

Annex II to the CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

EC Number: 231-159-6

CAS Number: 7440-50-8

Index Number: -

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"Section 5.4, Aquatic Toxicity" of CLH Report (2017), Proposal for harmonised classification and labelling, Substance name: Copper, granulated, February, 2017.

This Annex provides references to ecotoxicity studies used in the classification

(For "section 7, Annexes" of CLH Report (2017), Proposal for harmonised classification and labelling, Substance name: Copper, granulated, February, 2017, see Annex III. Other sections of the same CLH report, are not included in this dossier.)

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP
Regulation), Annex VI, Part 2**

Substance Name: Copper, granulated

EC Number: 231-159-6

CAS Number: 7440-50-8

Index Number: -

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5.4 AQUATIC TOXICITY

A large copper database was taken into account to determine the classification proposal. All available ecotoxicity data on soluble copper compounds were compiled and the results (EC₅₀, NOEC/EC₁₀ values) are expressed as soluble Cu²⁺. All data used for classification purposes are presented in the annex at the end of this document. Only new data submitted after the EU RAR (2008) publication are described.

The reliability of data coming from the EU RAR (2008) were evaluated in depth by the relevant industry experts and reviewed by the pre-REACH CAs⁴.

Data submitted in the reports of Heijerick and Van Sprang, 2016a and Heijerick and Van Sprang, 2016b were also evaluated in depth by the relevant industry experts and the new data issued from these reports are presented in this CLH dossier.

In accordance to the CLP guidance, Heijerick and Van Sprang, 2016a and 2016b, have proposed to use data from standardized methods. Nevertheless, valid data on other species at the same trophic level have also been considered. Considering the difficulty in assessing “equivalence” and recognizing the high number of copper ecotoxicity data available on standard species only the “standard species” – representing three main trophic levels - and endpoints are considered when

⁴ Italy has been acting as a reviewing Member State for the substance and the risk assessment report has been reviewed by the Technical Committee on New and Existing Substances (TC NES) according to standard operational procedures of the Committee

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deriving ERVs. This approach was also followed for earlier copper classification and is also supported in this dossier.

The general methodology followed by Heijerick and Van Sprang, 2016a and 2016b, are described in their reports. Only a summary is presented below.

- Species selection and test duration:

For acute database, the following test guidelines and standard species/endpoints are considered relevant for classification purposes:

- for fish: 96h LC₅₀ values generated according to OECD 203 conducted with juvenile fish (0.1 – 5g) following the EU CLP recommendations. Moreover, as the RAC committee has retained *P. promelas* ELS studies at pH6 for classification of copper flake and copper compounds, the acceptance of the larvae data was considered to be compliant with the OECD 236, and the new proposed database includes all life stages.

- For daphnids, 48 h EC₅₀ values determined according to OECD TG 202 Part I are recommended and are used in this database.

Acute toxicity data for the invertebrate *Ceriodaphnia dubia*, which is not a standard species under CLP but is widely used in the United States, are also included in this assessment.

- For aquatic plants, algal growth inhibition tests consistent with OECD TG 201 and providing 72 or 96 h EC₅₀ values are included in the database. Only the growth rate reduction endpoint (i.e. ErC₅₀) is retained.

- For vascular plants (e.g. *Lemna* sp), EC₅₀ values obtained from tests consistent with OECD TG 221 and US EPA 850:4400 are retained for the database. The observational acute endpoint is the change (50% effect) in the number of fronds produced and the test duration is 7 days.

For chronic database, the following test guidelines and standard species/endpoints are considered relevant for classification purposes:

- for fish: chronic or long-term tests with fish were initiated with fertilized eggs, embryos, juveniles, or reproductively active adults. Tests consistent with OECD TG 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can be used. Durations varied anywhere from 7 days to 330 days. Observational endpoints include hatching success, growth (length and weight changes of the surviving fish), spawning success, and survival.

The data from 7 days *P. promelas* tests were also retained in the chronic data-base if carried out with a sensitive life stage. The use of 7 days *P. promelas* tests with sensitive life stages is justified from Nordberg et al., 1985. They compared the sensitivity of *P. promelas* (tested in similar test waters) to copper, zinc and Dursban (an organic chemical) from 7 days sub-chronic larvae tests, 32 days Early life stage tests and a 327 days full life cycle and concluded that the 7 days *P. promelas* larvae Maximum Acceptable Tolerable Concentrations (MATC) were similar. For copper the MATCs varied between 14 and 19 µg Cu/L.

- For daphnids, tests, consistent with OECD TG 211 (duration: 21 days) and/or OECD TG 202 Part II (duration: 14 days) are recommended and retained. Regarding toxicity study on *Ceriodaphnia dubia*, the results obtain at 7 days of exposure were rejected in the Joint Research Centre (JRC) report (page 20) “New criteria for environmental long-term aquatic hazard classification under the CLP Regulation (EC) N° 1272/2008 (2nd ATP) - Screening of Annex VI substances with harmonised classifications”. Moreover, only 21 days data are used in the CLP guidance. Therefore, even if the Copper Task Force proposes to include these data

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in the current database as the 7 days test is widely used in the United States as typical test species for risk/hazard assessment purposes, these values (7days data) have not been included in the database for classification purposes.

- For algae, NOErC and ErC₁₀ values derived from 72 or 96 hours with OECD TG 201 are included in the database.

- For vascular plants (e.g. *Lemna sp*), NOEC/EC₁₀ values obtained from tests consistent with OECD TG 221 and US EPA 850:4400 were used for classification. In these international protocols, the observational endpoint is the change in the number of fronds produced and the test duration is 7 days.

- **Quality criteria:**

Tests have to be conducted with the above mentioned standard test species, endpoints, and guidelines. Sufficient information on the test (test design, test set-up, validity criteria) has to be available. Only test data that meet the criteria for a Klimisch scoring of 1 (reliable without restrictions) or 2 (reliable with restrictions) (Klimisch et al, 1997) have been used for the hazard assessment of copper. Reported adverse effect levels have to be expressed as measured, dissolved copper concentrations. Nominal data are not acceptable.

- **Physico-chemical conditions of test media:**

- *Effect of pH:*

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. These pH categories have been defined in accordance to the Guidance on the Application of the CLP criteria (version 4.1, June 2015) section IV.2. to be in line with the UN GHS transformation/dissolution protocol (T/Dp) which specifies a pH range of 6-8.5 for the 7days test and 5.5 to 8.5 for the 28 days test. Thus, both T/Dp and ecotoxicity data could be compared at a similar pH since both parameters will vary with pH.

- *Effect of dissolved organic carbon (DOC)*

The registrants have also investigated the impact of DOC on the derivation of the ERV. Their reports proposed a normalisation to a DOC level of 2 mg/L which was performed for all studies in the database for which DOC data were available or could reliably be estimated.

Therefore, two different ERVs for each pH group were derived:

- for acute classification:
 - ERV_{acute} based on all short-term data,
 - ERV_{acute} based on all toxicity data after normalization to a DOC of 2 mg/L, which is the limit value in the OECD 202 guideline;
 - for chronic classification:
 - ERV_{chronic} based on all long-term data,
 - ERV_{chronic} based on all toxicity data after normalization to a DOC of 2mg/L.
- *Effect of water hardness*

- In the acute toxicity studies, the hardness of the test medium does not influence the sensitivity of algae to Cu and reduces the sensitivity of invertebrates and fish to Cu.

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The table below summarizes the characteristics of the test-waters retained in this report. If the reported DOC values were below detection limit, then half of the detection limit was used.

Table 67: Median test water characteristics of the algae, invertebrates and fish tests in the acute copper ecotoxicity database.

Trophic level	Parameter	pH 5.5-6.5	pH 6.5-7.5	pH 7.5-8.5
Algae	DOC (mg/L)	7.7	10.2	6.1
	Hardness (mg CaCO ₃ /L)	100	250	244
Invertebrates	DOC (mg/L)	2.6	3.2	2.5
	Hardness (mg CaCO ₃ /L)	31	50	134
Fish	DOC (mg/L)	1.3	0.5	1.5
	Hardness (mg CaCO ₃ /L)	48	54	102

For invertebrate tests, the reported median hardness is, for each pH class, below the OECD recommendations for *D. magna* (140-250 mg CaCO₃/L). The median hardness of the fish tests is, for each pH class, in the lower range of the OECD recommendations (10-250 mg CaCO₃/L). Therefore, the acute ecotoxicity data used in this report are generally conservative with regards to the hardness of the test media.

- In the chronic toxicity studies, the hardness of the test medium does not influence the sensitivity of algae to Cu, has little influence on the sensitivity of invertebrates to Cu and reduces the sensitivity of fish to Cu.

The table below summarized the characteristics of the test-waters retained in this report. If the reported DOC were below detection limit, then half of the detection limit was used

Table 68: Median values for the reported test-water characteristics of the chronic algae, invertebrates and fish tests.

Trophic level	Parameter	pH 5.5-6.5	pH 6.5-7.5	pH 7.5-8.5
Algae	DOC (mg/L)	9.8	10.3	6.1
	Hardness (mg CaCO ₃ /L)	101	251	214
Invertebrates	DOC (mg/L)	2.5	1.8	5
	Hardness (mg CaCO ₃ /L)	129	41.3	129
Fish	DOC (mg/L)	1.5	1	0.6
	Hardness (mg CaCO ₃ /L)	12.6	38.5	137

The median hardness of the fish tests is, for each pH class, in the lower range of the OECD recommendations (10-250 mg CaCO₃/L) and the Foregs database (<http://weppi.gtk.fi/publ/foregsatlas/>). The median hardness of the algal and invertebrate tests is somewhat higher but has no or little influence on the copper toxicity. Therefore, the chronic ecotoxicity data used in this report are generally conservative with regards to the hardness of the test media.

- Data treatment:

The registrants considered that taking into account the data-richness of the toxicity copper database, the split-up by pHs, the median low hardness measured for critical species (fish) and the normalizations to DOC, geometric mean values could be calculated, also when less than 4 acceptable L(E)C₅₀ values are available for the same species. Indeed, the CLP guidance (version 4.1, p. 500-501, section 4.1.3.2.4.3) further mentions that geometric means can be used if four or more data points are available for a species. The registrants raised that the CLP guidance does not mention that there must be four data points within each pH band and therefore think that it is most appropriate to continue using geometric means after splitting up the database by pH band, as long as at least four data points are available across all pH values. Not using geometric means would lead to a double conservatism: the dataset is split-up according to pH because of data-richness, whereas within each pH band (less data-points), the lowest value is selected due to data scarcity.

Nevertheless, there is no reason to deviate from the CLP Guidance which indicates that the geometric mean have to be used if at least 4 data points on the same species and endpoint are available. However, to analyse the impact of the use of geomean or lowest value if less than 4 data points are available, both approach are presented in this CLH report.

5.4.1 FISH

5.4.1.1 Short-term toxicity to fish

According to the EU RAR (2008), 249 individual data points for fish were selected for 5 standard species (*Oncorhynchus mykiss*, *Pimephales promelas*, *Lepomis macrochirus*, *Brachydanio rerio* and *Cyprinus carpio*).

In the database submitted in the updated REACH registration dossier detailed in Heijerick and Van Sprang, 2016a, 94 new individual data points for fish were included for the 4 standard species (*Oncorhynchus mykiss*, *Pimephales promelas*, *Danio rerio* and *Cyprinus carpio*). These new data are presented below and details are given in the table 69.

- *Oncorhynchus mykiss*: Five different publications (Calfee et al, 2014; Ingersoll and Mebane, 2014; Little et al, 2012; Ng et al, 2010; Vardy et al, 2013) were identified until the EU RAR publication. A total of 28 reliable LC₅₀ values for copper were reported in these studies, with acute effect levels situated between 5.9 and 63 µg Cu/L. New data were available for each of the three classification pH-classes. An LC₅₀ of 19.4 µg/L determined by Ng et al (2010) was excluded of the database as it was obtained at pH 5. It should also be noted that the information on the testing methodology that is provided by Ng et al (2010) indicated that no real replicates were used and no acclimation was carried out to lower pHs; such a deficiency has an impact on the reliability of this study, but the generated acute effect levels in soft test water with hardness of 14-22 mg/L as CaCO₃ were taken into account for the derivation of acute ERVs. Another study by Dwyer et al (2005) reports an LC₅₀ of 80 µg/L. This value was not included in the database, as it was based on nominal copper levels.
- *Cyprinus carpio*: One publication (Dehghani et al, 2012) was identified for the common carp *C. carpio* given an LC₅₀ value for copper equal to 820 µg/L which is in the same range than the previous available dataset. The test was conducted at a pH of 7.6 and a hardness of 220 mg/L as CaCO₃.
- *Danio rerio*: One publication (Alsop and Wood, 2011) were identified for the zebra fish *D. rerio* given 96h-LC₅₀ ranging between 11.7 and 212.1 µg/L. It was mentioned, in the Heijerick and Van Sprang report, that the lowest value was obtained in a very soft water test

medium (hardness below 10 mg/L as CaCO₃), resulting in an LC₅₀ that was more than one order of magnitude lower than acute levels that were generated in test media with medium hardness (141 mg /L as CaCO₃). The difference in pH among those tests was less than 0.5 pH units. It can thus be assumed that the difference in hardness was responsible for the observed variation – either by decreased Cu-bioavailability (competition between Ca/Mg and Cu), or by a reduced overall health condition of the fish in very soft waters.

- *Pimephales promelas*: six new different publications (Johnson et al, 2008 ; Nimmo et al, 2006; Ryan et al, 2004 ; Van Genderen et al, 2007, 2008 ; Vardy et al, 2013) given 59 LC₅₀ values ranged from 5.9 to 2034 µg/L have been included in the database. As for *O. mykiss*, another study by Dwyer et al (2005) reports an LC₅₀ of 470 µg/L which was not included in the database, as it was based on nominal copper levels. The lowest pH-class (5.5-6.5) is poorly represented in the new literature data-set and questions were raised on increased sensitivity in the lower pH range. Therefore, new acute and chronic ELS toxicity tests with *P.promelas* at pH 6, 6.5 and 7 (OSU, 2016a) were included in the updated registration dossier. The effect on mortality after 96h of exposure was negligible (or well below 50%) in any of the highest test concentrations, resulting in unbounded LC₅₀ values of >12.2 µg/L, >13.0 µg/L (pH 6.0) and > 24.4 µg/L (pH 6.5). Due to the limited number of data for this species and pH-class, the unbounded values were included in the database as a worst-case estimate for the actual LC₅₀. The OSU (2016) study also reported an unbounded 96h-LC₅₀ of >55.1 µg/L at a pH of 7.0. However, as a large number of bounded LC₅₀-values are already available for this species and pH-category (n=46), this unbounded value was not retained.

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Table 69: Overview of the E(L)C50 values for fish published or generated since the EU RAR (2008)

	E(L)C50 Value	E(L)C50 Normalised value		DOC	Hardness	Temperature			
Species	µg/L	µg/L	pH	mg/L	mg/L as CaCO ₃	°C	Type of water	Reference	Lifestage
<i>Danio rerio</i>	11,7	26	7,34	0,9	7,8	28	Soft water	Alsop and Wood, 2011	larvae
<i>Danio rerio</i>	148,4	94,7	7,8	3,5	141	28	Hard water from Lake Ontario	Alsop and Wood, 2011	larvae
<i>Danio rerio</i>	212,1	146,9	7,8	3,5	141	26,5	Hard water from Lake Ontario	Alsop and Wood, 2011	Adult
<i>Oncorhynchus mykiss</i>	5,9	8,4	6,2	1,4	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
<i>Oncorhynchus mykiss</i>	9,2	13,1	7,1	1,4	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
<i>Oncorhynchus mykiss</i>	21	19,1	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	swim-up larvae
<i>Oncorhynchus mykiss</i>	22	20	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	juvenile
<i>Oncorhynchus mykiss</i>	24	21,8	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	juvenile
<i>Oncorhynchus mykiss</i>	40	36,4	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	yolk sac age
<i>Oncorhynchus mykiss</i>	60	127,2	7,84	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	1d post-hatch
<i>Oncorhynchus mykiss</i>	56,6	120,5	7,97	0,4	104	12	well water + deionized water	Ingersoll & Mebane, 2014	18d post-hatch
<i>Oncorhynchus mykiss</i>	40,8	99,0	8	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	60d post-hatch
<i>Oncorhynchus mykiss</i>	42,4	101,2	8	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	60d post-hatch
<i>Oncorhynchus mykiss</i>	50,1	110,9	8,04	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
<i>Oncorhynchus mykiss</i>	59	122,4	8,04	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
<i>Oncorhynchus mykiss</i>	19,1	55,7	8,05	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	95d post-hatch
<i>Oncorhynchus mykiss</i>	60,6	112,7	8,05	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	74d post-hatch
<i>Oncorhynchus mykiss</i>	63	127,2	8,05	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
<i>Oncorhynchus mykiss</i>	8,5	13,1	8,1	1,3	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
<i>Oncorhynchus mykiss</i>	30,9	70,1	8,1	0,5	108	12	Reconstituted water	Little et al., 2012	160d post-hatch
<i>Oncorhynchus mykiss</i>	36,5	78,9	8,1	0,5	107,7	12	Reconstituted water	Little et al., 2012	30d post-hatch
<i>Oncorhynchus mykiss</i>	59,9	122,4	8,1	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	32d post-hatch
<i>Oncorhynchus mykiss</i>	62,9	126,3	8,11	0,4	95	12	well water + deionized water	Ingersoll & Mebane, 2014	1d post-hatch

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<i>Oncorhynchus mykiss</i>	6,7	8,9	8,5	1,5	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
<i>Oncorhynchus mykiss</i>	56,6	107,8	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	18d post-hatch
<i>Oncorhynchus mykiss</i>	62,9	115,5	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	1d post-hatch
<i>Oncorhynchus mykiss</i>	59,9	111,8	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	32d post-hatch
<i>Oncorhynchus mykiss</i>	59	110,8	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	46d post-hatch
<i>Oncorhynchus mykiss</i>	42,4	90,1	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	60d post-hatch
<i>Oncorhynchus mykiss</i>	60,6	124,2	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	74d post-hatch
<i>Oncorhynchus mykiss</i>	19,1	64,6	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	95d post-hatch
<i>Pimephales promelas</i>	12,2	14,9	6	1,58	48	25	reconstituted water	OSU, 2016	larvae
<i>Pimephales promelas</i>	13	21,8	6	1,29	44	25	reconstituted water	OSU, 2016	larvae
<i>Pimephales promelas</i>	24,4	40,1	6,5	0,98	48	25	reconstituted water	OSU, 2016	larvae
<i>Pimephales promelas</i>	5,9	22,6	7,01	0,5	17,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	12,8	44,0	7,01	0,5	23,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	7,8	29,7	7,13	0,5	17,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	180	232,6	7,2	0,7	1213	n.r.	Natural water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	13,2	45,2	7,22	0,5	28,4	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	30,2	75,4	7,28	0,5	108,5	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	7,5	29,1	7,29	0,5	4,2	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	10,2	37,4	7,29	0,5	19,3	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	7,2	27,6	7,3	0,5	10,9	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	21,4	62,6	7,31	0,5	52,3	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	102	94,4	7,5	2,2	57	22	Carbon & biofiltered city water	Vardy et al., 2013	yolk sack stage
<i>Pimephales promelas</i>	15,3	50,7	7,58	0,5	19,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	17,4	52,8	7,6	<0,5	1245	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	31,7	82,2	7,8	0,5	97	25	Reconstituted water	Nimmo et al., 2006	larvae
<i>Pimephales promelas</i>	337,6	97,5	7,9	8,5	107	25	Natural water	Nimmo et al., 2006	larvae
<i>Pimephales promelas</i>	24,4	65,9	7,94	0,5	23,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
<i>Pimephales promelas</i>	769	552,6	8	6,9	438	n.r.	Natural water	Van Genderen et al., 2007	larvae

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<i>Pimephales promelas</i>	684	658,3	8,1	2,5	187	n.r.	Natural water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	1870	1669,4	8,1	4,4	66	n.r.	Natural water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	300	384,9	8,2	<0,5	287	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	906	550,2	8,2	9,8	288	n.r.	Natural water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	544	580,2	8,2	1,2	294	n.r.	Natural water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	1390	1185,1	8,2	5,4	794	n.r.	Natural water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	250	330,8	8,3	<0,5	156	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	262	341,7	8,3	<0,5	284	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	315	395,8	8,3	<0,5	767	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	197	273,8	8,4	<0,5	70	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	473	562,5	8,5	<0,5	445	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
<i>Pimephales promelas</i>	137	104,3	7,85-8,17	2,96	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	96,6	159,1	7,85-8,17	0,42	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	106,1	170,6	7,85-8,17	0,41	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	259,5	178,8	7,85-8,17	3,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	129	192,0	7,85-8,17	0,51	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	597,7	245,5	7,85-8,17	9,53	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	283,6	263,7	7,85-8,17	2,42	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	494,8	332,5	7,85-8,17	5,14	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	363,6	339,8	7,85-8,17	2,46	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	499,5	344,2	7,85-8,17	4,97	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	468,1	349,4	7,85-8,17	4,26	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	395,5	356,9	7,85-8,17	2,73	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	402,4	378,1	7,85-8,17	2,45	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	383,7	379,9	7,85-8,17	2,07	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae

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<i>Pimephales promelas</i>	429	380,9	7,85-8,17	2,89	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	422,1	388,9	7,85-8,17	2,61	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	596,3	394,0	7,85-8,17	5,68	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	613,3	418,3	7,85-8,17	5,47	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	774,3	531,4	7,85-8,17	5,95	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	726,5	542,1	7,85-8,17	4,98	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	671,2	610,5	7,85-8,17	2,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	2034	825,5	7,85-8,17	18,23	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1405	843,9	7,85-8,17	9,56	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1526	903,0	7,85-8,17	10,16	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1564	957,0	7,85-8,17	9,77	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1681	1039,0	7,85-8,17	9,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1959	1192,1	7,85-8,17	10,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1514	1251,9	7,85-8,17	5,01	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	2013	1260,1	7,85-8,17	10,58	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Pimephales promelas</i>	1949	1327,5	7,85-8,17	8,93	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
<i>Cyprinus carpio</i>	820		7,6		220	24	dechlorinated well water	Dehghani et al, 2012	Adult
<i>Pimephales promelas</i>	230		7,7		80-120	23	Reconstituted water	Johnson et al., 2008	< 24h old

Table 70: Summary of the acute toxicity data for fish for the three pH classes

Test organism	L(E)C ₅₀ ($\mu\text{g Cu/L}$)		
	pH: 5.51-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Oncorhynchus mykiss</i>			
n	8	22	47
Min	4.2	2.8	6.7
Max	82.2	890	516
Geometric mean	24.2	47.4	63.6
Lowest value (only when data<4)	/	/	/
<i>Danio rerio</i>			
n	/	2	3
Min	/	11.7	148.4
Max	/	35	212.1
Geometric mean	/	20.3	167.4
Lowest value (only when data<4)		11.7	148.4
<i>Cyprinus carpio</i>			
n	/	/	3
Min	/	/	800
Max	/	/	820
Geometric mean	/	/	810
Lowest value (only when data<4)	/	/	800
<i>Pimephales promelas</i>			
n	5	46	207
Min	4.4	5.9	12.4
Max	24.4	1400	2034
Geometric mean	12.1	96.7	255.9
Lowest value (only when data<4)	/	/	/
<i>Lepomis macrochirus</i>			
n		2	3
Min	/	1000	4250
Max	/	1100	9150
Geometric mean	/	1049	5509
Lowest value (only when data<4)	/	1000	4250

Table 71: Summary of the acute toxicity data for fish for the three pH classes considering DOC normalisation at 2mg/L

Test organism	L(E)C ₅₀ ($\mu\text{g Cu/L}$)		
	pH: 5.51-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Oncorhynchus mykiss</i>			
n	8	8	25
Min	6.28	13.1	8.9
Max	99.3	336.3	561.9
Geometric mean	40.6	45.9	94.7
Lowest value (only when data<4)	/	/	/
<i>Danio rerio</i>			
n	/	1	2
Min	/	26	94.7
Max	/	/	146.9
Geometric mean	/	/	117.9
Lowest value (only when data<4)		2	94.7
6			
<i>Pimephales promelas</i>			
n	3	11	47
Min	14.9	22.6	50.7
Max	40.1	232.6	1669.4
Geometric mean	23.5	49.3	382.86
Lowest value (only when data<4)	14.9	/	/

It should be noted that in the Heijerick et al., 2016 reportndata points for juveniles has been added. Nevertheless, as the significant number of studies did not provide sufficient information on the life stage, the number of data points was limited. Moreover, with the available data, the lowest E(L)C₅₀ retained for juveniles was in general somewhat higher than the overall geometric mean. Therefore, only data from all life stage are considered for ERV derivation.

As expected, an increased LC₅₀ with increasing pH was noted for these fish species. It should be noted that the previous lowest geomean LC50 value 8.1 $\mu\text{g/L}$ was recorded for *P. promelas* tested in ecotoxicity media with low pH (between 5.5 and 6.5) was challenged during the discussions on the previous copper CLH reports. These discussions are reflected in the opinion of the copper flakes (CLH-O-0000001412-86-30/F adopted 04 December 2014) and are reported here below. However, according to the improvement of database described before, **the current lowest LC50 value for fish was calculated to be 12.1 $\mu\text{g/L}$ corresponding to the geomean also recorded for *P. promelas* at pH between 5.5 and 6.5.**

If the geomean is used whatever the number of available data, the lowest LC50 value for fish would be 11.7 $\mu\text{g/l}$ recorded for *D. rerio* at pH between 6.5 and 7.5. If the normalisation with the DOC is taking into account, **the lowest LC50 value for fish was calculated to be 14.9 $\mu\text{g/L}$ corresponding to the lowest value recorded for *P. promelas* at pH between 5.5 and 6.5.**

If the geomean is used whatever the number of available data, the lowest LC50 value for fish would be 23.5 $\mu\text{g/l}$ recorded for *P. promelas* at pH between 6.5 and 7.5.

Extract from CLH-O-0000001412-86-30/F adopted 04 December 2014: "The lowest geometric mean LC₅₀ reported [in the CLH report] is 8.1 $\mu\text{g/L}$ (as copper) for fathead minnow *P. promelas* at pH 5.5-

6.5 (...). This is based on two values, both for larval fish, 15.0 µg/L and 4.4 µg/L. During PC (commenting period), industry indicated that the test medium in the study which resulted in the lowest EC₅₀ (cited as Erickson et al., 1996) used a high flow-through rate, had low hardness (22 mg CaCO₃/L) and low DOC concentration (not stated), and used larvae that were less than 24 hours' old. Although not mentioned in the CLH report, in the original paper the lowest LC₅₀ was determined at the minimum pH, i.e. 6.0. Industry therefore considered this test to represent a worst case, and suggested that the sensitivity of this species at pH 6 versus pH 7 was unexpected and may be related to insufficient adaptation to low pH conditions. The data were therefore not considered reliable and not used for classification in the REACH registrations as well as the vRAR. Nevertheless, RAC notes that other minimum acute fish LC₅₀s are of the same order of magnitude (e.g. *O. mykiss* at all pHs, and *P. promelas* at pH 6.5-7.5). The OECD TG 203 permits testing in waters with total hardness as low as 10 mg CaCO₃/L, and a preferred minimum pH of 6.0, so the conditions used in the Erickson (1996) study were within the validity criteria of the guidelines and cannot be considered a worst case. In addition, this species can tolerate poor conditions such as turbid, hot, poorly oxygenated, intermittent streams, which are unsuitable for most fishes

(<http://www.fishbase.org/Summary/speciesSummary.php?ID=4785&AT=fathead+minnow>).

Further papers provided by industry stakeholders following public consultation (Mount, 1973 and Zischke et al., 1983) indicate that *P. promelas* can survive at pHs as low as 4.5, so that a pH of

6.0 does not appear to be intolerable over short exposures. RAC also notes that the replacement test for acute fish toxicity (OECD TG236) involves embryos, so the life stage argument was not considered relevant either. It is also unclear why the dossier submitter decided to include them in the CLH report if they had been previously rejected. RAC accepts that an acute toxicity test with fish larvae may be more sensitive than one with older fish if they were not properly acclimated, but does not find the other reasons for rejection convincing.

Data for other species show trend of increasing acute fish toxicity with declining pH, presumably due to increasing bioavailability. The acute LC₅₀ for *Danio rerio* at pH 6.5-7.5 (35 µg/L, n=3 so a geometric mean is not appropriate) is similar to that of *O. mykiss* at pH 5.5-6.5 (geometric mean 29 µg/L, based on n=6), implying that the sensitivity of *D. rerio* at the lower pH could be higher. Rather than ignoring the *P. promelas* data completely, the geometric mean LC₅₀ of 8.1 µg/L is considered to be relevant for hazard classification as it takes account of uncertainties about the sensitivity of fish at acidic pH, although this is a conservative approach given the life stages that were tested (N.B. if the most sensitive value of 4.4 µg/L were used the consequence for classification would be the same for coated copper flakes). RAC has not considered how DOC or hardness affect the observed pattern in ecotoxicity data, as such an analysis was not presented in the CLH report.

5.4.1.2 LONG-TERM TOXICITY TO FISH

According to the EU RAR (2008), 29 individual data points for fish were selected for 3 standard species (*Oncorhynchus mykiss*, *Pimephales promelas* and *Salvelinus fontinalis*).

In the updated database summaries by Heijerick and Van Sprang, 2016b, 25 new individual data points for fish were included for the 2 standard species (*Oncorhynchus mykiss* and *Pimephales promelas*). The paper also re-evaluated some endpoint on existing studies to derived EC₁₀ instead of NOEC when possible. These new and re-evaluated data are presented below and details are given in the table 72.

- *Oncorhynchus mykiss*: three new publications (Besser et al., 2005 ; Ingersoll and Mebane, 2014 ; Ng et al., 2010) were identified until the EU RAR (2008) publication. A total of 12 reliable chronic endpoints were reported at pH varying between 6.2 and 8.3. The information on the testing methodology that is provided by Ng et al (2010) indicated that no real replicates were used and no acclimation was carried out to lower pHs; such a deficiency has an impact on the reliability of this study. There were also a number of tests (reported by Ng et al., 2010) that were conducted at a pH that fell outside the pH-range that is considered relevant for classification purposes (5.5-8.5). In addition, Ng et al. (2010) published EC₁₀ values for survival and growth.

No concentration-response relationship was found when growth (biomass/surviving fish) was used as parameter.

However, to complete the database on chronic toxicity to fish at low pH values, Oregon State University (OSU, 2016) conducted a 56 days early life stage test (OECD 210, 2013) with the rainbow trout, *O. Mykiss*, at pH 6 and pH 7 which is in line with the OECD 210 guideline. The survival and growth (wet weight) endpoints of the OSU (2016b) study were retained. These endpoints result in EC₁₀ values of 28.5 (survival) and 36.0 (wet weight) µg Cu/L at pH 6, and 49.3 (survival) and 47.3 (wet weight) µg/L at pH 7. This test was carried out in a test water similar to that of the Ng et al. (2010) study, but with replicates and corresponding statistical assessments, with organisms acclimated to low pH conditions, and with pre-equilibrated test waters. This OSU (2016) study may therefore replace the Ng et al., 2010 data.

A fourth publication by Welsh et al (2008) only reported chronic LC₅₀ values (n=6). These values cannot be used for the chronic hazard assessment of copper, and were therefore not further considered.

Moreover, the Heijerick and Van Sprang, 2016b report proposed to replace some of the NOEC-values that have been used until now from the previous study published in Seim et al (1984) and Marr et al (1996) by EC₁₀ values. This approach is agreed as EC₁₀ values are generally considered more reliable than NOEC-values in the current hazard assessment. The data that were provided in Seim et al (1984) also allowed the derivation of an EC₁₀ for an endpoint (survival) that had not been determined for this study.

Table 72: Proposed re-evaluated chronic data for the rainbow trout *O.mykiss*

Reference	Testing conditions	Existing NOEC	New EC ₁₀	Endpoint
Marr et al, 1996	pH: 7.5 DOC: 0.2 mg/L	2.2 µg/L	8.1 µg/L 3.3 µg/L	Growth Growth (length) Growth (weight)
Seim et al, 1984	pH: 7.65 DOC: 1.30 mg/L	16 µg/L	23.5 µg/L 53.3 µg/L	Growth (DW) Mortality (new)

- *Pimephales promelas*: a new publication (Besser et al, 2005) conducted a number of 30 days chronic tests (pH 8 class) with the fathead minnow *P. promelas*, and studied the long-term effects of copper exposure on mortality, weight-based growth of newly hatched larvae.

To complete the database at pH 6-7, the registrants have conducted a chronic ELS toxicity test with *P.promelas* at pH 6, 6.5 and 7 (OSU, 2016) as available in the updated REACH registration dossier of January 2017. The data allowed to calculate reliable NOEC/L(E)C₁₀ values for mortality and growth at pH 6, 6.5 and 7. The estimated EC₁₀ values decrease from 22 µg/L at pH 7 to 10 µg/L at pH 6, i.e. an estimated factor 2. However, given that the uncertainty on these EC₁₀ estimates is larger than a factor 2, these data cannot confirm nor exclude a pH effect on chronic copper toxicity to *P. promelas*. This study is considered reliable.

Moreover, as for *O. mykiss*, the Heijerick and Van Sprang, 2016b has proposed to re-evaluate the existing studies with the fathead minnow (Scudder et al, 1988; Mount and Stephan, 1969; Pickering et al, 1977; Brungs et al, 1976; Spehar and Fiandt, 1985) resulted in the identification of some additional data points that has not been picked up in the original evaluation of these studies. Secondly, some of the NOEC-values that have been used until

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now for *P.promelas* can be replaced by EC₁₀ values that were determined with available raw data.

Table 73: Proposed re-evaluation of chronic data for the fathead minnow *P.promelas* (Heijerick and Van Sprang, 2016b)

Reference	Testing conditions	Existing NOEC	New EC ₁₀	Endpoint
Spehar & Fiandt, 1985	pH: 7.05 DOC: 1.0 mg/L	4.8 µg/L 4.8 µg/L 16 µg/L (new)	3.8 µg/L 5.9 µg/L ---	Growth Mortality Reproduction
Mount & Stephan, 1969	pH: 6.90 DOC: 0.55 mg/L	10.8 µg/L ⁽¹⁾ 10.8 µg/L ⁽¹⁾ 10.8 µg/L ⁽¹⁾	--- ⁽⁵⁾ 13.1 µg/L 14.9 µg/L 16.7 µg/L	Reproduction ⁽²⁾ Mortality Growth _{length} Growth _{length,male} Growth _{length,female}
Pickering et al, 1977	pH: 7.85 DOC: 0.55 mg/L	37 µg/L (new) 22.5 µg/L 22.5 µg/L 25.5 µg/L 25.5 µg/L	22.3 µg/L --- ⁽⁵⁾ 16.3 µg/L --- ⁽⁵⁾	Growth _{length} Reproduction ⁽³⁾ Reproduction ⁽⁴⁾ Reproduction ⁽³⁾ Reproduction ⁽⁴⁾
Brungs et al, 1976	pH: 8.10 DOC: 5.9 mg/L	66 µg/L	47.6 µg/L	Reproduction ⁽³⁾
Scudder et al, 1988	pH: 8.17 DOC: 1.30 mg/L		53.7 µg/L 30.3 µg/L --- ⁽⁵⁾	Abnormalities Growth _{weight} Mortality

⁽¹⁾: the original value of 10.6 µg/L was the average of three analytical methods; the value that was based on AAS-measured Cu-concentrations was 10.8 µg/L

⁽²⁾: #spawnings/female

⁽³⁾: #eggs/female

⁽⁴⁾: #eggs/spawning

⁽⁵⁾: NOEC was retained as the range of the 95% Confidence Interval around the EC₁₀ was more than one order of magnitude.

- *Salvelinus fontinalis*: Heijerick and Van Sprang, 2016b has also re-evaluate the existing studies with the brook trout (Sauter et al., 1976). EC₁₀ values which could be determined could replace the previously used NOEC values. The new values are presented in table 74.

Table 74: Proposed re-evaluation of chronic data for the brook trout *S.fontinalis* (Heijerick and Van Sprang, 2016b)

Reference	Testing conditions	Existing NOEC	New EC ₁₀	Endpoint
Sauter et al, 1976	pH: 6.85 ⁽¹⁾ DOC: 1.3 mg/L pH: 6.90 ⁽²⁾ DOC: 1.3 mg/L	< 5 µg/L 13 µg/L 6.4 µg/L 21 µg/L 21 µg/L 49 µg/L	11.2 µg/L 12.4 µg/L 6.4 µg/L 44.4 µg/L 41.3 µg/L 36.4 µg/L	Growth Mortality Reproduction Growth Mortality

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				Reproduction
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⁽¹⁾: 60d exposure ; ⁽²⁾: 30d exposure

Table 75: Overview of the NOEC/EC10 values for fish published or generated since the EU RAR (2008) and re-evaluated chronic toxicity data for fish

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/E C10	Normalise d	pH	DOC	Alk	Medium	Reference
				(µg/L)	(µg/L)		mg/L	mg/L as CaCO ₃		
<i>Oncorhynchus mykiss</i>	juveniles	51 d	Mortality	28,5	43,75	6	1,15	40	Laboratory water	OSU, 2016
<i>Oncorhynchus mykiss</i>	juveniles	51 d	Growth - wet biomass	28,2	43,33	6	1,15	40	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	larvae	7 d	Mortality	10,1	17,1	6	1,10	12,0	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	larvae	7 d	Mortality	11,9	14,6	6	1,59	48,5	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	larvae	7 d	Mortality	13,8	23,5	6,5	1,14	15,0	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	larvae	7 d	Growth - dw	9	15,3	6	1,10	12,0	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	larvae	7 d	Growth - dw	11,9	14,6	6	1,59	48,5	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	larvae	7 d	Growth - dw	13,8	23,5	6,5	1,14	15,0	Laboratory water	OSU, 2016
<i>Oncorhynchus mykiss</i>	juveniles	51 d	Growth - biomass	38,8	57,67	7	1,26	40	Laboratory water	OSU, 2016
<i>Oncorhynchus mykiss</i>	juveniles	51 d	Mortality	54,2		7	1,26	40	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	fry (10 - 20 mm)	327 d	Growth - length male	14,9	45,2	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
<i>Pimephales promelas</i>	fry (10 - 20 mm)	327 d	Growth - length female	16,7	49,3	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
<i>Pimephales promelas</i>	embryo-larval	32 d	Growth	3,8	7,5	7,05	1	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
<i>Pimephales promelas</i>	larvae	7 d	Growth - dw	22	31,5	7	1,37	32,5	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	fry (10 - 20 mm)	327 d	Mortality	13,1	40,9	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
<i>Pimephales promelas</i>	embryo-larval	32 d	Mortality	5,9	11,6	7,05	1	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
<i>Pimephales promelas</i>	larvae	7 d	Mortality	22,5	32,2	7	1,37	32,5	Laboratory water	OSU, 2016
<i>Pimephales promelas</i>	embryo-larval	32 d	Reproduction (hatching)	16	30,6	7,05	1	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
<i>Pimephales promelas</i>	fry (10 - 20 mm)	327 d	Reproduction	10,8	34,9	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
<i>Salvelinus fontinalis</i>	fry	60 d	Growth	11,2	17,11	6,85	1,3	27,8	Well	Sauter et al., 1976

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<i>Salvelinus fontinalis</i>	fry	60 d	Mortality	12,4	18,91	6,85	1,3	27,8	Well	Sauter et al., 1976
<i>Salvelinus fontinalis</i>	fry	60 d	Reproduction	6,4	10,7	6,85	1,3	27,8	Well	Sauter et al., 1976
<i>Oncorhynchus mykiss</i>	fry (0.12 g; 2.6 cm)	60 d	Growth - length	8,1	52,7	7,5	0,2	27,7	Well + deionised water	Marr et al., 1996
<i>Oncorhynchus mykiss</i>	fry (0.12 g; 2.6 cm)	60 d	Growth - weight	3,3	28,5	7,5	0,2	27,7	Well + deionised water	Marr et al., 1996
<i>Oncorhynchus mykiss</i>	eggs	63 d	growth (dry wt)	23,5	34,02	7,65	1,3	126,0	Well	Seim et al., 1984
<i>Oncorhynchus mykiss</i>	larvae (26 days post hatch)	28 d	Growth (weight)	13	43,3	7,95	0,4	92,9	well water + deionized water	Ingersoll & Mebane, 2014
<i>Oncorhynchus mykiss</i>	eyed embryo	30 d	Growth (weight)	8,4	26,2	8,3	0,5	115,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Oncorhynchus mykiss</i>	swim-up fry	30 d	Growth (weight)	12	35	8,3	0,5	115,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Oncorhynchus mykiss</i>	eggs	63 d	Mortality	53,3	70,92	7,65	1,3	126,0	Well	Seim et al., 1984
<i>Oncorhynchus mykiss</i>	larvae (26 days post hatch)	21 d	Mortality	37	83,2	7,87	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
<i>Oncorhynchus mykiss</i>	larvae (1 day post hatch)	21 d	Mortality	41	88,4	7,92	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
<i>Oncorhynchus mykiss</i>	larvae (1 day post hatch)	52 d	Mortality	34	78,9	7,92	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
<i>Oncorhynchus mykiss</i>	larvae (26 days post hatch)	28 d	Mortality	34	78,9	7,95	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
<i>Oncorhynchus mykiss</i>	eyed embryo	30 d	Mortality	17	45,6	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Oncorhynchus mykiss</i>	swim-up fry	30 d	Mortality	22	54,8	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Pimephales promelas</i>	larvae	28 d	Abnormalities	53,7	69,9	8,17	1,3	211,9	Ground water	Scudder et al., 1988
<i>Pimephales promelas</i>	newly hatched larvae	30 d	Growth (weight)	10	31,4	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005

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<i>Pimephales promelas</i>	newly hatched larvae	30 d	Growth (weight)	16	45	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Pimephales promelas</i>	larvae	28 d	growth (weight)	30,3	41,79	8,17	1,3	211,9	Ground water	Scudder et al., 1988
<i>Pimephales promelas</i>	4 weeks old	187 d	growth/length	37	86,1	7,85	0,5 5	15,4	Spring+ deionised tap	Pickering et al., 1977
<i>Pimephales promelas</i>	newly hatched larvae	30 d	Mortality	19	51,3	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Pimephales promelas</i>	newly hatched larvae	30 d	Mortality	19	51	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Pimephales promelas</i>	newly hatched larvae	30 d	Mortality	24	59,8	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
<i>Pimephales promelas</i>	larvae (4 weeks old)	187 d	reproduction (#eggs/female)	16,3	50,3	7,85	0,5 5	15,4	Spring+ deionised tap	Pickering et al., 1977
<i>Pimephales promelas</i>	larvae (7 month old)	7 d	reproduction (#eggs/female)	22,3	62,92	7,85	0,5 5	15,4	Spring+ deionised tap	Pickering et al., 1977
<i>Pimephales promelas</i>	juvenile (32 - 38 mm; 5 months old)	270 d	reproduction (#spawnings/female)	47,6	47,6	8,1	2	183,0	River	Brungs et al., 1976

Table 76: Summary of the chronic toxicity data for fish for the 3 pH classes

Test organism	NOEC/EC10 ($\mu\text{g Cu/l}$)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Oncorhynchus mykiss(mortality)</i>			
n	1	3	8
Min	28.5	24	7.6
Max	/	54.2	53.3
Geometric mean	28.5	33.2	28.2
Lowest value (only when data<4)	/	24	/
<i>Oncorhynchus mykiss(growth)</i>			
n	1	4	5
Min	28.2	3.3	8.4
Max	/	45	23.5
Geometric mean	/	14.7	12.9
Lowest value (only when data<4)	28.2	/	/
<i>Pimephales promelas (Mortality)</i>			
n	3	3	5
Min	10.1	5.9	19
Max	13.8	22.5	61
Geometric mean	11.8	12.02	28.1
Lowest value (only when data<4)	10.1	5.9	/
<i>Pimephales promelas (Growth)</i>			
n	3	4	6
Min	8.7	3.4	10
Max	13.8	22	59.5
Geometric mean	11.4	12	26.6
Lowest value (only when data<4)	8.7	/	/
<i>Pimephales promelas (Reproduction)</i>			
n	/	2	9
Min	/	10.8	14.5
Max	/	16	66
Geometric mean	/	17.3	25.8
Lowest value (only when data<4)	/	10.8	/
<i>Salvelinus fontinalis (Mortality)</i>			
n	/	4	1
Min	/	9.5	22.3
Max	/	44.4	/
Geometric mean	/	16.9	/
Lowest value (only when data<4)	/	/	22.3
<i>Salvelinus fontinalis (Growth)</i>			
n	/	4	1
Min	/	9.5	22.3
Max	/	41.3	/
Geometric mean	/	17.1	/
Lowest value (only when data<4)	/	/	22.3
<i>Salvelinus fontinalis (Reproduction)</i>	n	/	/
		3	/

Min	/	6.4	/
Max	/	36.4	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	6.4*	/

* No geomean could be apply as only 3 data are available considering all pH-categories

Table 77: Summary of the chronic toxicity data for fish for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	NOEC/EC10 ($\mu\text{g Cu/l}$)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Oncorhynchus mykiss(mortality)</i>	n	1	2
	Min	43.75	16.5
	Max	/	19.3
	Geometric mean	/	17.5
	Lowest value (only when data<4)	43.75	16.5
<i>Oncorhynchus mykiss(growth)</i>	n	1	4
	Min	43.33	28.5
	Max	/	57.67
	Geometric mean	/	40.52
	Lowest value (only when data<4)	43.33	/
<i>Pimephales promelas (Mortality)</i>	n	3	3
	Min	14.6	11.6
	Max	23.5	40.9
	Geometric mean	17.9	24.8
	Lowest value (only when data<4)	14.6	11.6
<i>Pimephales promelas (Growth)</i>	n	3	4
	Min	14.6	7.5
	Max	23.5	49.3
	Geometric mean	17.3	27
	Lowest value (only when data<4)	14.6	/
<i>Pimephales promelas (Reproduction)</i>	n	/	2
	Min	/	30.6
	Max	/	34.9
	Geometric mean	/	32.7
	Lowest value (only when data<4)	/	30.6
<i>Salvelinus fontinalis (Mortality)</i>	n	/	4
	Min	/	18.7
	Max	/	55.3
	Geometric mean	/	28.4
	Lowest value (only when data<4)	/	/

<i>Salvelinus fontinalis</i> (Growth)			
n	/	4	1
Min	/	17.1	42.3
Max	/	58.9	/
Geometric mean	/	28.2	/
Lowest value (only when data<4)	/	/	42.3
<i>Salvelinus fontinalis</i> (Reproduction)			
n	/	3	/
Min	/	10.7	/
Max	/	49.38	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	10.7*	/

* No geomean could be apply as only 3 data are available considering all pH-categories

The improvement of database allowed to obtain chronic toxicity values for fish at pH 5.5-6.5. These values show that no more toxicity for fish is expected for long term exposure at pH 5.5-6.5 compared to toxicity observed at pH 6.5-7.5.

According to the improvement of database described before, **the current lowest NOEC/EC₁₀ value for fish was calculated to be 5.9 µg/L corresponding to the lowest value recorded for *P.promelas* at pH between 6.5 and 7.5 for mortality.**

If the geomean is used whatever the number of available data, the lowest NOEC/EC₁₀ value for fish would be 6.4 µg/l recorded for *S. fontinalis* at pH between 6.5 and 7.5.

If the normalisation with the DOC is taking into account, **the lowest NOEC/EC₁₀ value for fish was calculated to be 10.7 µg/L corresponding to the lowest value recorded for *S. fontinalis* at pH between 6.5 and 7.5 for reproduction effect.**

If the geomean is used whatever the number of available data, .

5.4.2 AQUATIC INVERTEBRATES

5.4.2.1 Short-term toxicity to aquatic invertebrates

According to the EU RAR (2008), 91 individual data points for aquatic invertebrates were selected for 2 standard species (*Ceriodaphnia dubia* and *Daphnia magna*).

In the updated database submitted by the registrants of the REACH registration dossier (as detailed in Heijerick and Van Sprang, 2016a), 239 new individual data points for invertebrates were included for the 2 standard species (*Daphnia magna* and *Ceriodaphnia magna*). These new data are presented below and details are given in the table 78.

- *Daphnia magna*: New acute data for *D.magna* available for each of the three classification pH-classes were identified in eleven different publications (Bossuyt et al, 2004 ; De Schamphelaere et al, 2004, 2007 ; Fulton and Meyer, 2014 ; Johnson et al, 2008 ; Kramer et al, 2004 ; Long et al, 2004 ; Rodriguez and Arbildua, 2012 ; Ryan et al, 2009;

Villavicencio et al, 2005 ; Yim et al, 2006), resulting in 180 new reliable E(L)C₅₀ values.

- *Ceriodaphnia dubia*: Eight different publications reported new information on the acute toxicity of copper for *C. dubia* (Cooper et al, 2009; De Schamphelaere et al, 2007 ; Hyne et al, 2005 ; Johnson et al, 2008 ; Markich et al, 2005 ; Nimmo et al, 2006 Van Genderen et al, 2007; Wang et al, 2011), resulting in 59 new, reliable and relevant E(L)C₅₀ values. The data from Cooper et al., 2009 were rejected because an Australian native strain was used as test species, and the calculated EC₁₀ (0.5 µg Cu/L)/NOEC (1.3 µg Cu/L) is below/at the copper concentration in the control (1 µg Cu/L).

Table 78: Overview of the E(L)C50 values for invertebrates published since the EU RAR (2008)

	E(L)C50V Alue	E(L)C50 Normalised value		DOC	Hardness	Temperature		
Species	µg/L	µg/L	pH	mg/L	mg/L as CaCO ₃	°C	Type of water	Reference
<i>Ceriodaphnia dubia</i>	9,0	7,4	6,1	2,5	375	25	Natural water	Markich et al., 2005
<i>Ceriodaphnia dubia</i>	12,0	9,8	6,1	2,5	140	25	Natural water	Markich et al., 2005
<i>Ceriodaphnia dubia</i>	1,6	16,4	6,5	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	1,6	11,6	6,5	0,1	374	25	Reconstituted soft water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	73	14,3	6,5	10	44	25	Reconstituted soft water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	23	18,4	7	2,5	25	25	Natural water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	30	24,2	7	2,5	374	25	Natural water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	32	25,7	7	2,5	140	25	Natural water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	5,38	14,1	7,2	0,7	1213	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	50	4,9	7,2	15,5	6,3	25	Natural water	Nimmo et al., 2006
<i>Ceriodaphnia dubia</i>	2,2	27,7	7,5	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	2,8	32,3	7,5	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	5,02	17,9	7,6	0,5	1245	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	23,6	7,0	7,7	6,5	114	25	Natural water	Nimmo et al., 2006
<i>Ceriodaphnia dubia</i>	39,0	31,3	7,8	2,5	140	25	Natural water	Markich et al., 2005
<i>Ceriodaphnia dubia</i>	42	33,7	7,8	2,5	25	25	Natural water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	44,0	35,5	7,8	2,5	375	25	Natural water	Markich et al., 2005
<i>Ceriodaphnia dubia</i>	14	3,1	7,9	8,5	110	25	Natural water	Nimmo et al., 2006
<i>Ceriodaphnia dubia</i>	42,2	9,5	7,9	8,5	107	25	Natural water	Nimmo et al., 2006
<i>Ceriodaphnia dubia</i>	77,4	28,0	8	5,8	159	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	148	55,2	8	6,4	268	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	279	131,5	8	6,9	438	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	257	100,5	8	7,7	349	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	10,4	31,2	8	0,5	158	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	6,5	48,0	8,1	0,1	44	25	Reconstituted soft water	Hyne et al., 2005

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<i>Ceriodaphnia dubia</i>	44,1	36,4	8,1	2,5	187	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	131	68,8	8,1	4,4	66	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	79,2	26,4	8,1	6,3	149	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	17,7	44,5	8,1	0,5	305	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	36,3	51,4	8,2	1,2	294	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	78,5	49,4	8,2	3,5	223	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	207	105,0	8,2	5,4	509	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	302	91,5	8,2	9,8	288	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	23,1	53,0	8,2	0,5	287	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	26,2	58,6	8,2	0,5	220	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	147	55,8	8,3	5,8	235	25	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	157	60,6	8,3	5,8	238	25	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	115,0	23,4	8,3	9,8	198	20	Natural water	Bossuyt et al., 2004
<i>Ceriodaphnia dubia</i>	267	61,3	8,3	10	249	25	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	14,5	42,2	8,3	0,5	164	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	20,3	51,3	8,3	0,5	156	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	29,3	63,1	8,3	0,5	260	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	37	72,7	8,3	0,5	284	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	39,5	80,3	8,3	0,5	767	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	16	65,5	8,4	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
<i>Ceriodaphnia dubia</i>	25	68,2	8,4	0,4	105	25	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	58,5	48,8	8,4	2,5	249	n.r.	Natural water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	10,4	32,6	8,4	0,5	70	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	29,8	65,6	8,4	0,5	349	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Ceriodaphnia dubia</i>	30	77,8	8,5	0,3	174	25	Reconstituted water (ASTM hard water)	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	24,8	55,6	8,5	0,5	445	n.r.	Reconstituted water	Van Genderen et al., 2007
<i>Daphnia magna</i>	1	2,3	5,96	0,8	19,8	25	Reconstituted water	Ryan et al., 2009
<i>Daphnia magna</i>	3,2	2,9	6,02	2,2	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	4,9	4,5	6,11	2,2	22,4	25	Reconstituted water with natural DOC	Ryan et al., 2009

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<i>Daphnia magna</i>	0,5	1,6	6,16	0,6	10,6	25	Reconstituted water	Ryan et al., 2009
<i>Daphnia magna</i>	7	6,1	6,17	2,3	39,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	4	3,1	6,19	2,6	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	8,6	6,6	6,23	2,6	19,8	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	7,4	7,4	6,28	2	21	20		Rodriguez & Arbildua, 2012
<i>Daphnia magna</i>	29,1	29,1	6,28	2	394	20		Rodriguez & Arbildua, 2012
<i>Daphnia magna</i>	25,2	5,3	6,28	9	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	16,5	15,8	6,29	2,1	169	20		Rodriguez & Arbildua, 2012
<i>Daphnia magna</i>	8,3	6,4	6,29	2,6	42,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	25,9	5,6	6,3	8,7	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	22,8	5,3	6,33	8,4	44,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	18,8	4,0	6,33	8,7	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	26,6	5,8	6,33	8,7	22,4	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	1,6	5,9	6,34	0,5	42,2	25	Reconstituted water	Ryan et al., 2009
<i>Daphnia magna</i>	47,3	10,6	6,42	8,7	42,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	81,8	56,9	6,9	3,04	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	128	62,8	6,92	4,53	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	311	98,9	6,92	8,54	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	53,8	54,9	6,94	1,95	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	192	86,9	6,97	5,35	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	86,6	87,5	6,98	1,97	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	607	101,7	6,98	16,9	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	332	74,9	6,99	10,8	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	638	198,8	6,99	11,7	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	542	101,3	6,99	15,4	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	261	139,4	7	5,11	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004

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<i>Daphnia magna</i>	101,8	22,2	7	9,01	40	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	129	130,8	7,01	1,95	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	275	71,0	7,03	9,22	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	60,6	48,1	7,06	2,58	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	212	31,7	7,07	13,7	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	50,6	48,8	7,08	2,08	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	9,2	16,6	7,1	1,1	81	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	372	44,7	7,1	17,8	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	34,4	8,7	7,13	7,28	8	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	21	9,9	7,21	4,1	9	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	6,183	23,1	7,3	0,5	42	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	4,9	12,2	7,41	0,81	9	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	118,5	27,0	7,43	8,47	14	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	77,3	15,3	7,45	9,4	12	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	3,7	21,3	7,5	0,34	12	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	50	30,5	7,55	3,27	18	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	5,5	20,6	7,57	0,53	20	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	14	14,7	7,6	1,9	75	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	61,82	10,2	7,6	11,4	50	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	47	34,2	7,62	2,75	12	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	39	34,7	7,64	2,25	12	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	7,3	23,9	7,65	0,6	18	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	19	23,9	7,65	1,59	13	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	6,596	23,7	7,65	0,5	72	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	11,4	23,7	7,7	0,96	14	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	35,23	22,8	7,7	3,1	80	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	3,976	15,4	7,71	0,5	42	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014

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<i>Daphnia magna</i>	3,8	20,1	7,78	0,37	14	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	30,0	83,4	7,8	0,38	250	20	Reconstituted water	Bossuyt et al., 2004
<i>Daphnia magna</i>	40,6	98,6	7,8	0,38	250	20	Reconstituted water	Bossuyt et al., 2004
<i>Daphnia magna</i>	53,2	115,3	7,8	0,38	250	20	Reconstituted water	Bossuyt et al., 2004
<i>Daphnia magna</i>	7,8	21,5	7,8	0,72	19	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	7,7	17,0	7,8	0,9	79	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	6,4	40,9	7,81	0,25	28	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	6,2	30,4	7,83	0,38	23	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	8,4	35,3	7,83	0,44	20	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	7,4	23,9	7,83	0,6	22	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	8,9	24,1	7,83	0,73	18	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	30,5	52,4	7,84	1,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	30,8	28,0	7,84	2,2	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	56,1	53,6	7,85	2,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	9,46	33,0	7,85	0,5	72	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	7,2	30,5	7,86	0,44	22	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	7,4	30,8	7,86	0,45	22	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	38,2	29,6	7,86	2,6	19,8	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	45,1	35,1	7,87	2,6	43,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	72	47,2	7,87	3,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	100	38,9	7,87	5,2	42,7	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	108,8	24,1	7,87	8,9	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	6,03	22,8	7,87	0,5	46	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	87,39	22,4	7,88	7,8	84	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	155,7	24,2	7,88	12,5	54	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	8,4	59,5	7,9	0,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	5,7	33,2	7,9	0,3	26	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	5,2	29,2	7,91	0,3	9,2	25	Reconstituted water	Ryan et al., 2009
<i>Daphnia magna</i>	88,8	43,0	7,91	4,2	42,7	20	Reconstituted water	Villavicencio et al., 2005

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<i>Daphnia magna</i>	15,3	13,3	7,92	2,3	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	12,1	52,4	7,94	0,31	132	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	187,6	47,5	7,94	8,3	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	9,3	28,2	7,95	0,6	42,2	25	Reconstituted water	Ryan et al., 2009
<i>Daphnia magna</i>	16,7	13,9	7,95	2,4	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	106,4	24,7	7,95	8,5	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	130,5	31,3	7,96	8,4	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	2,6	15,6	7,97	0,3	18,5	25	Reconstituted water	Ryan et al., 2009
<i>Daphnia magna</i>	21,1	20,1	7,98	2,1	40,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	122,5	28,7	7,98	8,6	44,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	159	37,1	7,98	8,8	40,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
<i>Daphnia magna</i>	208,0	41,0	8	10,4	198	20	Natural water	Bossuyt et al., 2004
<i>Daphnia magna</i>	68,45	23,4	8,02	5,8	42	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	19,28	53,8	8,02	0,5	100	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	8,3	40,8	8,03	0,31	61	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	68,31	12,5	8,03	10,5	60	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	96,23	25,0	8,06	7,8	106	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	304	49,5	8,1	14,7	229	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	27,8	45,1	8,11	1,18	38	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	156,1	76,9	8,11	4,65	85,2	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	9,2	57,9	8,12	0,14	32	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	104,5	61,7	8,12	3,63	85,2	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	19,24	54,3	8,13	0,5	96	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	12,1	46,5	8,15	0,38	88	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	32,3	67,8	8,16	0,71	270	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	24,3	54,4	8,17	0,76	68	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	141,6	28,4	8,19	10	54	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	116,3	21,6	8,19	10,7	90	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	25,3	84,8	8,2	0,1	85,2	20	Reconstituted water	Villavicencio et al., 2005

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<i>Daphnia magna</i>	60,3	98,1	8,2	0,87	85,2	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	69	42,4	8,2	3,5	230	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	71	37,9	8,2	4	189	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	87	37,9	8,2	5	218	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	93	37,8	8,2	5,3	174	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	314	58,8	8,2	17,3	591	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	10,14	34,6	8,2	0,5	80	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	78,4	85,3	8,22	1,78	85,2	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	109,7	87,6	8,23	2,69	85,2	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	32	64,7	8,24	0,79	72	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	135,5	22,2	8,24	12,3	102	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	81,06	14,5	8,27	11	104	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	37,78	30,6	8,29	2,5	88	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	104	53,9	8,3	4,3	203	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	170,0	43,5	8,3	8,2	236	20	Natural water	Bossuyt et al., 2004
<i>Daphnia magna</i>	178,0	37,3	8,3	9,8	198	20	Natural water	Bossuyt et al., 2004
<i>Daphnia magna</i>	308	49,8	8,3	18	481	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	15,58	47,8	8,31	0,5	98	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
<i>Daphnia magna</i>	60	123,5	8,36	0,1	166,9	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	48,2	74,6	8,36	1,07	120	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	28,3	89,7	8,39	0,07	88	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	239,8	167,7	8,39	3,94	166,9	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	109	60,1	8,4	4,1	195	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	207	33,3	8,4	14,2	294	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	59,9	91,6	8,43	0,98	112	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	31,65	19,7	8,44	3,2	48	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	100	141,0	8,45	0,84	166,9	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	66,2	75,4	8,45	1,67	128	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	235,9	187,6	8,45	3,25	166,9	20	Reconstituted water	Villavicencio et al., 2005

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<i>Daphnia magna</i>	125,2	140,4	8,46	1,57	166,9	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	206	193,1	8,46	2,33	166,9	20	Reconstituted water	Villavicencio et al., 2005
<i>Daphnia magna</i>	172,8	22,8	8,48	15,7	154	20	natural water	Fulton & Meyer, 2014
<i>Daphnia magna</i>	43,1	71,1	8,5	0,91	271	20	Natural water	Villavicencio et al., 2005
<i>Daphnia magna</i>	99	70,3	8,5	3,1	234	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	124	56,3	8,5	5	193	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	151	24,7	8,5	13,3	298	20	Filtered natural water	Kramer et al., 2004
<i>Daphnia magna</i>	354	58,1	8,5	15,1	186	20	Filtered natural water	Kramer et al., 2004
<i>Ceriodaphnia dubia</i>	1		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Ceriodaphnia dubia</i>	6,7		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Ceriodaphnia dubia</i>	9,7		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Ceriodaphnia dubia</i>	11,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Ceriodaphnia dubia</i>	12		7		112	25	Reconstituted water	Nimmo et al., 2006
<i>Ceriodaphnia dubia</i>	18		7,5		82,4	25,3	Reconstituted water (US EPA)	Cooper et al., 2009
<i>Ceriodaphnia dubia</i>	7,3		7,8		97	25	Reconstituted water	Nimmo et al., 2006
<i>Ceriodaphnia dubia</i>	42		8		80-110	23	Reconstituted water	Johnson et al., 2008
<i>Daphnia magna</i>	2		5,6		7,1	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	2,5		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	4,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	17,8		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	19,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	21,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	25,9		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	28,9		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	29,4		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
<i>Daphnia magna</i>	2,8		7		7,1	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	7,4		7		20,6	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	5		7,7		80-110	23	Reconstituted water	Johnson et al., 2008
<i>Daphnia magna</i>	4		7,8		44	25	Reconstituted water	Yim et al., 2006

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<i>Daphnia magna</i>	12		8		150	25	Reconstituted water	Yim et al., 2006
<i>Daphnia magna</i>	21		8		211	20	Reconstituted water	Kramer et al., 2004
<i>Daphnia magna</i>	25		8		211	20	Reconstituted water	Kramer et al., 2004
<i>Daphnia magna</i>	34		8		211	20	Reconstituted water	Kramer et al., 2004
<i>Daphnia magna</i>	40		8		211	20	Reconstituted water	Kramer et al., 2004
<i>Daphnia magna</i>	2		8,2		11,1	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	11,1		8,2		50,7	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	2		8,3		7,9	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	10		8,3		22,2	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	6,5		8,5		20,6	25	Reconstituted water	Long et al., 2004
<i>Daphnia magna</i>	14,1		6,1-6,3		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	14,7		6,17-6,25		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	21,6		6,95-7,05		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	19,4		6,98-7,03		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	67,3		7,96-8,06		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
<i>Daphnia magna</i>	68,4		7,99-8,02		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004

Sufficient data for the 3 pH classes were found for these 2 invertebrate species.

Table 79: Summary of the acute toxicity data for invertebrates for the 3 pH classes

Test organism	L(E)C ₅₀ (µg Cu/L)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Daphnia magna</i> (mortality)			
n	29	57	196
Min	0.5	2.5	2
Max	465	1213	826
Geometric mean	16.3	66.2	45.4
Lowest value (only when data<4)	/	/	/
<i>Ceriodaphnia dubia</i> (mortality)			
n	9	17	54
Min	1.6	1	5.02
Max	73	84	302
Geometric mean	12.6	14	40
Lowest value (only when data<4)	/	/	/

Table 80: Summary of the acute toxicity data for invertebrates for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	L(E)C ₅₀ (µg Cu/L)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Daphnia magna</i> (mortality)			
n	26	39	130
Min	1.6	8.7	102
Max	204.2	418	398.4
Geometric mean	11	63	49.2
Lowest value (only when data<4)	/	/	/
<i>Ceriodaphnia dubia</i> (mortality)			
n	8	10	42
Min	7.4	4.9	3.1
Max	52	76	131.5
Geometric mean	16	24.1	43.9
Lowest value (only when data<4)	/	/	/

According to the improvement of database described before, **the current lowest E(L)C₅₀ value for invertebrate was calculated to be 12.6 µg/L corresponding to the geomean recorded for *C. dubia* at pH between 5.5 and 6.5 for mortality.**

If the normalisation with the DOC is taking into account, **the lowest E(L)C₅₀ value for invertebrate was calculated to be 11 µg/L corresponding to the geomean recorded for *D. magna* at pH between 5.5 and 6.5 for mortality.**

5.4.2.2 LONG-TERM TOXICITY TO AQUATIC INVERTEBRATES

According to the EU RAR (2008), 19 individual data points for aquatic invertebrates were selected for 2 standard species (*Ceriodaphnia dubia* and *Daphnia magna*).

In the updated database detailed in the Heijerick and Van Sprang, 2016b, 25 new individual data points for invertebrates were included for the 2 standard species (*Daphnia magna* and *Ceriodaphnia dubia*). These new data are presented below and details are given in the table 81.

- *Daphnia magna*: New reliable chronic data for the invertebrate *D. magna* were identified in five different publications (Adam et al, 2015; Bossuyt and Janssen, 2004 ; Van Regenmortel et al, 2013, 2015 ; Rodriguez and Arbildua, 2012), and a total of 14 new relevant EC₁₀ or NOEC for reproduction situated between 4.7 and 300 µg/L were withheld for classification purposes. Villavicencio et al (2011) reported an extensive data set of chronic EC₅₀ values (endpoint: reproduction), but as no EC₁₀ could be derived from this study, it cannot be used for classification purposes.
- *Ceriodaphnia dubia*: Two publications (Wang et al, 2011 ; Cooper et al, 2009) reported on 12 different tests with *C. dubia*. Relevant endpoints were mortality (6 data points) and reproduction (6 data points). EC₁₀ values were available for 11 tests, whereas a NOEC was given for the remaining test. Values ranged between 2.4 and 200 µg/L for mortality, and between 1.3 and 46 µg/L for reproduction. The data from Cooper et al., 2009 were rejected because the NOEC/calculated EC₁₀ value for reproduction (1.3/0.5 µg Cu/L) was at or below the copper concentration in the control (1 µg Cu/L). Schwartz and Vigneault (2007) also reported a number of chronic data, but values represented an EC₂₅ which could not be considered for classification.

In addition, an extra EC₁₀-value of 14 µg/L (endpoint: reproduction) was calculated by Heijerick and Van Sprang, 2016b from the data that were published in Belanger et al (1989).

Nevertheless, as mentioned before, in the section of the selection of data, results obtain at 7 days of exposure were rejected in the Joint Research Centre (JRC) in their Report “New criteria for environmental long-term aquatic hazard classification under the CLP Regulation (EC) N° 1272/2008 (2nd ATP) - Screening of Annex VI substances with harmonised classifications” (p.20). Therefore, these values are only presented in italic in the table 81 for information but not used for classification purposes.

Table 81: Overview of the NOEC/EC10 values for invertebrates published since the EU RAR (2008)

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/EC10	Normalised	pH	DOC	Alk	Medium	Reference
				(µg/L)	(µg/L)		mg/L	mg/L as CaCO ₃		
<i>Daphnia magna</i>	Neonates	21 d	Reproduction	8,2	8,2	6,27	2	13,4	Reconstituted EPA water	Rodriguez & Arbildua, 2012
<i>Daphnia magna</i>	Neonates	21 d	Reproduction	5,9	5,9	6,28	2	13,4	Reconstituted EPA water	Rodriguez & Arbildua, 2012
<i>Daphnia magna</i>	Neonates	21 d	Reproduction	6,7	6,5	6,28	2,1	13,4	Reconstituted EPA water	Rodriguez & Arbildua, 2012
<i>Daphnia magna</i>	Neonates	21 d	Reproduction	32,9	7,3	6,41	6,1	96,2	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
<i>Daphnia magna</i>	Neonates	21 d	Reproduction	10,7	16,2	6,41	6,1	96,2	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Mortality	20	11,3	8,3	3	91,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Mortality	65	19,6	8,3	5,8	94,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Mortality	91	28,5	8,3	5,8	93,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Mortality	200	37,8	8,3	10	96,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Mortality	10	32,92	8,4	0,4	100,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Reproduction	34	19,95	8,3	3	91,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Reproduction	29	8,15	8,3	5,8	94,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Reproduction	46	13,4	8,3	5,8	93,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Reproduction	25	3,74	8,3	10	96,0	natural well water	Wang et al., 2011
<i>Ceriodaphnia dubia</i>	< 24 h	7 d*	Reproduction	8	31,1	8,4	0,4	100,0	natural well water	Wang et al., 2011
<i>Daphnia magna</i>	Neonates (< 24 h old)	21 d	Reproduction	56,1	22,4	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
<i>Daphnia magna</i>	Neonates (< 24 h old)	21 d	Reproduction	57,1	22,8	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
<i>Daphnia magna</i>	Neonates (< 24 h old)	21 d	Reproduction	70,5	28,2	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
<i>Daphnia magna</i>	Neonates (< 24 h old)	21 d	Reproduction	46,5	18,5	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
<i>Daphnia magna</i>	Neonates (< 24 h old)	21 d	Reproduction	50,2	20	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
<i>Daphnia magna</i>	Neonates (< 24 h old)	21 d	Reproduction	56,2	22,4	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
<i>Daphnia magna</i>	< 24 h	21 d	Reproduction	17	54,12	7,92	0,5	18,9	Reconstituted water (ISO medium)	Adam et al., 2015

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<i>Daphnia magna</i>	Neonates	21 d	Reproductio n	76,9	5,5	8,44	6,1	186,4	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
<i>Daphnia magna</i>	Neonates	21 d	Reproductio n	55,4	8	8,45	6,1	186,5	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
<i>Ceriodaphnia dubia</i>	<i>neonates (2-8 h)</i>	<i>7 d*</i>	<i>Reproductio n</i>	<i>14</i>	<i>7,66</i>	<i>8,31</i>	<i>3,7</i>	<i>140,0</i>	<i>River (Clinch River)</i>	<i>Belanger et al., 1989</i>

*in italics: not retained for classification purposes

Table 82: Summary of the chronic toxicity data for invertebrates for the 3 pH classes

Test organism	NOEC ($\mu\text{g Cu/L}$)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Daphnia magna</i> (mortality)	n	/	/
	Min	/	/
	Max	/	/
	Geometric mean	/	/
	Lowest value (only when data<4)	/	/
<i>Daphnia magna</i> (reproduction)	n	7	2
	Min	5.9	181
	Max	32.9	300
	Geometric mean	13.2	233
	Lowest value (only when data<4)	/	181
<i>Daphnia magna</i> (growth)	n	/	/
	Min	/	/
	Max	/	/
	Geometric mean	/	/
	Lowest value (only when data<4)	/	/

Table 83: Summary of the chronic toxicity data for invertebrates for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	NOEC ($\mu\text{g Cu/L}$)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Daphnia magna</i> (mortality)	n	/	/
	Min	/	/
	Max	/	/
	Geometric mean	/	/
	Lowest value (only when data<4)	/	/
<i>Daphnia magna</i> (reproduction)	n	7	2
	Min	5.9	16.5
	Max	20.6	34.4
	Geometric mean	10.5	23.8
	Lowest value (only when data<4)	/	16.5
<i>Daphnia magna</i> (growth)	n	/	/
	Min	/	/
	Max	/	/
	Geometric mean	/	/
	Lowest value (only when data<4)	/	/

According to the improvement of database described before, **the current lowest NOEC/EC₁₀ value for invertebrate was calculated to be 12.6 µg/L corresponding to the lowest value recorded for *D. magna* at pH between 7.5 and 8.5 for growth effect.**

If the normalisation with the DOC is taking into account, **the lowest NOEC/EC₁₀ value for invertebrate was calculated to be 10.5 µg/L corresponding to the lowest value recorded for *D. magna* at pH between 6.5 and 7.5 for reproduction effect.**

5.4.3 ALGAE AND AQUATIC PLANTS

According to the EU vRAR (2008), 17 individual acute data points for algae were selected for 1 standard species (*Pseudokirchneriella subcapitata*).

In the updated database detailed by Heijerick et al., 2016a, 38 new individual data points for algae were included for the standard species *Pseudokirchneriella subcapitata*. New individual data points are included for 2 other standard species *Chlamydomonas reinhardtii* and *Chlorella sp.* These new data are presented below and details are given in the table 84.

- *Pseudokirchneriella subcapitata*: new reliable acute data for green algae *P. subcapitata* were identified in 4 different publications (Heijerick et al, 2005; De Schamphelaere & Janssen, 2006; De Schamphelaere, 2005; De Schamphelaere et al, 2005). A total of 31 new relevant EbC50 and ErC50 between 16.5 and 824 mg/L. Levy et al (2009) also published two 72h-ErC50 values, but these tests did not meet the OECD-test guidelines and were therefore not retained for hazard classification purposes. As all data issued from Heijerick et al. (2005) publication related on biomass, therefore not retained for classification. Only data on ErC50 were retained for classification and were presented in the table below.
- *Chlamydomonas reinhardtii*: De Schamphelaere & Janssen (2006) reported 3 ErC50 varying between 146 µg/l and 380 µg/L at 3 different pH (6.02 ; 7.03 ; 8.11) which were included in the acute classification dataset.
- *Chlorella sp.*: Also in a publication of De Schamphelaere & Janssen (2006), 16 new ErC50 were reported and added in the acute classification dataset.

Table 84: Overview of the ErC50 values for algae/plants published since the EU RAR (2008)

		Value µg/L	Normalised value µg/L	DOC mg/L	Hardness mg/L as CaCO ₃	Temperature °C	Type of water	Reference
Species				pH				
<i>Chlamydomonas reinhardtii</i>	ErC50	380	143,2	6,02	9,84	250	NA	field water
<i>Chlamydomonas reinhardtii</i>	ErC50	315	80,4	7,03	9,84	250	NA	field water
<i>Chlamydomonas reinhardtii</i>	ErC50	146	31,4	8,11	9,84	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	602	305,2	5,5	10,27	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	440	319,3	6,01	5,03	400	NA	field water
<i>Chlorella vulgaris</i>	ErC50	333	199,9	6,03	5,17	100	NA	field water
<i>Chlorella vulgaris</i>	ErC50	773	236,8	6,04	15,49	100	NA	field water
<i>Chlorella vulgaris</i>	ErC50	987	404,7	6,05	15,24	400	NA	field water
<i>Chlorella vulgaris</i>	ErC50	254	59,4	7,01	10,03	500	NA	field water
<i>Chlorella vulgaris</i>	ErC50	296	63,7	7,03	10,81	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	364	87,5	7,03	10,81	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	60	74,3	7,04	1,5	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	208	43,6	7,04	10,23	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	446	49,3	7,05	19,9	250	NA	field water
<i>Chlorella vulgaris</i>	ErC50	238	48,1	7,07	10,26	25	NA	field water
<i>Chlorella vulgaris</i>	ErC50	111	44,2	7,88	5,31	100	NA	field water
<i>Chlorella vulgaris</i>	ErC50	380	54,1	7,88	15,66	100	NA	field water
<i>Chlorella vulgaris</i>	ErC50	99	42,1	7,92	5,04	400	NA	field water
<i>Chlorella vulgaris</i>	ErC50	506	89,1	7,97	15,82	400	NA	field water
<i>Pseudokirchneriella subcapitata</i>	ErC50	756	411,2	5,68	9,84	250	25	Synthetic-Ankeveen
<i>Pseudokirchneriella subcapitata</i>	ErC50	205	113,1	5,99	5,64	400	25	Synthetic-Ankeveen
<i>Pseudokirchneriella subcapitata</i>	ErC50	368	65,2	6,17	14,9	100	25	Synthetic-Ankeveen
<i>Pseudokirchneriella subcapitata</i>	ErC50	122	56,5	6,18	5,07	100	25	Synthetic-Ankeveen
<i>Pseudokirchneriella subcapitata</i>	ErC50	230	122,0	6,19	5,23	100	25	Synthetic-Bihain
<i>Pseudokirchneriella subcapitata</i>	ErC50	199	97,5	6,2	5,31	100	25	Synthetic-Ossenkolk
<i>Pseudokirchneriella subcapitata</i>	ErC50	811	205,9	6,2	15,6	100	25	Synthetic-Ossenkolk

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<i>Pseudokirchneriella subcapitata</i>	ErC50	824	227,7	6,22	15,8	100	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	174	18,9	6,95	18,2	250	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	100	19,7	7,01	10,2	250	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	30	30,7	7,02	1,95	250	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	105	20,6	7,02	10,4	500	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	102	20,5	7,03	9,98	250	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	99	19,6	7,04	10,1	250	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	190	42,0	7,04	9,89	500	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	35	31,8	7,05	2,21	250	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	35	34,0	7,08	2,06	250	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	156	32,5	7,09	9,99	250	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	178	33,1	7,09	11,1	250	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	685	94,2	7,11	19,9	250	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	281	63,2	7,12	10,5	500	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	462	57,7	7,17	18,5	250	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	193	37,1	7,19	10,4	25	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	151	20,0	7,78	15,2	400	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	37	37	7,8	2			Reconstituted medium	De Schampelaere et al, 2005
<i>Pseudokirchneriella subcapitata</i>	ErC50	51	19,2	7,92	5,46	400	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	93	32,7	7,92	5,99	400	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	268	38,8	8,01	15,1	400	25	Synthetic-Bihain	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	59	22,2	8,02	5,42	100	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	209	27,9	8,05	15,3	100	25	Synthetic-Ankeveen	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	346	48,7	8,05	16,1	400	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	92	34,0	8,07	5,75	400	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	161	33,8	8,25	10,3	250	25	Synthetic-Ossenkolk	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	219	49,8	8,37	10,3	250	25	Synthetic-Bihain	De Schampelaere, 2006
<i>Pseudokirchneriella subcapitata</i>	ErC50	18		8,5	<2		25	Reconstituted medium	De Schampelaere et al, 2005
<i>Pseudokirchneriella subcapitata</i>	ErC50	46		5,9	<2		25	Reconstituted medium	De Schampelaere et al, 2005

Table 85: Summary of the acute toxicity data for algae/plants for the 3 pH classes

Test organism	ErC ₅₀ (µg Cu/L)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Pseudokirchneriella subcapitata</i>			
Growth rate			
n	9	15	12
Min	46	30	18
Max	824	685	346
Geometric mean	277.6	131.6	104.9
Lowest value (only when data<4)	/	/	/
<i>Chlamydomonas reinhardtii</i>			
Growth rate			
n	1	1	1
Min	380	315	146
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data <4)	380	315	146
<i>Chlorella vulgaris</i>			
Growth rate			
n	5	7	4
Min	333	60	99
Max	987	446	506
Geometric mean	582.9	232.7	214.4
Lowest value (only when data<4)	/	/	/

Table 86: Summary of the acute toxicity data for algae/plants for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	ErC ₅₀ (µg Cu/L)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Pseudokirchneriella subcapitata</i>			
Growth rate			
n	8	15	11
Min	56	18.9	19.2
Max	411.2	94.2	19.8
Geometric mean	132.6	33	31.6
Lowest value (only when data<4)	/	/	/
<i>Chlamydomonas reinhardtii</i>			
Growth rate			
n	1	1	1
Min	143.2	80.4	31.4
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data <4)	143.2	80.4	31.4
<i>Chlorella vulgaris</i>			
Growth rate			
n	5	7	4
Min	199.9	43.6	42.1
Max	404.7	87.5	89.1
Geometric mean	284.6	59.2	54.7
Lowest value (only when data <4)	/	/	/

According to the improvement of database described before, **the current lowest ErC₅₀ value for algae/plants was calculated to be 104.9 µg/L corresponding to the lowest geomean recorded for *P. subcapitata* at pH between 7.5 and 8.5.**

If the normalisation with the DOC is taking into account, **the lowest ErC₅₀ value for algae/plants was calculated to be 31.4 µg/L corresponding to the lowest value recorded for *C. reinhardtii* at pH between 7.5 and 8.5.**

According to the EU vRAR (2008), 29 individual chronic data points for algae were selected for 3 standard species (*Pseudokirchneriella subcapitata*, *lemona minor* and *Chlorella vulgaris*).

In the updated database detailed by Heijerick and Van Sprang, 2016b, 37 new individual data points for algae/plants were included for the 2 standard species (*Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*).

- *Pseudokirchneriella subcapitata*: De Schamphelaere and Janssen (2006) reported 34 reliable ErC₁₀ values on copper for the green alga *P. subcapitata*. One additional value was obtained at a pH > 8.5, and was therefore not considered for hazard assessment purposes. The ErC₁₀ values ranged from 16.7 to 337 µg/L. Only data based on growth rate are retained for chronic classification purposes.

A test conducted by Levy et al (2009) was rejected for the chronic hazard assessment of copper as it was conducted in axenic conditions which is not in line with the OECD test protocol.

- *Chlamydomonas reinhardtii*: three new chronic data available for 3 different pH values are extracted from a publication of De Schamphelaere and Janssen (2006).

These new data are presented in the table below.

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Table 87: Overview of the ErC10 values for algae/plants published since the EU RAR (2008)

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/E C10	Normalised	pH	DOC	Alk	Medium	Reference
				(µg/L)	(µg/L)		mg/L	mg/L as CaCO ₃		
<i>Chlamydomonas reinhardtii</i>	Inoculum: 10,000 c/ml	3 d	growth	178	61,7	6,02	9,84	0,5	Reconstituted	De Schampelaere et al., 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	161,6	50,6	5,68	9,84	0,3	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	49,4	20,5	5,99	5,64	0,5	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	148,4	24,5	5,99	15,3	0,5	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	155,9	22,8	6,17	14,9	1,0	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	52,3	21,9	6,18	5,07	1,0	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	110,3	49,1	6,19	5,23	1,1	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	97,7	41,2	6,2	5,31	1,1	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	336,9	53,7	6,2	15,6	1,1	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	337,0	56,1	6,22	15,8	1,1	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Chlamydomonas reinhardtii</i>	Inoculum: 10,000 c/ml	3 d	Growth	108	27,4	7,03	9,84	9,9	Reconstituted	De Schampelaere et al., 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	86,8	9,3	6,98	18,2	8,9	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	52,2	10,2	7,01	10,2	13,9	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	16,7	17,1	7,02	1,95	9,7	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	54,8	10,6	7,02	10,4	9,7	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	51,6	10,3	7,03	9,98	10,1	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	93,2	19,4	7,04	9,89	10,2	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10 ⁴ cells/ml	72 h	Growth rate	50,6	10,0	7,04	10,1	10,3	Reconstituted OECD medium	De Schampelaere & Janssen, 2006

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<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	18,0	16,4	7,05	2,21	10,5	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	18,1	17,6	7,08	2,06	11,2	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	67,2	13,6	7,09	9,99	11,4	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	62,1	11,1	7,09	11,1	11,4	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	202,6	20,3	7,11	19,9	12,1	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	78,4	15,1	7,12	10,5	12,3	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	105,7	11,4	7,17	18,5	13,8	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	74,9	14,0	7,19	10,4	15,4	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Chlamydomonas reinhardtii</i>	Inoculum: 10,000 c/ml	3 d	growth	96	24,7	8,11	9,84	92,5	Reconstituted	De Schampelaere et al., 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	99,6	13,2	7,78	15,2	13,5	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	40,6	13,9	7,92	5,99	18,6	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	25,3	9,4	7,92	5,46	18,6	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	121,1	16,6	8,01	15,1	23,0	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	19,9	7,4	8,02	5,42	23,5	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	171,5	22,2	8,05	16,1	25,2	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	81,6	10,6	8,05	15,3	25,2	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	87,0	32,2	8,07	5,75	26,4	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	52,6	10,5	8,25	10,3	40,2	Reconstituted OECD medium	De Schampelaere & Janssen, 2006
<i>Pseudokirchneriella subcapitata</i>	1*10^4 cells/ml	72 h	Growth rate	86,8	17,8	8,37	10,3	53,3	Reconstituted OECD medium	De Schampelaere & Janssen, 2006

Table 88: Summary of the chronic toxicity data for algae/plants for the 3 pH classes

Test organism	NOErC ($\mu\text{g Cu/L}$)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Pseudokirchneriella subcapitata</i> (growth rate)			
n	9	15	10
Min	49.4	16.7	19.9
Max	337	202.6	171.5
Geometric mean	131.6	56.1	65.1
Lowest value (only when data<4)	/	/	/
<i>Chlamydomonas reinhardtii</i>			
n	2	1	1
Min	22	108	96
Max	178	/	/
Geometric mean	62.6	/	/
Lowest value (only when data<4)	22	108	96
<i>Chlorella vulgaris</i>			
n	5	7	4
Min	108.3	36.4	31
Max	510.2	282.9	188
Geometric mean	279.6	106.3	86.5
Lowest value (only when data<4)	/	/	/
<i>Lemna minor</i>			
n	1	/	/
Min	30	/	/
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	30	/	/

Table 89: Summary of the chronic toxicity data for algae/plants for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	NOErC ($\mu\text{g Cu/L}$)		
	pH: 5.5-6.5	pH: >6.5-7.5	pH: >7.5-8.5
<i>Pseudokirchneriella subcapitata</i> (growth rate)	n	9	15
	Min	20.5	9.3
	Max	56.1	20.3
	Geometric mean	34.9	13.3
	Lowest value (only when data<4)	/	/
<i>Chlamydomonas reinhardtii</i>	n	2	1
	Min	61.7	27.4
	Max	64.8	/
	Geometric mean	63.2	/
	Lowest value (only when data<4)	61.7	27.4
<i>Chlorella vulgaris</i>	n	5	7
	Min	60.2	19
	Max	213	54.1
	Geometric mean	124.4	30.3
	Lowest value (only when data<4)	/	/
<i>Lemna minor</i>	n	1	/
	Min	75.8	/
	Max	/	/
	Geometric mean	/	/
	Lowest value (only when data<4)	75.8	/

According to the improvement of database described before, **the current lowest NOErC value for algae/plants was calculated to be 22 $\mu\text{g/L}$ corresponding to the lowest value recorded for *C. reinhardtii* at pH between 5.5 and 6.5.**

If the geomean is used whatever the number of available data, the lowest NOErC value for algae/plants would be 30 $\mu\text{g/l}$ recorded for *L. minor* at pH between 5.5 and 6.5.

If the normalisation with the DOC is taking into account, **the lowest NOErC value for algae/plants was calculated to be 13.3 $\mu\text{g/L}$ corresponding to the lowest geomean recorded for *P. subcapitata* at pH between 6.5 and 7.5 for growth rate effect.** The same value is retained if the geomean is used whatever the number of available data

5.4.4 OVERALL CONCLUSION OF AQUATIC TOXICITY AND DERIVATION OF THE RELEVANT ERV

Based on the result presented in the section 5.4.1 to 5.4.3, the table 90 and 91 below summarised the lowest values retained to derive the ERV values for acute and chronic classification respectively.

Table 90: Overview of all acute toxicity data available at 3 pH-categories with or without normalisation at 2mg/L DOC

	L(E)C ₅₀ (μ g Cu/L) (number of available data)					
	pH: 5.51-6.5		pH: >6.5-7.5		pH: >7.5-8.5	
	Non normalise d	Normalised	Non normalise d	Normalised	Non normalise d	Normalised
<i>Oncorhynchus mykiss</i> Lowest value or geomean	24.4 (8)	40.6 (8)	47.4 (22)	45.9 (8)	63.6 (47)	94.7 (25)
<i>Danio rerio</i> Lowest value or geomean	/	/	11.7 (2)	26 (1)	148.4 (3)	94.7 (2)
<i>Danio rerio</i> Geomean	/	/	20.24 (2)	26 (1)	167.4 (3)	117.9 (2)
<i>Cyprinus carpio</i> Lowest value or geomean	/	/	/	/	800 (3)	/
<i>Cyprinus carpio</i> Geomean	/	/	/	/	810 (3)	/
<i>Pimephales promelas</i> Lowest value or geomean	12.1 (5)	14.9 (3)	96.7 (46)	49.3 (11)	255.9 (207)	382.86 (47)
<i>Pimephales promelas</i> Geomean	12.1 (5)	23.5 (3)	96.7 (46)	49.3 (11)	255.9 (207)	382.86 (47)
<i>Lepomis macrochirus</i> Lowest value or geomean	/	/	1000 (2)	/	4250 (3)	/
<i>Lepomis macrochirus</i> Geomean	/	/	1048 (2)	/	5509 (3)	/
<i>Daphnia magna</i> (mortality) Lowest value or geomean	16.3 (29)	11 (26)	66.2 (57)	63 (39)	45.4 (196)	49.2 (130)
<i>Ceriodaphnia dubia</i> (mortality) Lowest value or geomean	12.6 (9)	16 (8)	14 (17)	24.1 (10)	40 (54)	43.9 (42)
<i>Pseudokirchneriella subcapitata</i> (growth rate) Lowest value or geomean	277.6 (9)	132.6 (8)	131.6 (15)	33 (15)	104.9 (12)	31.6 (11)
<i>Chlamydomonas reinhardtii</i> (growth rate) Lowest value or geomean	380 (1)	143.2 (1)	315 (1)	80.4 (1)	146 (1)	31.4 (1)
<i>Chlorella vulgaris</i> (growth rate) Lowest value or geomean	582.9 (5)	284.6 (5)	232.7 (7)	59.2 (7)	214.4 (4)	54.7 (4)

In grey, values used for classification proposal.

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Table 91: Overview of all chronic toxicity data available at 3 pH-categories with or without normalisation at 2mg/L DOC

	NOEC/EC10 ($\mu\text{g Cu/L}$) (number of available data)					
	pH: 5.51-6.5		pH: >6.5-7.5		pH: >7.5-8.5	
	Non normalise d	Normalised	Non normalise d	Normalised	Non normalise d	Normalised
<i>Oncorhynchus mykiss(mortality)</i> Lowest value or geomean	28.5 (1)	/	24 (3)	16.5 (2)	28.2 (8)	60.6 (8)
<i>Oncorhynchus mykiss(mortality)</i> Geomean	28.5 (1)	/	33.2 (3)	17.5 (2)	28.2 (8)	60.6 (8)
<i>Oncorhynchus mykiss(growth rate)</i> Lowest value or geomean	28.2 (1)	/	14.7 (4)	40.52 (4)	12.9 (5)	31.3 (5)
<i>Pimephales promelas</i> (mortality) Lowest value or geomean	10.1 (3)	14.6 (3)	5.9 (3)	11.6 (3)	28.1 (5)	60.5 (5)
<i>Pimephales promelas</i> (mortality) Geomean	11.8 (3)	17.9 (3)	12 (3)	24.8 (3)	28.1 (5)	60.5 (5)
<i>Pimephales promelas</i> (growth) Lowest value or geomean	8.7 (3)	14.6 (3)	12 (4)	27 (4)	26.6 (6)	63.8 (6)
<i>Pimephales promelas</i> (growth) Geomean	11.4 (3)	17.3 (3)	12 (4)	27 (4)	26.6 (6)	63.8 (6)
<i>Pimephales promelas</i> (reproduction) Lowest value or geomean	/	/	10.8 (2)	30.6 (2)	25.8 (9)	57 (9)
<i>Pimephales promelas</i> (reproduction) Geomean	/	/	13.14 (2)	32.7 (2)	25.8 (9)	57 (9)
<i>Salvelinus fontinalis</i> (Mortality) Lowest value or geomean	/	/	16.9 (4)	28.4 (4)	22.3 (1)	42.3 (1)
<i>Salvelinus fontinalis</i> (Growth) Lowest value or geomean	/	/	17.1 (4)	28.2 (4)	22.3 (1)	42.3 (1)
<i>Salvelinus fontinalis</i> (Reproduction) Lowest value or geomean	/	/	6.4 (3)	10.7 (3)	/	/
<i>Daphnia magna</i> (mortality)	/	/	/	/	36.8 (1)	36.8 (1)

Lowest value or geomean						
<i>Daphnia magna</i> (reproduction)	13.2 (7)	10.5 (7)	181 (2)	16.5 (2)	55 (15)	24.8 (15)
Lowest value or geomean						
<i>Daphnia magna</i> (reproduction)	13.2 (7)	10.5 (7)	233 (2)	23.8 (2)	55 (15)	24.8 (15)
Geomean						
<i>Daphnia magna</i> (growth)	/	/	/	/	12.6 (1)	12.6 (1)
Lowest value or geomean						
<i>Pseudokirchneriella</i> <i>a subcapitata</i> (growth rate)	131.6 (9)	34.9 (9)	56.1 (15)	13.3 (15)	65.1 (10)	14.1 (10)
Lowest value or geomean						
<i>Chlamydomonas</i> <i>reinhardtii</i>	22 (2)	61.7 (2)	108 (1)	27.4 (1)	96 (1)	24.7 (1)
Lowest value or geomean						
<i>Chlamydomonas</i> <i>reinhardtii</i>	62.6 (2)	63.2 (2)	108 (1)	27.4 (1)	96 (1)	24.7 (1)
Geomean						
<i>Chlorella vulgaris</i>	279.6 (5)	124.4 (5)	106.3 (7)	30.3 (7)	86.5 (4)	24 (4)
Lowest value or geomean						
<i>Lemna minor</i>	30 (1)	75.8 (1)	/	/	/	/
Lowest value or geomean						

In grey: values used for classification proposal.

Regarding the all data set presented in the sections above, the table below summarised the acute and chronic ERV retained for classification. These ERV correspond to the lowest EC50 and EC10/NOEC or geomean EC50 and EC10/NOEC for each pH-category.

Table 92: Acute and chronic ERV considering the geomean only when at least 4 data are available. If less than 4 data are available, the lowest data is retained.

Ecotoxicity Reference Values - µg dissolved Cu/L				
		pH: 5.51-6.5	pH: >6.5-7.5	pH:>7.5-8.5
Non normalised values				
Acute ERV	LC50	12.1 (<i>P. promelas</i> - 5)	11.7 (<i>D. rerio</i> -2)	40 (<i>C. dubia</i> - 54)
Chronic ERV	EC10/NOEC	8.7 (<i>P. promelas</i> -3)	5.9 (<i>P. promelas</i> -3)	12.6 (<i>D. magna</i> -1)
Values considering DOC normalisation to 2 mg/L				
Acute ERV	LC50	11 (<i>D. magna</i> -26)	24.1 (<i>C. dubia</i> -10)	31.4 (<i>C. reinhardtii</i> -1)
Chronic ERV	EC10/NOEC	10.5 (<i>D. magna</i> -7)	5.6 (<i>O. mykiss</i> -3)	12.6 (<i>D. magna</i> -11)

It could be noted that the normalisation of the EC50 and EC10/NOEC results in a similar ERV values than results without DOC normalisation. Therefore, only acute and chronic ERV without DOC normalisation are used for comparison with criteria for environmental classification.

Table 93: Acute and chronic ERV considering the geomean even when less 4 data are available

Ecotoxicity Reference Values - µg dissolved Cu/L				
		pH: 5.51-6.5	pH: >6.5-7.5	pH:>7.5-8.5
Non normalised values				
Acute ERV	LC50	12.1 (<i>P. promelas</i> - 5)	14 (<i>C. dubia</i> -17)	40 (<i>C. dubia</i> - 54)
Chronic ERV	EC10/NOEC	11.4 (<i>P. promelas</i> -3)	6.4* (<i>S. fontinalis</i> -3)	12.6 (<i>D. magna</i> -1)
Values considering DOC normalisation to 2 mg/L				
Acute ERV	LC50	11 (<i>D. magna</i> -26)	24.1 (<i>C. dubia</i> -10)	31.4 (<i>C. reinhardtii</i> -1)
Chronic ERV	EC10/NOEC	10.5 (<i>D. magna</i> -7)	10.7* (<i>S. fontinalis</i> -3)	12.6 (<i>D. magna</i> -11)

* No geomean could be apply as only 3 data are available considering all pH-categories

5.4.5 OTHER AQUATIC ORGANISMS (INCLUDING SEDIMENT)