ACUTE & CHRONIC ECOTOXICITY OF SOLUBLE COPPER SPECIES IN VIEW OF HAZARD CLASSIFICATION OF COPPER AND COPPER COMPOUNDS

UPDATE OF EU RA APPENDIX K1

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1. Introduction

Different copper compounds have a range of solubility and hence bioavailability. Copper metal (C°) is not soluble but needs to be transformed into Cu^{+} or Cu^{2+} to become dissolved and bioavailable.

In accordance to the DSD - EU hazard classification system, soluble copper compounds, sparingly soluble copper compounds and copper metal are hence evaluated as follows:

a. Soluble copper species ($CuSO_4$, Cu oxychloride) : Ecotoxicity data obtained from tests carried out with soluble copper species and expressed as copper ions (µg Cu/L) are used directly, after molecular weight correction.
   The classification of these soluble copper was agreed by the EU classification and labelling group and included in ANNEX VI of the CLP.
b. Sparingly soluble copper compounds (Cu_2O and CuO): Ecotoxicity data obtained from tests carried out with soluble copper species and expressed as copper ions (µg Cu/L) are compared to copper ions released (µg Cu/L) during transformation/dissolution (T/D) tests.
   The classification was agreed by the EU classification and labelling group and included in ANNEX VI of the CLP.
c. Copper powders and copper massives: Ecotoxicity data obtained from tests carried out with soluble copper species and expressed as copper ions (µg Cu/L) are compared to copper ions released (µg Cu/L) during the transformation/dissolution (T/D) tests. *The classification outcome was discussed at TCNES.*

The Global Harmonized System for Classification and Labelling recommended in its metal annex (Annex 10) the development of further guidance to assess bioaccumulations and removal from the water-column as input into the chronic classification for metals. This was recognized in the recent Guidance to Regulation (EC) No 1272/2008 Classification, Labelling and Packaging of substances and mixtures, (CLP guidance, 2009). The CLP guidance (2009) includes a metal-specific annex, that allows to assess bio-accumulation and removal from the water–column for metals. A few relevant extracts are provided below.

Bioaccumulation : "BCF and BAF *may be used to estimate metal accumulation:*

a) consider information on essentiality and homeostasis of metals/ metal compounds. Metals that are essential nutrients are actively regulated: removal and sequestration processes that minimise toxicity are complemented by an ability to up-regulate concentrations for essentiality. As a result, of such regulation, the "bioaccumulative" criterion is not applicable to these metals.

b) non- essential metals are also actively regulated to some extent and therefore also for nonessential metals, an inverse relationship between the metal concentration and the external concentration may be observed (Mc Geer et al., 2003). Bioconcentration factors, determined at/around the EC_{50} value from the acute toxicity, for the same taxonomic group, are therefore recommended."

Removal from the water column *: "Environmental transformation of one species of a metal to another species of the same does not constitute degradation as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. However as a result of naturally occurring geochemical processes metal ions can partition from the water column. Data on water column residence time, the processes* *involved at the water – sediment interface (i. e. deposition and re-mobilisation) are fairly extensive, but have not been integrated into a meaningful database. Nevertheless, using the principles and assumptions discussed above in Section IV.1, it maybe possible to incorporate this approach into classification. "*

…. The use of laboratory mesocosm and/or field tests for evaluating removal of soluble metal species through precipitation/partitioning processes over a range of environmentally relevant conditions are described in the guidance

The "bioaccumulation" and "removal from the water-column" characteristics have therefore been assesses for copper in view of refining the chronic classification entries.

The approach taken for selection the acute and chronic ecotoxicity data and defining ecotoxicity reference values for copper/copper compounds is described below. The approach is in-line with the previous assessments at EU level (eg official classification of Cu2O and K1 annex to the copper Risk Assessment Report) and is described below. Where small changes have been made (to align the approach to the CLP guidance (2009)) these are indicated.

- Because the data quality of the extracted information may, vary considerably between individual source documents the compiled ecotoxicity data was assessed with regard to their 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 here both the *reliability* of the available data and the *relevance* of the data for environmental classification purposes. These two basic elements are defined 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 classifying the metal/metal compound.

Only those data that can be considered relevant and of high quality were retained for the assessment and used for ecotoxicological benchmarking. Guidance on how to screen and select the most appropriate data is outlined hereunder.

2. Copper bibliography

The compilation of the acute toxicity data is based on an extensive literature search performed by J. Meyer, University of Wyoming (1999). The consulted articles, books, databases are listed in Annex 1.

To compile data on the effects of Cu and related compounds towards aquatic biota both extensive databases and scientific literature were consulted. The literature sources used for gathering the chronic toxicity data are reported in the EU RAR.

All selected records are included in the IUCLID database

3. Data selection criteria

For the selection of the toxicity data, the following reliability and relevance criteria were used:

3.1 Reliability criteria

- Soluble copper compounds have been used as test compound.
- Standard OECD approved tests and non standardised tests have been considered as suitable.
- Tests should be performed according standard operational procedures. A detailed description of methods employed in the study should be provided
- Clear dose response relationships should be observed and an NOEC/L(E)C_{50} should be derived according to appropriate statistical methods.
- Since the acute & chronic effect levels are often not far above reported copper background concentrations, only effect levels based on actual (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.

3.2 Relevance criteria

- Only data obtained for the standard OECD test species were selected as relevant to hazard classification.
- Only standardised OECD endpoints and exposure duration were selected.
- Fish: LC_{50} values from 96 hours 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, growth rate values were used for further evaluation.
- Cu-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 selection criteria but evaluated and discussed where appropriate.

4. Data treatment

Considering the large copper database, hazard classification based on the lowest recorded value would result in a hazard classification system that is more stringent than for new substances. Therefore, in agreement with the CLP guidance data summaries were carried out: when 4 or more acceptable L(E)C_{50} / NOEC values are available for the same species, the geometric mean of the toxicity values was used as representative toxicity value for that species instead of the lowest value for the species[1].

Considering the crucial importance of pH of the test media on the copper solubility and ecotoxicity, for the acute and chronic toxicity endpoints, 3 pH categories were distinguished within the acute and chronic ecotoxicity database: pH 5.5-6.5, >6.5-7.5 and >7.5-8.5.

The lowest species-specific acute $L(E)C_{50}$ and chronic NOEC was selected as final hazard classification entry at the three pH levels and across pHs.

In agreement with the CLP guidance data, the data were also skimmed into a data-set with Dissolved Organic Carbon (DOC) concentrations <2 mg/L[2].

5. Ecotoxicity of soluble copper species

5.1. Acute ecotox value to be used for hazard classification

All data and a synthesis of the species mean/lowest acute ecotoxicity data are given in Annex 2 (Table 2A) and Table 1. After data selection, as discussed above, 451 high quality acute data points were retained. For the algae 66 individual data points were selected for 3 standard species (*Pseudokirchnerella subcapitata*, *Chamydomonas reinhardtii* and *Chlorella vulgaris*). For the invertebrates 123 individual data points were selected for 2 standard species *(Ceriodaphnia dubia* and *Daphnia magna*) and for the fish 262 individual data points were selected for 5 standard species (*Oncorhynchus mykiss, Pimephales promelas, Lepomis macrochirus, Brachydanio rerio* and *Cyprinus carpio*).

High quality $L(E)C_{50}$ values range between 2.8 and 9150 µg Cu/l. Species specific geomean/lowest values range between 4.4 (*Pimephales promelas*) and 4250.0 µg Cu/l (*Lepomis macrochirus*). When evaluating the high quality $L(E)C_{50}$ values at the three pH classes, sufficient data for the 3 pH classes were found for 2 fish species (*O. mykiss, P. promelas*), 3 algae species (*R. subcapitata, P.subcapitata, C.reinhardtii* and *C.vulgaris*) and 2 invertebrate species (*D. magna* and *C. dubia*).

An overview of the selected high quality species mean acute toxicity data for the 3 different pH classes is provided in Table 1

[1] In the copper RA report , annex K1 (2008), the geomean was also used for 2 and 3 data-points per species. [2] In the copper RA report , no DOC selection was performed.

Table 1: Overview of the selected high quality acute toxicity data for the individual species (L(E)C_{50} values expressed as µg/l) for the 3 pH classes (lowest values in bold).

Test organism	**L(E)C_{50} (µg/l)**			
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**	**All pHs**
Algae				
Pseudokirchnerella subcapitata (former *Raphidocelis subcapitata*)				
n	11	18	18	47
Min.	92.0	30.0	36.9	30.0
Max.	824.0	685.0	346.0	824.0
Geometric mean/lowest value	263.5	120.1	106.8	138.9
Chamydomonas reinhardtii				
n	1	1	1	3
Min.	380.0	315.0	146.0	146.0
Max.	380.0	315.0	146.0	380.0
Geometric mean/lowest value	380.0	315.0	146.0	146.0
Chlorella vulgaris				
n	5	7	4	16
Min.	333.0	60.0	99.0	60.0
Max.	987.0	446.0	506.0	987.0
Geometric mean/lowest value	582.9	232.7	214.4	296.1
Invertebrates				
Daphnia magna				
n	8	19	75	102
Min.	33.8	7.0	9.8	7.0
Max.	465.0	1213.0	826.0	1213.0
Geometric mean/lowest value	101.4	129.1	68.8	79.8
Ceriodaphnia dubia				
n	4	4	13	21
Min.	9.5	28.0 84.0	8.5	8.5
Max.	56	47.3	200.0	200.0
Geometric mean/lowest value	**25.0**		**29.8**	**34.4**
Fish				
Oncorhynchus mykiss				
n	6	18	24	48
Min.	4.2	2.8	9.5	2.8
Max.	82.2	890	516.0	890
Geometric mean/lowest value	29.2	63.2	97.4	71.2
Brachydanio rerio				
n	/	1	1	2
Min.	/	35.0 35.0	149.0	35.0
Max.	/	**35.0**	149.0	149.0
Geometric mean/lowest value	/		149.0	35.0
Cyprinus carpio				

n Min. Max. Geometric mean/lowest value	/ / / /	/ / / /	2 800.0 810.0 800.0	2 800.0 810.0 800.0
Pimephales promelas				
Test organism	**L(E)C_{50} (µg/l)**			
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**	**All pHs**
n Min. Max. Geometric mean/lowest value	2 4.4 15.0 ***4.4***	37 5.6 1400.0 169.2	166 12.4 1060.0 199.8	205 4.4 1400.0 216.7
Lepomis macrochirus n Min. Max. Geometric mean/lowest value	 / / / /	 2 1000.0 1100.0 1000.0	 3 4250.0 9150.0 4250.0	 5 1000.0 9150.5 2837.5

/: no data available

The lowest species geomean/lowest L(E)C_{50} value from the database is 4.4 µg Cu/l. This ecotoxicity value is applicable to pH 6 media. The lowest species geometric mean/lowest L(E)C_{50} value applicable to pH 7 and 8 media are respectively 35.0 and 29.8 µg Cu/l. The lowest species mean/lowest value of 4.4 µg/l Cu is extracted from Erickson et al. (1996). This test was performed in a flow-through with a very short retention time (± 45 min.) using a diluted reconstituted medium (prepared from Lake Superior water through reverse osmosis) with a low hardness (22 mg/l $CaCO_3$) and DOC concentration (reverse osmosis). This test performed with larvae (< 24 h old) represent worst case conditions explaining therefore this low LC_{50} value. Moreover such discrepancy between the sensitivity of *P. promelas* at pH 6 and pH 7 is not expected (
Table 2). Indeed an acute sensitivity ratio (expressed as $LC50_{pHx}/LC50_{pHx-1}$) of 38.5 is found for that species while for the other species *O. mykiss* this ratio was 2.2. These observed ratios are also in accordance with the BLM-predicted values[2] for the same species. Indeed, the predicted sensitivity ratio for *P. promelas* at pH 6 and pH 7 is 2.2.

Table 2: Acute sensitivity ratio (expressed as $LC50_{pHx}/LC50_{pHx-1}$) for different fish species.

Fish species	**Sensitivity ratio ($LC50_{pHx}/LC50_{pHx-1}$)**	
	pH7/pH6	**pH8/pH7**
Observed values		
O. mykiss	2.2	1.5
O. kisutch	/	/
B. rerio	/	4.3
C. carpio	/	/
P. promelas	38.5	1.2
L. macrochirus	/	4.3
Predicted values (acute-BLM)		
P. promelas	2.2	1.9

[2] Acute BLM predictions for fathead minnow (Hydroqual) were performed in a worst case medium (cfr. Effects assessment in the EU RAR) where pH was the only variable.

/: could not be calculated

Moreover, a higher acute sensitivity of *P. promelas* compared to *O. mykiss* towards copper is not expected. According to the database *P. promelas* is ± 6 times more sensitive than *O. mykiss* at pH 6. An opposite trend is observed when comparing the sensitivities of both fish species at pH 7 and pH 8. At pH 7, *O. mykiss* was 2.9 times more sensitive than *P. promelas*, at pH 8 2.1 times. The higher sensitivity of *O. mykiss* compared to *P. promelas* could also be confirmed in the chronic effects assessment performed in this EU RAR. Indeed, based on chronic BLM predictions (performed at pH 6.6 on the endpoint mortality), *O. mykiss* was 2.3 times more sensitive than *P. promelas* (normalized species mean NOEC value for *O. mykiss* is 17.9 µg/l, for *P. promelas* 39.7 µg/l).

Based on the above performed analysis, the reliability of species mean/lowest value of 4.4 µg/l for *P. promelas* at pH 6 is questioned. Considering the whealth of information available for this species the 2 pH 6 data are considered as outliers and the pH 6 data were therefore not retained for setting the reference value at pH 6. It is therefore proposed to use the species mean value of 25.0 µg/l for *C. dubia* as the reference value at pH 6.

Table 3 summarizes the lowest recorded species-specific L(E)C50 for each taxonomic group and pH. The lowest species-specific value across taxonomic groups are proposed as reference values.

Table 3: The lowest recorded species-specific L(E)C50 for each taxonomic group and proposed reference values

	EC50 (µgCu/l)			
Test organisms	**pH 6**	**pH 7**	**pH 8**	**All pH**
Fish	29.2	35	97.4	35
Invertebrates	25	47.3	29.8	34.4
Algae/aq.plants	263.5	120.1	106.8	138.9
Proposed reference	**25.0**	**35.0**	**29.8**	**34.4**

In a second assessment, the acute toxicity data retained in Table 2 were further skimmed based on their DOC-content. Toxicity data points were only retained in case their DOC content ranged between 0 and 2 mg/L, or in case the studies were performed in laboratory or reconstituted water (for these waters, it is assumed that the DOC are < 2 mg/L). The results for the 3 pH classes are listed below in Table 4.

Table 4 : Overview of the selected high quality acute toxicity data with DOC-levels < 2 mg/L, for the individual species (L(E)C50 values - expressed as µgCu /l for the 3 pH classes.

Test organism	**L(E)C$_{50}$ (µg/l)**			
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**	**All pHs**
Algae				
Pseudokirchnerella subcapitata				
n	/	2	3	5
Min.	/	30.0	36.9	30.0
Max.	/	32.0	133.0	133.0
Geometric mean	/			45.4
Chlorella vulgaris				
Test organism	**L(E)C$_{50}$ (µg/l)**			
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**	**All pHs**
n	/	1	/	1
Min.	/	60.0	/	60.0
Max.	/	60.0	/	60.0
Geometric mean	/		/	
Invertebrates				
Daphnia magna				
n	/	3	49	52
Min.	/	92.6	20.0	20.0
Max.	/	117.0	152.0	152.0
Geometric mean	/		39.7	41.93
Ceriodaphnia dubia				
n	2	2	9	13
Min.	9.5	**28.0**	200.0	9.5
Max.	52	76.0	200.0	200.0
Geometric mean	**22.2**		**26.1**	**27.8**
Fish				
Oncorhynchus mykiss				
n	/	1	/	1
Min.	/	890	/	890
Max.	/	890	/	890
Geometric mean	/		/	
Pimephales promelas				
n		6	10	16
Min.		44.0	136.5	15.0
Max.		663.0	996.3	996.3
Geometric mean		265.8	473.6	315.26
Lepomis macrochirus				
n	/	/	1	1
Min.	/	/	9150.0	9150.0
Max.	/	/	9150.0	9150.0
Geometric mean	/	/		

/: no data available;

The selection of DOC<2 mg/L results in much smaller database. The comparison of with the full data-set is provided Table 5. Considering the relative small difference between the 2 datasets, the more robust reference values obtained with full data-set is retained for the classification

Table 5: Derived acute reference values with full and skimmed data-set

pH range	**Reference values**	
	All $L(E)C_{50}$ (µg/l)	**$L(E)C_{50}$ (µg/l) with low DOC**
pH 5.5-6.5	25.0 (n=37)	22.2* (n=2)
pH >6.5-7.5	35.0 (n=107)	28.0 (n=15)
pH >7.5-8.5	29.8 (n=307)	26.1 (n=72)
all pH	34.4 (n= 451)	27.8 (n=89)

* the geomean of the 2 values for *C. dubia* test were taken to allow comparison

5.2 Chronic ecotoxicity value to be used for hazard classification

All data and a synthesis of the chronic ecotoxicity data are given in Table 6 and in Annex 2 (Table 2B). After data selection, 87 high quality chronic data points were retained. For the algae/aquatic plants, 33 individual data points were selected for 4 standard species (*Raphidocelis subcapitata*, *Chlorella vulgaris*, *Chlamydomonas reinhardti and Lemna minor*). For the invertebrates 23 individual data points were selected for 3 standard species *(Ceriodaphnia dubia*, *Daphnia magna*). For the fish, 31 individual data points were selected for 3 standard species (*Oncorhynchus mykiss*, *Pimephales promelas* and *Salvelinus fontanilis*). NOEC values range between 2.2 µg Cu/L (*O. Mykiss*) and 510 µg Cu/l (*C. vulgaris*). Overall species specific geomean values (or lowest value if <4 data-points) range between 14.5 (*O.mykiss*) and 138 µg Cu/l *(C. vulgaris*).

When evaluating the NOEC values at the three pH classes from Table 6, sufficient data for the 3 pH classes were found for 2 algae species (*R. subcapitata* and *C. vulgaris*) and 2 invertebrate species (*D. magna* and *C. dubia*). No chronic toxicity values for fish were gathered at pH 5.5-6.5. Waiwood and Beamish (1978) assessed however 10 days growth rates of *O. Mykiss* growth at pH 6, with hardness varying between 30 and 360 mg $CaCO_3$/L – the observed EC20s were calculated by De Schamphelaere et al (2004) are respectively 23, 23 and 80 µg Cu/L. These data are included in the data-set.

A clear trend (lower toxicity at lower pH values) was observed for the algae. No clear pH trend was noticed for the invetebrates or fish.

Table 6: Overview of the selected high quality chronic toxicity data (NOEC values expressed as µg/l) or the 3 pH classes.

Test organism	NOEC (µg/l)			
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**	**All pHs**
Algae and higher plants				
Chlorella vulgaris				
n	5	7	5	17
Min.	108	36.4	31	31
Max.	510	283	188	510
Geometric mean/lowest value	280	106	98.1	138
Raphidocelis subcapitata				
n	1	3	8	12
Min.	94.7	52.9	15.7	15.7
Max.	94.7	65.5	164	164
Geometric mean/lowest value	94.7	52.9	34.5	43.1
Chlamydomonas rheinhardti				
n	1	2	/	3
Min.	178	22	/	22
Max.	178	188	/	188
Geometric mean/lowest value	178	22	/	22
Lemna minor				
n	1			1
Min.	30	/	/	30
Max.	30	/	/	30
Geometric mean/lowest value	30	/	/	30
Invertebrates				
Ceriodaphnia dubia				
n	1	4	9	14
Min.	20	4	6.3	4
Max.	20	19	122	122
Geometric mean/lowest value	20	7.4	19.8	14.9
Daphnia magna				
n	2	1	6	9
Min.	21.5	181	12.6	12.6
Max.	28	181	106	181
Geometric mean/lowest value	21.5	181	45.5	46.3
Fish				
Oncorhynchus mykiss				
n	3	4	3	10
Min.	23 (1)	2.2 (2)	11.4	2.2 (2)
Max.	80 (1)	45	16	45
Geometric mean/lowest value	23	16.1 (24 (3))	11.4	15.3 (19.0 (3))

Pimephales promelas				
n	/	5	9	14
Min.	/	4.8	14.5	4.8
Max.	/	10.6	66	66
Geometric mean/lowest value	/	7.7	33.3	19.7
Salvelinus fontanilis				
n	/	11	2	13
Min.	/	7	22.3	7
Max.	/	49	22.3	49
Geometric mean/lowest value	/	14.6	22.3	15.6

/: no data

(1) No NOECs available. EC20 values, as calculated by De schamphelaere et al (2004)
(2) The value of 2.2 µg Cu/L was derived by Marr et al 1999. The value was derived using extremely low DOC and Cu background levels in test and culturing water The resulting NOEC value is within the natural background range for copper in Europe. The other three values reported for the same species and pH range between 24 and 45 µg Cu/L. The value of 2.2 µg Cu/L is therefore considered as an outlier.
(3) Excluding the outlier

Table 7 summarizes the lowest recorded species-specific NOECs for each taxonomic group and pH. The lowest species-specific value across taxonomic groups are proposed as reference values.

Table 7: The lowest recorded species-specific NOECs for each taxonomic group and proposed reference values

Test organisms	**NOEC (µgCu/l)**			
	pH 6	**pH 7**	**pH 8**	**All pH**
Fish	23	7.7	11.4	15.6
Invertebrates	20	7.4	19.8	14.9
Algae/aq.plants	30	22	34.5	22
Proposed reference	**20**	**7.4**	**11.4**	**14.9**

Considering the relation between DOC and copper ecotoxcity, Table 8 provides an overview of the reliable NOECs from Table 7, but test-waters with low DOC (< 2 mg/L).

Table 8: Overview of the selected high quality chronic toxicity data (NOEC values expressed as µg/l), obataiained with test media with low DOC (<2 mg/L), for the 3 pH classes.

Test organism	NOEC (µg/l)			
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**	**All pHs, only if DOC<2 mg/L**
Algae and higher plants				
Chlorella vulgaris				
n	/	1	/	1
Min.	/	36.4	/	36.4
Max.	/	36.4	/	36.4
Geometric mean/lowest value	/	36.4	/	36.4
Raphidocelis subcapitata				
n	/	/	2	2
Min.	/	/	15.7	15.7
Max.	/	/	19.3	19.3
Geometric mean/lowest value	/	/	15.7	15.7
Chlamydomonas rheinhardti				
n	/	1	/	1
Min.	/	22	/	22
Max.	/	22	/	22
Geometric mean/lowest value	/	22	/	22
Lemna minor				
n	1	/		1
Min.	30	/	/	30
Max.	30	/	/	30
Geometric mean/lowest value	30	/	/	30
Invertebrates				
Ceriodaphnia dubia				
n	/	4	4	8
Min.	/	4	10	4
Max.	/	19	20	20
Geometric mean/lowest value	/	7.4	16.8	11.1
Daphnia magna				
n	/	/	4	4
Min.	/	/	12.6	12.6
Max.	/	/	68.8	68.8
Geometric mean/lowest value	/	/	32.9	32.9
Fish				
Oncorhynchus mykiss				
n	3	/	3	6
Min.	23 (1)	/	11.4	11.4
Max.	80 (1)	/	16	16
Geometric mean/lowest value	23	/	11.4	17.7

Pimephales promelas				
n	/	5	9	14
Min.	/	4.8	14.5	4.8
Max.	/	10.6	66	66
Geometric mean/lowest value	/	7.7	33.3	19.7
Salvelinus fontanilis				
n	/	11	2	13
Min.	/	7	22.3	7
Max.	/	49	22.3	49
Geometric mean/lowest value	/	14.6	22.3	15.6

Legend see table 5a

The selection of DOC<2 mg/L results in smaller database. The comparison of with the full data-set is provided Table 9. Considering the relative small difference between the 2 data-sets, the more robust reference values obtained with full data-set is retained for the classification

Table 9: Derived acute reference values with fill and skimmed data-set

pH range	**Reference values**	
	All NOEC (µg/l)	**NOEC (µg/l) with low DOC**
pH 5.5-6.5 pH >6.5-7.5 pH >7.5-8.5	20 (n=14) 7.4 (n=34) 11.4 (n=42)	30 (n=1) 7.4 (n=23) 11.4 (24)
all pH	14.9 (n= 90)	11.1 (48)

5.3. Summary on reference values

Table 10 gives a summary of the acute and chronic reference values for soluble copper ions.

The derived reference values are the same as the ones used for the classification of Cu_20 and the copper RA, except for a somewhat lower L(E)C50 at pH 5.5-6.5 (29.8 µg Cu/L in RA report versus 25 µg Cu/L in this report) and a some somewhat lower NOEC at pH 7.5-8.5 (16 µg Cu/L in RA report versus 11.4 µg Cu/L in this report). These small difference are related to data-treatment (no geomean values if less than 4 data-points).

Table 10: Acute and chronic reference values for soluble copper ions

pH range	Acute reference L(E)C50(µg Cu/l)	Chronic reference NOEC (µg Cu/l)
pH 5.5-6.5	**25.0**	**20**
pH >6.5-7.5	**35.0**	**7.4**
pH >7.5-8.5	**29.8**	**11.4**
Accross pHs	**34.4**	**14.9**

For classification purposes, these are to be translated to the respective soluble copper compounds using a molecular weight translation. They are translated to the classification of sparingly soluble copper compounds, copper powders and copper massives using the results of the transformation/dissolutions.

Annex 1. Bibliographies and databases consulted for the selection of acute ecotoxicity values for copper, applicable to the EU hazard classification system.

Major bibliographies
International Copper Association (in-house compilation)
North American Benthological Society (Current and Selected Bibliographies on Benthic Biology – 1991-1996 issues).
U.S. Environment Protection Agency (Fourth Annotated Bibliography on Biological Effects of Metals in Aquatic Environments)
World Health Organization (Copper Environmental Health Criteria)

Electronic Databases
Agricola
Applied Science and Technology Abstracts
AQUIRE
Article First
Biological Abstracts
Environment
Fish and Fisheries Worldwide
Papers First
Pollution Abstracts
Wildlife Worldwide
Wyoming Water Bibliography

Tables of contents of journals
Aquatic Toxicology (1991 – 1997)
Archives of Environmental Contamination and Toxicology (1990 – 1997)
Bulletin of Environmental Contamination and Toxicology (1991 – 1997)
Canadian Journal of Fisheries and Aquatic Sciences (1991 – 1997)

Environmental Pollution (1991 – 1997)
Environmental Toxicology and Chemistry (1982 – 1998)
Reviews of Environmental Contamination and Toxicology (1995 – 1997)
Toxicology and Environmental Chemistry (1990 – 1997)
Water, Air and Soil Pollution (1991 – 1997)
Water Research (1991 – 1996)

Major review articles and books

"Metal bioaccumulation in fishes and aquatic invertebrates: a literature review" (USEPA 1978)
"Metal speciation and bioavailability in aquatic systems" (Campbell 1995)
"The effects of environmental acid on freshwater fish with particular reference to the softwater lakes in Ontario and the modifying effects of heavy metals: a literature review" (Spry, Wood and Hodson 1981)

Annex 2: Summary of the individual high quality acute & chronic toxicity data

A list of abbreviations related to the tables listed herebelow:

Abbreviations for the life stage:
A: adult
ALV: alevins
EGG: eggs
EL: embryo-larval
F: fry
FGL: fingerlings
L: larvae
J: juveniles
N: neonates
P: parr
SA: sub-adult
SM: smolt
Y: yearling

Abbreviations for the water sources:
F: field water
L: lab water
T: tap water
R: reconstituted water
W: well water

Abbreviations for the flow mode:
S: static
SR: static renewal
FT: flow through

Table 2 A: overview of the individual acute toxicity data used for hazard classification. Abbrevations: see above; legend see also Table 2B

Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Algae										
Raphidocelis subcapitata				152,0	F	S	20-25	5,7	8,91	10,1
Raphidocelis subcapitata				194,0	F	S	20-25	6,1	10,3	7,9
Raphidocelis subcapitata				84,0	F	S	20-25	7,0	2,34	12,4
Raphidocelis subcapitata				32,0	F	S	20-25	7,1	1,55	27,8
Raphidocelis subcapitata				163,1	F	S	20-25	7,4	20,4	134,0
Raphidocelis subcapitata				52,7	F	S	20-25	7,7	2,52	48,5
Raphidocelis subcapitata				112,6	F	S	20-25	7,7	6,13	132,0
Raphidocelis subcapitata				245,3	F	S	20-25	7,8	17,8	166,0
Raphidocelis subcapitata				36,9	F	S	20-25	8,2	1,7	169,0
Raphidocelis subcapitata				41,0	F	S	20-25	7,9	1,99	191,0
Raphidocelis subcapitata				108,0	F	S	20-25	8,0	6,42	220,0
Raphidocelis subcapitata				65,4	F	S	20-25	7,8	8,24	238,0
Raphidocelis subcapitata				133,0	R	S		7,8		23,8
Heijerick et al., 200 2										
Pseudokirchnerella subcapitata				230				6,19	5,23	100
Pseudokirchnerella subcapitata				824				6,22	15,8	100
Pseudokirchnerella subcapitata				93				7,92	5,99	400
Pseudokirchnerella subcapitata				35				7,05	2,21	250
Pseudokirchnerella subcapitata				268				8,01	15,1	400
Pseudokirchnerella subcapitata				156				7,09	9,99	250
Pseudokirchnerella subcapitata				190				7,04	9,89	500
Pseudokirchnerella subcapitata				219				8,37	10,3	250

Pseudokirchnerella subcapitata				462				7,17	18,5	250
Pseudokirchnerella subcapitata				199				6,2	5,31	100
Pseudokirchnerella subcapitata				811				6,2	15,6	100
Pseudokirchnerella subcapitata				92				5,75	5,75	400
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Pseudokirchnerella subcapitata				35				7,08	2,06	250
Pseudokirchnerella subcapitata				346				8,05	16,1	400
Pseudokirchnerella subcapitata				178				7,09	11,1	250
Pseudokirchnerella subcapitata				281				7,12	10,5	500
Pseudokirchnerella subcapitata				161				8,25	10,3	250
Pseudokirchnerella subcapitata				685				7,11	19,9	250
Pseudokirchnerella subcapitata				122				6,18	5,07	100
Pseudokirchnerella subcapitata				368				6,17	14,9	100
Pseudokirchnerella subcapitata				51				7,92	5,46	400
Pseudokirchnerella subcapitata				30				7,02	1,95	250
Pseudokirchnerella subcapitata				151				7,78	15,2	400
Pseudokirchnerella subcapitata				99				7,04	10,1	250
Pseudokirchnerella subcapitata				105				7,02	10,4	500
Pseudokirchnerella subcapitata				55				8,58	10,5	250
Pseudokirchnerella subcapitata				174				6,98	18,2	250
Pseudokirchnerella subcapitata				205				5,99	5,64	400
Pseudokirchnerella subcapitata				59				8,02	5,42	100
Pseudokirchnerella subcapitata				209				8,05	15,3	100
Pseudokirchnerella subcapitata				756				5,68	9,84	250
Pseudokirchnerell				193				7,19	10,4	25

a subcapitata										
Pseudokirchnerella subcapitata				102				7,03	9,98	250
Pseudokirchnerella subcapitata				100				7,01	10,2	250
De Schamphelaere, 2003										
Chlamydomonas reinhardtii				380	F	S		6,02	9,84	250
Chlamydomonas reinhardtii				315	F	S		7,03	9,84	250
Chlamydomonas reinhardtii				146	F	S		8,11	9,84	250
De Schamphelaere, 2006										
Chlorella vulgaris				333				6,03	5,17	100
Chlorella vulgaris				773	F	S		6,04	15,49	100
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Chlorella vulgaris				99	F	S		7,92	5,04	400
Chlorella vulgaris				506	F	S		7,97	15,82	400
Chlorella vulgaris				296	F	S		7,03	10,81	250
Chlorella vulgaris				254	F	S		7,01	10,03	500
Chlorella vulgaris				60	F	S		7,04	1,5	250
Chlorella vulgaris				200	F	S		8,75	9,9	250
Chlorella vulgaris				446	F	S		7,05	19,09	250
Chlorella vulgaris				440	F	S		6,01	5,03	400
Chlorella vulgaris				987	F	S		6,05	15,24	400
Chlorella vulgaris				111	F	S		7,88	5,31	100
Chlorella vulgaris				380	F	S		7,88	15,66	100
Chlorella vulgaris				602	F	S		5,5	10,27	250
Chlorella vulgaris				238	F	S		7,07	10,26	25
Chlorella vulgaris				364	F	S		7,03	10,81	250
Chlorella vulgaris				208	F	S		7,04	10,23	250
De Schamphelaere, 2006										
Invertebrates										
Daphnia magna	N			100	R			7,59	1,5	
Daphnia magna	N			200	R			7,29	3,4	
Daphnia magna	N			106	R			6,4	2,2	
Daphnia magna	N			276	R			7,65	3,6	
Daphnia magna	N			292	R			6,11	6,9	
Daphnia magna	N			92,6	R			6,77	1,4	
Daphnia magna	N			210	R			8,39	3,8	
Daphnia magna	N			152	R			7,67	1,7	
Daphnia magna	N			526	R			7,71	7,8	
Daphnia magna	N			826	R			8,27	14,2	
Daphnia magna	N			388	R			7,74	4,0	

Daphnia magna	N			157	R			8,46	2,5	
Daphnia magna	N			136	R			6,95	2,5	
Daphnia magna	N			229	R			7,35	3,7	
Daphnia magna	N			244	R			8,39	4,4	
Daphnia magna	N			100	R			6,85	1,3	
Daphnia magna	N			1213	R			7,41	14,8	
Daphnia magna	N			421	R			6,29	9,0	
Daphnia magna	N			300	R			7,43	4,6	
Daphnia magna	N			289	R			7,81	4,8	
Daphnia magna	N			117	R			6,98	1,4	
Daphnia magna	N			109	R			7,0	2,2	
Daphnia magna	N			465	R			6,31	8,3	
Daphnia magna	N			798	R			8,46	10,9	
Daphnia magna	N			380	R			7,83	4,2	
Daphnia magna	N			35,2	F	S		5,5	10,3	8,0
Daphnia magna	N			37,9	F	S		6,3	2,72	10,1
Daphnia magna	N			33,8	F	S		6,1	2,34	12,4
Daphnia magna	N			40,9	F	S		6,4	4,87	18,1
Daphnia magna	N			295,0	F	S		7,0	10,1	52,7
Daphnia magna	N			648,0	F	S		7,5	20,4	133,4
Daphnia magna	N			792,0	F	S		7,4	22,8	139,7
Daphnia magna	N			686,0	F	S		7,3	17,8	165,4
Daphnia magna	N			281,0	F	S		7,6	7,81	69,7
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Daphnia magna	N			484,0	F	S		7,7	10,0	92,0
Daphnia magna	N			366,0	F	S		7,7	7,88	108,1
Daphnia magna	N			332,0	F	S		7,9	11,8	112,7
Daphnia magna	N			399,0	F	S		7,9	14,3	116,6
Daphnia magna	N			529,0	F	S		7,6	18,4	125,1
Daphnia magna	N			175,0	F	S		7,6	6,13	131,7
Daphnia magna	N			276,0	F	S		7,9	6,60	135,7
Daphnia magna	N			119,0	F	S		8,1	1,98	190,2
Daphnia magna	N			188,0	F	S		8,3	6,42	219,3
Daphnia magna	N			257,0	F	S		8,3	8,24	236,4
De Schampelaere et al, 2002										
Daphnia magna				7,0	T	S	25,0	7,5		45,0
Daphnia magna				10,0	T	S	20,0	7,5		45,0
Daphnia magna				40,0	T	S	15,0	7,5		45,0
Daphnia magna				70,0	T	S	10,0	7,5		45,0
Daphnia magna				90,0	T	S	5,0	7,5		45,0
Cairns et al., 1978										
Daphnia magna	N			26,0	W	S	21,6	7,5		143,0
Lewis, 1983										

Daphnia magna	N			9,8	F	S	18,0	7,7		45,3
Daphnia magna	N			60,0	F	S	18,0	7,7		45,3
Biesinger & Christe nsen, 1972										
Daphnia magna	N			26,0	W	S	18,2	7,8		52,0
Daphnia magna	N			30,0	W	S	20,3	7,9		105,0
Daphnia magna	N			38,0	W	S	19,7	8,1		106,0
Daphnia magna	N			69,0	W	S	19,9	8,3		207,0
Chapman et al., 198 0										
Daphnia magna	N			52,0	R	S	20,0	8,2		170,0
Daphnia magna	N			51,0	R	S	20,0	8,2		170,0
Daphnia magna	N			65	R	S	20,0	8,2		170,0
Daphnia magna	N			58	R	S	20,0	8,2		170,0
Daphnia magna	N			35	R	S	20,0	8,2		170,0
Daphnia magna	N			37	R	S	20,0	8,2		170,0
Daphnia magna	N			46	R	S	20,0	8,2		170,0
Daphnia magna	N			28	R	S	20,0	8,2		170,0
Daphnia magna	N			31	R	S	20,0	8,2		170,0
Daphnia magna	N			50	R	S	20,0	8,2		170,0
Daphnia magna	N			39	R	S	20,0	8,2		170,0
Daphnia magna	N			31	R	S	20,0	8,2		170,0
Daphnia magna	N			38	R	S	20,0	8,2		170,0
Daphnia magna	N			20,0	R	S	20,0	8,2		170,0
Daphnia magna	N			22,0	R	S	20,0	8,2		170,0
Daphnia magna	N			23,0	R	S	20,0	8,2		170,0
Daphnia magna	N			25,0	R	S	20,0	8,2		170,0
Daphnia magna	N			26,0	R	S	20,0	8,2		170,0
Daphnia magna	N			27,0	R	S	20,0	8,2		170,0
Daphnia magna	N			27,0	R	S	20,0	8,2		170,0
Daphnia magna	N			28,0	R	S	20,0	8,2		170,0
Daphnia magna	N			28,0	R	S	20,0	8,2		170,0
Daphnia magna	N			32,0	R	S	20,0	8,2		170,0
Daphnia magna	N			33,0	R	S	20,0	8,2		170,0
Daphnia magna	N			34,0	R	S	20,0	8,2		170,0
Test organism	**Life stage**	**Lengt h (cm)**	**Weight (g ww)**	**LC$_{50}$ (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardne ss mg/l CaCO3**
Daphnia magna	N			36,0	R	S	20,0	8,2		170,0
Daphnia magna	N			37,0	R	S	20,0	8,2		170,0
Daphnia magna	N			39,0	R	S	20,0	8,2		170,0
Daphnia magna	N			42,0	R	S	20,0	8,2		170,0
Daphnia magna	N			43,0	R	S	20,0	8,2		170,0
Daphnia magna	N			44,0	R	S	20,0	8,2		170,0
Daphnia magna	N			46,0	R	S	20,0	8,2		170,0
Lazorchak, 1987										
Daphnia magna	N		0,0095	30,0	R	SR	19,0	8,2		170,0

Daphnia magna	N		0,0083	31,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0094	31,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0134	35,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0113	37,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,011	38,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0122	39,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0109	46,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0156	50,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0136	51,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0142	52,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0164	58,0	R	SR	19,0	8,2		170,0
Daphnia magna	N		0,0155	63,0	R	SR	19,0	8,2		170,0
Lazorchak, 1993										
Daphnia magna	N			31,8	R	S	20,0	8,4		
Borgmann & Ralph, 1983										
Ceriodaphnia dubia	N			66,0	F	FT	25,0	8,2		100,0
Spehar & Fiandt, 19 85										
Ceriodaphnia dubia	<48 h			9,5	R	S	25,0	6,3		290,0
Ceriodaphnia dubia	<48 h			28,0	R	S	25,0	7,3		290,0
Ceriodaphnia dubia	<48 h			200,0	R	S	25,0	8,3		290,0
Schubauer-Berigan et al., 1993										
Ceriodaphnia dubia				8,5	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				8,5	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				10,8	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				10,8	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				39,6	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				39,0	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				46,9	R	SR	23,0	7,6		85,0
Ceriodaphnia dubia				46,3	R	SR	23,0	7,6		85,0
Cerda & Olive, 199 3										
Ceriodaphnia dubia				14,0	F	S	25,0	6,0	2.9 (2)	97,6
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**

Ceriodaphnia dubia				52,0	F	S	25,0	6,0	2 (3)	113,6
Ceriodaphnia dubia				56,0	F	S	25,0	6,0	3 (4)	182,0
Ceriodaphnia dubia				28,0	F	S	25,0	7,0	2.9 (2)	97,6
Ceriodaphnia dubia				76,0	F	S	25,0	7,0	2 (3)	113,6
Ceriodaphnia dubia				84,0	F	S	25,0	7,0	3 (4)	182,0
Ceriodaphnia dubia				31,0	F	S	25,0	8,0	2.9 (2)	97,6
Ceriodaphnia dubia				91,0	F	S	25,0	8,0	2 (3)	113,6
Ceriodaphnia dubia				93,0	F	S	25,0	8,0	3 (4)	182,0
Belanger & Cherry, 1990										
Fish										
Oncorhynchus mykiss	ALV			80,0	W	FT	12,0	7,7		120,0
Seim et al., 1984										
Oncorhynchus mykiss	F		2,7	4,2	W	FT	15,8	5,7		9,2
Oncorhynchus mykiss	F		2,65	2,8	W	FT	15,8	7,0		9,2
Cusimano et al., 19 86										
Oncorhynchus kisutch	A			57,0	W	FT	9,0	7,6		42,0
Chapman & Steven s, 1978										
Oncorhynchus mykiss	F		0,17	17,0	F	FT	12,2	7,1		22,0
Oncorhynchus mykiss	P		6,96	18,0	F	FT	12,2	7,1		22,0
Oncorhynchus mykiss	ALV			28,0	F	FT	12,2	7,1		22,0
Oncorhynchus mykiss	SM		68,19	29,0	F	FT	12,2	7,1		22,0
Chapman, 1978										
Oncorhynchus mykiss			2,2	28,9	W	FT	15,0	6,0		31,0
Oncorhynchus mykiss			1,1	22,4	W	FT	15,0	6,0		32,0
Oncorhynchus mykiss			3,2	40,0	W	FT	15,0	6,0		101,0
Oncorhynchus mykiss			1	70,0	W	FT	15,0	6,0		366,0
Oncorhynchus mykiss			1,7	82,2	W	FT	15,0	6,0		371,0
Oncorhynchus mykiss			0,75	31,9	W	FT	15,0	7,0		99,0

Oncorhynchus mykiss			10	81,1	W	FT	15,0	7,0		100,0
Oncorhynchus mykiss			1,55	47,4	W	FT	15,0	7,0		101,0
Test organism	**Life stage**	**Lengt h (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardne ss mg/l CaCO3**
Oncorhynchus mykiss			6,6	298,0	W	FT	15,0	7,0		361,0
Oncorhynchus mykiss			1,4	30,0	W	FT	15,0	8,0		31,0
Oncorhynchus mykiss			0,9	309,0	W	FT	15,0	8,0		360,0
Oncorhynchus mykiss			1,8	516,0	W	FT	15,0	8,0		371,0
Howarth & Sprague , 1978										
Oncorhynchus mykiss			2,1	83,3	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss				85,3	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			2,5	103,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			4,3	128,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			11,5	165,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			1	169,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			18,7	197,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			9,4	221,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			25,6	243,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			2,6	274,0	W	FT	12,8	7,8		194,0
Oncorhynchus mykiss			24,9	514,0	W	FT	12,8	7,8		194,0
Chakoumakos et al., 1979										
Oncorhynchus mykiss			1.2-7.9	102,0	W	FT	15	8,2		362,0
Fogels & Sprague, 1977										
Oncorhynchus mykiss	J		6	164,0	F	SR	13,5	7,3		33,0
Oncorhynchus mykiss	J		6	286,0	F	SR	13,5	7,5		41,0
Buckley, 1983										
Oncorhynchus mykiss	P			94,0	F	FT	6,0	6,8		18,3

Oncorhynchus mykiss	P			89,0	F	FT	9,5	7,1		18,6
Oncorhynchus mykiss	P			93,0	F	FT	4,4	6,9		23,7
Oncorhynchus mykiss	P			68,0	F	FT	15,3	7,2		28,6
Mudge et al., 1993										
Oncorhynchus mykiss				890,0	L	FT	15,3	7,4		300,0
Calamari & Marche tti, 1973										
Oncorhynchus mykiss	2 mo.			253,0	F	FT		7,4		
Test organism	**Life stage**	**Lengt h (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardne ss mg/l CaCO3**
Hale, 1977										
Oncorhynchus mykiss	F		0,36	9,5	W	FT	10,0	7,6		24,2
Oncorhynchus mykiss	F		0,36	10,5	W	FT	10,0	7,6		24,2
Oncorhynchus mykiss	F		0,36	12,5	W	FT	10,0	7,6		24,2
Oncorhynchus mykiss	F		0,36	16,5	W	FT	10,0	7,6		24,2
Oncorhynchus mykiss	F		0,36	21,5	W	FT	10,0	7,6		24,2
Marr et al., 1999										
Oncorhynchus mykiss	F			18,0	W	FT	9,8	7,5		25,1
Marr et al., 1998										
Oncorhynchus mykiss	J		29,1	190,0	T	FT	10,0	7,8		125,0
Oncorhynchus mykiss	J		3,9	200,0	T	FT	10,0	7,8		125,0
Oncorhynchus mykiss	A		176	210,0	T	FT	10,0	7,8		125,0
Spear, 1977										
Brachydanio rerio			0.2-0.6	149,0	W	FT	25,0	8,2		362,0
Fogels & Sprague, 1977										
Brachydanio rerio	6 mo.			35,0	R	SR	24,0	7,2		100,0
Bresch, 1982										
Cyprinus carpio				800,0	F		28,0	8,0		55,0
Rehwoldt et al., 197 2										
Cyprinus carpio				810,0	F	S	17,0	7,8		53,0
Rehwoldt et al., 197 1										
Pimephales promelas	L 1-d			15,0	R	S	25,0	6,3		290,0
Schubauer-Berigan et al., 1993										

Pimephales promelas	F			75,0	F	FT	22,0	7,1		31,4
Pimephales promelas	F			84,0	F	S	22,0	7,1		31,4
Mount & Stephan, 1 969										
Pimephales promelas	30 d		0,15	96,0	F	FT	25,0	7,4		43,9
Spehar & Fiandt, 19 85										
Pimephales promelas	L			171,0	F	S	25,0	7,4		36,0
Nelson et al., 1985										
Pimephales promelas		2.0-6.9		600,0	F	S	9,5	8,5		212,0
Pimephales promelas		2.0-6.9		690,0	F	S	21,5	8,3		120,0
Pimephales promelas		2.0-6.9		750,0	F	S	6,5	8,2		244,0
Pimephales promelas		2.0-6.9		830,0	F	S	17,5	8,1		228,0
Pimephales		2.0-6.9		930,0	F	S	21,5	8,1		150,0
Test organism	**Life stage**	**Lengt h (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardne ss mg/l CaCO3**
promelas										
Pimephales promelas		2.0-6.9		980,0	F	S	18,0	8,0		224,0
Pimephales promelas		2.0-6.9		820,0	F	S	12,5	8,1		260,0
Pimephales promelas		2.0-6.9		630,0	F	S	25,5	8,2		280,0
Pimephales promelas		2.0-6.9		750,0	F	S	3,0	8,0		280,0
Pimephales promelas		2.0-6.9		770,0	F	S	21,5	8,4		280,0
Pimephales promelas		2.0-6.9		730,0	F	S	23,5	8,0		294,0
Pimephales promelas		2.0-6.9		860,0	F	S	22,5	8,3		298,0
Pimephales promelas		2.0-6.9		840,0	F	S	26,0	8,3		308,0
Pimephales promelas		2.0-6.9		870,0	F	S	18,5	8,2		310,0
Brungs et al., 1976										
Pimephales promelas				390,0	F	S	22,0	7,6		44,0
Curtis & Ward, 198 1										
Pimephales promelas	L 1-d			44,0	R	S	25,0	7,3		290,0
Pimephales	L 1-d			>200, 0	F	S	25,0	8,3		290,0

promelas										
Schubauer-Berigan et al., 1993										
Pimephales promelas	F			430,0	F	S	20,8	7,9		198,0
Pimephales promelas	F			470,0	F	FT	20,8	7,9		198,0
Mount, 1968										
Pimephales promelas	SA			460,0	F	FT	22,0	7,8		202,0
Pimephales promelas	F			490,0	F	FT	22,0	7,8		202,0
Pickering et al., 197 7										
Pimephales promelas				1090,0	F	S	11,0	7,4		260,0
Pimephales promelas				920,0	F	S	15,0	7,5		240,0
Pimephales promelas				>610, 0	F	S	24,5	7,4		266,0
Pimephales promelas				<640, 0	F	S	25,5	7,5		280,0
Pimephales promelas				>780, 0	F	S	21,5	7,5		280,0
Pimephales promelas				960,0	F	S	24,5	7,5		282,0
Pimephales promelas				1400,0	F	S	25,0	7,4		298,0
Pimephales promelas				970,0	F	S	26,0	7,2		308,0
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Pimephales promelas				810,0	F	S	18,5	7,3		310,0
Pimephales promelas				780,0	F	S	21,0	7,5		324,0
Pimephales promelas				>800, 0	F	S	23,5	7,3		336,0
Pimephales promelas	FGL		1,6	465,0	L	FT	24,0	8,0		200,0
Pimephales promelas				600,0	F	S	3,0	8,2		206,0
Pimephales promelas				640,0	F	S	8,5	8,1		242,0
Pimephales promelas				660,0	F	S	9,5	8,5		212,0
Pimephales promelas				690,0	F	S	3,0	8,0		210,0
Pimephales promelas				750,0	F	S	6,5	8,0		244,0

Pimephales promelas				820,0	F	S	17,5	7,9		228,0
Pimephales promelas				940,0	F	S	21,5	7,9		150,0
Pimephales promelas				1060,0	F	S	18,0	8,0		224,0
Pimephales promelas				580,0	F	S	6,5	7,8		252,0
Pimephales promelas				830,0	F	S	23,0	7,6		252,0
Pimephales promelas				820,0	F	S	4,5	7,9		260,0
Pimephales promelas				950,0	F	S	12,5	8,0		260,0
Pimephales promelas				680,0	F	S	2,5	8,1		262,0
Pimephales promelas				560,0	F	S	18,5	8,1		272,0
Pimephales promelas				645,0	F	FT	17,0	8,3		274,0
Pimephales promelas				650,0	F	S	19,5	8,0		276,0
Pimephales promelas				750,0	F	S	3,0	8,0		280,0
Pimephales promelas				820,0	F	S	21,0	7,6		284,0
Pimephales promelas				830,0	F	S	24,0	7,7		284,0
Pimephales promelas				490,0	F	S	15,0	7,9		290,0
Pimephales promelas	FGL		0,6	1000,0	F	FT	24,0	8,0		303,0
Pimephales promelas				750,0	F	S	7,0	7,8		308,0
Pimephales promelas				810,0	F	S	22,5	7,9		310,0
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Pimephales promelas				760,0	F	S	14,5	7,6		312,0
Pimephales promelas	FGL			540,0	F	FT	24,0	8,0		314,0
Pimephales promelas				865,0	F	FT	19,0	8,2		316,0
Pimephales promelas	FGL			650,0	F	FT	15,0	8,2		318,0
Pimephales promelas				920,0	F	S	8,0	8,0		322,0
Geckler et al., 1977										

Pimephales promelas	A			136,5	R	S	22,0	7,6		44,0
Curtis et al., 1979										
Pimephales promelas	L			12,4	F/L	FT	22,0	7,0		24,0
Pimephales promelas	L			4,4	F/L	FT	22,0	6,0		22,5
Pimephales promelas	L			21,0	F	FT	22,0	7,2		45,0
Pimephales promelas	L			19,7	F	FT	22,0	7,4		45,5
Pimephales promelas	L			18,2	F	FT	22,0	7,2		46,0
Pimephales promelas	L			5,9	F	FT	22,0	6,53		49,0
Pimephales promelas	L			79,7	F/L	S	22,0	7,3		85,1
Pimephales promelas	L			71,2	F/L	S	22,0	7,1		87,1
Pimephales promelas	L			663,0	L	S	22,0	7,2		251,2
Pimephales promelas	L			20,5	F/L	S	22,0	7,5		19,0
Pimephales promelas	L			26,9	F/L	FT	22,0	8,0		23,0
Pimephales promelas	L			12,4	F/L	S	22,0	7,6		25,0
Pimephales promelas	L			17,2	F	S	22,0	7,9		45,5
Pimephales promelas	L			20,3	F/L	S	22,0	7,6		27,0
Pimephales promelas	L			21,6	F	S	22,0	7,9		45,5
Pimephales promelas	L			23,2	F/L	S	22,0	7,5		34,0
Pimephales promelas	L			26,7	F/L	S	22,0	7,5		27,0
Pimephales promelas	L			26,7	F/L	S	22,0	7,6		30,0
Pimephales promelas	L			28,0	F/L	S	22,0	7,6		29,0
Pimephales promelas	L			35,0	F/L	S	22,0	7,5		51,0
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Pimephales promelas	L			36,9	F	FT	22,0	8,2		47,0
Pimephales promelas	L			41,1	F	FT	22,0	8,0		44,0

Pimephales promelas	L			42,1	F/L	S	22,0	7,7		27,0
Pimephales promelas	L			42,6	F	S	22,0	8,0		44,5
Pimephales promelas	L			44,7	F	FT	22,0	7,8		44,0
Pimephales promelas	L			46,3	F	FT	22,0	8,0		44,5
Pimephales promelas	L			52,7	F	FT	22,0	8,1		45,0
Pimephales promelas	L			58,5	F	FT	22,0	8,4		48,0
Pimephales promelas	L			62,3	F	S	22,0	7,9		45,5
Pimephales promelas	L			62,5	F/L	S	22,0	7,8		41,0
Pimephales promelas	L			68,6	F	S	22,0	8,0		44,5
Pimephales promelas	L			70,1	F/L	S	22,0	7,9		50,0
Pimephales promelas	L			70,5	F	FT	22,0	8,1		47,0
Pimephales promelas	L			70,8	F	FT	22,0	8,0		45,0
Pimephales promelas	L			75,8	F	FT	22,0	8,1		45,0
Pimephales promelas	L			77,0	F	FT	22,0	8,0		44,0
Pimephales promelas	L			77,5	F/L	S	22,0	8,0		51,0
Pimephales promelas	L			78,9	F	S	22,0	7,8		37,0
Pimephales promelas	L			81,6	F/L	S	22,0	8,0		51,0
Pimephales promelas	L			83,9	F	S	22,0	8,0		45,5
Pimephales promelas	L			84,3	F/L	S	22,0	8,0		52,0
Pimephales promelas	L			92,9	F	FT	22,0	8,2		43,0
Pimephales promelas	L			94,0	F	S	22,0	8,0		44,5
Pimephales promelas	L			96,7	F/L	S	22,0	8,1		60,1
Pimephales promelas	L			97,9	F/L	S	22,0	8,0		51,0
Pimephales promelas	L			99,6	F/L	S	22,0	7,6		88,1
Pimephales promelas	L			101,5	F/L	S	22,0	7,9		59,1

Test organism	Life stage	Length (cm)	Weight (g ww)	LC_{50} (µg/l)	Water source	Flow mode	Temp °C	pH	DOC mg/l	Hardness mg/l CaCO3
Pimephales promelas	L			103,8	F	S	22,0	7,8		45,0
Pimephales promelas	L			110,9	F/L	S	22,0	8,0		53,0
Pimephales promelas	L			111,8	F	S	20,0	7,8		47,0
Pimephales promelas	L			112,0	F	FT	22,0	8,1		45,0
Pimephales promelas	L			113,4	F	S	22,0	7,8		47,0
Pimephales promelas	L			114,6	F	S	22,0	7,9		46,0
Pimephales promelas	L			117,4	F	S	22,0	7,9		75,1
Pimephales promelas	L			122,9	F	S	22,0	7,8		138,1
Pimephales promelas	L			122,9	F	FT	22,0	8,1		243,2
Pimephales promelas	L			123,3	F	S	22,0	8,0		46,5
Pimephales promelas	L			125,4	F	S	22,0	7,8		60,1
Pimephales promelas	L			125,7	F	S	22,0	7,9		46,0
Pimephales promelas	L			126,6	F	S	22,0	7,9		74,1
Pimephales promelas	L			127,1	F/L	S	22,0	8,1		91,1
Pimephales promelas	L			129,9	F	S	22,0	7,8		45,0
Pimephales promelas	L			131,1	F	S	22,0	7,8		45,0
Pimephales promelas	L			132,8	F/L	S	22,0	7,7		87,1
Pimephales promelas	L			137,2	F/L	S	22,0	8,1		87,1
Pimephales promelas	L			143,0	F	S	22,0	8,0		45,5
Pimephales promelas	L			148,7	F/L	S	22,0	8,2		90,1
Pimephales promelas	L			150,3	F/L	S	22,0	8,2		91,1
Pimephales promelas	L			151,0	F	S	22,0	7,8		46,0
Pimephales promelas	L			152,0	F/L	S	22,0	8,0		53,0
Pimephales	L			155,7	F	S	22,0	7,8		76,1

promelas										
Pimephales promelas	L			158,1	F	S	22,0	7,8		107,1
Pimephales promelas	L			160,3	F	S	22,0	7,8		45,0
Pimephales promelas	L			166,7	F	S	22,0	7,8		103,1
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Pimephales promelas	L			167,3	F	S	22,0	7,8		103,1
Pimephales promelas	L			167,4	F	S	22,0	7,9		76,1
Pimephales promelas	L			167,4	F	S	22,0	7,8		45,0
Pimephales promelas	L			168,4	F	S	22,0	8,0		44,5
Pimephales promelas	L			172,4	F	S	22,0	7,8		151,1
Pimephales promelas	L			172,6	F	S	22,0	7,9		43,0
Pimephales promelas	L			172,8	F	S	22,0	7,9		133,1
Pimephales promelas	L			175,4	F/L	S	22,0	7,9		52,0
Pimephales promelas	L			176,9	F	S	22,0	7,8		46,0
Pimephales promelas	L			181,0	F	S	22,0	7,8		45,0
Pimephales promelas	L			182,4	F/L	S	22,0	8,4		87,1
Pimephales promelas	L			183,0	F	S	22,0	7,8		44,0
Pimephales promelas	L			183,0	F/L	S	22,0	8,0		90,1
Pimephales promelas	L			183,0	F/L	S	22,0	8,1		120,1
Pimephales promelas	L			189,1	F/L	S	22,0	8,1		87,1
Pimephales promelas	L			190,6	F	S	22,0	7,9		45,5
Pimephales promelas	L			190,8	F/L	S	22,0	8,1		180,2
Pimephales promelas	L			191,7	F	S	22,0	7,8		189,2
Pimephales promelas	L			193,0	F	FT	22,0	8,1		45,5
Pimephales promelas	L			199,8	F	S	22,0	7,8		134,1

Pimephales promelas	L			223,7	F/L	S	22,0	8,1		93,1
Pimephales promelas	L			226,8	F	S	22,0	7,8		134,1
Pimephales promelas	L			229,9	F	FT	22,0	8,3		47,0
Pimephales promelas	L			230,0	F	FT	22,0	8,1		45,0
Pimephales promelas	L			242,6	F	S	22,0	7,8		139,1
Pimephales promelas	L			253,5	F/L	S	22,0	8,1		92,1
Pimephales promelas	L			256,2	F	FT	22,0	8,1		45,5
Test organism	**Life stage**	**Length (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardness mg/l CaCO3**
Pimephales promelas	L			262,3	F/L	S	22,0	8,0		90,1
Pimephales promelas	L			268,4	F	S	22,0	8,1		138,1
Pimephales promelas	L			271,4	F/L	S	22,0	8,1		107,1
Pimephales promelas	L			283,3	F/L	S	22,0	8,1		92,1
Pimephales promelas	L			289,1	F	S	22,0	8,1		43,0
Pimephales promelas	L			289,1	F	S	22,0	7,8		45,0
Pimephales promelas	L			292,3	F/L	S	22,0	8,0		88,1
Pimephales promelas	L			370,3	F/L	S	22,0	8,0		140,1
Pimephales promelas	L			405,1	F/L	S	22,0	8,0		140,1
Pimephales promelas	L			496,0	F/L	S	22,0	8,1		194,2
Pimephales promelas	L			521,0	L	S	22,0	8,1		235,2
Pimephales promelas	L			644,9	F/L	S	22,0	8,4		144,1
Pimephales promelas	L			646,8	F/L	S	22,0	8,3		218,2
Pimephales promelas	L			653,8	F/L	S	22,0	8,3		217,2
Pimephales promelas	L			758,1	F/L	S	22,0	8,1		200,2
Pimephales promelas	L			940,4	F/L	S	22,0	8,3		212,2
Pimephales	L			953,0	F/L	S	22,0	8,4		226,2

promelas										
Pimephales promelas	L			892,1	L	S	22,0	8,3		251,2
Pimephales promelas	L			905,4	L	S	22,0	7,6		252,2
Pimephales promelas	L			996,3	L	S	22,0	7,9		252,2
Pimephales promelas	L			38,4	F	FT	22,0	8,1		255,7
Pimephales promelas	L			698,0	F/L	S	22,0	8,3		292,3
Pimephales promelas	L			752,9	F/L	S	22,0	8,2		440,4
Erickson et al., 1996										
Pimephales promelas				297,0	R		12,0	8,2		164,0
Pimephales promelas				311,0	R		22,0	8,2		164,0
Pimephales promelas				450,0	R		5,0	8,2		164,0
Pimephales				513,0	R		32,0	8,2		164,0
Test organism	**Life stage**	**Lengt h (cm)**	**Weight (g ww)**	**LC_{50} (µg/l)**	**Water source**	**Flow mode**	**Temp °C**	**pH**	**DOC mg/l**	**Hardne ss mg/l CaCO3**
promelas										
Richards & Beiting er, 1995										
Pimephales promelas	L			232,0	F	S	25,0	7,5		55,0
Pimephales promelas	L			363,0	F	S	25,0	7,5		68,0
Pimephales promelas	L			>449, 0	F	S	25,0	7,3		82,0
Pimephales promelas	L			427,0	F	S	25,0	7,3		90,0
Pimephales promelas	L			52,0	F	S	25,0	7,7		52,0
Pimephales promelas	L			171	F	S	25,0	7,4		36,0
Nelson et al., 1985										
Pimephales promelas	A			210,0	R	S	22,0	7,4		103,0
Pimephales promelas	A			360,0	R	S	22,0	7,4		103,0
Pimephales promelas	A			390,0	R	S	22,0	7,4		262,5
Pimephales promelas	A			410,0	R	S	22,0	7,4		262,5
Birge et al., 1983										

Lepomis macrochirus	FGL			4250,0	F	FT	15,0	8,2		318,0
Lepomis macrochirus	FGL			4300,0	F	FT	19,0	8,2		316,0
Lepomis macrochirus	FGL		18,9	9150,0	L	FT	24,0	8,0		200,0
Geckler et al., 1976										
Lepomis macrochirus				1000,0	T	FT	22,0	7,2		40,2
Thompson et al., 19 80										
Lepomis macrochirus	J		35	1100,0	F	FT	20,0	7,5		45,0
Benoit, 1975										
Lepomis macrochirus			1-9	710,0	R	S	20,0	5,4		46,0
Lepomis macrochirus			1-9	770,0	R	S	20,0	5,4		46,0
Trama, 1954										

Table 2 B: overview of the individual chronic toxicity data used for hazard classification.

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC µg Cu/L	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Oncorhynchus mykiss	parr	61 d	growth	**45**	FT	/	T: 9.5 °C; pH: 7.2; H: 24.4 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus mykiss	eggs	63 d	growth	**16**	FT	3	T: 12 °C; pH: 7.65; H: 120 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Seim et al., 1984 (29)
Oncorhynchus mykiss	parr	61 d	mortality	**24**	FT	/	T: 9.5 °C; pH: 7.15; H: 24.4 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus mykiss	parr	61 d	mortality	**28**	FT	/	T: 8.7 °C; pH: 7.0; H: 28.7 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus mykiss	embryo	45 d	Growth	**11.4**	FT	3	T: 10.8 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Oncorhynchus mykiss	embryo	45 d	mortality	**11.4**	FT	3	T: 10.8 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Oncorhynchus mykiss	fry (0.12 g; 2.6 cm)	60 d	growth	**2.2**	FT	<0.45*	T: 9.8 °C; pH: 7.5; H: 24.6 mg/l CaCO3; DOC: 0.2 mg/l (12)	Well + deionised water	Marr et al., 1996 (28)

Pimephales promelas	fry (10 - 15 mm)	330 d	growth	**33**	FT	3.5	T: 21°C; pH: 8.0; H: 198 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount, 1968 (34)
Pimephales promelas	fry (10 - 20 mm)	327 d	growth	**10.6**	FT	4.4	T: 22°C; pH: 6.9; H: 31.4 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount & Stephan, 1969 (35)
Pimephales promelas	larvae (4 weeks old)	187 d	growth	**59.5**	FT	4.2	T: 23°C; pH: 7.85; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ demineralised tap	Pickering et al., 1977 (36)
Pimephales promelas	embryo-larval	32 d	growth	**4.8**	FT	1.25*	T: 25°C; pH: 7.05; H: 44 mg/l CaCO3; DOC: 1 mg/l (13)	Lake (Lake Superior)	Spehar & Fiandt, 1985 (37)
Pimephales promelas	fry (10 - 15 mm)	330 d	mortality	**33**	FT	3.5	T: 21°C; pH: 8.0; H: 198 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount, 1968 (34)
Pimephales promelas	fry (10 - 20 mm)	327 d	mortality	**10.6**	FT	4.4	T: 22°C; pH: 6.9; H: 31.4 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount & Stephan, 1969 (35)
Pimephales promelas	larvae	28 d	mortality	**61**	FT	0.6	T: 21°C; pH: 8.17; H: 202 mg/l CaCO3; DOC: 1.3 mg/l (9)	Ground water	Scudder et al., 1988 (38)
Pimephales promelas	embryo-larval	32 d	mortality	**4.8**	FT	1.25*	T: 25°C; pH: 7.05; H: 44 mg/l CaCO3; DOC: 1 mg/l (13)	Lake (Lake Superior)	Spehar & Fiandt, 1985 (37)
Pimephales promelas	juvenile (32 - 38 mm; 5 months old)	270 d	reproduction	**66**	FT	7	T: 23°C; pH: 8.1; H: 274 mg/l CaCO3; DOC: 2 mg/l (3)	River	Brungs et al., 1976 (39)
Pimephales promelas	fry (10 - 15 mm)	330 d	reproduction	**14.5**	FT	3.5	T: 21°C; pH: 8.0; H: 198 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount, 1968 (34)
Pimephales promelas	fry (10 - 20 mm)	327 d	reproduction	**10.6**	FT	4.4	T: 22°C; pH: 6.9; H: 31.4 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount & Stephan, 1969 (35)
Pimephales promelas	larvae (4 weeks old)	187 d	reproduction	**25.5**	FT	4.2	T: 23°C; pH: 7.9; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Pickering et al., 1977 (36)
Pimephales promelas	larvae (4 weeks old)	97 d	reproduction	**23**	FT	4.2	T: 23°C; pH: 7.9; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Pickering et al., 1977 (36)
Pimephales promelas	larvae (4 weeks old)	7 d	reproduction	**22.5**	FT	4.2	T: 23°C; pH: 7.9; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Pickering et al., 1977 (36)
Salvelinus fontinalis	embryo	60 d	Growth	**22.3**	FT	/	T: 5.6 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)

Salvelinus fontinalis	embryo	60 d	mortality	**22.3**	FT	/	T: 5.6 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Salvelinus fontinalis	Alevins/juveniles	189 d	Growth	**9.5**	FT	/	T: 10.6 °C; pH: 7.5; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	Alevins/juveniles	189 d	mortality	**9.5**	FT	/	T: 10.6 °C; pH: 7.5; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	yearling	244 d	growth	**17.4**	FT	/	T: 10.6 °C; pH: 7.5; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	fry	30 d	Growth	**7**	FT	3	T: 10 °C; pH: 6.85; H: 37.5 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	fry	30 d	growth	**21**	FT	3	T: 10 °C; pH:6.9; H: 187 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	yearling	244 d	mortality	**17.4**	S	1.9	T: 10.6 °C; pH: 7.45; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	fry	60 d	mortality	**13**	FT	3	T: 10 °C; pH: 6.85; H: 37.5 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	fry	30 d	mortality	**21**	FT	3	T: 10 °C; pH:6.9; H: 187 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	yearling	244 d	reproduction	**17.4**	FT	1.9	T: 10.6 °C; pH: 7.45; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	fry	60 d	reproduction	**7**	FT	3	T: 10 °C; pH: 6.85; H: 37.5 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	fry	30 d	reproduction	**49**	FT	3	T: 10 °C; pH:6.9; H: 187 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproduction	**10**	R	0.5*	T: 23°C; pH: 7.6; H: 85 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitued	Cerda & Olive, 1993 (5)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	mortality	**20**	R	0.5*	T: 23°C; pH: 7.6; H: 85 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitued	Cerda & Olive, 1993 (5)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproduction	**10**	S	1.5*	T: 25°C; pH: 9.0; H: 98 mg/l CaCO3; DOC: 2.9 mg/l (2)	River (New River)	Belanger & Cherry, 1990 (6)

Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproductio n	**20**	S	1.5*	T: 25°C; pH: 8.0; H: 114 mg/l CaCO3; DOC: 2 mg/l (3)	River (Amy Bayou)	Belanger & Cherry, 1990 (6)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproductio n	**20**	S	1.5*	T: 25°C; pH: 9.0; H: 114 mg/l CaCO3; DOC: 2 mg/l (3)	River (Amy Bayou)	Belanger & Cherry, 1990 (6)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproductio n	**20**	S	1.5*	T: 25°C; pH: 6.0; H: 182 mg/l CaCO3; DOC: 3 mg/l (4)	River (Clinch River)	Belanger & Cherry, 1990 (6)
Ceriodaphnia dubia	neonates (< 8 h)	7 d	mortality	**19**	S	/	T: 25°C; pH: 7.0; H: 22 mg/l CaCO3; DOC: 2 mg/l (3)	River	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 8 h)	7 d	mortality	**4**	S	/	T: 25°C; pH: 6.95; H: 20 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitut ed	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	mortality	**122**	R	3.4	T: 25°C; pH: 8.25; H: 100 mg/l CaCO3; DOC: 5.7 mg/l (5)	River (Lester River)	Spehar & Fiandt, 1985 (8)
Ceriodaphnia dubia	neonates (2-8 h)	7 d	reproductio n	**6.3**	S	1.5	T: 25°C; pH: 8.15; H: 94 mg/l CaCO3; DOC: 2.9 mg/l (2)	River (New River)	Belanger et al., 1989 (9)
Ceriodaphnia dubia	neonates (2-8 h)	7 d	reproductio n	**24.1**	S	4.7	T: 25°C; pH: 8.31; H: 179 mg/l CaCO3; DOC: 3 mg/l (4)	River (Clinch River)	Belanger et al., 1989 (9)
Ceriodaphnia dubia	neonates (< 8 h)	7 d	reproductio n	**4**	S	/	T: 25°C; pH: 6.3-7.6; H: 20 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitut ed	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 8 h)	7 d	reproductio n	**10**	S	/	T: 25°C; pH: 6.6-7.4; H: 22 mg/l CaCO3; DOC: 2 mg/l (3)	River	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproductio n	**31.6**	S	3.4	T: 25°C; pH: 8.25; H: 100 mg/l CaCO3; DOC: 5.7 mg/l (5)	River (Lester River)	Spehar & Fiandt, 1985 (8)
Daphnia magna	neonates	21 d	growth	**12.6**	R	2.6	T: 20°C; pH: 8.1; H: 225 mg/l CaCO3; DOC: 2 mg/l (3)	Lake (Lake Ijssel)	Van Leeuwen et al., 1988 (10)
Daphnia magna	neonates	21 d	mortality	**36.8**	R	2.6	T: 20°C; pH: 8.1; H: 225 mg/l CaCO3; DOC: 2 mg/l (3)	Lake (Lake Ijssel)	Van Leeuwen et al., 1988 (10)

Daphnia magna	neonates	21 d	population growth	**36.8**	FT	2.6	T: 20°C; pH: 8.1; H: 225 mg/l CaCO3; DOC: 2 mg/l (3)	Lake (Lake Ijssel)	Van Leeuwen et al., 1988 (10)
Daphnia magna	neonates	21 d	reproductio n	**28**	R	0.5*	T: 20°C; pH: 6.31; H: 10 mg/l CaCO3; DOC: 2.72 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	21 d	reproductio n	**21.5**	R	0.5*	T: 20°C; pH: 6.1; H: 12.4 mg/l CaCO3; DOC: 2.34 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	21 d	reproductio n	**71.4**	R	0.5*	T: 20°C; pH: 8.3; H: 238 mg/l CaCO3; DOC: 8.24 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	21 d	reproductio n	**68.8**	R	0.5*	T: 20°C; pH: 8.06; H: 191 mg/l CaCO3; DOC: 1.99 mg/l	River	Heijerick et al., 2002 (11)
Daphnia magna	neonates	21 d	reproductio n	**106**	R	0.5*	T: 20°C; pH: 7.55; H: 132 mg/l CaCO3; DOC: 6.13 mg/l	River	Heijerick et al., 2002 (11)
Daphnia magna	neonates	21 d	reproductio n	**181**	R	0.5*	T: 20°C; pH: 7.5; H: 134 mg/l CaCO3; DOC: 20.4 mg/l	Lake	Heijerick et al., 2002 (11)
Chlamydomonas reinhardtii	Inoculum: 1,000 c/ml	10 d	growth	**22**	FT	0.5*	T: 24°C; pH: 6.6; H: 25 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitut ed	Schäfer et al., 1994 (1)

3 d

Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml		growth	**178**	S	0.5*	T: 20°C; pH: 6.02; H: 23 mg/l CaCO3; DOC: 9.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	growth	**108**	S	0.5*	T: 20°C; pH: 7.03; H: 23 mg/l CaCO3; DOC: 9.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	growth	**96**	S	0.5*	T: 20°C; pH: 8.11; H: 23 mg/l CaCO3; DOC: 9.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**108.3**	S	0.5*	T: 20°C; pH: 6.03; H: 97 mg/l CaCO3; DOC: 5.17 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**407.4**	S	0.5*	T: 20°C; pH: 6.04; H: 99 mg/l CaCO3; DOC: 15.5 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**55.6**	S	0.5*	T: 20°C; pH: 7.92; H: 388 mg/l CaCO3; DOC: 5.0 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**36.4**	S	0.5*	T: 20°C; pH: 7.04; H: 242 mg/l CaCO3; DOC: 1.5 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**172.9**	S	0.5*	T: 20°C; pH: 7.97; H: 389 mg/l CaCO3; DOC: 15.8 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**98.9**	S	0.5*	T: 20°C; pH: 7.03; H: 244 mg/l CaCO3; DOC: 10.8 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**85.4**	S	0.5*	T: 20°C; pH: 7.01; H: 486 mg/l CaCO3; DOC: 10.0 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)

3 d

Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**161.9**	S	0.5*	T: 20°C; pH: 8.75; H: 243 mg/l CaCO3; DOC: 9.9 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml		growth	**282.9**	S	0.5*	T: 20°C; pH: 7.05; H: 244 mg/l CaCO3; DOC: 19.10 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**187.8**	S	0.5*	T: 20°C; pH: 6.01; H: 389 mg/l CaCO3; DOC: 5.0 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**510.2**	S	0.5*	T: 20°C; pH: 6.05; H: 390 mg/l CaCO3; DOC: 15.2 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**31**	S	0.5*	T: 20°C; pH: 7.88; H: 98 mg/l CaCO3; DOC: 5.3 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**188**	S	0.5*	T: 20°C; pH: 7.88; H: 99 mg/l CaCO3; DOC: 15.7 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**404.1**	S	0.5*	T: 20°C; pH: 5.5; H: 244 mg/l CaCO3; DOC: 10.3 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**158.7**	S	0.5*	T: 20°C; pH: 7.07; H: 25 mg/l CaCO3; DOC: 10.3 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**83.9**	S	0.5*	T: 20°C; pH: 7.03; H: 244 mg/l CaCO3; DOC: 10.8 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	**132.3**	S	0.5*	T: 20°C; pH: 7.04; H: 246 mg/l CaCO3; DOC: 10.2 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**52.9**	S	0.5*	T: 20°C; pH: 6.74; H: 10.0 mg/l CaCO3; DOC: 2.72 mg/l	Lake	Heijerick et al., 2002 (3)

3 d

Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**61.8**	S	0.5*	T: 20°C; pH: 7.0; H: 12.4 mg/l CaCO3; DOC: 2.34 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**94.7**	S	0.5*	T: 20°C; pH: 6.14; H: 7.9 mg/l CaCO3; DOC: 12 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml		growth	**17.9**	S	0.5*	T 20°C; pH: 7.66; H: 48.7 mg/l CaCO3; DOC: 2.52 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**49**	S	0.5*	T: 20°C; pH: 8.0; H: 220 mg/l CaCO3; DOC: 6.42 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**35.4**	S	0.5*	T: 20°C; pH: 7.84; H: 238 mg/l CaCO3; DOC: 8.24 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**23.1**	S	0.5*	T: 20°C; pH: 7.93; H: 191 mg/l CaCO3; DOC: 1.99 mg/l	River	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**19.3**	S	0.5*	T: 20°C; pH: 7.93; H: 191 mg/l CaCO3; DOC: 1.99 mg/l	River	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**56.4**	S	0.5*	T: 20°C; pH: 7.69; H: 132 mg/l CaCO3; DOC: 6.13 mg/l	River	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**164**	S	0.5*	T: 20°C; pH: 7.84; H: 166 mg/l CaCO3; DOC: 17.8 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**65.5**	S	0.5*	T: 20°C; pH: 7.35; H: 134 mg/l CaCO3; DOC: 20.4 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	growth	**15.7**	S	0.5*	T: 20°C; pH: 8.16; H: 169 mg/l CaCO3; DOC: 1.7 mg/l	Lake	Heijerick et al., 2002 (3)
Lemna minor	Double fronded colonies	7 d	growth	**30**	S	0.5*	T: 25°C; pH: 6.5; H : 26.8 mg/l CaCO3; DOC: 0.5 mg/l (1)	artificial	Teisseire et al., 1998 (4)

Annex 3: composition of the OECD 203 (ISO 6341) aqueous test medium

Component	mg/l	Mg^{2+}	Ca^{2+}	Cl^-	Alkalinity $CaCO_3$	SO_4^{2-}	Na^+
$CaCl_2.2H_2O$	293.8		79.9	141.7			
$MgSO_4.7H_2O$	123.3	12				48.1	
$NaHCO_3$	64.8				47.1		17.7
KCl	5.8			2.8			
totals		12	79.9	144.5	64	48.1	17.7

The total hardness of the test mediun is 250 mg/l $CaCO_3$. According to the GHS (2001) the total organic carbon in the test media should not exceed 2 mg/l.

References

See IUCLID reference list