Sulfate

EQS data overview

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ACES report number 14
Department of Environmental Science and Analytical Chemistry, Stockholm University
2018

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Preface

The Department of Environmental Science and Analytical Chemistry (ACES) was commissioned, by the Swedish Agency for Marine and Water Management and the Swedish Environmental Protection Agency, to perform a literature overview and possible EQS derivation for the specific pollutant sulfate. The work was performed under the Water Framework Directive (2000/60/EC) using the European Communities's guidance document "Technical Guidance for Deriving Environmental Quality Standards".

The report was prepared by Sara Sahlin and Marlene Ågerstrand.

Stockholm, April 23rd, 2018
The Department of Environmental Science and Analytical Chemistry (ACES)
Stockholm University

Förtydligande från Havs- och vattenmyndigheten

Havs- och vattenmyndigheten planerar att ta med sulfat bland de ämnen som regleras i Havs- och vattenmyndighetens föreskrifter (HVMFS 2013:19) om klassificering och miljökvalitetsnormer avseende ytvatten[1]. Stockholms Universitet har därför på uppdrag av Havs- och vattenmyndigheten och Naturvårdsverket tagit fram beslutsunderlag för att kunna etablera bedömningsgrunder för sulfat. Utifrån litteratursökning och granskning av underlag har förslag på värden beräknats utifrån de riktlinjer som ges i CIS 27 (European Communities, 2011). I denna rapport har flera alternativa värden tagits fram utifrån olika beräkningssätt. Slutgiltigt val av värden att utgå ifrån vid statusklassificering har föreslagits av Havs- och vattenmyndigheten efter dialog med deltagare i en arbetsgrupp (representanter från Kemikalieinspektionen, Naturvårdsverket och Läkemedelsverket). Alternativ som baseras på probabilistiska beräkningar har förordats över värden baserade på deterministiska beräkningar, vilket är i linje med CIS 27. Granskning av vissa studiers tillförlitlighet och relevans har även diskuterats med deltagare i arbetsgruppen samt inkopplad forskningsexpertis.

I enlighet med detta föreslås för limnisk miljö **34 mg/L som årsmedelvärde och 73 mg/L som maximal tillåten koncentration.** Värdena är framtagna utifrån en probabilistisk beräkning och en ”added risk” approach, vilket innebär att de har tagits fram för att man i samband med utvärderingen ska beakta naturlig bakgrundshalt om den annars hindrar efterlevnaden av värdet. Probabilistiskt beräknade värden för toxicitet vid olika vattenhårdhet har inte kunnat beräknas då det saknas data för dagsländor, den känsligaste organismen, vid lägre hårdhet. Det föreslagna värdet avser därför alla vatten oavsett hårdhet och är baseras på tester utförda vid lägre hårdhet än 100 mg $CaCO_3$/L, vilket speglar de hårdhetsförhållanden som normalt råder i Sverige. Maximal tillåten koncentration är baserat på studier som gjorts vid ungefär 100 mg $CaCO_3$/L, vilket är högre hårdhet än vad som normalt råder i Sverige. Detta motiveras genom att kortvariga toppar av sulfat ofta sammanfaller med koncentrationstoppar i hårdhet. Något värde för marin miljö föreslås inte.

Notera att bedömningsgrunder för sulfat ännu inte har beslutats.

[1] https://www.havochvatten.se/hav/vagledning--lagar/foreskrifter/register-vattenforvaltning/klassificering-och-miljokvalitetsnormer-avseende-ytvatten-hvmfs-201319.html

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1. METHOD CONSIDERATIONS

Legal frameworks

The work was performed under the Water Framework Directive (2000/60/EC) using the European Communities's (2011) guidance document "Technical Guidance for Deriving Environmental Quality Standards".

Environmental Quality Standards (EQS) for pelagic communities were derived to cover long-term (Annual Average: AA-EQS) and short-term (Maximum Acceptable Concentration: MAC-EQS) exposure. Risks for benthic communities or secondary poisoning for pelagic biota or top predators were not addressed in the EQS derivation (not identified as potential receptors at risk).

EQS derivation

The EQS derivation was based on ecotoxicity data conducted with sodium sulfate (Na_2SO_4 CAS 7757-82-6). Other salts of sulfate were not included due to that the cation (e.g. K^+, Mg^+) may contribute to the toxicological response (Mount et al 1997). Ca_2SO_4 was not considered toxic, but is not as soluble as Na_2SO_4 (Mount et al. 1997) and are not frequently used in ecotoxicological tests.

The following databases were used when searching for data: Scopus, Web of science, Google Scholar, ETOX, Ekotoxzentrum, UBA, INERIS, RIVM, IRIS, UK TAG, OECD, USEPA. The following keywords were used: sulfate, sulphate, sodium sulfate, sodium sulphate* toxicity, ecotoxicity, aquatic toxicity, ecotoxicology, NOEC, EC10, EC50, LC50. The literature search was conducted in February 2017.

Due to time restrictions, reliability and relevance evaluation was only performed on a selected number of the ecotoxicity studies using the CRED evaluation method. The result from the evaluations can be found in table S4. The studies were scored as; R1 (Reliable without restrictions), R2 (Reliable with restriction), R3 (Not Reliable), R4 (Not assignable), C1 (Relevant without restriction), C2 (Relevant with restrictions), C3 (Not Relevant), C4 (Not assignable) (Moermond et al. 2016).

According to European Communities (2011), chronic values reported as LOEC and EC_{50} and acute values reported as NOEC should not be included in the derivation of EQS. EC_{20} values were divided by 2 and tabulated as NOEC. MATC values were divided by $\sqrt{2}$ and tabulated as NOEC. One value per species (and endpoint) was used in the derivation. In case of multiple values for the same species and the same endpoint (at approximately same water hardness and ionic composition of the test media), the values were aggregated (geometric mean). According European Communities (2011), toxicity values higher or lower than the range of test concentrations (e.g. $LC_{50} > x$ or $LC_{50} < x$) should not be used in the derivation. However, one EQS proposal was based on an effect data with lower toxicity value than the tested concentration (NOEC<x), since it suggests higher toxicity at softer water compared to other available data.

Several of the studies found in the literature investigate mortality as endpoint during long-term exposures (see supportive information, table S3). When comparing chronic studies to the mortality studies with long-term exposure, LC_{10} values for embryos of *P. promelas* (Wang et al. 2016a) suggest higher toxicity (382.05 mg/L) compared to data reported for larvae of *P. promelas* and endpoint growth, EC_{10} 760 (Elphick et al. 2011) and NOEC 1397 mg/L (PESC, 2013), all studies conducted in hardness 80-100 mg $CaCO_3$/L with 7 days exposure. Wang et al. (2016a) reported that reduced survival during hatching period was the primarily effect of sulfate, and no growth effect was found in their 7-14 days study. Though, in their 34 days study they received EC_{20} values for the endpoint biomass of

185 and 106 mg/L but the authors stated that there were uncertainties in the growth response (therefore not included in the EQS derivation). The LC_{10} values for *P. promelas* (Wang et al. 2016a) were therefore included in the derivation. The LC_{10} values reported by Kennedy et al. (2012) for eyed eggs *of O. mykiss* was included since the data suggest evidence that early life stage were sensitive to sulfate exposure. Kennedy et al. (2012) also investigated growth of the fry and the effect was minimal and statistical effect values could not be calculated. Though, it is not well-defined in European Communities (2011) if LC_{10} values may be used in the derivation.

When sufficient data was available both deterministic derivation (applying an assessment factor (AF) to the lowest effect value) and probabilistic derivation (performing a species sensitivity distribution (SSD)) were used to enable comparison between the methods. The software ETX 2.1 (provided by the Netherlands National Institute for Public Health and the Environment (RIVM)) was used for modelling the SSD. Normal distribution and goodness-fit of the model were calculated with three different tests: Anderson-Darling, Kolmogorov-Smirnov, and Cramer von Mises.

Only one marine ecotoxicological study was found in the literature search (the algae *Nitzschia linearis* with a LC_{50} of 1284 mg/L). When deriving EQS values for marine ecosystems in the absence of marine data, larger AF are necessary to take into account additional uncertainties associated with the extrapolation (European Communities, 2011). The relative toxicity to the marine crustacean *Americamysis bahia* was F^- > K^+ > $HCO3^-$ > Ca^{2+} > Mg^{2+} > Br^- > SO_4^2 (Unpublished data, SETAC 2004). Elevated ion concentrations may cause toxicity to freshwater organisms due to osmotic stress. Freshwater organisms are hyper-osmotic regulators and as the salinity increase they tend to take up more ions, consequently, they lose water from cells causing adverse effects. However, marine species are generally hypo-osmotic regulators and have physiological mechanisms to maintain a proper balance of water and dissolved ions (SETAC 2004; Hart et al. 1991), for this derivation it was therefore assumed that marine species have higher (or equal) tolerance to sulfate.

Hardness dependent EQS

Initially, all data were categorized based on the water hardness (mg $CaCO_3$/L): Very soft (<36), Soft (36-89), Moderate hard (89-178), Hard (178-374), and Very hard (>374). However, no distinct hardness-related relationship could be established. This could be due to that the available studies include different durations, statistical criterion, endpoints, and water chemistry (ionic composition). Since several studies only use on level of hardness (typical 100 mg $CaCO_3$/L), sensitive species were not present in the lower categories of hardness, which resulted in stringent effect values at higher hardness. When only data that investigated hardness as a modifying factor were used, it was possible to distinguish a hardness related response. Three different scenarios for deriving EQS were proposed:

(1) Derive hardness dependent EQS based on studies that investigated hardness as a modifying factor (deterministic derivation).
(2) Derive EQS based on data of water hardness of approximately 100 mg $CaCO_3$/L (deterministic and probabilistic derivation).
(3) Derive EQS based on realistic worst-case data, with data of hardness representing Swedish water (≤ 50 mg $CaCO_3$/L) (only AA-EQS).

The British Columbia Ministry of Environment has established water quality guidelines for sulfate at different categories of water hardness (BC, 2013). The water quality guidelines were based on LC_{20} values in a deterministic derivation using AF 2. However, the use of LC_{20} values and such low AF in a deterministic derivation is not in line with European Communities (2011).

2. PROPOSED ENVIRONMENTAL QUALITY STANDARDS FOR SULFATE

Proposals of MAC-EQS for sulfate (MAC-EQS_{added})					
Hardness (mg $CaCO_3$/L) / Method	**Scenario 1**				**Scenario 2**
	≥25	***40-50***	**75-100**	**≥160**	**≈100**
Deterministic (mg/L)	59.6 (57.6)	95.7 (88.3)	158.0 (154.0)	317.8 (270.8)	65.3 (63.3)
Probabilistic (mg/L)	-				73.9 (72.5)

Proposals of AA-EQS for sulfate (AA-EQS_{added})						
Hardness (mg $CaCO_3$/L) / Method	**Scenario 1**				**Scenario 2**	**Scenario 3**
	6-15	**40-50**	**80-100**	**>160**	**≈100**	**Realistic worst-case**
Deterministic (mg/L)	-	15.0	41.9	56.0	12.9 (7.2)	12.9 (7.2)
Probabilistic (mg/L)	-				35.0 (25.6)	43.7 (34.1)

3. MEASURED ENVIRONMENTAL CONCENTRATIONS IN SWEDEN

Freshwater monitoring data divided into number of monitoring stations with different sulfate concentrations are presented in table 1. Table 2 and 3 presents sulfate measurements at different water hardness. Table 2 provides measurements for recipient controls (SRK) (areas affected by human activity), and table 3 from national and regional monitoring from 2012-2016. The data were collected from the Swedish University of Agricultural Sciences (SLU) database of environmental monitoring data. It was not possible to determine precise background concentrations. However, the majority (66%) of the measurements were below 5 mg/L.

Table 1. Monitoring data for 2010-1016 from rivers and lakes in Sweden (SLU database of monitoring data).

Sulfate concentration (mg/L)	Number of stations
<5	24 352
5–10	6 879
10–25	3 527
25–50	1 386
50–100	514
100–200	94
>200	81

Table 2. Measured sulfate concentrations from Swedish recipient controls (SRK) at different hardness (SLU database of monitoring data).

Hardness (mg $CaCO_3$/L) / SO_4^{2-} (mg/L)	<15	15-25	25-50	50-100	100-150	150-200	200-250	250-300	>300
Mean	4.8	8.7	12.6	27.4	52.2	87.4	193.5	285.1	389.9
Max	110	154	52.9	100	153.7	211.4	400	459.3	524.5
Min	0.3	2.6	3.6	6.4	14	19	29	190.3	279.7
Nr of samples	3024	578	493	361	146	85	32	27	24

Table 3. Measured sulfate concentrations from Swedish national and regional monitoring at different hardness (SLU database of monitoring data).

Hardness (mg $CaCO_3$/L) / SO_4^{2-} (mg/L)	<15	15-25	25-50	50-100	100-150	150-200	200-250	250-300	>300
Mean	3.0	7.3	12.0	19.4	32.0	43.6	45.8	42.8	77.8
Max	38.3	91.2	139.2	164.0	315.2	427.9	146.4	94.1	424.6
Min	0.2	0.2	0.2	1.4	1.8	2.7	6.4	9.4	27.5
Nr of samples	24861	4194	2360	986	676	459	206	91	32

4. AQUATIC ECOTOXICITY OF SULFATE

Mechanisms of major-ion toxicity to aquatic organisms are due to osmoregulatory stress, specific ion toxicity (concentration exceeding toxic levels), or imbalance of the ionic composition (SETAC 2004; Goetsch and Palmer 1997). Mount et al. (1997) investigated the toxicity of varying combinations of major ions to *P. promelas*, *D. magna* and *C. dubia*. In general, the ionic toxicity was $K^+ > HCO3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$. The toxicity of SO_4^{2-} was reduced in waters containing more than one cation when *C. dubia* and *D. magna* were exposed. No described mode of action for sulfate has been found.

Modifying factors

Several studies have demonstrated that increased water hardness decreases the toxicity of sulfate in both acute and chronic exposures. The exceptions of this general trend were chronic exposures to *B. calyciflorus* (rotifer), *P. regilla* (amphibian), and *H. azteca* (crustacea). When very hard water was used (e.g. 320 mg $CaCO_3$/L) the sensitivity in some cases increases, this may be due to the overall ionic strength in the test dilutions, which may result in osmotic stress to the organisms (Elphick et al., 2011).

Chloride has also been identified as a modifying factor. Soucek (2007b) demonstrated that increasing chloride concentrations from 5 to 25 mg/L increased the tolerance of sulfate to *H. azteca*. However, the toxicity to *C. dubia* was not significantly correlated within that range of chloride. Chloride concentrations ranging between 25-500 mg/L resulted in an opposite trend for both species and increased the mortality. The results from the study suggest evidence that chloride and sulfate toxicity were additive at higher concentrations of chloride. Soucek (2007b) also concluded that the conductivity was highly positive correlated with survival of *H. azteca* and *C. dubia* during sulfate exposure. Likewise, Soucek and Kennedy (2005) observed that the toxicity to *H. azteca* decreased with increasing chloride concentrations from 1.6 to 60 mg/L. Other findings suggest that increased molar ratio of calcium and magnesium may influence the toxicity (Davies and Hall, 2007; Davies, 2002). In exposures of similar hardness but with higher chloride concentrations and higher calcium-magnesium ratio, the toxicity to *H. azteca* and *C. dubia* decreased (Soucek ad Kennedy, 2005). The LC_{50} varied from 2050 to 2526 mg/L for *C. dubia,* and from 512 to 2855 mg/L for *H. Azteca,* in diluents with Ca:Mg of 0.88 and chloride levels of 1.9 mg/L compared to Ca:Mg of 3.25 and chloride levels of 3.25 mg/L respectively (Soucek and Kennedy, 2005; Soucek 2007a). The same trend was observed in exposures to *D. magna* (Davies, 2002; Davies and Hall, 2007). Davies (2002) stated that most natural waters have Ca:Mg ratios above 0.7 and that toxicity data based on water with low Ca:Mg ratios should be considered conservative. However, Wang et al. (2016) concluded that the toxicity to embryos of *P. promelas* did not decrease with increased Ca:Mg ratio or increased chloride concentrations (10 to 25 mg/L). Instead they suggested that the decreased toxicity could be explained by increased potassium concentrations (from 1 to 3 mg/L).

Data used in the derivation for crustacean was conducted in molar ratio of Ca:Mg of 1.7-3.2 and chloride concentrations below 10 mg/L. Regarding acute *P. promelas* (embryo) study by Wang et al. (2016a), data conducted in potassium concentrations of 1 mg/L was used in the derivation since concentrations of 3 mg/L was assessed as not realistic in relation to Swedish waters.

5. ACUTE FRESHWATER TOXICITY

In total, 17 acute ecotoxicity studies with 101 effect values were found (table S1). The study showing lowest effect value was Goetsch and Palmer (1997) with the insect *Tricorythus sp*. and a LC_{50} of 446 mg/L conducted in water hardness 69.4 mg $CaCO_3$/L (table S1). However, the study was not included in the derivation since the study did not investigated hardness as a modifying factor, or was not conducted in hardness of approximately 100 mg $CaCO_3$/L. Additionally, there are some uncertainties in the study; Goetsch and Palmer (1997) used field-collected organisms, river water as experimental medium instead of synthetic medium, and the effect value was not statistically confirmed (only observed experimentally). In addition, other studies demonstrate that *N.triangulifer* (different species but same order) requires food during acute ecotoxicity tests (Struewing et al. 2015; Weaver et al. 2015; Soucek and Dickinson 2015). Soucek and Dickinson (2015) conducted a fed acute ecotoxicity test using *N. triangulifer* and received an effect value of 1227 mg/L, the differences in the results may be due to different sensitivity to sulfate, that *Tricorythys sp.* was not fed or due to different water hardness used in the tests.

Scenario 1: Hardness dependent MAC-EQS, based on studies investigating hardness as a modifying factor

Using data from studies investigating hardness as a modifying factor it was possible to distinguish a hardness-related response. The dataset provides effect data for three different categories of hardness representing two or three trophic levels (table 4). Note that scenario 1 does not include the most sensitive species *P. promelas* (embryo) with LC_{50} of 653 mg/L since this was not a study investigating hardness as a modifying factor (Wang et al. 2016, see table 5).

Deterministic derivation

The data showing lowest effect value for hardness 10-25 was *H. azteca* with a LC_{50} of 596 mg/L, for hardness 40-50 *P. promelas* with a LC_{50} of 957 mg/L, for hardness 75-100 *H. azteca* with an LC_{50} of 1580mg/L, for hardness ≥160 *P. promelas* with a LC_{50} of 3178 mg/L. AF 10 was applied since the dataset includes data for species of three trophic levels (except for hardness 10-25) and the standard deviation of the ecotoxicity data was not higher than 3 in both directions (European Communities, 2011). The MAC-EQS was set to 59.6, 95.7, 158.0 and 317.8 mg/L for hardness 10-25, 40-50, 75-100 and ≥160 respectively (Table 7).

Table 4. Acute ecotoxicity data from studies investigating hardness as a modifying factor.

Species	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration	Effect value SO_4^{2-}(mg/L)	Reference
Hardness 10-25[1]				
D. magna	25	48h LC_{50}	1194	Davies and Hall 2007
	25	48h LC_{50}	1563	Davies and Hall 2007
	25	48h LC_{50}	957	Davies 2002
	25	48h LC_{50}	1571	Davies 2002
H. azteca	25	96h LC_{50}	596	Davies and Hall 2007
P. subcapitat	10	72h EC_{50}	1430	Elphick et al. 2011
Hardness 40–50[2]				
P. promelas (larvae)	40	7d LC_{50}	1649	Elphick et al. 2011
	50	7d LC_{50}	957	PESC et al. 2013
H. azteca	50	96h LC_{50}	1448	Davies and Hall 2007
D. magna	50	48h LC_{50}	1551	Davies and Hall 2007
	50	48h LC_{50}	1768	Davies 2002
Hardness 75–100				
P. promelas (larvae)	80	7d LC_{50}	2938	Elphick et al. 2011
D. magna	75	48h LC_{50}	3342	Davies and Hall 2007
	75	48h LC_{50}	3155	Davies 2002
	100	48h LC_{50}	3203	Davies and Hall 2007
	100	48h LC_{50}	3808	Davies and Hall 2007
	100	48h LC_{50}	3839	Davies 2002
H. azteca	75	96h LC_{50}	1580	Davies and Hall 2007
	100	96h LC_{50}	2240	Davies and Hall 2007
	100	96h LC_{50}	2971	Davies 2002
P. subcapitat	80	72h EC_{50}	2742	Elphick et al. 2011
Hardness ≥160[2]				
P. promelas	160	7d LC_{50}	4553	Elphick et al. 2011
	250	7d LC_{50}	3178	PESC 2013
H. azteca	250	96h LC_{50}	5259	Davies and Hall 2007

1 = EQS was derived for hardness 10-25 mg $CaCO_3$/L although it lacked data for fish (i.e. not in accordance with European Communities, 2011) to ensure protection of *H. azteca.* 2 = base set assumed to represent three trophic levels even though the lack of algae (algae EC_{50} for hardness of 10-25 mg $CaCO_3$/L were not among the most sensitive taxonomic group).

Scenario 2: MAC-EQS based on studies with hardness ≈ 100 mg $CaCO_3$/L

The data from studies conducted in approximately 100 (80-110) mg $CaCO_3$ /L, is presented in table 5. The datasets includes one order of fish and algae, two orders of crustacean and insects, and four orders of mollusca (a total of 15 species).

Table 5. Acute ecotoxicity studies for sulfate at water hardness ≈ 100 mg $CaCO_3$/L used in the MAC-EQS derivation.

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration	Effect value SO_4^{2-}(mg/L)	Reference
Fish				
Pimephales promelas (embryos)	102-110	7d LC_{50}	653[1]	Wang et al. 2016a
Invertebrates				
Ceriodaphnia dubia (neonates)	100	48h EC_{50}	2441	Wang et al. 2016a
Daphnia magna (<24h)	100	48h LC_{50}	3823	Geometric mean
Hyalella azteca (2-11d old)	80-100	96h $L(E)C_{50}$	2415	Geometric mean
Insecta				
Chironomus dilutus (larvae)	100	96h EC_{50}	5992	Wang et al. 2016a
Chironomus tentans (10d old)	94	48h LC_{50}	14134	Soucek and Kennedy 2005
Neocleon triangulifer (nymph)	99	96h LC_{50}	1227	Soucek and Dickinson 2015
Mollusca				
Lampsilis abrupta (juveniles)	100	96h EC_{50}	2362	Wang et al. 2016a
Lampsilis siliquoidea (juvenile)	106	96h EC_{50}	2325	Wang et al 2016b
Ligumia recta	92	96h LC_{50}	1483	US EPA 2010
Margaritifera falcata (juvenile)	106	96h EC_{50}	1378	Wang et al 2016b
Megalonaias nervosa (juvenile)	103	96h EC_{50}	2279	Wang et al 2016b
Sphaerium simile (juvenile)	94	96h LC_{50}	2078	Soucek and Kennedy 2005
Utterbackia imbecillis (juvenile)	103	96h EC_{50}	2709	Wang et al 2016b
Algae				
Pseudokirchneriella subcapitata	80	72h EC_{50}	2742	Elphick et al. 2011

1 = Potassium concentrations of approximately 1 mg/L, this study has been evaluated to be of sufficient reliability and relevance for EQS derivation (see table S4).

Deterministic derivation

The study showing lowest effect values was Wang et al. (2016a) with embryos of *P. promelas* and a LC_{50} of 653 mg/L. AF 10 was applied since the dataset includes three trophic levels and the standard deviation of the ecotoxicity data was not higher than 3 in both directions (European Commiunities, 2011). The MAC-EQS was set to 65.3 mg/L (table 7).

Probabilistic derivation

The dataset does not fulfil the criteria to perform a SSD due to the absence of taxonomic groups for higher aquatic plants and a second family in the phylum Chordata (European Communities, 2011) (table 5). However, there are supportive information for higher aquatic plants (chronic), fish (acute), and amphibians (chronic) suggesting low toxicity (table S1 and S2). Despite the lack of data, a SSD was performed as a comparison to the deterministic derivation (figure 1). Normal distribution was accepted at significance level 0.05 in the Anderson-Darling and Cramer von Mises tests, and at level 0.025 in the Kolmogorov-Smirnov test. The HC5 was 739.18 mg/L (table 6). AF 10 was applied (European Communities 2011), resulting in a MAC-EQS of 73.9 mg/L (table 7).

Table 6. The results of HC5 from the SSD of acute sulfate ecotoxicity data conducted in hardness ≈ 100 mg $CaCO_3$/L.

Type of HC5	Value (mg/L)	log10(Value)(mg/L)	Description
LL HC5	396.26	2.60	Lower estimate of the HC5
HC5	739.18	2.87	Median estimate of the HC5
UL HC5	1101.89	3.04	Upper estimate of the HC5
sprHC5	2.83	0.44	Spread of the HC5 estimate

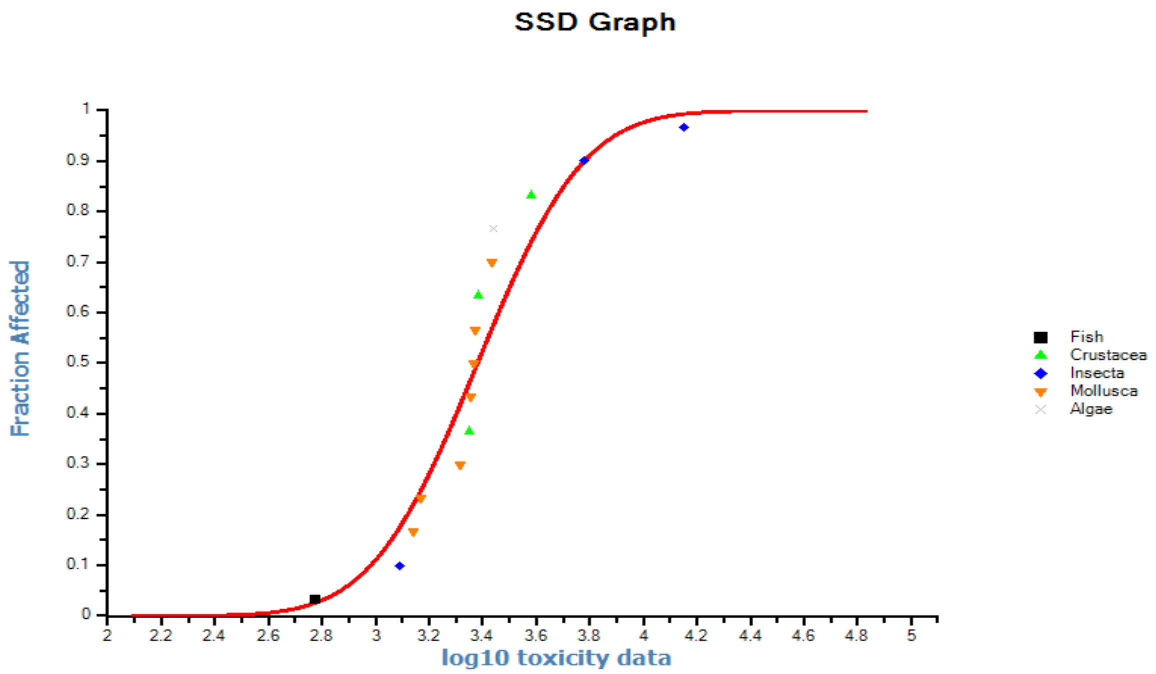

Figure 1. SSD (ETX 2.1) for acute freshwater ecotoxicity studies of sulfate conducted in hardness ≈ 100 mg $CaCO_3$/L. The most sensitive species was the fish *P. promelas* (embryos). The HC5 was 739.18 mg/L.

Table 7. Proposals of MAC-EQS for sulfate based on different methods and/or hardness.

Hardness (mg $CaCO_3$/L) / Method	Scenario 1				Scenario 2
	≥25	*40-50*	75-100	≥160	Hardness ≈100
Deterministic (mg/L)	59.6	95.7	158.0	317.8	65.3
Probabilistic (mg/L)	-				73.9[1]

1 = Based on incomplete dataset (lacked data for higher aquatic plants and a second family in the phylum Chordata).

6. CHRONIC FRESHWATER TOXICITY

The total dataset includes two orders of fish, crustacean, insects, higher aquatic plants and one order of amphibians, mollusca, rotifer and algae. In total, 9 chronic ecotoxicity studies with 95 effect values were found (table S2 and S3).

Scenario 1: Hardness dependent AA-EQS, based on studies investigating hardness as a modifying factor

Table 8 summarizes the chronic studies investigating hardness as modifying factor. Water hardness higher than 250 mg $CaCO_3$/L was omitted since it have shown higher toxicity which may be a result of the overall ionic strength (Elphick et al. 2011). Note that scenario 1 does not include the most sensitive species *N. triangulifer* (mayfly) with a NOEC of 129 mg/L since this study did not investigate hardness as a modifying factor (Soucek and Dickinson 2015, see table 9). The lack of mayflies studies has previously been stressed when setting water quality guidelines due to preliminary work indicating sensitivity to sulfate (BC, 2013). In addition, Vellemu et al. (2017) provide supporting information of mayflies (*Adenophlebia auriculata*) being sensitive with 10 day LC_{10} of 129 mg/L (not considered in the derivation due to short duration).

Deterministic derivation

The data showing lowest effect value for hardness 6-15 was *O. mykiss* with a LC_{10} of 175.4 mg/L, for hardness 40-50 *C. dubia* with a NOEC of <150 mg/L, for hardness 80-100 *O. mykiss* with a LC_{10} of 419.2 mg/L and for hardness >160 *B. calyciflorus* with a NOEC of 560.0 mg/L. The hardness category 6-15 lacked ecotoxicity data for the trophic level crustacean (which represented the most sensitive species for hardness 40-50), EQS was therefore not derived. The AA-EQS was set to 15.0, 41.9 and 56.0 mg/L at hardness 40-50, 80-100 and >160mg $CaCO_3$/L respectively (table 13). According to European Communities (2011), EQS values should not be based on effect data with higher toxicity than the tested concentration (e.g. *C. dubia,* Elphick et al. 2011). An alternative was therefore to base EQS for hardness <50 on *O. mykiss* with a LC_{10} of 175.4 resulting in an AA-EQS of 17.5 mg/L.

Table 8. Chronic studies investigating hardness as a modifying factor.

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-} (mg/L)	Reference
Hardness 6-15[1]					
Oncorhynchus mykiss (eyed eggs)	6	Survival	21d LC_{10}	175	Kennedy 2012
Pseudacris regilla (tadpoles)	15	Survival/ growth	21d NOEC	1075	Elphick et al. 2011
Pseudokirchneriella subcapitata	10	Growth	72h NOEC	1100	Elphick et al. 2011
Hardness 40-50					
Oncorhynchus mykiss (eyed eggs)	50	Survival	21d LC_{10}	300	Kennedy 2012
Ceriodaphnia dubia (neonates)	40	Reproduction	7d NOEC	<150[2]	Elphick et al. 2011
Brachionus calyciflorus (<4h old)	40	Reproduction	48h NOEC	950	Elphick et al. 2011
Lemna minor	50	Frond increase	7d EC_{10}	2143	PESC 2013
Hardness 80-100					
Oncorhynchus mykiss (eyed eggs)	100	Survival	21d LC_{10}	419	Kennedy 2012
Pseudacris regilla (tadpoles)	80	Survival/ growth	21d NOEC	978	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	80	Reproduction	7d NOEC	645	Elphick et al. 2011
Brachionus calyciflorus (<4h old)	80	Reproduction	48h NOEC	510	Elphick et al. 2011
Pseudokirchneriella subcapitata	80	Growth	72h NOEC	1200	Elphick et al. 2011
Lemna minor	100	Frond increase	7d EC_{10}	2243	PESC 2013
Hardness ≥160					
Oncorhynchus mykiss (eyed eggs)	250	Survival	21d LC_{10}	674	Kennedy 2012
Ceriodaphnia dubia (neonates)	160	Reproduction	7d NOEC	775	Elphick et al. 2011
Brachionus calyciflorus (<4h old)	160	Reproduction	48h NOEC	560	Elphick et al. 2011
Lemna minor	250	Frond increase	7d EC_{10}	2314	PESC 2013

1 = Hardness of 6-15 lacked ecotoxicity data for invertebrates. 2 = Large confidential interval.

Scenario 2: AA-EQS based on studies with hardness ≈ 100 mg $CaCO_3$/L

The data from studies conducted in approximately 100 (80-105) mg $CaCO_3$ /L is presented in table 9 and includes two orders of fish, crustacean, insects and higher aquatic plants, and one order of amphibian, mollusca, rotifer and algae (a total of 12 species).

Table 9. Chronic ecotoxicity data of sulfate conducted in hardness ≈ 100 mg CaCO3/L used in the AA-EQS derivation.

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-} (mg/L)	Reference
Fish					
Oncorhynchus mykiss (eyed eggs)	100	Survival	31d LC_{10}	419	Kennedy et al. 2012
Pimephales promelas (embryos)	100	Survival	34d LC_{10}	430	Wang et al. 2016a[1]
Amphibians					
Pseudacris regilla (tadpoles)	80	Survival/ growth	21d NOEC	978	Elphick et al. 2011
Invertebrates - Crustacean					
Ceriodaphnia dubia (neonates)	80-100	Reproduction	7d NOEC	632	Geometric mean
Hyalella azteca	100	Growth	28d EC_{10}	682	PESC 2013
Invertebrates -Mollusca					
Lampsilis abrupta (juveniles)	100	Dry weight	28d EC_{10}	320	Wang et al. 2016a
Invertebrates- Insecta					
Chironomus dilutus (larvae)	100	Dry weight	7d EC_{10}	489	Wang et al. 2016a
Neocleon triangulifer (nymph)	99	Development delay	36d NOEC	129[2]	Soucek and Dickinson 2015[1]
Rotifers					
Brachionus calyciflorus (<4h old)	80	Reproduction	48h NOEC	510	Elphick et al. 2011
Algae					
Pseudokirchneriella subcapitata	80	Growth	72h NOEC	1200	Elphick et al. 2011
Higher aquatic plants					
Fontinalis antipyretica	105	Shoot length	21d NOEC	1000	Davies 2007
Lemna minor	100	Frond increase	7d EC_{10}	2243	PESC 2013

1 = The study has been evaluated to be of sufficient reliability and relevance for EQS derivation (see table S4). 2 = NOEC was not reported, the concentration below the statistically significant concentration was set as NOEC.

Deterministic derivation

The most sensitive species was the *N. triangulifer* with the endpoint “percent of pre-emergent nymph” (i.e. developmental effects) with a NOEC of 129 mg/L (Soucek and Dickinson 2015). AF 10 was applied since the dataset include chronic data for three trophic levels (European Communities, 2011). The AA-EQS was set to 12.9 mg/L.

Probabilistic derivation

The dataset fulfilled the criteria to perform a SSD (European Communities, 2011). Normal distribution was accepted at all significance levels in all tests. The SSD graph is presented in figure 2. The median estimate of the HC5 was 175.12 (table 10). AF 5 was used (European Communities 2011), resulting in an AA-EQS of 35.0 mg/L.

Table 10. The results of HC5 from the SSD of chronic sulfate ecotoxicity data conducted in hardness ≈ 100 mg $CaCO_3$/L.

Type of HC5	Value (mg/L)	log10(Value) (mg/L)	Description
LL HC5	81.94	1.91	lower estimate of the HC5
HC5	175.12	2.24	median estimate of the HC5
UL HC5	276.47	2.44	upper estimate of the HC5
sprHC5	3.37	0.53	spread of the HC5 estimate

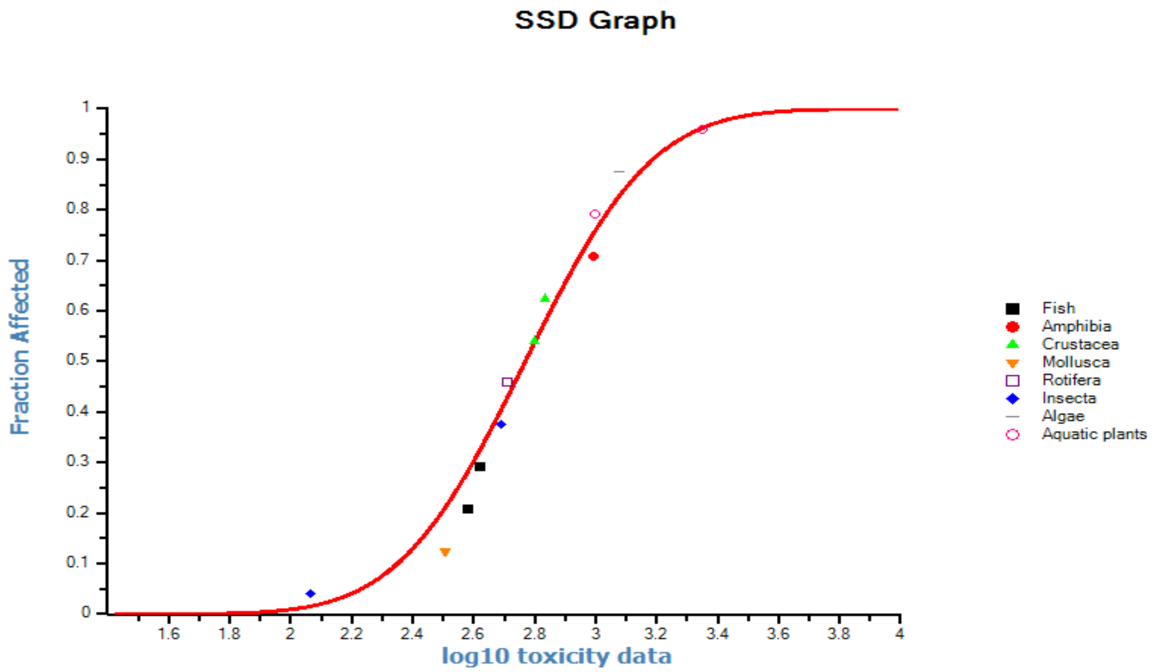

Figure 2. SSD (ETX 2.1) for chronic freshwater ecotoxicity studies of sulfate conducted in hardness ≈ 100 mg $CaCO_3$/L. The most sensitive species was the insect *N. triangulifer.* The HC5 was set to 175.12 mg/L.

Scenario 3: AA-EQS based on realistic worst-case data for Sweden

Studies conducted in hardness that represent Swedish water (≤50 mg $CaCO_3$/L) are presented in table 11. The *P. promelas* study by Wang et al. (2016a) (100 mg $CaCO_3$/L) was included since available studies with softer water were conducted with shorter duration (i.e. showed lower toxicity). Species of which there only were available studies conducted in hardness 100 mg $CaCO_3$/L were included to gain sufficient effect values for a SSD. The dataset includes two orders of fish, crustacean, insects and higher aquatic plants, and one order of amphibian, mollusca, rotifer and algae (a total of 12 species).

Table 11. Chronic ecotoxicity data with realistic worst-case data for Sweden used in the AA-EQS derivation.

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-} (mg/L)	Reference
Fish					
Oncorhynchus mykiss (eyed eggs)	6	Survival	31d LC_{10}	175	Kennedy et al. 2012
Pimephales promelas (embryos)	100	Survival	34d LC_{10}	430	Wang et al. 2016a[1]
Amphibians					
Pseudacris regilla (tadpoles)	15-80[2]	Survival/ growth	21d NOEC	1025	Elphick et al. 201l
Invertebrates - Crustacean					
Ceriodaphnia dubia (neonates)	40-44	Reproduction	7d NOEC	266	Geometric mean
Hyalella azteca	50-100[2]	Growth	28d EC_{10}	893	PESC 2013
Invertebrates -Mollusca					
Lampsilis abrupta (juveniles)	100	Dry weight	28d EC_{10}	320	Wang et al. 2016b
Invertebrates- Insecta					
Chironomus dilutus (larvae)	100	Dry weight	7d EC_{10}	489	Wang et al. 2016a
Neocleon triangulifer (nymph)	99	Development/ Survival	36d NOEC	129[3]	Soucek and Dickinson 2015[1]
Rotifers					
Brachionus calyciflorus (<4h old)	40-80[2]	Reproduction	48h NOEC	696	Elphick et al. 2011
Algae					
Pseudokirchneriella subcapitata	10	Growth	72h NOEC	1100	Elphick et al. 2011
Higher aquatic plants					
Fontinalis antipyretica	15	Growth	21d NOEC	628	Elphick et al. 2011 Geometric mean
Lemna minor	50	Growth	7d EC_{10}	2143	PESC 2013

1 = The study has been evaluated to be of sufficient reliability and relevance for EQS derivation (see table S4). 2 = The effect value for harder water was included in the geometric mean since it suggests higher toxicity. 3 = NOEC was not reported, the concentration below the statistically significant concentration was set as NOEC.

Deterministic derivation

The same AA-EQS as in scenario 2. The most sensitive species was the *N. triangulifer* with the endpoint "percent of pre-emergent nymph" (i.e. developmental effects) with a NOEC of 129 mg/L (Soucek and Dickinson 2015). AF 10 was applied since the dataset include chronic data for three trophic levels (European Communities, 2011). The AA-EQS was set to 12.9 mg/L.

Probabilistic derivation

The dataset fulfilled the criteria to perform a SSD (European Communities, 2011). Normal distribution was accepted at all significance levels in all tests. The median estimate of the HC5 was 130.94 mg/L (table 12). The graph of the SSD is presented in figure 3. AF 3 was used since the derivation was based on a large dataset that showed good taxonomic representativeness. In addition, the data were based on worst-case data. The AA-EQS was set to 43.7 mg/L. All AA-EQS are summarized in table 13.

Table 12. The results of HC5 from the SSD of chronic sulfate ecotoxicity based on realistic worst-case data for Sweden.

Type of HC5	Value (mg/L)	log10(Value) (mg/L)	Description
LL HC5	55.90	1.75	lower estimate of the HC5
HC5	130.94	2.12	median estimate of the HC5
UL HC5	218.44	2.34	upper estimate of the HC5
sprHC5	3.91	0.59	spread of the HC5 estimate

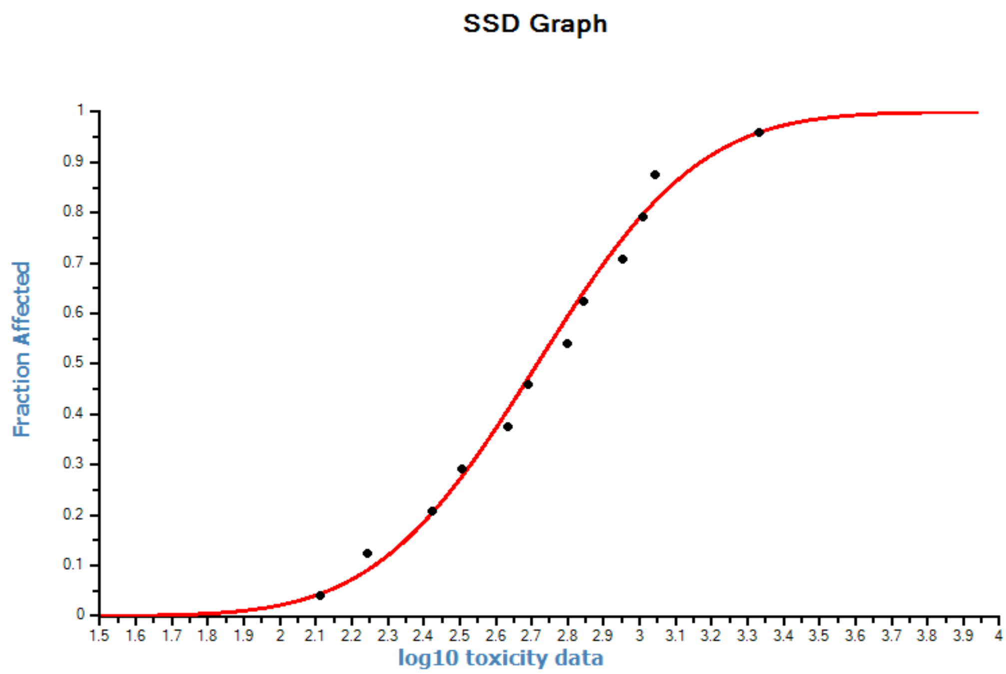

Figure 3. SSD (ETX 2.1) for chronic freshwater toxicity studies with realistic worst-case data for Sweden. The most sensitive species was the insect *N. triangulifer.* The HC5 was set to 130.94 mg/L.

Table 13. Proposed AA-EQS for sulfate based on different hardness scenarios and method.

Hardness ($mg\ CaCO_3/L$) / Method	Scenario 1				Scenario 2	Scenario 3
	6-15	**40-50**	**80-100**	**>160**	**Hardness≈100**	**Realistic worst- case**
Deterministic (mg/L)	-	15.0[1]	41.9	56.0	12.9	12.9
Probabilistic (mg/L)	-				35.0	43.7

1 = 17.5 mg/L if excluding NOEC of <150 mg/L for *C. dubia.*

7. ADDED RISK APPROACH

Added effect values (e.g. $NOEC_{added}$) was calculated by subtracting the sulfate concentration used in the control medium from the effect value (European Communities, 2011). Added risks (EQS_{added}) was calculated for all MAC-EQS scenarios (table 14), and for scenario 2 (hardness ≈ 100 mg $CaCO_3$/L) and 3 (realistic worst-case data) for the AA-EQS (table 15). Effect $values_{added}$ for MAC-EQS_{added} can be found in supportive information table S5 (scenario 1), S6 (scenario 2) and for AA-EQS_{added} in table S8 (scenario 2) and S10 (scenario 3). The MAC-EQS_{added} did not differ considerably from MAC-EQS (except for hardness ≥160 mg $CaCO_3$/L). The AA-EQS_{added} were approximately 6-10 mg/L lower compared to AA-EQS.

Table 14. MAC-EQS_{added} for sulfate based on different hardness scenarios and method.

Hardness (mg $CaCO_3$/L) / Method	Scenario 1				Scenario 2
	≥25	***40-50***	**75-100**	**≥160**	**Hardness ≈100**
Deterministic (mg/L)	57.6	88.3	154.0	270.8	63.3
Probabilistic (mg/L)	-				72.5[1]

1 = HC5 results in table S7, SSD graph in figure S1.

Table 15. AA-EQS_{added} for sulfate (mg/L) based on different hardness scenarios and method.

Hardness (mg $CaCO_3$/L) / Method	Scenario 2	Scenario 3
	Hardness≈100	**Realistic worst- case**
Deterministic (mg/L)	7.2	7.2
Probabilistic (mg/L)	25.6[1]	34.1[2]

1 = HC5 results in table S9, SSD graph in figure S2. 2 = HC5 results in table S11, SSD graph in figure S3.

8. IDENTIFICATION OF ISSUES RELATING TO UNCERTAINTY IN RELATION TO THE EQSs DERIVED

Different molar ratio of calcium, magnesium, chloride, and possibly potassium concentrations may influence the toxicity of sulfate. The complexity of imbalance toxicity of major ion entails uncertainties given the large numbers and combinations of ions.

The most critical studies setting the base for the deterministic derivations (scenario 2 and 3) have been evaluated for their reliability and relevance (supportive information table S4). Due to time restrictions, evaluations were not conducted for entire datasets used in scenario 1 or in the probabilistic derivations.

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10. SUPPORTIVE INFORMATION – Ecotoxicity studies

Table S1. Acute ecotoxicity studies for sulfate (na= not available).

Species (life stage)	**Order**	**Guideline**	**Cl (mg/L)**	**Ca:Mg ratio**	**Hardness $CaCO_3$ (mg/L)**	**Endpoint & Duration**	**Effect value SO_4^{2-}(mg/L)**	**Control SO_4^{2} (mg/L)**	**Reference**
Fish									
Pimephales promelas	Cypriniformes	US EPA	na	na	na	96h LC_{50}	5384	na	Mount et al. 1997
Pimephales promelas (juveniles)	Cypriniformes	ASTM	9.5	1.7	100	96h LC_{50}	10869[1]	20	Wang et al. 2016a
Pimephales promelas (larvae)	Cypriniformes	ASTM	9.5	1.7	100	96h LC_{50}	4833[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.5	1.7	105	7d LC_{50}	534[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.2	1.8	108	7d LC_{50}	508[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	11	1.8	102	7d LC_{50}	645[1,2]	19	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.8	1.8	108	7d LC_{50}	718[1,2]	19	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	23	1.8	109	7d LC_{50}	637[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	3.7	0.8	103	7d LC_{50}	>1719[3]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	11	1.8	110	7d LC_{50}	600[1,2]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	2.7	0.8	100	7d LC_{50}	1780[3]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	4.7	0.8	108	7d LC_{50}	>1613[3]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	na	1.8	109	7d LC_{50}	1612[3]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.5	1.7	105	10d LC_{50}	478[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.2	1.8	108	10d LC_{50}	508[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	11	1.8	102	10d LC_{50}	645[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.8	1.8	108	14d LC_{50}	692[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	23	1.8	109	14d LC_{50}	644[1]	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	3.7	0.8	102	14d LC_{50}	>1986[3]	20	Wang et al. 2016a
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	40*	7d LC_{50}	1649	23	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	80*	7d LC_{50}	2938	45	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	160*	7d LC_{50}	4553	91	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	320*	7d LC_{50}	>5250	182	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	50*	7d LC_{50}	946	74	PESC 2013
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	100*	7d LC_{50}	1843	125	PESC 2013

Species (life stage)	Order	Guideline	Cl (mg/L)	Ca:Mg ratio	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration	Effect value SO_4^{2-}(mg/L)	Control SO_4^{2} (mg/L)	Reference
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	250*	7d LC_{50}	3178	470	PESC 2013
Lepomis macrochirus	Perciformes	na	na	na	na	24h LC_{50}[4]	11824	na	Dowden and Bennett 1965
Lepomis macrochirus	Perciformes	na	na	na	38	96h LC_{50}	9121	na	Trama 1954
Lepomis macrochirus	Perciformes	na	na	na	na	96h LC_{50}[4]	9121	na	Patrick et al. 1968
Invertebrates- Crustacean									
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	1.9	0.88	89	48h LC_{50}	2050	90	Soucek and Kennedy 2005
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	33.9	3.25	107	48h LC_{50}	2526	59	Soucek and Kennedy 2005
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	1.9	0.88	92	48h LC_{50}	2500	90	Soucek 2007a
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	33.9	3.25	92	48h LC_{50}	3000	59	Soucek 2007a
Ceriodaphnia dubia	Cladocera	US EPA	na	na	100	48h LC_{50}	2083	na	Mount et al. 1997
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	1.9	0.88	194*	48h LC_{50}	3000	na	Soucek and Kennedy 2005
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	1.9	0.88	288*	48h LC_{50}	2946	na	Soucek and Kennedy 2005
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	1.9	0.88	390*	48h LC_{50}	3174	na	Soucek and Kennedy 2005
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	1.9	0.88	484*	48h LC_{50}	3516	na	Soucek and Kennedy 2005
Ceriodaphnia dubia (<24h old)	Cladocera	ASTM	1.9	0.88	578*	48h LC_{50}	3288	na	Soucek and Kennedy 2005
Ceriodaphnia dubia (neonates)	Cladocera	na	9.5	1.7	100	48h EC_{50}	2441	20	Wang et al. 2016a
Ceriodaphnia dubia (neonates)	Cladocera	na	na	na	na	48h EC_{50}	3150	na	Warne and Schifko 1999
Daphnia magna (<24h old)	Cladocera	US EPA	na	na	100	48h LC_{50}	3098	na	Mount et al. 1997
Daphnia magna (<24h old)	Cladocera	US EPA	na	0.7	25*	48h LC_{50}	1194	na	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	na	0.7	50*	48h LC_{50}	1551	na	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	na	0.7	75*	48h LC_{50}	3342	na	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	na	0.7	100*	48h LC_{50}	3203	59	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	na	0.7	25	48h LC_{50}	1194	na	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	na	1.8	25	48h LC_{50}	1563	na	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	na	7	25	48h LC_{50}	1985	na	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	16.8	0.7	100	48h LC_{50}	3203	102	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	16.8	1.8	100	48h LC_{50}	3808[5]	102	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	US EPA	16.8	7	100	48h LC_{50}	4395	102	Davies and Hall 2007
Daphnia magna (<24h old)	Cladocera	na	na	na	na	96h LC_{50}[4]	3072	na	Dowden and Bennett 1965

Species (life stage)	Order	Guideline	Cl (mg/L)	Ca:Mg ratio	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration	Effect value SO_4^{2-} (mg/L)	Control SO_4^{2} (mg/L)	Reference
Daphnia magna (adult)	Cladocera	na	na	na	na	96h LC_{50}[4]	426	na	Dowden and Bennett 1965
Daphnia magna (neonates)	Cladocera	EPS	na	na	25*	48h LC_{50}	957	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	na	50*	48h LC_{50}	1768	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	na	75*	48h LC_{50}	3155	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	0.7	25	48h LC_{50}	1285	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	3.8	25	48h LC_{50}	1571	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	7	25	48h LC_{50}	1993	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	0.7	100	48h LC_{50}	3146	54	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	3.8	100	48h LC_{50}	3839[5]	na	Davies 2002
Daphnia magna (neonates)	Cladocera	EPS	na	7	100	48h LC_{50}	4414	115	Davies 2002
Hyalella azteca (juvenile)	Amphibods	EPS	na	na	80	96h EC_{50}	2461[5]	45	Elphick et al. 2011
Hyalella azteca (7-14 d old)	Amphibods	ASTM	25	1.41	100*	96h LC_{50}	1900	na	Soucek 2007b
Hyalella azteca (7-14 d old)	Amphibods	ASTM	25	1.41	500*	96h LC_{50}	4000	na	Soucek 2007b
Hyalella azteca (7-14d old)	Amphibods	ASTM	1.9	0.88	94	96h LC_{50}	512	90	Soucek and Kennedy 2005
Hyalella azteca (7-14d old)	Amphibods	ASTM	33.9	3.25	107	96h LC_{50}	2855	59	Soucek and Kennedy 2005
Hyalella azteca (2-9d old)	Amphibods	US EPA	na	3	25*	96h LC_{50}	569	na	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	na	3	50*	96h LC_{50}	1448	na	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	na	3	75*	96h LC_{50}	1580	na	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	na	3	123*	96h LC_{50}	3144	na	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	na	3	250*	96h LC_{50}	5259	na	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	2.4	0.7	100	96h LC_{50}	2101	58	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	2.4	1.8	100	96h LC_{50}	2240[5]	58	Davies and Hall 2007
Hyalella azteca (2-9d old)	Amphibods	US EPA	2.4	7	100	96h LC_{50}	2725	58	Davies and Hall 2007
Hyalella azteca (7-11d old)	Amphibods	ASTM	5	3.2	84	96h LC_{50}	2084[5]	52	Soucek et al. 2015
Hyalella azteca (7-11d old)	Amphibods	ASTM	25	3.2	84	96h LC_{50}	1882	52	Soucek et al. 2015
Hyalella azteca (7-11d old)	Amphibods	ASTM	50	3.2	84	96h LC_{50}	1919	52	Soucek et al. 2015
Hyalella azteca (2-9d old)	Amphibods	EPS	na	na	100*	96h LC_{50}	2971[5]	43	Davies 2002
Hyalella azteca (2-9d old)	Amphibods	EPS	na	na	250*	96h LC_{50}	4864	109	Davies 2002

Invertebrates- Insecta									
Chironomus tentans (10d old)	Diptera	US EPA	1.9	0.8	94	48h LC_{50}	14134	90	Soucek and Kennedy 2005
Chironomus dilutus (larvae)	Diptera	ASTM	9.5	1.7	100	96h EC_{50}	5992	20	Wang et al. 2016a
Culex sp. (larvae)	Diptera	na	na	na	na	48h LC_{50}[4]	9122	na	Dowden and Bennett 1965
Neocleon triangulifer (nymph)	Ephemeroptera	ASTM	na	na	99	96h LC_{50}	1227	57	Soucek and Dickinson 2015
Tricorythus sp.	Ephemeroptera	na	na	na	69.4	96h LC_{50}	446	na	Goetsch and Palmer 1997
Invertebrates- Mollusca									
Idioteuthis latipinna	Teuthida	na	na	na	na	48h LC_{50}[4]	10808	na	Dowden and Bennett 1965
Lampsilis abrupta (juveniles)	Unionoida	ASTM	9.5	1.7	100	96h EC_{50}	2362	20	Wang et al. 2016a
Lampsilis siliquoidea (juvenile)	Unionidae	ASTM	na	na	106	96h EC_{50}	2325	na	Wang et al 2016b
Ligumia recta	Unionoida	USEPA	na	na	92	96h LC_{50}	1483	na	US EPA 2010
Lymnaea sp. (eggs)	Basommatophora	na	na	na	na	96h LC_{50}[4]	2401	na	Dowden and Bennett 1965
Margaritifera falcata (juvenile)	Unionidae	ASTM	na	na	106	96h EC_{50}	1378	na	Wang et al 2016b
Megalonaias nervosa (juvenile)	Unionidae	ASTM	na	na	103	96h EC_{50}	2279	na	Wang et al 2016b
Megalonaias nervosa	Unionoida	USEPA	na	na	92	96h LC_{50}	3378	na	US EPA, 2010
Sphaerium simile (juvenile)	Verioida	ASTM	1.9	0.88	94	96h LC_{50}	2078	90	Soucek and Kennedy 2005
Utterbackia imbecillis (juvenile)	Unionidae	ASTM	na	na	103	96h EC_{50}	2709	na	Wang et al 2016b
Algae									
Nitzschia linearis (marine)	Bacillariales	na	na	na	na	120h LC_{50}[4]	1284	na	Patrick et al. 1968
Pseudokirchneriella subcapitata	Sphaeropleales	EPS	na	na	10*	72h EC_{50}	1430	6	Elphick et al. 2011
Pseudokirchneriella subcapitata	Sphaeropleales	EPS	na	na	80*	72h EC_{50}	2742	45	Elphick et al. 2011
Pseudokirchneriella subcapitata	Sphaeropleales	EPS	na	na	320*	72h EC_{50}	2510	182	Elphick et al. 2011
Pseudokirchneriella subcapitata	Sphaeropleales	na	na	na	na	96h EC_{50}	1054	na	Simmons 2012
Aquatic plants									
Lemna minor	Alismatales	na	na	na	na	48h EC_{50}	2264	na	Simmons 2012

1 = potassium concentration approximately 1 mg/L. 2 = data used to calculate geometric mean (some data was excluded after reliability evaluation and due to high potassium concentration of 3 mg/L). 3 = potassium concentration approximately 3 mg/L. 4 = TLm tabulated as LC50. 5 = data used to calculate geometric mean (based on hardness, chloride concentrations, and Ca:Mg ratio. * = Studies investigating hardness as a modifying factor.

Table S2. Chronic ecotoxicity studies for sulfate (na= not available).

Species (life stage)	Order	Guideline	Cl (mg/L)	Ca:Mg ratio	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-} (mg/L)	Control SO_4^{2-} (mg/L)	Reference
Fish										
Oncorhynchus kisutch (embryos)	Salmoniformes	EPS	na	na	15	Development	10d NOEC	825	na	Elphick et al. 2011
Oncorhynchus mykiss (embryos)	Salmoniformes	EPS	na	na	15	Development	21d NOEC	205	na	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	40*	Growth	7d NOEC	595	23	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	80*	Growth	7d NOEC	760	45	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	160*	Growth	7d NOEC	1300	91	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	320*	Growth	7d NOEC	820	182	Elphick et al. 2011
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	50*	Growth	7d EC_{10}	931	74	PESC 2013
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	100*	Growth	7d EC_{10}	1397	125	PESC 2013
Pimephales promelas (larvae)	Cypriniformes	EPS	na	na	250*	Growth	7d EC_{10}	2969	470	PESC 2013
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.5	1.7	100	Biomass	34d EC_{10}[1]	92.5	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.5	1.7	100	Biomass	34d EC_{10}[1]	53	20	Wang et al. 2016a
Amphibans										
Pseudacris regilla (tadpoles)	Anura	OECD	na	na	15*	Survival/ growth	21d NOEC	1075	na	Elphick et al. 2011
Pseudacris regilla (tadpoles)	Anura	OECD	na	na	80*	Survival/ growth	21d NOEC	978	45	Elphick et al. 2011
Invertebrates- Crustacean										
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	40*	Reproduction	7d NOEC	<150	23	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	80*	Reproduction	7d NOEC	645[2]	45	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	160*	Reproduction	7d NOEC	775	91	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	320*	Reproduction	7d NOEC	420	182	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPA	na	na	44	Reproduction	7d NOEC	500	21	Lasier and Hardin 2009
Ceriodaphnia dubia (neonates)	Cladocera	EPA	na	na	44	Reproduction	7d NOEC	250	21	Lasier and Hardin 2010
Ceriodaphnia dubia	Cladocera	ASTM	1.9	0.88	92	Reproduction	7d EC_{50}	1148	90	Soucek 2007a
Ceriodaphnia dubia	Cladocera	ASTM	33.9	3.25	92	Reproduction	7d EC_{50}	1458	59	Soucek 2007a

Species (life stage)	Order	Guideline	Cl (mg/L)	Ca:Mg ratio	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-}(mg/L)	Control SO_4^{2-} (mg/L)	Reference
Ceriodaphnia dubia (neonates)	Cladocera	EPA	na	na	93	Reproduction	7d NOEC	1000[2]	46	Lasier and Hardin 2009
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	1.9	0.88	92	Survival/ reproduction	7d LOAEC	2216	90	Soucek 2007a
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	33.9	3.25	92	Survival/ reproduction	7d LOAEC	3000	59	Soucek 2007a
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	1.9	0.88	92	Reproduction	7d LOAEC	1000	90	Soucek 2007a
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	9.5	1.7	100	Reproduction	7d EC_{10}[1]	466[2]	20	Wang et al. 2016a
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	9.5	1.7	100	Reproduction	7d EC_{10}[1]	532[2]	20	Wang et al. 2016a
Hyalella azteca (juvenile)	Amphibods	EPS	na	na	80	Survival/ reproduction	14d NOEC	1637	81	Elphick et al. 2011
Hyalella azteca	Amphibods	na	na	na	50*	Growth	28d EC_{10}	1170	34	PESC 2013
Hyalella azteca	Amphibods	na	na	na	100*	Growth	28d EC_{10}	682	57	PESC 2013
Hyalella azteca	Amphibods	na	na	na	250*	Growth	28d EC_{10}	437	164	PESC 2013
Invertebrates- Mollusca										
Lampsilis abrupta (juveniles)	Unionoida	ASTM	9.5	1.7	100	Dry weight	28d EC_{10}[1]	319.5	20	Wang et al. 2016a
Invertebrates- Rotifers										
Brachionus calyciflorus (<4h old)	Rotifer	na	na	na	40*	Reproduction	48h NOEC	950	23	Elphick et al. 2011
Brachionus calyciflorus (<4h old)	Rotifer	na	na	na	80*	Reproduction	48h NOEC	510	45	Elphick et al. 2011
Brachionus calyciflorus (<4h old)	Rotifer	na	na	na	160*	Reproduction	48h NOEC	560	91	Elphick et al. 2011
Brachionus calyciflorus (<4h old)	Rotifer	na	na	na	320*	Reproduction	48h NOEC	1800	182	Elphick et al. 2011
Invertebrates- Insecta										
Chironomus dilutus (larvae)	Diptera	ASTM	9.5	1.7	100	Dry weight	7d EC_{10}[1]	488.5	20	Wang et al. 2016a
Chironomus dilutus (larvae)	Diptera	ASTM	9.5	1.7	100	Reproduction	41d EC_{10}[1]	1293.5	20	Wang et al. 2016a
Neocleon triangulifer (nymph)	Ephemeroptera	na	na	na	99	% pre-emergent nymph	36d NOEC[3]	129	57	Soucek and Dickinson 2015
Neocleon triangulifer (nymph)	Ephemeroptera	na	na	na	99	No of eggs per female	36d EC_{10}[1]	140.5	57	Soucek and Dickinson 2015
Algae										
Pseudokirchneriella subcapitata	Sphaeropleales	EPS	na	na	10*	Growth	72h NOEC	1100	6	Elphick et al. 2011

Species (life stage)	Order	Guideline	Cl (mg/L)	Ca:Mg ratio	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-}(mg/L)	Control SO_4^{2-} (mg/L)	Reference
Pseudokirchneriella subcapitata	Sphaeropleales	EPS	na	na	80*	Growth	72h NOEC	1200	45	Elphick et al. 2011
Pseudokirchneriella subcapitata	Sphaeropleales	EPS	na	na	320*	Growth	72h NOEC	1300	182	Elphick et al. 2011
Pseudokirchneriella subcapitata	Sphaeropleales	na	na	na	na	Flourescence	96h EC_{10}	426	na	Simmons 2012
Pseudokirchneriella subcapitata	Sphaeropleales	na	na	na	na	Cell density	96h EC_{10}	810	na	Simmons 2012
Higher aquatic plants										
Fontinalis antipyretica	Hypnales	na	na	na	15	Growth	21d NOEC	603	na	Elphick et al. 2011
Fontinalis antipyretica	Hypnales	na	na	na	15	Growth	21d NOEC	654	na	Elphick et al. 2011
Fontinalis antipyretica	Hypnales	na	na	na	15	Clorofyll	21d NOEC	145	na	Elphick et al. 2011
Fontinalis antipyretica	Hypnales	na	na	na	15	Clorofyll	21d NOEC	654	na	Elphick et al. 2011
Fontinalis antipyretica	Hypnales	na	na	2.4	19	Shoot length	21d NOEC	200	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	6.7	26	Shoot length	21d NOEC	600	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	6.7	105	Shoot length	21d NOEC	1000	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	2.4	19	Growth	21d NOEC	400	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	67	26	Growth	21d NOEC	1000	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	6.7	105	Growth	21d NOEC	200	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	2.4	19	Chlorophyll reduction	21d NOEC	200	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	6.7	26	Chlorophyll reduction	21d NOEC	400	na	Davies 2007
Fontinalis antipyretica	Hypnales	na	na	6.7	105	Chlorophyll reduction	21d NOEC	800	na	Davies 2007
Lemna minor	Alismatales	EPS	na	na	50*	Frond increase	7d EC_{10}	2143	103	PESC 2013
Lemna minor	Alismatales	EPS	na	na	100*	Frond increase	7d EC_{10}	2243	217	PESC 2013
Lemna minor	Alismatales	EPS	na	na	250*	Frond increase	7d EC_{10}	2314	248	PESC 2013
Lemna minor	Alismatales	na	na	na	na	No. of live thalli	7d EC_{10}	345	na	Simmons 2012

1 = EC20 divided by 2, tabulated as EC10. 2 = data used to calculate geometric mean. 3 = NOEC was not reported, the concentration below the statistically significant concentration was set as NOEC. * = Studies investigating hardness as a modifying factor.

Table S3. Ecotoxicity studies with long-term exposure and endpoint survival (na= not available).

Species (life stage)	**Order**	**Guideline**	**Cl (mg/L)**	**Ca:Mg ratio**	**Hardness $CaCO_3$ (mg/L)**	**Endpoint & Duration**		**Effect value SO_4^{2-}(mg/L)**	**Control SO_4^{2-} (mg/L)**	**Reference**
Oncorhynchus mykiss (eyed eggs)	Salmoniformes	EPS	na	na	6	Survival	21d LC_{10}	175.4	2	Kennedy 2012
Oncorhynchus mykiss (eyed eggs)	Salmoniformes	EPS	na	na	50	Survival	21d LC_{10}	299.5	44	Kennedy 2012
Oncorhynchus mykiss (eyed eggs)	Salmoniformes	EPS	na	na	100	Survival	21d LC_{10}	419.2	89	Kennedy 2012
Oncorhynchus mykiss (eyed eggs)	Salmoniformes	EPS	na	na	250	Survival	21d LC_{10}	673.7	206	Kennedy 2012
Oncorhynchus mykiss (fry)	Salmoniformes	EPS	na	na	6	Survival	30d LC_{10}	363.2	2	Kennedy 2012
Oncorhynchus mykiss (fry)	Salmoniformes	EPS	na	na	50	Survival	30d LC_{10}	367.9	44	Kennedy 2012
Oncorhynchus mykiss (fry)	Salmoniformes	EPS	na	na	100	Survival	30d LC_{10}	771.7	89	Kennedy 2012
Oncorhynchus mykiss (fry)	Salmoniformes	EPS	na	na	250	Survival	30d LC_{10}	1224.7	206	Kennedy 2012
Oncorhynchus mykiss (embryos)	Salmoniformes	EPS	11.7	5.3	50	Survival	21d LC_{10}	123[1]	28	PESC 2013
Oncorhynchus mykiss (embryos)	Salmoniformes	EPS	24	5.3	100	Survival	21d LC_{10}	162[1]	53	PESC 2013
Oncorhynchus mykiss (embryos)	Salmoniformes	EPS	60	5.3	250	Survival	21d LC_{10}	191[1]	140	PESC 2013
Oncorhynchus tshawytscha	Salmoniformes	EPS	na	na	250	Survival	21d LC_{10}	1287	189	PESC 2013
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.5	1.7	100	Survival	34d LC_{10}	430	20	Wang et al. 2016a
Pimephales promelas (embryos)	Cypriniformes	ASTM	9.5	1.7	100	Survival	34d NOEC	245	20	Wang et al. 2016a
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	40	Survival	7d NOEC	610	23	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	80	Survival	7d NOEC	1250	45	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	160	Survival	7d NOEC	1300	91	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	EPS	na	na	320	Survival	7d NOEC	1450	182	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	9.5	1.7	100	Survival	7d LC_{20}	1592	20	Wang et al. 2016a
Ceriodaphnia dubia (neonates)	Cladocera	ASTM	9.5	1.7	100	Survival	7d LC_{20}	1751	20	Wang et al. 2016a
Hyalella azteca	Amphibods	na	na	na	50	Survival	28d LC_{10}	1430	na	PESC 2013
Elliptio complanata	Unionoida	ASTM	na	na	50	Survival	28d LC_{10}	139[2]	34	PESC 2013
Elliptio complanata	Unionoida	ASTM	na	na	250	Survival	28d LC_{10}	676	158	PESC 2013
Lampsilis abrupta (juveniles)	Unionoida	ASTM	9.5	1.7	100	Survival	28d LC_{20}	1759	20	Wang et al. 2016a
Chironomus dilutus (larvae)	Diptera	ASTM	9.5	1.7	100	Survival	7d LC_{20}	>6160	20	Wang et al. 2016a
Neocleon triangulifer (nymph)	Ephemeroptera	ASTM	na	na	99	% survival to nymph stage	21d EC_{20}	289	57	Soucek and Dickinson 2015

1 = Suggests higher toxicity than Kennedy et al. (2012). However, Kennedy et al. (2012) used an increased sample size and received a more robust result. 2 = Suggest low toxicity at hardness 50, but was not included in the derivation due to large confidence interval (12-1640).

11. SUPPORTIVE INFORMATION – Reliability and relevance evaluations

The reliability and relevance of studies by Wang et al. (2016a) and Soucek and Dickinson (2015) were evaluated using the CRED evaluation method (Moermond et al. 2016) (table S4). The studies were assessed to be of sufficient reliability and relevance for use in the EQS derivation, although, some of the values from Wang et al. (2016a) were eliminated (because of technical error and reduced survival in control).

Table S4. Evaluation of the reliability and relevance of Wang et al. (2016a) and Soucek and Dickinson (2015) using the CRED evaluation method (Moermond et al. 2016).

	Wang et al. 2016a				**Soucek and Dickinson 2015**	
	CHRONIC	**Comments**	**ACUTE**	**Comments**	**CHRONIC**	**Comments**
Species	*P. promelas* (embryos)	Study 2011 (test 1, 2 and 3). Data from test 1 and 2 excluded (see criteria 3).	*P. promelas* (embryos)	Study 2012 and 2013	*N. triangulifer*	
Endpoint	Survival	Other endpoints investigated: Biomass and growth (but uncertainties in the results)	Survival		(1) % of pre-emergent nymph (development delay) (2) % survival to pre-emergent nymph stage	Other endpoints investigated: No. of days to pre-emergent nymph stage (NOEC 209 mg/L); %e emergence (NOEC 209 mg/L); pre-egg laying weight (NOEC 359 mg/L); No. Of eggs per female (not significant); No of eggs per original female (EC20 281mg/L, no dose-response)
Effect value (mg/L)	LC10: 430	NOEC: 245, LOEC:468, LC20:477 (This study also calculated LC50 for 7days of 645 mg/L (test 3))	LC50: 625.55	Geometric mean of 645, 718 and 600 (potassium ≈1 mg/L)	NOEC: 129	MATC: 164 (LOEC= 209), EC20: 170 MATC: 164 (LOEC=209), EC20: 289
Reliability evaluation						
Is the guideline method (OECD/ISO) or modified guideline used?	Yes	ASTM E1241-05 and E729-96	Yes	ASTM E1241-05 and E729-96	No	Based on DOI: 10.1016/j.chemosphere.2014.04.092, with several modifications and DOI: 10.1016/j.chemosphere.2014.04.096.
Is the test performed under GLP conditions?	No		No		No	
If applicable, are validity criteria fulfilled (e.g., control survival, growth)?	Partly fulfilled.	Poor survival in test 1 (data not used); technical error in test 2 (data not used); 87% control survival in test 3; (data used); No information about temperature between chambers; DO ok; Analytic measures performed. Chambers were held in	Partly fulfilled	95% control survival (2012), 98% control survival (2013); Constant conditions; DO ok; Results based on measured concentration Chambers were held in temperature-controlled baths, no information about if temperatures	Yes	"Control survival was evaluated as no. of organisms surviving to pre-emergent nymph stage". "Percentage of survival to pre-emergent nymph stage was high for the controls and up to 51 mg/L". ≥80 %

		temperature-controlled baths, no information about if temperatures varied over time or between chambers.		varied over time or between chambers.		
Are appropriate controls performed (e.g., solvent control, negative and positive control)?	Yes		Yes		Yes	
Is the test substance identified with name or CAS number? Are test results reported for the appropriate compound?	Yes		Yes		Yes	
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	Yes	99%; Sigma- Aldrich	Yes	99%; Sigma-Aldrich	Not reported	
If a formulation is used or if impurities are present: Do, other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	No formulation/ mixture, etc.		No formulation/ mixture, etc.		No formulation/ mixture, etc.	
Are the organisms well described (e.g., scientific name, weight, length, growth, age/Life stage, strain/clone, gender if appropriate)?	Yes	<24h old	Yes	<24h old	Yes	Age: <24h,

Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	Yes	Cultured in control water; US Geological survey Columbia environmental research center in Columbia, MO, USA.	Yes	Cultured in control water; US Geological survey Columbia environmental research center in Columbia, MO, USA.	Yes	Source: Stroud Water Research Center Clone #WCC-2; Tests were conducted in Duluth 100 hard water, this was also the mayfly culture water, and eggs were stored in this water, so no acclimation was required.
Is the experimental system appropriate for the test substance. taking into account its physicochemical characteristics?	Yes	Flow-through, 250 ml/chamber/30min	Yes	Static-renewal	Yes	Static/renewal. Renewal Days 0–4: none; day 5—end of test: three times weekly
Is the experimental system appropriate for the test organism (e.g., choice of medium or test water, feeding, water characteristics, temperature, light/dark conditions, pH, oxygen content)? Have conditions been stable during the test?	Yes	Temp 25; DO 7,7-8,4; pH 8,2; Hardness 103-106; Photoperiod 16:8 Fed 3 times a day (2 times/day on weekends)	Yes	Temp 25; DO 7,3 ; pH 7,9; hardness 108 (2012); DO 8,2; pH 8,2; hardness 110 (2013)	Yes	Temperature, pH, dissolved oxygen, alkalinity, and hardness were 25.0±0.38C, 8.4±0.1mg/L, 7.3±0.4 (lowest value= 6.1) mg/L, 83± 3 mg/L as $CaCO_3$, and 95± 4 mg/L as $CaCO_3$, respectively; Diatoms used to feed mayflies included Mayamea sp. and Nitzschia sp. ; Photoperiod 16:8
Were exposure concentrations below the limit of water solubility (taking the use of a solvent into account)? If a solvent is used, is the solvent within the appropriate range and is a solvent control included?	Yes		Yes		Yes	
Is correct spacing between exposure concentrations applied?	Yes	Mean concentrations: 19 (control), 74, 132, 245, 468 and 958.	Yes	Mean concentrations: 19 (control), 121, 249, 476, 830 and 1580 (2012); 20 (control), 108, 242, 442, 781 and 1555 (2013)	Yes	Nominal SO42– concentrations were as follows: 59 mg/L (control), 136 mg/L, 214 mg/L, 369 mg/L, 679 mg/L, and 1300 mg/L.

Is the exposure duration defined?	Yes	34d	Yes	14 days (2012) and 7 days (2013)	Yes	30 days
Are chemical analyses adequate to verify concentrations of the test substance over the duration of the study?	Yes	% of nominal: 93-120 %	Yes	Renewed once on day 2. Measured on day 0, 7 and 14 (2012)	Yes	"For the Na2SO4 test, measured sulfate averaged 97% of nominal (range, 90–106%)" (ion chromatography)
Is the biomass loading of the organisms in the test system within the appropriate range (e.g., <1 g/L)?	Yes	30 embryos/ 1000ml later removed to 7L chambers (flow-through)	Unclear	30 embryos/ 280 ml (1000ml on day 5) (2012), no information about 2013	Yes	2 organisms/ 30ml (larger volume from day 14)
Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?	Yes	4 replicates with 30 embryos	Yes	2-3 replicates	Yes	10 replicates with 2 organisms per replicate
Are appropriate statistical methods used?	Yes	Toxicity response analysis program; Dunett´s test; Steel´s test; TOXSTAT; SAS/STAT.	Yes	Toxicity response analysis program; Dunett´s test; Steel´s test; TOXSTAT; SAS/STAT.	Yes	Fisher's exact test, Tukey's honest significant difference, TRAP software
Is a concentration–response curve observed? Is the response statistically significant?	Yes	Possible to determine dose-response.	Yes	Possible to determine dose-response	Yes	Possible to determine dose-response
Are sufficient data available to check the calculation of endpoints and (if applicable) validity criteria (e.g., control data, concentration–response curves)?	Yes		Yes		Partly fulfilled	
Reliability results	**R2**		**R2**		**R2**	

Relevance evaluation						
Is the species tested relevant for the compartment under evaluation?	Yes		Yes		Yes	
Are the organisms tested relevant for the tested compound?	Yes		Yes		Yes	
Are the reported endpoints appropriate for the regulatory purpose?	Yes	Survival	Yes		Yes	
Are the reported endpoints appropriate for the investigated effects or the mode of action of the test substance?	Yes	No known mode of action	Yes	No known mode action	Yes	No known mode of action
Is the effect relevant on a population level?	Yes		Yes		Partly fulfilled	Endpoint (1): development delay, Unclear; Endpoint (2): Yes, survival relevant on population level (but endpoint do not fulfil criteria for chronic effect values)
Is the magnitude of effect statistically significant and biologically relevant for the regulatory purpose (e.g., EC10, EC50)?	Yes	Survival relevant on a population level (but endpoint do not fulfil criteria for chronic effect values).	Yes		Yes	
Are appropriate life stages studied?	Yes	Juveniles and larvae was not as sensitive as embryos (same study)	Yes		Yes	
Are the experimental conditions relevant for the tested species?	Yes		Partly fulfilled		Yes	
Is the exposure duration relevant and appropriate for the studied endpoints and species?	Yes		Yes		Yes	

If recovery is studied, is this relevant for the framework for which the study is evaluated?	Not studied		Yes		Not studied	
In case of a formulation, other mixture, salts, or transformation products, is the substance tested representative and relevant for the substance being assessed?	No formulation/ mixture etc.		No formulation/ mixture, etc.		No formulation/ mixture, etc.	
Is the tested exposure scenario relevant for the substance?	Yes		Yes		Yes	
Is the tested exposure scenario relevant for the species?	Yes		Yes		Yes	
Relevance results	**C1**		**C1**		**C1**	

12. SUPPORTIVE INFORMATION – Added risk and SSD results

Scenario 1: Hardness dependent MAC-EQS, based on studies investigating hardness as a modifying factor

Table S5 $LC_{50\ added}$ used in the deterministic derivation for $MAC\text{-}EQS_{added}$ (scenario 1: hardness depended EQS).

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration	Effect value SO_4^{2-}(mg/L)	SO_4^{2-} control (mg/L)	Effect value added SO_4^{2-} (mg/L)	Reference
Hardness 10-25						
H. azteca	25	96h LC_{50}	596	20	576	Davies and Hall 2007
Hardness 40–50						
P. promelas (larvae)	50	7d LC_{50}	957	74	883	PESC et al. 2013
Hardness 75–100						
H. azteca	75	96h LC_{50}	1580	40	1540	Davies and Hall 2007
Hardness >160						
P. promelas	250	7d LC_{50}	3178	470	2708	PESC 2013

Scenario 2: MAC-EQS based on studies with hardness≈ 100 mg $CaCO_3$/L

Table S6. $L(E)C_{50\ added}$ used in the deterministic and probabilistic derivation of $MAC\text{-}EQS_{added}$ (scenario 2: hardness ≈ 100 mg $CaCO_3$/L).

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration	Effect value SO_4^{2-}(mg/L)	SO_4^{2-} control (mg/L)	Effect value added SO_4^{2-} (mg/L)	Reference
Pimephales promelas (embryos)	102-110	7d LC_{50}	652[1]	20	633	Wang et al. 2016a
Ceriodaphnia dubia (neonates)	100	48h EC_{50}	2441	20	2421	Wang et al. 2016a
Daphnia magna (<24h, neonates)	100	48h LC_{50}	3823	102	3721	Geometric mean
Hyalella azteca (2-11d old)	80-100	96h $L(E)C_{50}$	2415	43-58	2665	Geometric mean
Chironomus dilutus (larvae)	100	96h EC_{50}	5992	20	5972	Wang et al. 2016a
Chironomus tentans (10d old)	94	48h LC_{50}	14134	90	14044	Soucek and Kennedy 2005
Neocleon triangulifer (nymph)	99	96h LC_{50}	1227	57	1170	Soucek and Dickinson 2015
Lampsilis abrupta (juveniles)	100	96h EC_{50}	2362	20	2342	Wang et al. 2016a
Lampsilis siliquoidea (juvenile)	106	96h EC_{50}	2325	20*	2305	Wang et al 2016b
Ligumia recta	92	96h LC_{50}	1483	40*	1443	US EPA 2010
Margaritifera falcata (juvenile)	106	96h EC_{50}	1378	20*	1358	Wang et al 2016b
Megalonaias nervosa (juvenile)	103	96h EC_{50}	2279	20*	2229	Wang et al 2016b
Sphaerium simile (juvenile)	94	96h LC_{50}	2078	90	1988	Soucek and Kennedy 2005
Utterbackia imbecillis (juvenile)	103	96h EC_{50}	2709	20*	2689	Wang et al 2016b
Pseudokirchneriella subcapitata	80	72h EC_{50}	2742	45	2697	Elphick et al. 2011

* Sulfate concentrations in control medium estimated.

Table S7. HC5 results for probabilistic derivation of MAC-EQS_{added} (scenario 2: hardness ≈ 100 mg $CaCO_3$/L).

Type of HC5	Value (mg/L)	log10(Value) (mg/L)	Description
LL HC5	386.07	2.59	lower estimate of the HC5
HC5	725.20	2.86	median estimate of the HC5
UL HC5	1085.88	3,04	upper estimate of the HC5
sprHC5	2.81	0.45	spread of the HC5 estimate

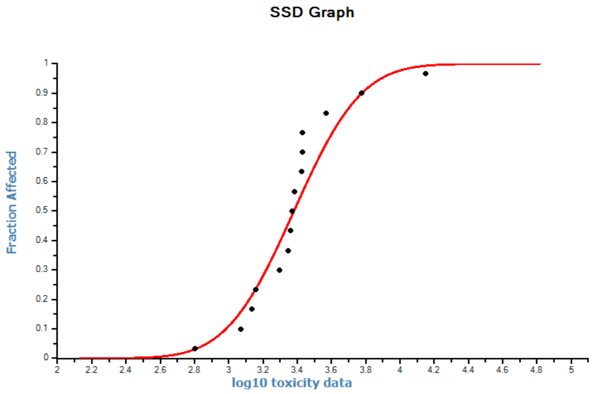

Figure S1. SSD graph for MAC-EQS-added (scenario 2: hardness ≈ 100 mg $CaCO_3$/L). *P. promelas* was the most sensitive species. HC5 was set to 725.20. The normality was rejected in the Kolmogorov-Smirnov test at 0.05-0.1 significance level.

Scenario 2: AA-EQS based on studies with hardness ≈ 100 mg $CaCO_3$/L

Table S8. $NOEC_{added}$ and $L(E)C_{10\ added}$ used in the deterministic and probabilistic derivation of $AA\text{-}EQS_{added}$ (scenario 2: hardness ≈ 100 mg $CaCO_3$/L).

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-} (mg/L)	SO_4^{2-} control (mg/L)	Effect value added SO_4^{2-} (mg/L)	Reference
Oncorhynchus mykiss (eyed eggs)	100	Survival	31d LC_{10}	419	89	330	Kennedy et al. 2012
Pimephales promelas (embryos)	100	Survival	34d LC_{10}	430	20	410	Wang et al. 2016a
Pseudacris regilla (tadpoles)	80	Survival/ growth	21d NOEC	978	45	933	Elphick et al. 2011
Ceriodaphnia dubia (neonates)	80-100	Reproduction	7d NOEC	632	20-46	589	Geometric mean
Hyalella azteca	100	Growth	28d EC_{10}	682	57	625	PESC 2013
Lampsilis abrupta (juveniles)	100	Dry weight	28d EC_{10}	320	20	300	Wang et al. 2016a
Chironomus dilutus (larvae)	100	Dry weight	7d EC_{10}	489	20	469	Wang et al. 2016a
Neocleon triangulifer (nymph)	99	Development/ survival	36d NOEC	129	57	72	Soucek and Dickinson 2015
Brachionus calyciflorus (<4h old)	80	Reproduction	48h NOEC	510	45	465	Elphick et al. 2011
Pseudokirchneriella subcapitata	80	Growth	72h NOEC	1200	45	1155	Elphick et al. 2011
Fontinalis antipyretica	105	Shoot length	21d NOEC	1000	40	960	Davies 2007
Lemna minor	100	Frond increase	7d EC_{10}	2243	217	2026	PESC 2013

Table S9. HC5 results for probabilistic derivation of $AA\text{-}EQS_{added}$ (scenario 2: hardness ≈ 100 mg $CaCO_3$/L).

Type of HC5	Value (mg/L)	log10(Value) (mg/L)	Description
LL HC5	53.31	1.73	lower estimate of the HC5
HC5	128.20	2.11	median estimate of the HC5
UL HC5	217.26	2.34	upper estimate of the HC5
sprHC5	4.08	0.61	spread of the HC5 estimate

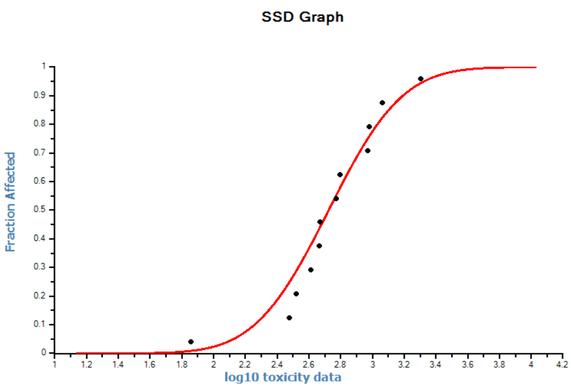

Figure S2. SSD graph for $AA\text{-}EQS_{added}$ (scenario 2: hardness ≈ 100 mg $CaCO_3$/L). *N. triangulifer* was the most sensitive species. HC5 was set to 128.20. The normality was accepted at all significance levels in all tests.

Scenario 3: AA-EQS based on realistic worst-case data for Sweden

Table S10. $NOEC_{added}$ and $L(E)C_{10\ added}$ used in the deterministic and probabilistic derivation of $AA\text{-}EQS_{added}$ (scenario 3: realistic worst-case data).

Species (life stage)	Hardness $CaCO_3$ (mg/L)	Endpoint & Duration		Effect value SO_4^{2-} (mg/L)	SO_4^{2-} control (mg/L)	Effect value added SO_4^{2-} (mg/L)	Reference
Oncorhynchus mykiss (eyed eggs)	6	Survival	31d LC_{10}	175	2	173	Kennedy et al. 2012
Pimephales promelas (embryos)	100	Survival	34d LC_{10}	430	20	410	Wang et al. 2016a
Pseudacris regilla (tadpoles)	15-80	Survival/ growth	21d NOEC	1025	45	980	Elphick et al. 201l
Ceriodaphnia dubia (neonates)	40-44	Reproduction	7d NOEC	266	21-23	244	Geometric mean
Hyalella azteca	50-100	Growth	28d EC_{10}	893	34-57	848	PESC 2013
Lampsilis abrupta (juveniles)	100	Dry weight	28d EC_{10}	320	20	300	Wang et al. 2016a
Chironomus dilutus (larvae)	100	Dry weight	7d EC_{10}	489	20	469	Wang et al. 2016a
Neocleon triangulifer (nymph)	99	Development / Survival	36d NOEC	129	57	72	Soucek and Dickinson 2015
Brachionus calyciflorus (<4h old)	40-80	Reproduction	48h NOEC	696	23-45	662	Elphick et al. 2011
Pseudokirchneriella subcapitata	10	Growth	72h NOEC	1100	6	1094	Elphick et al. 2011
Fontinalis antipyretica	15	Growth	21d NOEC	628	6*	622	Elphick et al. 2011 Geometric mean
Lemna minor	50	Growth	7d EC_{10}	2143	103	2040	PESC 2013

* Sulfate concentrations in control medium estimated.

Table S11. HC5 results for probabilistic derivation of $AA\text{-}EQS_{added}$ (scenario 3: realistic worst-case data).

Type of HC5	Value (mg/L)	log10(Value) (mg/L)	Description
LL HC5	39,50	1,60	lower estimate of the HC5
HC5	102,31	2,01	median estimate of the HC5
UL HC5	181,31	2,26	upper estimate of the HC5
sprHC5	4,59	0,66	spread of the HC5 estimate

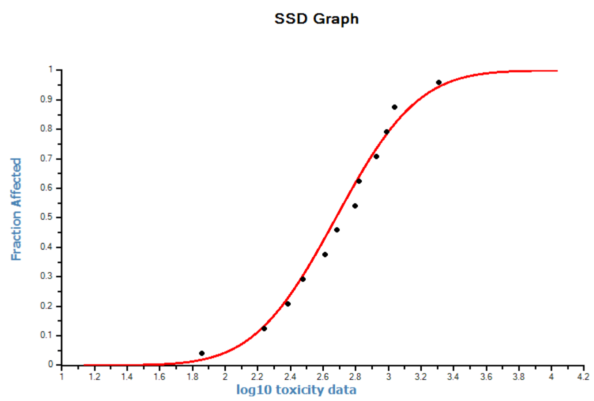

Figure S3. SSD graph for $AA\text{-}EQS_{added}$ (scenario 3: realistic worst-case data). *N. triangulifer* was the most sensitive species. HC5 was set to 102.31. The normality was accepted at all significance levels in all tests.

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