicate. Adult *G. pulex* were obtained from the River Culm, Devon, UK, and were kept in dechlorinated, aerated, Exeter tap water at 10°C. Toxicity tests were performed in triplicate, and each container designated for silver exposures was pre-soaked for 24h in a solution containing 12.7 µg Ag(I)/L to ensure the saturation of Ag(I) binding sites of the plastic. To 110 mL of medium 10 G. pulex were added and mortalities were assessed at 24, 48, 72, and 96h. All reported silver levels are nominal concentrations. LC50 values were calculated by Log probit analysis.
Result:	Q2: NR if test concentrations were measured
pH	7.6 (*D. magna* and *pulex*); 6.8 (*G. pulex*)
DOC (mg/L)	
Ca (mg/L)	17.6 (*D. magna*); 14 (*D. pulex*); 2 (*G. pulex*)
Mg (mg/L)	11.7 (*D. magna*); 12.1 (*D. pulex*); 0.24 (*G. pulex*)
Na (mg/L)	26.2 (*D magna* and *pulex*); 11.5 (*G. pulex*)
Total Hardness (mg/L)	92 (*D. magna*); 85 (*D. pulex*); 6 (*G. pulex*)

Reference:	Campbell et al., 2002
Type of test:	Acute algal test/static
Species	*Chlamydomonas reinhardtii*
Exposure period:	96h

Unit:	µg/L
NOEC:	
E(L)C50:	IC50= 2.5 (growth inhibition)
Limit test:	No
Analytical monitoring:	Measured (?)
Test procedure:	NR
GLP:	NR
Test substance:	Ag^+
Test method:	*C. reinhardtii* was obtained from University of Toronto Culture Collection (UTCC11). The mid-exponential cells are suspended in defined medium containing the test metal and various test ligands (e.g. chloride, thiosulfate, citrate, or alanine). Nominal Ag concentrations were 10, 20, 30, 50, and 80 nM (expressed as total silver). Growth rates were calculated on the basis of the dilution rate of the turbidostats (continuous cultures).
Result:	IC50 value for growth inhibition by silver in the absence of any complexing ligand was 21 nM (2.3 µg/L) expressed as free Ag^+. Q3: test medium and test design not fully defined, no statistics described
pH	NR
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	NR

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:	Diamond et al., 1997
Type of test:	Acute fish and invertebrate test/static renewal

Species	*Ceriodaphnia dubia* and *Pimephales promelas*
Exposure period:	48h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 0.79 (*C. dubia*); 5.7 (*P. promelas*)
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Test was conducted in moderately hard reconstituted water (20°C). Neonates (<24h) were used in *Ceriodaphnia* tests and larval fish (1-7d) were used in minnow tests. Two replicate chambers, each with 10 organisms were used for each test treatment. At least six including control metal concentrations were tested. 48h-LC50 were computed using an EPA-supplied computer program (EMSL, Cincinnati, OH, USA).
Result:	Q3: no standard endpoint for fish (test duration 48h)
pH	NR
DOC (mg/L)	<1
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	70-90

Reference:	Erickson et al., 1998
Type of test:	Acute invertebrate and fish test/static renewal
Species	*Daphnia magna, Pimephales promelas*
Exposure period:	48h; 96h
Unit:	µg/L
NOEC:	
E(L)C50:	48h-LC50= 0.58 96h-LC50= 10.4

Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Fathead minnows were from cultures maintained at the University of Wisconsin-Superior Lake Superior Research Institute (LSRI) in dechlorinated municipal tap water (Superior, WI, USA). Juvenile (30d) fish were exposed in duplicate chambers at each of five to seven silver concentrations (0.5 dilution factor) with 10 fish per chamber in laboratory water of 25°C. *Daphnia magna* were from cultures maintained at LSRI in hard reconstituted water. Each test consisted of duplicated control chambers and duplicated chambers at each of five silver concentrations (0.5 dilution factor) with 10 organisms (<24h) per chamber (20°C). LC50 were calculated using the trimmed Spearman-Karber method.
Result:	The article report also results of the effects of added hardness, pH, and organic carbon on acute toxicity of silver nitrate to juvenile fathead minnows in a figure.
pH	8
DOC (mg/L)	1.5
Ca (mg/L)	17
Mg (mg/L)	3.8
Na (mg/L)	11
Total Hardness (mg/L)	48

Reference:	Fjällborg et al., 2006
Type of test:	Acute invertebrate and plant test/static
Species	*Daphnia magna* and *Lactuca sativa*
Exposure period:	48h, 96h
Unit:	µg/L
NOEC:	
E(L)C50:	48h-EC84= 0.06 (soft water) ; 0.15 (hard water,*D. magna*, immobility) 96h-EC50= 1,000 (*L. sativa*, root elongation)
Limit test:	No
Analytical monitoring:	NR

Test procedure:	Standardized method (ISO, 1996) for *D. magna*
GLP:	NR
Test substance:	$AgNO_3$
Test method:	*D. magna* test: the acute toxicity for *D. magna* was determined in replicates using a standardized method (ISO 1996). Plastic, six-well Nunc plates were used as test vessel, with 10 mL of sample in each well. The dilution series was made with reconstituted water (ISO 1996) with hard water and soft water (22°C). Each test was accompanied by a positive control. Tested concentrations were: 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, 2 µg/L. *L. sativa* test: the dilution series were conducted in pure water (Milli Q). Test concentrations were: 62.5, 125, 250, 500, 1000, 2000, 4000 µg/L. Replicates with 50 mL of each sample were poured into Petri dishes. Approximately ten lettuce seeds were added to each Petri dish. The seeds were then incubated at 22°C in darkness for 96h. As negative controls, two Petri dishes with deionized water were used. EC84 and EC50 values were determined by the moving average method.
Result:	Q3: no useful endpoint for *Daphnia magna*
pH	8.0-8.2 (hard water) 7.2-7.6 (soft water)
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	250 (hard water, *D. magna*) 45 (soft water, *D. magna*)

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:	(Q1) All fish were marked before the start of the test. 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:	Glover et al., 2005
Type of test:	Acute invertebrate test/static renewal
Species	*Daphnia magna*
Exposure period:	48h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 0.23

Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	A laboratory culture of *Daphnia magna* was established from adult gravid animals acquired from Aquatic Research Organisms (ARO strain, Hampton, USA). Test medium was reconstituted synthetic Lake Ontario water (20-22°C). Ten daphnid neonates (age <24h) were added to dark chambers. There is a 24h-renewal of test medium and samples were taken to determine both total and dissolved Ag. LC50 was determined using probit analysis.
Result:	
pH	8
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	100

Reference:	Grosell et al., 2000
Type of test:	Acute fish test/flow-through
Species	*Oncorhynchus mykiss* and *Anguilla anguilla*
Exposure period:	96h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 10.2 (*O. mykiss*); 34.4 (*A. anguilla*)
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$

Test method:	*A. anguilla* (45-75 g, mean 60 g) were caught in fyke nets in Roskilde Fjord (brackish water). All fish were yellow stage eels (adult, non-migrating). *O. mykiss* (20-30 g, mean 25 g) were obtained from Reerso fish farm, Kalundborg, Denmark. A total of eight fish (eel or trout) were transferred to each of five 25 l glass tanks supplied with a flow-through of 0.1 L/min 'low chloride' softwater (14°C). For both species, one tank served as control. LC50 values were based on measured silver concentrations. LC50 values were calculated by probit analysis.
Result:	
pH	NR
DOC (mg/L)	1.3
Ca (mg/L)	0.4
Mg (mg/L)	<0.0234
Na (mg/L)	11.5
Total Hardness (mg/L)	NR

Reference:	Hiriart-Baer et al., 2006
Type of test:	Acute algae test/static
Species	*Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata*
Exposure period:	96h
Unit:	µg/L
NOEC:	
E(L)C50:	EC50= 1.6 (*C. reinhardtii*); 2.4 (*P. subcapitata*) (growth inhibition)
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	Ag^{+}(?)

Test method:	Cultures of *C. reinhardtii* (UTCC 11) and *P. subcapitata* (UTCC 37) were obtained from the University of Toronto Culture Collection, Toronto, Ont., Canada. A constant illumination of 100 +/-10 $\mu Em^{-2}s^{-1}$ at 20°C. Batch cultures of the algae in exponential growth were transferred to three replicates. The algal growth rate was monitored, and once it had stabilized (normally within 18-24h) the Ag concentration was increased to 20 nM. The algal growth rate was again monitored until it had stabilized (usually 18-24h), following which the Ag concentration was again increased. In this manner we were able to monitor algal growth rates as the Ag concentration was increased stepwise in the sequence 10, 20, 30, 50, and 80 nM Agtot.
Result:	Q3: it is not clearly reported with test compound is used
pH	7
DOC (mg/L)	NR
Ca (mg/L)	2.7
Mg (mg/L)	2.0
Na (mg/L)	2.3
Total Hardness (mg/L)	15 (calculated from Ca and Mg concentrations)

Reference:	Hogstrand et al., 1996
Type of test:	Acute fish test/static renewal
Species	*Oncorhynchus mykiss*
Exposure period:	96h; 7d
Unit:	µg/L
NOEC:	
E(L)C50:	96h-LC50= 11.8 ($AgNO_3$); 161,000 ($Ag(S_2O_3)_n$) 7d-LC50= 9.1 ($AgNO_3$); 137,000 ($Ag(S_2O_3)_n$) no mortality up to 100,000 ($AgCl_n$)
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$; $Ag(S_2O_3)_n$; $AgCl_n$

Test method:	Rainbow trout (1-4g) were purchased from a local hatchery (Rainbow Springs Hatchery, Thamesford, ON, Canada). Test medium was dechlorinated Hamilton city tap water (15°C). $AgNO_3$-test: fourteen 50-L tanks with total Ag concentrations ranging from 0.1 to 1,000 µg/L in a semilogarithmic series. Ten juvenile fish were put into each tank. There was no measurable change in the total dissolved Ag concentrations between water renewal. $Ag(S_2O_3)_n$-test: experimental setup was largely like $AgNO_3$-test. The 16 concentrations tested ranged from 32 to 1,000,000 µg Ag/L. There was on average a 7% decrease in the measured total dissolved Ag concentrations between water renewal. $AgCl_n$-test: nine concentrations were tested ranging nominally from 0.1 to 1,000 µg Ag/L. The low solubility of $AgCl_n$ was reflected in a 20% decrease in the concentrations of total/suspended Ag between water renewals. To overcome this problem, fish were acclimated for 2 weeks to freshwater supplemented with 50 mM NaCl, and then exposed to $AgNO_3$ in this medium. A total of 11 concentrations, ranging from 100 to 100,000 µg Ag/L, were tested under these conditions.
Result:	Q2/Q3: age test organism, hardness?
pH	7.9-8.2
DOC (mg/L)	NR
Ca (mg/L)	40
Mg (mg/L)	NR
Na (mg/L)	13.8
Total Hardness (mg/L)	NR

Reference:	Hook and Fisher, 2001
Type of test:	Acute invertebrate test/static
Species	Freshwater cladocerans *Simocephalus sp.* and *Ceriodaphnia dubia*
Exposure period:	48h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 27
Limit test:	No
Analytical monitoring:	NR
Test procedure:	ASTM protocol
GLP:	NR

Test substance:	Ag
Test method:	*Simocephalus sp*. was collected April through November from a pond on the campus of SUNY, Stony Brook (Stony Brook, NY, USA), *Ceriodaphnia dubia* was obtained from Aquatic Bio Systems (Fort Collins, CO, USA). To determine lethal toxicity, animals were continuously exposed to Ag solutions ranging from 0.1 to 1,000 nM for 48h following ASTM protocols. Background Ag levels were estimated at 0.001 µg/L. Exposure solution was deionized water (20°C) with WCL-1 salts added.
Result:	Q3: measured concentrations not reported, no distinction is made between the two different cladocerans used in the experiment
pH	7
DOC (mg/L)	0.2
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	16

Reference:	Karen et al., 1999
Type of test:	Acute fish and invertebrate test/static and static renewal
Species	*Oncorhynchus mykiss* and *Pimephales promelas* and *Daphnia magna*
Exposure period:	48h, 96h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50 ranges (different hardness, Cl, and DOC concentrations tested) *O. mykiss*: 1.48-28.42 *P. promelas*: 1.97-13.35 *D. magna*: 0.58-3.8
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$

Test method:	48h-*D. magna* test: static renewal test (22°C). Six tested silver concentrations: 0, 0.5, 1.0, 2, 3.5, and 5 µg/L. Tests were blocked around chloride levels (3, 10, 40, and 60 mg/L), hardness (as CaCO3/L; 100 and 200 mg/L) and humic acid (DOC; 0, 2, 5, and 10 mg/L) were used together with six silver concentrations in a complete factorial design totalling 48 treatments per blocked test. Test medium is reconstituted water. Five neonates (<24h) were added to each of the six replicates. 96h-*P. promelas* test: fathead minnow larvae less than 24h old were purchased from Charles River Aquatic Research Organisms (Hampton, NH, USA). The static, nonrenewal test was conducted at 22°C. Tests were blocked around chloride levels (3, 20, 40, and 60 mg/L), hardness (as $CaCO_3$; 50, 100, and 200 mg/L) and humic acid (0, 5, and 10 mg/L) were used in conjunction with six concentrations of silver (0, 2, 5, 10, 20, and 40 µg/L) in a complete factorial design, totalling 54 treatments per blocked test. Each treatment had three replicates containing 10 fish. Test medium is reconstituted water. 96h-*O.mykiss* test: rainbow trout were obtained from one of three local hatcheries (Walhalla, SC, USA; Erwin, TN, USA; or Brevard, NC, USA). Nine experiments were conducted to determine the effects of varying water quality parameters on silver toxicity. Chloride (0, 3, 20, and 40 mg/L) and humic acid (0, 2.5, and 5 mg/L) were used in conjunction with six silver nitrate concentrations (0, 2, 5, 10, 20, and 40 µg/L). Each test began with trout of the same age (20 +/-3 d). Flow-through test with reconstituted water (hardness 60 mg/L) of 10 to 12 °C. Tanks (54L) were subdivided into three sections, each containing 10 fish. Thus, each treatment and control contained 60 fish, for a total of 360 fish per test. The Shapiro-Wilk's test for normality was conducted before calculation of LC50. Probit, logit, and log10 transformations were used for normally distributed data, whereas those data found to lack normal distribution were subjected to trimmed Spearman-Karber analysis.
Result:	Different hardness, DOC and Cl concentrations are tested. Details about the results are found in the article. Here, only the ranges are given.
pH	8.2 (*D. magna* and *P. promelas*); 9 (*O. mykiss*)
DOC (mg/L)	Tested concentrations: 0, 5, 10
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	Tested concentrations: 50, 100, 200

Reference:	Lee et al., 2005
Type of test:	Acute algae test/static
Species	*Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*
Exposure period:	6h
Unit:	µg/L

NOEC:	
E(L)C50:	EC50= 2.8 (*P. subcapitata*); 1.3 (*C. reinhardtii*) algal growth
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	Ag^+
Test method:	Tests were performed with two green algae, *C. reinhardtti* was obtained from University of Toronto Culture Collection, UTCC11, UTCC, Ont., Canada and *P. subcapitata* formerly known as *S. capricornutum*, UTCC37. All exposure solutions were initially prepared from ultrapure water. Algal cultures in mid-exponential growth phase were used with initial density of ~ 50,000 cells/mL for *C. reinhardtti* and ~ 120,000 cells/mL for *P. subcapitata*. Tests were carried out at 20°C and a light regime of 100 $\mu Em^{-2}s^{-1}$. Under the first experimental regime, growth assays were conducted over a wide range of the nominal total silver concentrations and a low fixed chloride concentration (Cl= 0.18 mg/L). Since this low level of chloride does not affect silver speciation (silver exists almost entirely as free Ag^+). Nominal Agtot or Ag^+ concentrations tested were: 0, 1.1, 2.2, 3.2, 5.4 µg/L for *C. reinhardtti* and 0, 1.1, 3.2, 5.4, 8.6 µg/L for *P. subcapitata*. Aqueous silver concentration at the end of 6h period or final measured Agtot concentrations were: 0, 0.14, 0.35, 1.18, 2.64 µg/L for *C. reinhardtti* and 0, 0.02, 0.35, 1.67, 3.88 µg/L for *P. subcapitata*. Tests were performed in duplicate. The Excel macro REGTOX was used to calculate EC50s.
Result:	
pH	7
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	NR

Reference:	Lima et al., 1982
Type of test:	Acute fish and invertebrate test/static
Species	*Jordanella floridae* (flagfish), *Pimephales promelas* (fathead minnows), *Gammarus pseudolimnaeus* (scud), *Tanytarsus dissimilis* (midges)
Exposure period:	48h (only midges), 96h

Unit:	µg/L
NOEC:	
E(L)C50:	96h-LC50= 10.7 (*P. promelas*); 9.2 (*J. floridae*); 4.5 (*G. pseudolimnaeus*) 48h-LC50= 4.7 (*G. pseudolimnaeus*); 3160 (*T. dissimilis*)
Limit test:	No
Analytical monitoring:	Measured
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	All test organisms were reared in Lake Superior water. Flagfish, fathead minnows and midges were obtained from the culture unit at the U.S. EPA Environmental Research Laboratory-Duluth (ERL-D). Scuds were collected from the Eau Claire River near Gordon, Wisconsin. Each test consisted of five concentrations of silver and one control with a duplicate of each. Fish: fifteen 30 day old flagfish (0.044g)/replicate and twenty 30 day old fathead minnows (0.079g)/replicate. Temperature was 25°C. Mean silver concentrations were 3.4, 8.4, 13.7, 29.6, and 67 µg/L and <0.2 µg/L in the control. Scud: test organisms were selected based upon size uniformity (length=0.67 +/-0.24 cm) with no attempt to determine age. Ten scuds were used per replicate. Temperature was 20°C. Mean silver concentrations were 0.80, 2.15, 4.9, 15.3, 35.6 µg/L and <0.2 µg/L in the control. Midges: Ten 3rd instar midge larvae with total length of 2.5-2.75 mm were used per replicate. Temperature was 20°C. Mean silver concentrations were 371, 842, 1870, 3350, and 7190 µg/L and <0.2 µg/L in the control. LC50 values were determined by the Trimmed Spearman-Karber Method.
Result:	
pH	7.4
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	44

Reference:	Mann et al., 2004
Type of test:	Acute fish test/static renewal
Species	*Oncorhynchus mykiss*

Exposure period:	96h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 15 Agtot; 13.2 Agdis
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Juvenile rainbow trout (weight <1.0 g) were obtained from Humber Springs Hatchery (Orangeville, ON, Canada). Ten animals were allocated impartially to each of seven exposure tanks; the average biomass loading was 30.8 mg/L. Seven nominal concentrations of Ag were used: 0, 65, 93, 130, 185, 260, and 371 nM as $AgNO_3$. Test solution was dechlorinated Hamilton tap water (14°C). The Spearman-Karber statistic and Probit analysis were used to generate estimates of the LC50.
Result:	Q2/Q3: test method and test solution not reported in detail
pH	NR
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	NR

Reference:	Morgan and Wood, 2004
Type of test:	Acute fish test/flow-through
Species	*Oncorhynchus mykiss*
Exposure period:	96h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 13.3 Agtot; 3.3 Agdis
Limit test:	No

Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$
Test method:	Juvenile rainbow trout were obtained from Humber Springs Trout Hatchery (Orangeville, ON, Canada). Synthetic soft water was generated by reverse osmosis of Hamilton city dechlorinated tap water (10°C). Fish (mean weight 6.3 g) were exposed to nominal silver concentrations of 0, 5, 20, 25, 30, and 40 µg of total silver/L. Exposure tanks contained 32 fish in 20 L synthetic soft water. LC50 values were calculated using the trimmed Spearman-Karber method.
Result:	Q2: replicates?
pH	7
DOC (mg/L)	0.7
Ca (mg/L)	2.0
Mg (mg/L)	0.49
Na (mg/L)	1.15
Total Hardness (mg/L)	10

Reference:	Pitcher and McNamara, 1972
Type of test:	Acute invertebrate test/static
Species	*Aphelenchoides ritzemabosi* (nematode)
Exposure period:	24h
Unit:	µg/L
NOEC:	NOEC 10 LOEC 100 Endpoint is mortality
E(L)C50:	
Limit test:	No
Analytical monitoring:	NR
Test procedure:	NR
GLP:	NR
Test substance:	Ag^+

Test method:	*A. ritzemabosi* were extracted from dry chrysanthemum leaves. The available nematodes were distributed equally over all treatments by pipetting 15mL of an aqueous suspension (glass-distilled water) into each Petri-dish. An equal volume of metallic salt solution was added and the dishes were placed in a refrigerator at 5°C. The dishes were withdrawn 18-24h later, one replicate at a time, and left at room temperature (20°C) for 30 min to allow the nematodes to recover motility after chilling. Trials usually comprised five replicates of 100 individuals of *A. ritzemabosi*.
Result:	
pH	NR
DOC (mg/L)	NR
Ca (mg/L)	NR
Mg (mg/L)	NR
Na (mg/L)	NR
Total Hardness (mg/L)	NR

Reference:	VanGenderen et al., 2003
Type of test:	Acute fish test/static renewal
Species	*Pimephales promelas*
Exposure period:	96h
Unit:	µg/L
NOEC:	
E(L)C50:	LC50= 6.2 Agtot; 4.5 Agdis
Limit test:	No
Analytical monitoring:	Measured (AAS)
Test procedure:	NR
GLP:	NR
Test substance:	$AgNO_3$

Test method:	Acute toxicity of silver nitrate to fathead minnow was determined in the presence of natural DOM from different sources. Tests were conducted in US Environmental Protection Agency moderately hard reconstituted laboratory water (25°C). Larval were <24h old at start of exposure. The larvae were from an in-house laboratory culture maintained at the Clemson Institute of Environmental Toxicology (Pendleton, SC, USA. Triplicate beakers were prepared for each of five Ag concentrations plus a control with 10 larvae per beaker. Test media were renewed at 48h and fish was fed 0.2 mL *Artemia* nauplii 2h before water renewal. LC50 was calculated using trimmed Spearman-Karber method.
Result:	
pH	8.2
DOC (mg/L)	4.4 +/-0.8
Ca (mg/L)	$CaSO_4.2H_2O$: 60
Mg (mg/L)	$MgSO_4$: 60
Na (mg/L)	$NaHCO_3$: 96
Total Hardness (mg/L)	79.2