ACES REPORT 13

Nitrate

EQS data overview

Sara Sahlin, Marlene Ågerstrand

Department of Environmental Science and Analytical Chemistry (ACES)

Stockholm University

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EQS DATA OVERVIEW

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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 nitrate. The derivation aims to protect pelagic communities from direct toxic effects of nitrate and does not consider indirect effects as a result of eutrophication.

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". Nitrate is regulated in the Nitrate Directive (91/676/EEG) and the Groundwater Directive (2006/118/EC). These two directives specify that nitrate concentrations in surface- and groundwater should not exceed 50 mg/L NO_3 (11,5 mg/L NO_3-N).

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 nitrat 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 nitrat. 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 från 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 **2,1 mg/L NO_3-N** som årsmedelvärde, vilket motsvarar 9,1 mg/L NO_3. Värdet är framtaget utifrån en probabilistisk beräkning, trots att det saknas data för alger och högre växter. Eftersom nitrat är ett växtnäringsämne har avsaknaden av sådan data inte påverkat valet av osäkerhetsfaktorn (AF). Årsmedelvärdet är ett ”added risk”- värde, vilket innebär att det har tagits fram för att man i samband med utvärderingen ska beakta naturlig bakgrundshalt om den annars hindrar efterlevnaden av värdet. Som maximal tillåten koncentration föreslås **11 mg/L NO3-N** (motsvarande 47 mg/L NO3) baserat på probabilistisk beräkning.

Motsvarande värden för marin miljö föreslås till **10** respektive **11 mg/L NO_3-N** som årsmedelvärde respektive maximal tillåten koncentration, i båda fallen baserat på deterministiska beräkningar. Marina värden samt maximal tillåten koncentration för limnisk miljö är dock inte framtagna för att naturlig bakgrund ska beaktas.

Notera att bedömningsgrunder för nitrat 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". Nitrate is regulated in the Nitrate Directive (91/676/EEG) and the Groundwater Directive (2006/118/EC). These two directives specify that nitrate concentrations in surface- and groundwater should not exceed 50 mg/L NO_3 (11.5 mg/L NO_3-N).

Environmental Quality Standards (EQS) for pelagic communities are 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).

Lessons from previous work

In 2013, ITM (now ACES) proposed EQS for nitrate and the AA-EQS was set to 0.16 mg/L (ITM, 2013). The value has been criticized for being low, possibly below background levels, and therefore difficult to implement in regulatory work. In a report from SLU (Fölster and Djodjic, 2015) the authors stated that an assessment factor (AF) was not necessary for nitrate since it is an essential nutrient. However, according to European Communities (2011) the AF is required to take account uncertainties e.g. inter- and intraspecies variation and laboratory to field extrapolation.

EQS derivation

The EQS derivation was based on ecotoxicity data conducted with sodium nitrate ($NaNO_3$, CAS 7631-99-4). Other nitrate salts were not included due to that the cation (e.g. K^+, NH_4^+) may contribute to the toxicological response (Mount et al 1997, Schuytema and Nebeker 1999a). The concentration of nitrate is reported as nitrate-nitrogen (NO_3-N). Nitrate reported as NO_3 in the ecotoxicological literature has been converted to NO_3-N by multiplying with a factor of 0.23 (molar ratio of nitrogen: nitrate). The proposed EQS are presented as NO_3-N and the corresponding NO_3 concentration (NO_3-N divided by 0.23).

The following databases were used: Scopus; Web of science; Google Scholar; ETOX; Ekotoxzentrum; UBA; INERIS; RIVM; IRIS; UK TAG; OECD; USEPA. The following keywords were used: nitrate* sodium nitrate* inorganic nitrogen* toxicity, ecotoxicity* ecotoxicology* aquatic toxicity* NOEC* EC50* LC50. The literature search was conducted in January 2017.

Chronic toxicity values reported as LOEC, $E(L)C_{50}$ or IC_{25} and acute values reported as NOEC were not included. Values with toxicity higher or lower than the range of test concentrations (e.g. $LC_{50} > x$ or $LC_{50} < x$) were excluded. One value per species and endpoint were used in the derivation. In case of multiple values for the same species and the same endpoint, the values were aggregated (geometric mean). When multiple values for different endpoints were available for the same species, the most sensitive endpoint or life-stage was used (European Communities, 2011). Studies investigating mortality during long-term exposure (supportive information, table S3) were not included in the final derivation. However, if these data were to be included it would not have affected the outcome of the EQS derivation considerably.

There was no available toxicity data for algae or higher plants. However, there was unpublished data suggesting a NOEC of 625 mg/L for the macroalgae *Pseudokirchneriella subcapitata* (NIWA, 2013). Since nitrate and algae are primarily associated with eutrophication (and not toxicity) datasets were considered as complete even though ecotoxicity studies for algae were absent (and higher plants in the case of species sensitivity distribution (SSD)).

When sufficient data was available both deterministic derivation (applying AF) and probabilistic derivation (using 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. Since marine species tend to be less sensitive to nitrate than freshwater species (Camargo and Alonso, 2006), these were handled separately and not pooled together.

Due to time restrictions, reliability and relevance evaluation was only performed on chronic freshwater ecotoxicity studies. The studies were evaluated using the CRED evaluation method (Moermond et al. 2016). 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).

2. PROPOSED ENVIRONMENTAL QUALITY STANDARDS

Method and assessment factor (AF)	NO_3-N (mg/L)	Corresponding NO_3 (mg/L)
Proposed MAC-EQS$_{fw}$		
Deterministic (AF 10)	6.3	27.4
Probabilistic (AF 10)	10.7	46.7
Proposed AA-EQS$_{fw}$		
Deterministic (AF 10)	2.5	10.8
Probabilistic (AF 5)	2.2	9.6
Probabilistic added risk (AF 5)	2.1	9.1
Proposed MAC-EQS$_{sw}$		
Deterministic (AF 50)	11.4	49.7
Proposed AA-EQS$_{sw}$		
Deterministic (AF 10)	10.0	44.3

3. MEASURED CONCENTRATIONS

In 2014 the Board of Agriculture (Jordbruksverket), in accordance with the Nitrate Directive, complied monitoring data of nitrate for 376 and 336 stations of watercourses and lakes, respectively (vulnerable zones) (table 1).

Table 1. Monitoring data for nitrate in watercourses and lakes. Numbers of stations with nitrate concentration classified by average and maximum concentration (period 2010-2012) (Jordbruksverket, 2014).

Nitrate concentration mg NO_3-N/L (corresponding mg NO_3/L)	**Number of stations (watercourses)**		**Number of stations (lakes)**	
	Average 2010-2012	**Max 2010-2012**	**Average 2010-2012**	**Max 2010-2012**
0.46 (<2)	293	207	331	307
0.46- 1.15 (2-5)	52	88	4	20
1.15- 4.6 (5-20)	30	70	1	9
4.6- 9.2 (20-40)	1	8	-	-
9.2-11.5 (40-50)	-	3	-	-
11.5 (>50)	-	-	-	-

4. AQUATIC ECOTOXICITY OF NITRATE

In aquatic ecosystems, the most common form of inorganic nitrogen is nitrate, nitrite and ammonium. Nitrite and ammonia (unionized form of ammonium) is more toxic to aquatic organisms compared to nitrate. In fish and crayfish, the proposed toxic action of nitrite is inhibition of the oxygen-carrying capacity of haemoglobin and hemocyanin. The toxicity of nitrate is likely due to reduction of nitrate to nitrite in the blood, causing the same effect as nitrite (Camargo and Alonso, 2006). The uptake of nitrate in aquatic organisms appears to be less frequent than the uptake of nitrite and ammonium, resulting in relative low toxicity (Camargo et al., 2005).

Baker et al. (2016) investigated the ecotoxicity of nitrate in different water hardness. Both acute (using *Oncorhynchus mykiss* and *Hyalella azteca*) and chronic (using *Charaxes dilutus, Ceriodaphnia dubia* and *Pimephales promelas*) effects were correlated with water hardness and the result show decreased toxicity with increased hardness. However, major ions (other than calcium and magnesium) co-varied in the test dilutions and could have influence the toxicity of nitrate. Baker et al. (2016) concluded that the ionic strength and the ionic composition influenced the toxicity of nitrate. Likewise, Hickey et al. (2013) and Nautilus Environment (2012) observed a hardness related-response in chronic fish toxicity. However, the acute response was the opposite, increased toxicity with increasing hardness (Hickey et al. 2013).

Chloride levels have also been identified to influence nitrate toxicity in acute exposures to *H. Azteca.* The observed trend for *H. azteca* was decreased toxicity with increased concentrations of chloride and the LC_{50} varied from 210 to 736 mg/L NO_3-N at 9.9 and 97.6 mg/L Cl respectively (Soucek et al 2015; Soucek and Dickenson, 2016). However, nitrate toxicity to *C. dubia* did not significantly correlate with chloride concentrations either in acute or chronic exposures (Soucek and Dickenson, 2016). Though, mechanisms regarding hardness (and ion composition) are not fully understood and there is no clear pattern of the relationship between hardness and effect concentrations.

5. ACUTE FRESHWATER ECOTOXICITY

In total, 28 acute ecotoxicity studies with 72 effect values were found in the literature (table S1). The data used in the derivation are presented in table 2 and includes three orders of crustacean and mollusca, six orders of fish, one order of amphibians, and four orders of insect (a total of 35 species). In general, invertebrates were more sensitive than fish. The most sensitive species were the crustaceans *Echinogammarus echinosetosus* and *Eulimnogammarus toletanus,* and the insect *Hydropsyche occidentalis*.

Table 2. Summary of the acute data used in the deterministic and probabilistic derivation of freshwater EQS (na= not available).

Species (life stage)	Hardness (mg $CaCO_3$/L)	Endpoint & Duration	Effect value (mg NO_3-N/L)	Reference
Fish				
Acipenser baeri	260	96h LC_{50}	374	Hamlin 2006
Carassius auratus	na	24h LC_{50}	2040	Dowden and Bennett 1965
Coregonus clupeaformis (fry)	10-16	96h LC_{50}	1903	McGurk et al. 2006
Cyprinus carpio	na	24h LC_{50}	234.7	Geometric mean (see table S1 for all data)
Danio rerio (swim-up larvae)	na	96h LC_{50}	1250	Learmonth and Carvalho 2015
Galaxias maculatus (Juvenile)	14-100	96h EC_{50}	1840.2	Geometric mean (see table S1 for all data)
Gambusia holbrooki (juvenile)	na	96h LC_{50}	1116.5	Wallen et al. 1957
Ictalurus punctatus (fingerlings)	102	96h LC_{50}	1426	Colt and Tchobanoglous 1976
Lepomis macrochirus (fingerlings)	45-50	96h LC_{50}	2060.2	Geometric mean (see table S1 for all data)
Micropterus treculi (fry)	310	96h LC_{50}	1261	Tomasson and Carmichael 1976
Notropis topeka	210-230	96h LC_{50}	1354	Adelman et al 2009
Oncorhynchus mykiss	11-164	96h LC_{50}	1468	Geometric mean (see table S1 for all data)
Oncorhynchus tshawytscha (fingerlings)	na	96h LC_{50}	1310[a]	Westin 1974
Pimephales promelas	12-168	7d LC_{50}	269.9	Geometric mean (see table S1 for all data)
Salvelinus namaycush (fry)	10-16	96h LC_{50}	1121	McGurk et al. 2006
Amphibians				
Hypsiboas faber (embryo-larval)	na	48h LC_{50}	1245.4	Bellezi et al. 2015
Pseudacris regilla (embryos)	76.0	96h LC_{50}	643	Schuytema and Nebeker 1999b
Xenopus laevis (embryos)	36.2	120h LC_{50}	438	Schuytema and Nebeker 1999b

Invertebrates – Crustacean				
Austropotamobius italicus	na	96h LC_{50}	2950	Benítez-Mora et al., 2014
Ceriodaphnia dubia (neonates)	na	48h LC_{50}	374	Scott and Crunkilton 2000
Daphnia magna (neonates)	na	48h LC_{50}	462	Scott and Crunkilton 2000
Eulimnogammarus toletanus (adults)	293	96h LC_{50}	85	Camargo et al. 2005
Echinogammarus echinosetosus (adults)	293	96h LC_{50}	63	Camargo et al. 2005
Hyalella azteca	44-164	96h LC_{50}	215.2	Geometric mean (see table S1 for all data)
Invertebrates - Insecta				
Amphinemura delosa (juvenile)	88-92	96h LC_{50}	456	US EPA 2010, Soucek and Dickinson 2012
Allocapnia vivipara (juvenile)	99	96h LC_{50}	836	Soucek and Dickinson 2012
Cheumatopsyche pettiti (early instar larvae)	42.7	96h LC_{50}	128	Camargo and Ward 1995
Chironomus dilutus	84-136	48h LC_{50}	278	US EPA 2010
Hydropsyche occidentalis (early instar larvae)	42.7	96h LC_{50}	90	Camargo and Ward 1995
Hydropsyche exocellata (last instar larvae)	293	96h LC_{50}	270	Camargo et al. 2005
Neocleon triangulifer (nymph)	99	96h LC_{50}	179	Soucek and Dickinson 2015
Invertebrates - Mollusca				
Lampsilis siliquoidea (juvenile)	91	96h LC_{50}	357	US EPA 2010, Soucek and Dickinson 2012
Megalonaias nervosa (juvenile)	91	96h LC_{50}	937	US EPA 2010, Soucek and Dickinson 2012
Potamopyrgus antipodarum	na	96h LC_{50}	1042	Alonso and Camargo 2003
Sphaerium simile (juvenile)	91	96h LC_{50}	371	US EPA 2010, Soucek and Dickinson 2012

a = Reported as TLm (median tolerance limit).

5.1 Deterministic derivation

The critical acute data was the LC_{50} for *E. echinosetosu*s of 63 mg/L (Camargo et al., 2005). By applying the lowest possible AF of 10 the deterministic MAC-EQS was set to 6.30 mg/L. The AF was selected since the dataset includes more than three trophic levels and the standard deviation of the ecotoxicity data for all species was below a factor of 3 in both directions (European Communities, 2011).

5.2 Probabilistic derivation

The dataset fulfils the criteria for a probabilistic derivation (European Communities, 2011). Data in table 1 was used in the probabilistic derivation and the SSD is graphically shown in figure 1. Normal distribution was accepted at a 0.05 significance level in the Anderson-Darling and Cramer von Miseds tests (accepted at 0.025 in the Kolmogorov-Smirnov test). The median estimate of the HC5 (50% effect concentration for 5% of the species) was 107.37 mg/L (table 3). The median HC5 was divided by AF 10 (default) and the MAC-EQS was set to 10.74 mg/L. All proposals of MAC-EQS are summarized in table 4.

Table 3. Results from the HC5 estimation for nitrate for acute ecotoxicity studies.

Type of HC5	Value (mg/L)	log10 (Value) (mg/L)	Description
LL HC5	63.24	1.816039023	Lower estimate of the HC5
HC5	107.37	2.037330889	Median estimate of the HC5
UL HC5	159.98	2.204804384	Upper estimate of the HC5

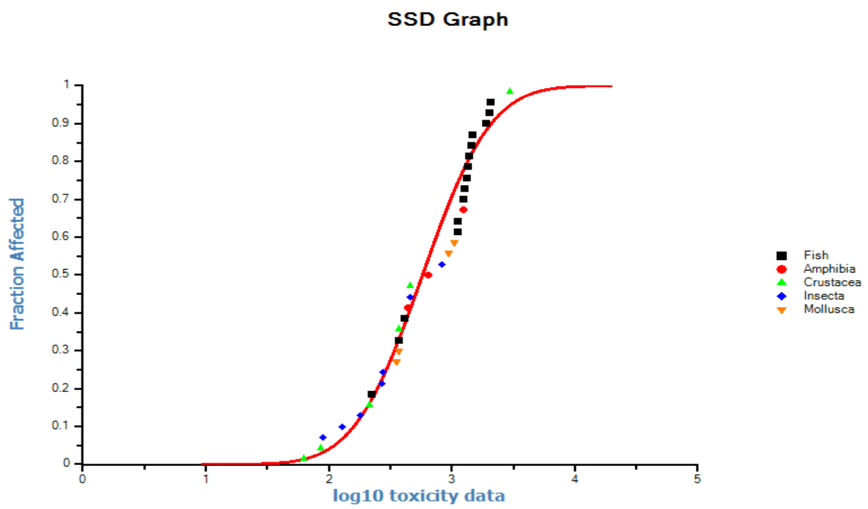

Figure 1. SSD (ETX 2.1) for acute freshwater toxicity of nitrate. The most sensitive species were the crustaceans *E. echinosetosus* and *E. toletanus,* and the insect *H. occidentalis*. The HC5 was 107.37 mg/L.

Table 4. Proposed MAC-EQS freshwater for nitrate using deterministic and probabilistic derivation.

Method (assessment factor, AF)	NO_3-N (mg/L)	Corresponding NO_3 (mg/L)
Deterministic derivation (AF 10)	6.3	27.4
Probabilistic derivation (AF 10)	10.7	46.7

6. CHRONIC FRESHWATER ECOTOXICITY

In total, 19 chronic ecotoxicity studies with 61 effect values were found in the literature search (table S2). The most sensitive endpoints and species were developmental delay in early life stage of the fish *Salvelinus namaycush* (McGurk et al. 2006) and growth and survival of juveniles of the fish *Galaxias maculatus* (Hickey et al. 2013). The most sensitive invertebrates were *Neocleon triangulifer* (Soucek and Dickinson, 2015) and *C. dubia* (Scott and Crunkilton, 2000) with the endpoint development delay and reproduction, respectively. The studies showing lowest effect values were conducted in water with hardness ≤16 mg CaCO3/L, but there are no mechanisms explaining why hardness would influence the toxicity of nitrate.

The studies above showed unclearness regarding their reliability and relevance for this assessment (see Supportive information - Comments on Reliability and Relevance). The study showing highest reliability (it was possible to determine a dose-response of an ecological relevant endpoint and low NH_3-N concentrations in the system) was *Xenopus laevis* (embryos) with a 5d NOEC of 24.8 mg/L (Schuytema and Nebeker 1999b). This study was used as the critical data in the deterministic derivation. It should be noted, however, that the effect (LOEC) was seen at 56.7 mg/L which is considered as non-realistic environment concentration.

A probabilistic derivation was also conducted to compare outcomes (table 5). This derivation includes the lowest effect values, regardless of reliability and relevance evaluation[2]. However, the study from McGurk et al. (2006) (NOEC of 1.6 mg/L) was excluded (see justification in Supportive information - Comments on Reliability and Relevance).

An added risk approach was also undertaken. Added effect values (e.g. $NOEC_{added}$) were calculated by subtracting the NO_3-N concentration used in the control medium from the effect value (European Communities, 2011). Data used in the derivation are presented in table 5 and includes three orders of fish and crustacean, and one order of amphibian, insecta and mollusca (a total of 14 species).

[2] Not possible to use the probabilistic approach (European Communities, 2011) when eliminating studies assessed as not reliable or not relevant.

Table 5. Chronic freshwater ecotoxicity data for nitrate (as NO_3-N) used in the probabilistic derivation of AA-EQS for freshwater (na= not available).

Species (life stage)	Hardness (mg $CaCO_3$/L)	Endpoint & Duration		Effect value (mg NO_3-N/L)	Effect $value_{added}$	Evaluation	Reference
Fish							
Galaxias maculatus (Juvenile)	14-39	Weight, Survival	40d NOEC	11.20	10.22	R2-3/C2	Hickey et al. 2013
Notropis Topeka (juvenile)	210-230	Growth	30d NOEC	268	267.25	R2/C2	Adelman et al. 2009
Oncorhynchus mykiss (early life stage)	40	Length	42d NOEC	99	98.65	R2/C2	Hickey et al. 2013
Pimephales promelas (embryo-larvae)	132-180	Growth, Survival	32d EC_{10}	49	48,59[a]	R2/C2	US EPA 2010
Amphibians							
Pseudacris regilla (embryos)	75	Growth	10d NOEC	56.7	56.6	R2/C2	Schuytema and Nebeker 1999b
Rana aurora (embryo/Larvae)	25.5	Weight	16d NOEC	116.8	116.2	R2/C2	Schuytema and Nebeker 1999c
Xenopus laevis (embryos)	36.2	Weight	5d NOEC	24.8	24.7	R2/C2	Schuytema and Nebeker 1999b
Invertebrates – Crustacean							
Austropotamobius italicus (juvenile)	na	Food consumption	14d NOEC	100	99.33	R2/C4	Benítez-Mora et al. 2014
Ceriodaphnia dubia	150-184	Reproduction	7d NOEC	21.3	19.1	R2-3/C2	Scott and Crunkilton 2000
Daphnia magna	150-184	Reproduction	7d NOEC	358	355.8	R2-3/C2	Scott and Crunkilton 2000
Gammarus pseudolimnaeus	na	Growth	21d NOEC	128	127.64	R2/C2	Stelzer and Joachim, 2010
Macrobrachium rosenbergii (larvae)	na	Development, weight	16d NOEC	180	179.59[a]	R4/C2	Mallasen et al. 2008
Invertebrates – Insecta							
Neocleon triangulifer (nymph)	99	% Pre-emergent nymph	30d NOEC[b]	26	25.97	R2/C2	Soucek and Dickinson 2015
Invertebrates – Mollusca							
Potamopyrgus antipodarum	92	Behaviour-velocity (No of live newborn)	35d NOEC (35d LOEC)	21.4	20.99[a]	R2/C4	Alonso and Camargo, 2013

a = Concentrations in test medium was not given, the control medium concentration was estimated based on the geometric mean of all reported test medium concentrations (0.41 mg/L.)
b = NOEC was not reported, the concentration below the significant concentration was set as NOEC (MATC=36, LOEC=51).

6.1 Deterministic derivation

The *Xenopus laevis* (embryos) with a 5d NOEC of 24.8 mg/L (Schuytema and Nebeker 1999b) for the endpoint reduced weight was used as the critical data in the derivation. The lowest possible AF of 10 was used according to European Communities (2011), giving an AA-EQS of 2.5 mg/L.

6.2 Probabilistic derivation

Data from the following taxonomic groups were needed to perform a SSD: two chordata (e.g. fish and amphibians), crustacea, insecta, a phylum other than arthropoda or chrodata (e.g. rotifer, annelida, mollusca), an additional family in any order of insect or any phylum not already included, algae and higher plants (European Communities 2011). The dataset for nitrate lacked one additional phylum or an additional order of insects (and algae and higher plants). However, there was supporting information of an additional insect order (Lepidoptera) in the study by Baker et al. (2016) suggesting lower or equal sensitivity of that order (10d IC_{25} = 48.8 mg/L) compared to the order Ephemeroptera (table S2). This data was not used in the derivation since the effect value was reported as IC_{25}. Despite the lack of data, a SSD was preformed (figure 2). Normal distribution was accepted at all significance level in all tests. The result of the median HC5 (no-effect concentration for 5% of the species) was 10.92 and 10.34 mg/L for NO_3-N and NO_3-N_{added}, respectively (table 6). AF 5 (default) was applied and the AA-EQS and AA-EQS_{added} was set to 2.2 and 2.1 mg/L, respectively. A lower AF would be justified to avoid EQS below background levels (European Communities, 2011). The proposed AA-EQSs for freshwater are summarized in table 7.

Table 6. The results from the HC5 estimation for nitrate as NO_3-N and NO_3-N_{added}.

Type of HC5	NO_3-N (mg/L)	NO_3-N_{added} (mg/L)	Description
LL HC5	4.08	3.79	Lower estimate of the HC5
HC5	10.92	10.34	Median estimate of the HC5
UL HC5	20.39	19.45	Upper estimate of the HC5
SprHC5	4.97	4.14	Spread of the HC5 estimate

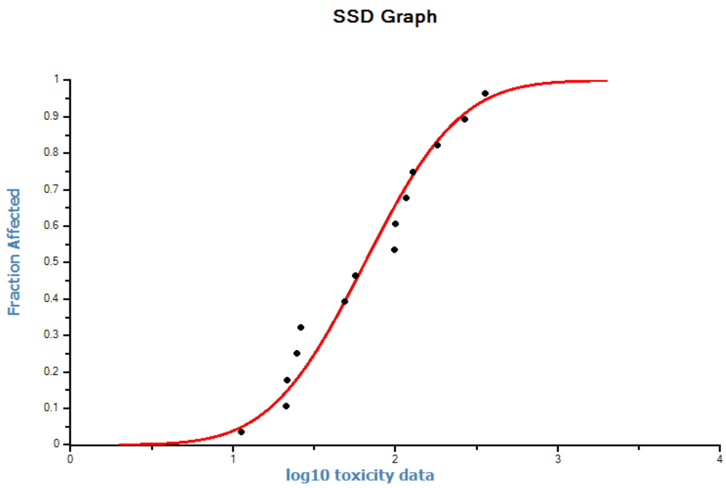

Figure 2. SSD (ETX 2.1) for chronic freshwater toxicity studies of nitrate (NO_3-N). The most sensitive species were the fish. *G. maculatus*. The HC5 was 10.92 mg/L.

Table 7. Proposals of AA-EQS freshwater for nitrate.

Method (Assessment factor, AF)	NO_3-N (mg/L)	Corresponding NO_3 (mg/L)
Deterministic (AF 10)	2.5	10.8
Probabilistic (AF 5)[a]	2.2	9.6
Probabilistic added risk (AF 5)	2.1	9.1

a = When results from McGurk et al. (2006) (*S. namaycush* with NOEC of 1.6 mg/L) and effect values only from hardness 14 mg $CaCO_3$/L in Hickey et al. (2013) (NOEC of 6 mg/L) were included the AA-EQS_{fw} (as worst-case data) was 0.7 NO_3-N mg/L, corresponding to 3.01 NO_3 mg/L using AF 5 (i.e. probably below background concentrations).

7. ACUTE MARINE TOXICITY

7.1 Deterministic derivation

The acute marine toxicity dataset includes data for fish, *Crustacean* and *Mollusca* (table 8). The study with lowest acute toxicity was Pierce et al. (1993) using *Monacanthus hispidus* resulting in a LC_{50} of 572 mg/L. According to European Communities (2011), AF 50 should be applied since the dataset include acute data for three trophic levels (in this case only two due to the absence of algae), one additional specific marine taxonomic group, and if the standard deviation of the data for all species is not higher than a factor of 3 in both directions. Using AF 50 results in a $MAC\text{-}EQS_{sw}$ of 11.44 mg/L (table 9).

7.2 Probabilistic derivation

The dataset does not fulfil the criteria for probabilistic derivation (European Communities, 2011).

Table 8. All acute marine toxicity data for nitrate (as NO_3-N).

Species (life stage)	Order	Endpoint & Duration	Effect value (mg NO_3-N/L)	Reference
Fish				
Centropristis striata	Perciformes	96h LC_{50}	2400	Pierce et al. 1993
Diplodus saegus (fingerlings)	Perciformes	24h LC_{50}	3560	Brownell 1980
Heteromycteris capensis	Pleuronectiformes	24h LC_{50}	5050	Brownell 1980
Lithognathus mormyrus (fingerlings)	Perciformes	24h LC_{50}	3450	Brownell 1980
Monacanthus hispidus	Tetraodontiformes	96h LC_{50}	573	Pierce et al. 1993
Pomacentrus leucostictus	Perciformes	96h LC_{50}	>3000	Pierce et al. 1993
Rachycentron canadum (juvenile)	Perciformes	24h LC_{50}	2407	Rodrigues et al. 2011
Rachycentron canadum (juvenile)	Perciformes	96h LC_{50}	1829	Rodrigues et al. 2011
Raja eglanteria	Rajiformes	96h LC_{50}	>960	Pierce et al. 1993
Trachinotus carolinus	Perciformes	96h LC_{50}	1000	Pierce et al. 1993
Invertebrates – Crustacean				
Penaeus monodon (juvenile)	Decapoda	96h LC_{50}	1575	Tsai and Chen 2002
Penaeus aztecus	Decapoda	48h LC_{50}	3400	Wickins 1976
Penaeus japonicu	Decapoda	48h LC_{50}	3400	Wickins 1976
Penaeus occidentalis	Decapoda	48h LC_{50}	3400	Wickins 1976
Penaeus orientalis	Decapoda	48h LC_{50}	3400	Wickins 1976
Penaeus setiferus	Decapoda	48h LC_{50}	3400	Wickins 1976
Portunus pelagicus (juvenile)	Decapoda	96h LC_{50}	2449	Romano and Zeng 2007
Scylla serrata (juvenile)	Decapoda	96h LC_{50}	2629	Romano and Zeng 2007
Invertebrates - Mollusca				
Argopecten irradians (juvenile)	Ostreoida	96h LC_{50}	4453.4	Widman et al. 2008
Crossostrea virginica (juvenile)	Ostreoida	96h LC_{50}[a]	3794	Epifano and Srna 1975

a = Reported as TLm.

Table 9. Proposal of MAC-EQS saltwater for nitrate.

Method (Assessment factor, AF)	**NO_3-N (mg/L)**	**Corresponding NO_3 (mg/L)**
Deterministic (AF 50)	11.4	49.7

8. CHRONIC MARINE TOXICITY

8.1 Deterministic derivation

The chronic dataset includes fish, crustacean, mollusca and echinodermata (table 10). The study showing lowest effect values was Basuyaux and Mathieu (1999) using *Paracentrotus lividus* and *Haliotis tuberculata* with NOEC of 100 mg/L for the endpoint growth. According to European Communities (2011), AF 10 should be applied since the dataset include chronic data for three trophic levels (in this case only two due to the absence of algae), and two additional specific saltwater taxonomic groups. The AA-EQS was set to 10 mg/L (table 11).

8.2 Probabilistic derivation

The dataset does not fulfil the criteria for a probabilistic derivation (European Communities, 2011).

Table 10. All chronic marine toxicity data for nitrate (as NO_3-N).

Species (life stage)	**Order**	**Endpoint & Duration**		**Effect value (mg NO_3-N/L)**	**Reference**
Fish					
Psetta maxima (juvenile)	Pleuronectioformes	Condition factor, Survival, Food consumption and Splenic index	42d NOEC	125[a]	Bussel et al. 2012
Invertebrates - Crustacean					
Farfantepenaeus paulensis (juvenile)	Decapoda	Growth (weight) and biomass	30d LOEC	80.7[a]	Wasieleskya et al. 2016
Litopenaeus vannamei (juvenile)	Decapoda	Biomass and antennae length	42d NOEC	220	Kuhn et al. 2010
Litopenaeus vannamei (juvenile)	Decapoda	Survival and growth	42d NOEC	435	Kuhn et al. 2010
Invertebrates - Mollusca					
Haliotis tuberculata	Archaeogastropoda	Growth	15d NOEC	100	Basuyaux and Mathieu 1999
Invertebrates - Echinodermata					
Paracentrotus lividus	Echinoida	Growth	15d NOEC	100[b]	Basuyaux and Mathieu 1999

a = Not used in the derivation since LOEC (NOEC should be used according to European Communities 2011).
b = NOEC was not reported, the concentration below the significant concentration was set as NOEC.

Table 11. Proposal of AA-EQS saltwater for nitrate.

Method (Assessment factor, AF)	**NO_3-N (mg/L)**	**Corresponding NO_3 (mg/L)**
Deterministic (AF 10)	10.0	44.3

9. EXISTING WATER QUALITY GUIDELINES

New Zealand National Institute of Water and Atmospheric Research (NIWA) and The Canadian Council of Ministers of the Environment (CCME) revised their water quality guidelines for nitrate in 2013 (chronic values revised 2016) and 2012, respectively. The chronic values represent 95% protection. Their quality guidelines are compared to the proposals presented in this report in table 12.

Table 12. Comparison between the EQS values proposed in this report and the water quality guidelines derived by the Canadian Council of Ministers of the Environment (CCME) and New Zealand National Institute of Water and Atmospheric Research (NIWA).

	Freshwater NO_3-N (mg/L)		**Marine water NO_3-N (mg/L)**		**Comments**
	Chronic	**Acute**	**Chronic**	**Acute**	
CCME	3	124	45	339	Derived based on SSD. No AF applied on HC5. Includes IC25 values. Recalculates values to MATC (the geometric mean of NOEC and LOEC), which results in higher effect values. Critical studies used in the derivation for acute freshwater was Camargo and Ward 1995 (*Hydropsyche occidentalis*), chronic freshwater was McGurk et al. 2006, NOEC of 1.6 mg/L (MATC= 3,22), acute marine: *Strongylocentrotus purpuratus* (study not available for this derivation) and chronic marine: *Nereis grubei* (study not available for this derivation) (CCME 2012).
NIWA	2.1	20	-	-	Derived based on SSD. No AF applied on HC5. Includes values that showed no effect in the study (e.g. NOEC>x). Includes IC25 values. Critical study used in the chronic derivation is McGurk et al. 2006, NOEC of 1.6 mg/L. Includes long-term studies with endpoint survival. Used a chronic EC50 value as the critical acute data (54.9 mg/L) (NIWA 2013).
The present EQS proposals	2.5; 2.2; 2.1_{added}	10.7	10.0	11.5	Proposals based on deterministic and probabilistic (chronic freshwater) derivation.

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

Uncertainties related to MAC-EQS$_{fw}$, MAC-EQS$_{sw}$ and AA-EQS$_{sw}$

None of the ecotoxicity studies used in the derivation of MAC-QS$_{fw}$, MAC-QS$_{sw}$ or AA-QS$_{sw}$ have been evaluated for their reliability or relevance. However, the derived QSs indicate that the threshold level (11.5 mg NO_3-N/L) given in the Nitrate Directive would be reasonably protective against ecotoxicological effects.

Uncertainties related to AA-EQS$_{fw}$

The early life-stage studies with fish showed highest sensitivity to nitrate (Hickey et al. 2013; Nautilus Environment, 2012; McGurk et al. 2006). McGurk et al. (2011) and Nautilus Environment (2012) observed a small delay of yolk sac absorption of *Salvelinus namaycush* in very soft water (10 mg $CaCO_3$/L). The results were not included in the derivations since then endpoint is not directly linked to effects at population level. Also, inclusion would generate an AA-QS$_{fw}$ possibly below background levels. Likewise, results from Hickey et al. (2013) was excluded in the deterministic derivation due to elevated ammonia concentrations (although it was not possible to draw any conclusion about actual contribution of ammonia toxicity). It is not possible to rule out potential toxicity to early life-stages of fish, especially under conditions with soft water. However, the proposed AA-EQSs for freshwater (2.1-2.5 mg/L) are below the LOEC of 6.25 mg/L reported in McGurk et al. (2006).

Uncertainties related to tested concentrations in ecotoxicity studies

All proposed EQS are derived from effect values exceeding realistic environmental concentrations (which is lowering the relevance category from C1 to C2). The lowest available LOELs were 20 and 56.7 mg NO_3-N/L for chronic freshwater ecotoxicity, this can be compared to the Swedish measurements (table 1) that rarely exceed 9.2 mg NO_3-N/L (three stations with maximum concentrations of 9.2-11.5 and one station with average concentration of 4.6- 9.2 NO_3-N/L).

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12. SUPPORTIVE INFORMATION - Ecotoxicity studies

Table S1. Acute freshwater ecotoxicity studies for nitrate (as NO_3-N) (na= not available).

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration	Effect value (mg NO_3-N/L)	Reference
Fish								
Acipenser baeri	Acipenseriformes	21-23.5	Sat ≥ 95%	7.9	260	96h LC_{50}	374	Hamlin 2006
Carassius auratus	Cypriniformes	na	na	na	na	24h LC_{50}	2040	Dowden and Bennett 1965
Coregonus clupeaformis	Salmoniformes	7-8	10.4-12.5	6-7.4	10-16	96h LC_{50}	1903	McGurk et al. 2006
Cyprinus carpio	Cypriniformes	na	na	na	na	24h LC_{50}	247	Tilak et al., 2007
Cyprinus carpio	Cypriniformes	na	na	na	na	24h LC_{50}	223	Tilak et al., 2007
Cyprinus carpio	Cypriniformes					24h LC_{50}	234.7	Geometric mean
Danio rerio (Embryos)	Cypriniformes	28	Sat ≥85%	8.1-8.3	na	96h LC_{50}	1606	Learmonth and Carvalho (2015)
Danio rerio (Larvae)	Cypriniformes	28	Sat≥85%	8.1-8.3	na	96h LC_{50}	1987	Learmonth and Carvalho (2015)
Danio rerio (Swim-up larvae)	Cypriniformes	28	Sat ≥85 %	8.1-8.3	na	96h LC_{50}	1250	Learmonth and Carvalho (2015)
Galaxias maculatus (Juvenile)	Osmeriformes	15	9.1-10.6	6.6-7.9	14	96h EC_{50}	3171	Hickey et al. 2013
Galaxias maculatus (Juvenile)	Osmeriformes	15	9.1-10.6	6.6-7.9	39	96h EC_{50}	1378	Hickey et al. 2013
Galaxias maculatus (Juvenile)	Osmeriformes	15	9.1-10.6	6.6-7.9	100	96h EC_{50}	1426	Hickey et al. 2013
Galaxias maculatus (Juvenile)	Osmeriformes	15	9.1-10.6	6.6-7.9	14-100	96h EC_{50}	1840.2	Geometric mean
Gambusia holbrooki (juvenile)	Cyprinodontiformes	19-20	na	7.0-7.3	na	96h LC_{50}	1116.5	Wallen et al. 1957
Gobiocypris rarus (larvae)	Cypriniformes	na	na	na	na	3d NOEC	20	Luo et al., 2016
Gobiocypris rarus (larvae)	Cypriniformes	na	na	na	na	3d NOEC	99.8	Luo et al., 2016
Gobiocypris rarus (larvae)	Cypriniformes	na	na	na	na	3d NOEC	176	Luo et al., 2016
Ictalurus punctatus (fingerlings)	Siluriformes	22-30	na	8.6-8.8	102	96h LC_{50}	1426	Colt and Tchobanoglous 1976
Lepomis macrochirus (fingerlings)	Perciformes	22	4.8-8.3	7.5-8.4	45-50	96h LC_{50}	1975	Trama 1954

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration	Effect value (mg NO_3-N/L)	Reference
Lepomis macrochirus (fingerlings)	Perciformes	na	na	na	na	24h LC_{50}	2149	Dowden and Bennett 1965
Lepomis macrochirus (fingerlings)	Perciformes					24h LC_{50}	2060.2	Geometric mean
Micropterus treculi (fry)	Perciformes	22	na	7.9-8.4	310	96h LC_{50}	1261	Tomasson and Carmichael 1976
Notropis topeka (Juvenile)	Cypriniformes	23.9	>6	8.1-8.3	210-230	96h LC_{50}	1354	Adelman et al 2009
Oncorhynchus mykiss	Salmoniformes	15	na	6.9-7.3	11	96h LC_{50}	808	Baker et al. 2016
Oncorhynchus mykiss	Salmoniformes	15	na	7.3-7.7	54	96h LC_{50}	1446	Baker et al. 2016
Oncorhynchus mykiss	Salmoniformes	15	na	7.7-8	90	96h LC_{50}	1958	Baker et al. 2016
Oncorhynchus mykiss	Salmoniformes	15	na	7.6-7.9	164	96h LC_{50}	1913	Baker et al. 2016
Oncorhynchus mykiss (Juvenile)	Salmoniformes	12	Sat 66%	7.4-7.8	40-42	96h LC_{50}	1658	Buhl and Hamilton 2000
Oncorhynchus mykiss	Salmoniformes	13-17	na	na	na	96h LC_{50}	1380[a]	Westin 1974
Oncorhynchus mykiss	Salmoniformes				11-164	96h LC_{50}	1468	Geometric mean
Oncorhynchus tshawytscha (Fingerlings)	Salmoniformes	13-17	na	na	na	96h LC_{50}	1310[a]	Westin 1974
Pimephales promelas (Larvae)	Cypriniformes	25	7.9-8.3	7.9-8.3	156-172	96h LC_{50}	1341	Scott and Crunkilton 2000
Pimephales promelas (larvae)	Cypriniformes	24.2-25	5.8-8.4	7.2-8.1	90-92	96h LC_{50}	415	US EPA 2010
Pimephales promelas (adults)	Cypriniformes	23	5.5	8.2-8.3	na	96h LC_{50}	1559	Adelman et al. 2009
Pimephales promelas (juvenile)	Cypriniformes	23	5.5	8.2-8.3	na	96h LC_{50}	1354	Adelman et al. 2009
Pimephales promelas	Cypriniformes	25	na	6.9-7.3	12	7d LC_{50}	117	Baker et al. 2016
Pimephales promelas	Cypriniformes	25	na	7.3-7.7	50	7d LC_{50}	235	Baker et al. 2016
Pimephales promelas	Cypriniformes	25	na	7.7-8	94	7d LC_{50}	415	Baker et al. 2016
Pimephales promelas	Cypriniformes	25	na	7.6-7.9	168	7d LC_{50}	465	Baker et al. 2016
Pimephales promelas	Cypriniformes				12-168	7d LC_{50}	269.9	Geometric mean (Baker et al. 2016)
Salvelinus namaycush	Salmoniformes	7-8	10.4-12.5	6-7.4	10-16	96h LC_{50}	1121	McGurk et al. 2006
Amphibians								

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration	Effect value (mg NO_3-N/L)	Reference
Hypsiboas faber (embryo-larval)	Anura	na	na	na	na	48h LC_{50}	1245.4	Bellezi et al. 2015
Pseudacris regilla (Tadpoles)	Anura	22	7.2	7.0-7.6	58.4	96h LC_{50}	1749.8	Schuytema and Nebeker 1999a
Pseudacris regilla (embryos)	Anura	22	7.2	6.7	76.0	96h LC_{50}	643	Schuytema and Nebeker 1999b
Xenopus laevis (Tadpoles)	Anura	22	7.6	7	20.6	96h LC_{50}	1655.8	Schuytema and Nebeker 1999a
Xenopus laevis (embryos)	Anura	22	7.6	7	36.2	120h LC_{50}	438.4	Schuytema and Nebeker 1999b
Invertebrates – Crustacean								
Austropotamobius italicus	Decapoda	19	9.5	8.1	na	96h LC_{50}	2950	Benítez-Mora et al., 2014
Ceriodaphnia dubia (neonates)	Cladocera	25	7.9-8.3	7.9-8.3	na	48h LC_{50}	374	Scott and Crunkilton 2000
Daphnia magna (neonates)	Cladocera	25	7.9-8.3	7.9-8.3	na	48h LC_{50}	462	Scott and Crunkilton 2000
Eulimnogammarus toletanus (adults)	Amphiphods	17.9	7.7	7.8	293	96h LC_{50}	85	Camargo et al. 2005
Echinogammarus echinosetosus (adults)	Amphiphods	17.9	7.7	7.8	293	96h LC_{50}	63	Camargo et al. 2005
Hyalella azteca	Amphiphods	23	na	7.4-7.7	44	96h LC_{50}	168	Baker et al. 2016
Hyalella azteca	Amphiphods	23	na	7.7-8	100	96h LC_{50}	485	Baker et al. 2016
Hyalella azteca	Amphiphods	23	na	8-8.2	164	96h LC_{50}	921	Baker et al. 2016
Hyalella azteca (juveniles)	Amphiphods	22.5	8.1	8.0	117	96h LC_{50}	667	Soucek and Dickinson 2012
Hyalella azteca (juveniles)	Amphiphods	23	Sat >80%	7.8-8.3	80-84	96h LC_{50}	16.4	US EPA 2010
Hyalella azteca (adults)	Amphiphods	23	7.3	7.4	61.6	96h LC_{50} (food)	14.5	Pandey et al. 2011
Hyalella azteca (adults)	Amphiphods	23	7.3	7.4	61.6	96h (no food) LC_{50}	124.2	Pandey et al. 2011
Hyalella azteca (adults)	Amphiphods				44-164	96h LC_{50}	215.2	Geometric mean
Invertebrates – Insecta								
Allocapnia vivipara (juveniles)	Plecoptera	11	10.3	7.9	99	96h LC_{50}	836	Soucek and Dickinson 2012

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration	Effect value (mg NO_3-N/L)	Reference
Amphinemura delosa (juveniles)	Plecoptera	11.9-12.8	8.8-10	7.8-8	88-92	96h LC_{50}	456	US EPA 2010, Soucek and Dickinson 2012
Cheumatopsyche pettiti (last instar larvae)	Trichoptera	18	9.6	7.9	42.7	96h LC_{50}	153.8	Camargo and Ward 1995
Cheumatopsyche pettiti (early instar larvae)	Trichoptera	18	9.6	7.9	42.7	96h LC_{50}	128.3	Camargo and Ward 1995
Chironomus dilutus	Diptera	23	7.5-8.3	7.8-8	84-136	48h LC_{50}	278	US EPA 2010
Hydropsyche exocellata (last instar larvae)	Trichoptera	18	9.6	7.9	293	96h LC_{50}	270	Camargo and Ward 2005
Hydropsyche occidentalis (early instar larvae)	Trichoptera	18	9.6	7.9	42.7	96h LC_{50}	90.4	Camargo and Ward 1995
Hydropsyche occidentalis (last instar larvae)	Trichoptera	18	9.6	7.9	42.7	96h LC_{50}	105.2	Camargo and Ward 1995
Neocleon triangulifer (nymph)	Ephemeroptera	24.7	7.8	8.3	99	96h LC_{50}	179	Soucek and Dickinson 2015
Invertebrates – Mollusca								
Lampsilis siliquoidea (juveniles)	Unionoida	19.8-20.1	7.7-8.1	7.9-8	91	96h LC_{50}	357	US EPA 2010,Soucek and Dickinson 2012
Megalonaias nervosa (juveniles)	Unionoida	20.8-20.9	7.9-8.4	7.8-8.2	91	96h LC_{50}	937	US EPA 2010, Soucek and Dickinson 2012
Potamopyrgus antipodarum	Littorinimorpha	20.4	6.7	8.3	na	96h LC_{50}	1042	Alonso and Camargo 2003
	Veneroida	22.5-23	4.5-8.3	7.8-8.1	91	96h LC_{50}	371	US EPA 2010,Soucek and Dickinson 2012

a = Reported as TLm.

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

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration		Effect value (mg NO_3-N /L)	Reference
Fish									
Coregonus clupeaformis (embryo, fry, alevin)	Salmoniformes	7-8	10.4-12.5	6-7.4	10-16	Development delay	146d NOEC	6.3	McGurk et al. 2006
Danio rerio (larvae)	Cypriniformes	28	Sat≥ 85%	8.1-8.3	na	Weight/ Survival	23d NOEC	200	Learmonth and Carvalho (2015)
Galaxias maculatus (Juvenile)	Osmeriformes	14.4-15.1	9.8-10.4	6.8-7.9	14	Weight / Survival	40d NOEC	6	Hickey et al. 2013
Galaxias maculatus (Juvenile)	Osmeriformes	14.4-15.1	9.8-10.4	6.8-7.9	14	Weight/ Survival	40d LOEC	20	Hickey et al. 2013
Galaxias maculatus (Juvenile)	Osmeriformes	14-15.6	10.2-11.2	7.3-7.8	39	Weight/ Survival	40d NOEC	20.9	Hickey et al. 2013
Galaxias maculatus (Juvenile)	Osmeriformes	14-15.6	10.2-11.2	7.3-7.8	39	Weight/ Survival	40d LOEC	108	Hickey et al. 2013
Galaxias maculatus (sub-adults)	Osmeriformes	15	na	7.5-7.6	40	Growth/Survival	31d NOEC	>103	Martin and Thompson 2012
Notropis topeka (juvenile)	Cypriniformes	23.4	>6	8.2-8.3	210-230	Growth	30d NOEC	268	Adelman et al. 2009
Oncorhynchus mykiss (early life stage)	Salmoniformes	13.7-14	10.4-10.6	7.6-7.7	39	Length and Yolk	42d NOEC	99	Hickey et al. 2013
Oncorhynchus mykiss (early life stage)	Salmoniformes	13.7-14	10.4-10.6	7.6-7.7	39	Survival/Hatching	42d NOEC	389	Hickey et al. 2013
Pimephales promelas	Cypriniformes	25	na	6.9-7.3	12	Growth	7d IC_{25}	69.6	Baker et al. 2016
Pimephales promelas	Cypriniformes	25	na	7.3-7.7	50	Growth	7d IC_{25}	209	Baker et al. 2016
Pimephales promelas	Cypriniformes	25	na	7.7-8	94	Growth	7d IC_{25}	358	Baker et al. 2016
Pimephales promelas	Cypriniformes	25	na	7.6-7.9	168	Growth	7d IC_{25}	402	Baker et al. 2016
Pimephales promelas (larvae)	Cypriniformes	25	6.6	7.3-8.3	152-200	Growth	7d NOEC	358	Scott and Crunkilton et al. 2000
Pimephales promelas (embryos and larvae)	Cypriniformes	25	6.6	7.3-8.3	152-200	Growth/Survival	11d NOEC	358	Scott and Crunkilton et al. 2000
Pimephales promelas (breeding adults and offspring)	Cypriniformes	25	6.6	7.3-8.3	152-200	Growth/Survival	7d NOEC	>1434	Scott and Crunkilton et al. 2000

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration		Effect value (mg NO_3-N /L)	Reference
Pimephales promelas (juvenile)	Cypriniformes	23.1	5.5	8.2-8.3	210-230	Growth	30d NOEC	58	Adelman et al. 2009
Pimephales promelas (juvenile)	Cypriniformes	23.1	5.5	8.2-8.3	210-230	Growth	30d LOEC	121	Adelman et al. 2009
Pimephales promelas (embryo-larvae)	Cypriniformes	23.1	5.5	8.2-8.3	210-230	Weight	30d NOEC	157	Adelman et al. 2009
Pimephales promelas (embryo-larvae)	Cypriniformes	24.7-25.3	7.2-7.9	8-8.3	132-180	Growth/Survival	32d EC_{10}	49	US EPA 2010
Salvelinus namaycush (fry)	Salmoniformes	7.5	10.4-12.5	6-7.4	10-16	Developmental delay / Weight	132d NOEC	1.6	McGurk et al. 2006
Salvelinus namaycush (fry)	Salmoniformes	7.5	10.4-12.5	6-7.4	10-16	Developmental delay/ Weight	132d LOEC	6.25	McGurk et al. 2006
Salvelinus namaycush (fry)	Salmoniformes	7.5	10.4-12.5	6-7.4	10-16	Length	132d LOEC	25	McGurk et al. 2006
Salvelinus namaycush (alevin)	Salmoniformes	7.5	10.4-12.5	6-7.4	10-16	Hatching delay/ abnormal behaviours	132d NOEC	400	McGurk et al. 2006
Salvelinus namaycush (embryo, fry, alevin)	Salmoniformes	7	11-12.4	6.7-7.2	10	Growth	132d LC_{10}	>43.4	Nautilus Environment 2012
Salvelinus namaycush (embryo, fry, alevin)	Salmoniformes	7	11-12.4	7.2-7.6	80-100	Growth	132d LC_{10}	>329.8	Nautilus Environment 2012
Salvelinus namaycush (embryo, fry, alevin)	Salmoniformes	7	11-12.4	6.7-7.2	10	Swim up fry	132d LC_{10}	<2.9	Nautilus Environment 2012
Salvelinus namaycush (embryo, fry, alevin)	Salmoniformes	7	11-12.4	7.2-7.6	80-100	Swim up fry	132d LC_{10}	>329.8	Nautilus Environment 2012
Salvelinus namaycush (embryo, fry, alevin)	Salmoniformes	7	11-12.4	6.7-7.2	10	Delayed yolk sac absorption	132d NOEC	11[c]	Nautilus Environment 2012
Sander lucioperca (juvenile)	Perciformes	24	na	7.1-8	na	Growth (weight)	42d NOEC	>359	Schram et al. 2014
Amphibians									
Ambystoma jeffersonianum (eggs)	Urodela	5-10	na	na	na	Hatching success	23d NOEC	>9	Laposata and Dunson, 1998

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration		Effect value (mg NO_3-N /L)	Reference
Ambystoma maculatum (eggs)	Urodela	5-10	na	na	na	Hatching success	23d NOEC	>9	Laposata and Dunson, 1998
Bufo americanus (eggs)	Anura	5-10	na	na	na	Hatching success	23d NOEC	>9	Laposata and Dunson, 1998
Pseudacris regilla (tadpoles)	Anura	22	7.2	7-7.6	58.4	Weight	10d NOEC	<30	Schuytema and Nebeker 1999a
Pseudacris regilla (embryos)	Anura	22	7.6	6.7	75	Length and Weight	10d NOEC	56.7	Schuytema and Nebeker 1999b
Xenopus laevis (tadpoles)	Anura	22	7.2	6.7-7.6	20.6	Weight	10d NOEC	65.6	Schuytema and Nebeker 1999a
Rana aurora (embryo, Larvae)	Anura	15	8.7	6.8	25.5	Length	16d NOAC	<29.1	Schuytema and Nebeker 1999c
Rana aurora (embryo, Larvae)	Anura	15	8.7	6.8	25.5	Weight	16d NOEC	116.8	Schuytema and Nebeker 1999c
Rana sylvatica (eggs)	Anura	5-10	na	na	na	Hatching success	23d NOEC	>9	Laposata and Dunson, 1998
Xenopus laevis (embryos)	Anura	22	7.6	7	36.2	Weight	5d NOEC	24.8	Schuytema and Nebeker 1999b
Invertebrates – Crustacean									
Austropotamobius italicus (juvenile)	Decopoda	19	9.5	8.1	na	Food consumption	14d NOEC	100	Benítez-Mora et al. 2014
Ceriodaphnia dubia	Cladocera	25	na	7.6-7.9	44	Reproduction	7d IC_{25}	13.8	Baker et al. 2016
Ceriodaphnia dubia	Cladocera	25	na	8.1	98	Reproduction	7d IC_{25}	23.5	Baker et al. 2016
Ceriodaphnia dubia	Cladocera	25	na	8-8.4	166	Reproduction	7d IC_{25}	47.5	Baker et al. 2016
Ceriodaphnia dubia	Cladocera	25	8.2	7.5-8.6	150-184	Reproduction	7d NOEC	21.3	Scott and Crunkilton 2000
Daphnia magna	Cladocera	25	8.2	7.5-8.6	150-184	Reproduction	7d NOEC	358	Scott and Crunkilton 2000
Gammarus pseudolimnaeus	Amphipoda	15	na	8.55	na	Growth	21d NOEC	128	Stelzer and Joachim, 2010

Species (life stage)	Order	Temp C°	DO (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Endpoint & Duration		Effect value (mg NO_3-N /L)	Reference
Hyalella azteca	Amphipoda	23	na	7.4-7.7	46	Growth	14d IC_{25}	12.2	Baker et al. 2016
Hyalella azteca	Amphipoda	23	na	7.7-8	86	Growth	14d IC_{25}	116	Baker et al. 2016
Hyalella azteca	Amphipoda	23	na	8-8.2	172	Growth	14d IC_{25}	181	Baker et al. 2016
Macrobrachium rosenbergii (larvae)	Decopoda	30	6.0	8.0	na	Larvae development	16d NOEC	180	Mallasen et al. 2008
Invertebrates – Insecta									
Charaxes dilutus	Lepidoptera	23	na	7.4-7.7	46	Growth	10d IC_{25}	48.8	Baker et al. 2016
Charaxes dilutus	Lepidoptera	23	na	7.7-8	86	Growth	10d IC_{25}	102	Baker et al. 2016
Charaxes dilutus	Lepidoptera	23	na	8-8.2	172	Growth	10d IC_{25}	178	Baker et al. 2016
Neocleon triangulifer (nymph)	Ephemeroptera	25.2	7.5	8.3	99	% pre-emergent nymph; Nr of days to pre-emergent nymph	30d NOEC	26[c]	Soucek and Dickinson 2015
Invertebrates - Mollusca									
Potamopyrgus antipodarum	Littorinimorpha	18.5	7.6	8	92	Behaviour-velocity	35d NOEC	21.4	Alonso and Camargo, 2013
Potamopyrgus antipodarum	Littorinimorpha	18.5	7.6	8	92	Inactive snails	35d NOEC	>156.1	Alonso and Camargo, 2013
Potamopyrgus antipodarum	Littorinimorpha	18.5	7.6	8	92	Nr of live newborns	35d LOEC	21.4	Alonso and Camargo, 2013

a = Reported as TL. b = EC20 divided by 2. c = NOEC was not reported, the concentration below the statistically significant concentration was set as NOEC.

Table S3. Long-term ecotoxicity studies with endpoint survival for nitrate (as NO_3-N) (na= not available).

Species (life stage)	**Order**	**Hardness (mg $CaCO_3$/L)**	**Endpoint & Duration**	**Effect value (mg NO_3-N/L)**	**Reference**
Fish					
Coregonus clupeaformis (embryo, fry, alevin)	Salmoniformes	10-16	146d NOEC	25	McGurk et al. 2006
Danio rerio (Larvae)	Cypriniformes	107–142	29d NOEC	200	Learmonth and Carvalho 2015
Oncorhynchus mykiss	Salmoniformes	25	30d NOEC	1.15[c]	Kincheloe et al. 1979
Oncorhynchus tshawytscha (fry)	Salmoniformes	25	30d NOEC	2.3[c]	Kincheloe et al. 1979
Oncorhynchus tshawytscha	Salmoniformes	na	7d LC_{10}	1055[a]	Westin 1974
Pimephales promelas (larvae)	Cypriniformes	152-200	7d NOEC	717	Scott and Crunkilton et al. 2000
Pimephales promelas (larvae)	Cypriniformes	210-230	30d NOEC	157	Adelman et al. 2009
Salmo clarki (fry)	Salmoniformes	39	30d NOEC	4.6[b]	Kincheloe et al. 1979
Salmo clarki (egg)	Salmoniformes	39	30d NOEC	2[c]	Kincheloe et al. 1979
Salvelinus namaycush (embryo, fry, alevin)	Salmoniformes	10-16	146d NOEC	100	McGurk et al. 2006
Invertebrates – Crustacean					
Ceriodaphnia dubia	Cladocera	44	7d LC_{50}	62	Baker et al. 2016
Ceriodaphnia dubia	Cladocera	98	7d LC_{50}	120	Baker et al. 2016
Ceriodaphnia dubia	Cladocera	166	7d LC_{50}	127	Baker et al. 2016
Hyalella azteca	Amphipoda	46	14d LC_{50}	124	Baker et al. 2016
Hyalella azteca	Amphipoda	86	14d LC_{50}	275	Baker et al. 2016
Hyalella azteca	Amphipoda	172	14d LC_{50}	>622	Baker et al. 2016
Macrobrachium rosenbergii	Decopoda	na	21d LC_{50}	160	Wickins 1976
Invertebrates – Insecta					
Charaxes dilutus	Lepidoptera	46	10d LC_{50}	114	Baker et al. 2016
Charaxes dilutus	Lepidoptera	86	10d LC_{50}	222	Baker et al. 2016
Charaxes dilutus	Lepidoptera	172	10d LC_{50}	342	Baker et al. 2016
Deleatidium sp. (larvea)	Ephemeroptera	40	10d NOEC	18	Martin and Thompson 2012
Deleatidium sp. (larvea)	Ephemeroptera	40	20d EC_{50}	31	Martin and Thompson 2012
Neocleon triangulifer (nymph)	Ephemeroptera	99	30d NOEC	51	Soucek and Dickinson 2015
Invertebrates - Mollusca					
Pomacea paludosa (juvenile)	Architaenioglossa	na	14d EC_{50}	166	Carrao et al. 2006
Long-term marine toxicity studies with endpoint survival					
Farfantepenaeus paulensis (juvenile)	Decapoda	na	30d NOEC	323[c]	Wasieleskya et al. 2016

a = Reported as TL. b = NOEC was not reported, the concentration below the significant concentration was set as NOEC.

13. SUPPORTIVE INFORMATION - Reliability and relevance evaluations

Chronic Freshwater Ecotoxicity studies: General Comments

McGurk et al. 2006 - EPS guideline study (Evaluation result: R2 /C3)
The *Coregonus clupeaformis* was eliminated from the dataset due to poor survival in the control (according to CCME (2012) it may be due to high temperature). The S*alvelinus namaycush* study met validity criteria (survival of control and stability of experimental conditions) according to the guideline used (EPS 1/RM/28, 2nd edition). Dissolved oxygen was in the range of other studies found (varying between 10.4-12.5 mg/L), and the toxicity was therefore not assumed to be a result of elevated nitrite concentrations. Levels of ammonium was not reported thus, it was not possible to estimate the risk of ammonia toxicity.

In this *Salvelinus namaycush* study (conducted in hardness 10-16 mg CaCO3/L) fry showed reduced weight (NOEC 1.6 mg/L). The endpoint weight was not considered in the derivation for two reasons:

(i) The exposures in each test continued until the yolk was fully absorbed in all concentrations, consequently the exposure durations differed for the control and the concentrations (the fry that had lower wet weights had been reared for 8-28 days longer compared to the control fish).

(ii) No effect was seen on wet weight or length (IC_{10}> 43.4 mg/L) when the study was repeated (Nautilus Environment, 2012).

McGurk et al. (2006) demonstrated statistical significant delay of development in terms of days to fully absorb the yolk sac. In both controls (with hardness 10-16 and 136 mg CaCO3/L) and at concentration of 1.6 mg/L, swim-up (based on the number of days for >90% of trout larvae to reach the swim-up fry stage) occurred at day 120 and on day 128 at concentration 6.25 mg/L (LOEC) (with increased delay at higher concentrations indicating dose-response). The proportion of swim-up was only affected at the highest tested concentration of 400 mg/L. It is not clear if this endpoint (delayed yolk absorption) is considered adverse on a population level. McGurk et al. (2006) stated that the endpoint development delay (day of swim-up) was ecological relevant due to the increased time of early life stage, which is a vulnerable period of exposure to predators, but that the risk to the entire population was not necessary high. There was no effect on the remaining ten endpoints (surviving of different life stage, hatching and deformation) at concentrations below 100 mg/L.

The general weaknesses with the study:

- Standard error or confidential interval are not given so it was not possible to estimate statistical uncertainties related to the results.
- Exposure durations differed for the control and the tested concentrations.
- A spacing factor of approximately 4 was used between the concentrations. This is problematic because the distance between 1.6 and 6.0 mg/L, highly impact the derivation of AA-EQS. A general scaling factor of 3.2 is recommended (maximum factor of 10) (Moermond et al. 2016).

The *S. namaycush* study has been repeated (Nautilus Environment, 2012. See table S2) under same conditions as McGurk et al. (2006). Both studies provide data indicating that S. *namaycush* shows development delay in yolk sac absorption and swim-up. The result in Nautilus Environment (2012) was reported as scored yolk absorption (instead of number of days) with a NOEC of 11 mg/L. The proportion of swim-up at termination (132 days) was lower in all concentrations compared to the control with

LC_{10} and LC_{20} of <2.9 and 2.6 mg/L, respectively. However, these effect values (for proportion of swim-up) are not suitable for EQS-derivation since the effect (10% reduced swim-up at termination) occurred below the lowest concentration. In addition, since the study was terminated at day 132 it is not known how many days the swim-up was delayed (i.e. it is not possible to compare the results to McGurk et al. 2006).

Conclusion: It is not possible to estimate the potential effects at population level caused by delayed yolk adsorption and small delay of swim-up. Incorporation of McGurk et al. (2006) highly impact the derivation and would generate a low AA-EQS (possible below natural background levels) and consequently, difficulties when implemented in regulatory work. It is therefore not justified that this study should set the base for $AA\text{-}EQS_{fw}$.

Hickey et al. 2013 - OECD guideline study (Evaluation result: R1-3/C2)
This study investigated survival and growth of *Galaxias maculatus* (juveniles) in very soft (16 mg $CaCO_3$/L) and soft water (39 mg $CaCO_3$/L), and survival, hatching, growth and yolk development in early life-stage *of O. mykiss* (soft water). The study was well documented.

The *G. maculatus* study yielded a NOEC of 6 mg/L (11% weight reduction and 5.8% reduced survival) and LOEC of 20 mg/L (35% weight reduction and 20% reduced survival) in very soft water (16 mg $CaCO_3$/L). The NOEC and LOEC in soft water (39 mg $CaCO_3$/L) was 20 and 100 mg/L, respectively. The study met validity criteria (e.g. survival of control and stability of experimental conditions) according to OECD 215. Dissolved oxygen was in the range of other studies (9.1-10.6 mg/L), the toxicity was therefore not assumed to be a result of elevated nitrite concentrations due to low dissolved oxygen. Hickey et al. (2013) reported the ammonium-nitrogen (NH_4-N) concentrations which were used to calculate the ammonia-nitrogen (NH_3-N) concentrations and further investigate the risk of ammonia toxicity (table S4). All concentrations (including controls) were above the Swedish AA-EQS of NH_3-N (1.0 µg/L) and the LOEC of 20 mg/L (hardness 14 mg $CaCO_3$/L) had a maximum concentration of 7.7 µg/L. Increased ammonia-nitrogen concentration could explain the reduced survival. However, when comparing the NH_3-N concentrations from hardness of 14 with the 39 mg $CaCO_3$/L (and the *O. mykiss* study[3]), it was not possible to draw any conclusions about ammonia toxicity since these NH_3-N concentrations were greater i.e. not expected to cause less toxicity (e.g. see control NH_3-N concentrations).

[3] The *O. mykiss* study yielded statistically significant effects for growth (length) and yolk development, with a NOEC of 99 mg/L (no effect was seen on survival, hatching, or weight).

Table S4. Calculated ammonia-nitrogen (NH_3-N) concentrations at different NO_3-N concentrations in Hickey et al. (2013).

Nominal NO_3-N (mg/L)	**Hardness 14 (very soft)**		**Hardness 39 (soft)**	
	NH_3-N (µg/L) (mean)	**NH_3-N (µg/L) (max)**	**NH_3-N (µg/L) (mean)**	**NH_3-N (µg/L) (max)**
100 (LOEC hardness 39)	Not calculated	Not calculated	3.30	6.03
20 (LOEC hardness 14)	2.04	7.69	3.86	7.29
5	1.23	5.04	4.47	Not calculated
2	1.27	5.86	2.66	Not calculated
0 (control)	1.20	3.77	3.83	10.07

Scott and Crunkliton (2000) - Non-guideline study (Evaluation result: R2-3/C2)
Dose-response curve reported for *C. dubia* (NOEC and LOEC of 21.3 and 42.6 mg/L, respectively). Standard errors or confidential intervals for the chronic effect values for *P. promelas* or *D. magna* were not reported, thus it was not possible to estimate statistic uncertainties. Effect concentrations were above realistic environmental concentrations. Increased levels of ammonia (NH_3-N) with average of <1.0 mg/L (range 0–1.68 mg/L).

Soucek and Dickinson (2015) - Non- guideline study (Evaluation result: R2 /C2)
Ecotoxicity data reported for all concentrations. The response was statistically significant, although the effect for endpoint "percent pre-emergent nymph" was somewhat drastic with 100%, 60% and 0% at 26, 51 and 101 mg/L, respectively i.e. no clear dose-response due to 100% mortality at 101 mg/L. In addition, non-realistic environmental concentrations were used. Standard error or confidential interval were not reported for this endpoint. For endpoint "number of days to pre-emergent nymph" the effect was not assumed to be relevant on a population level since the delay was one day. NH_4-N and NH_3-N concentrations (pH 8.3 and 25°C) were not reported.

Schuytema and Nebeker (1999c) - ASTM guideline study (Evaluation result: R2/C2)
Toxicity data and standard errors reported for all concentrations. It was possible to determine dose-response relationship. Reduced weight was seen at 235 mg NO_3-N/L (LOEC) which is considered a non-realistic environmental concentration. Background NH_4-N concentrations in the well water ranged from 0.005 to 0.010 mg/L, which gives low NH_3-N concentrations of 0.002-0.004 µg/L (pH 6.2 and 15°C).

Schuytema and Nebeker (1999b) - ASTM guideline study (modifications) (Evaluation result: R2/C2)
Toxicity data and standard errors reported for all concentrations. It was possible to determine a clear dose-response relationship. Although, the effect (LOEC) occurred at 56.7 mg/L which is considered as a non-realistic environmental concentration. Did not report NH_4-N nor NH_3-N concentrations. Well water was collected at same locations as in Schuytema and Nebeker (1999c), using the same NH_4-N concentration gives NH_3-N of 0.02-0.05 µg/L (pH ≈7 and 22°C).

Alonso and Camargo (2013) - Non-guideline study (Evaluation result: R3/C3)
Concentrations of 44.9, 81.8 and 156.1 mg/L (non-realistic environmental concentrations) reduced the mean velocity of snails after 28 and 35 days (no permanent inactive snails) yielding a NOEC of 21.4 mg/L. However, this endpoint is not included in the current OECD guideline which is designed to assess effects on reproduction (i.e. difficult to predict ecological/population effects of this endpoint). Reproduction endpoints in the study investigated the mean number of live newborns which was significantly reduced in all concentrations compared to the controls (NOEC <21.4 mg/L). According to OECD 242 (*Potamopyrgus antipodarum* Reproduction Test) six replicates with six female snails are required for each concentration. In this study 12 replicates with one snail each was used (no information about gender). The endpoint in OECD 242 is based on the number of embryos (not number of live newborns). NH_4-N levels was <0.05 mg/L, giving NH_3-N of <1.7 µg/L.

Department of Environmental Science and Analytical Chemistry (ACES)

Stockholms universitet 106 91 Stockholm Tel 08-16 20 00
www.su.se info@su.se

Stockholm University