Aquatic Life Water Quality Standards Technical Support Document for Nitrate

Triennial Water Quality Standard Amendments to Minn. R. chs. 7050 and 7052

DRAFT For External Review, November 12, 2010

Minnesota Pollution Control Agency

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Authors
Phil Monson, M.S.

Contributors / acknowledgements
Angela Preimesberger, M.S.

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Minnesota Pollution Control Agency
520 Lafayette Road North | Saint Paul, MN 55155-4194 | www.pca.state.mn.us | 651-296-6300
Toll free 800-657-3864 | TTY 651-282-5332

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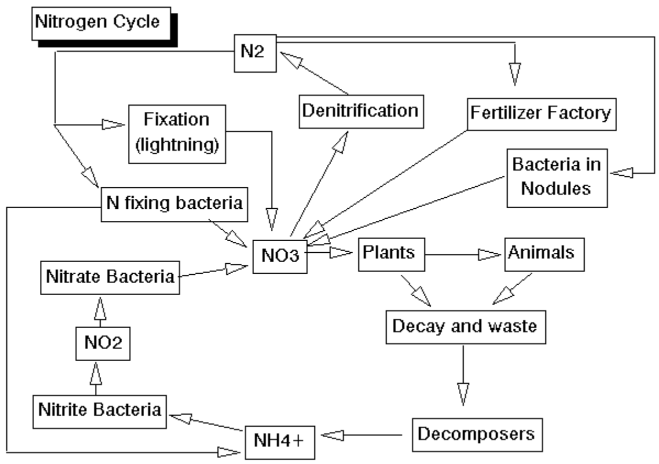
Introduction

Nitrate is formed as part of the breakdown of organic wastes, production by nitrogen-fixing plants, and through industrial production. Sources of excess nitrate in the environment can be linked to human activities on the landscape that result in the release of nitrogen to surface and ground waters. Nitrogen cycling in the environment results in nitrogenous compounds such as ammonia denitrifying into the more stable and conservative nitrate ion (NO_3).



Obtained from: www.marietta.edu/~biol/102/ecosystem.html

Concern regarding the toxicity of nitrate to aquatic organisms was brought to the attention of the MPCA from comments made during the preceding 2005-2008 rules revision by the Minnesota Center for Environmental Advocacy and concerns raised by the Minnesota Department of Natural Resources. The scientific literature has documented nitrate toxicity at concentrations that are environmentally relevant (Camargo and Alonso, 2006) to concentrations reported from Minnesota surface waters. In addition, the Minnesota State Legislature in 2010 approved funding for the MPCA to develop aquatic life standards for nitrogen and nitrate. Development of a nitrate standard is part of the effort to address these concerns. The MPCA is also engaged in developing a nitrogen budget for the state that focuses on total nitrogen in surface waters.

Natural sources of nitrate to surface waters in the state vary; however, when nitrate concentrations in surface water samples from "reference" areas (i.e., areas with relatively little human impact) are compared to samples from areas of greater human impact, the reference areas exhibit much lower nitrate concentrations. Nitrate concentrations in these reference areas are typically below 1 mg/L (Heiskary and Wilson, 2005). Still, elevated concentrations of nitrate have been documented in surface waters throughout the state. A comprehensive assessment of these data is beyond the scope of this document, but current trends in the data suggest that increased nitrate concentrations are associated with areas of higher human activity on the landscape.

In the surface water quality standards for Minnesota's Class 1 waters, protected as drinking water sources, human exposure to nitrates is regulated through the Federal Safe Drinking Water Act, with the Maximum Contaminant Level set at 10 milligrams/liter (mg/L), and a nitrite standard set at 1 mg/L. However, there is little guidance for protection of United States waters from the impacts of nitrate toxicity to aquatic organisms. The importance of nitrate toxicity to aquatic organisms has been a concern to aquaculture management for many years. In the environment, nitrate toxicity has not been a subject of scrutiny compared to the more toxic ammonia and nitrite. This document will present the technical discussion of surface water exposures and resulting toxicity of nitrate to aquatic organisms, and will propose a draft water quality standard necessary for the protection of aquatic life.

Why is nitrate not in a nutrient standard?

Nitrate is the form of nitrogen most available for use by plants. In freshwater systems, nitrogen is not a limiting nutrient for aquatic plant growth and excess nitrogen, primarily in the nitrate form, may accumulate in these systems. In contrast, growth of saltwater plants typically is limited by available nitrogen in the ecosystem. As such, the transport of excess nitrogen, predominantly as nitrate from freshwater systems, has been implicated – along with phosphorus – in the formation of oxygen-depleted areas in many marine sites including the Gulf of Mexico. The cause of these oxygen-depleted areas is largely the result of nutrient enrichment or eutrophication (excess algal growth and decay).

In Minnesota, water quality standards have been adopted to protect lakes from conditions of eutrophication, and the current rule revision includes draft standards to protect against eutrophication in rivers. Nutrient standards are based on phosphorus concentration as the primary cause of eutrophication, and efforts to develop these standards considered the roles of both phosphorus and nitrogen. In developing the eutrophication standards, monitoring data was examined and compared to a number of responses measured in the biological community like fish assemblages and abundances. No clear trend was established for the role of nitrogen in the response of these organisms or any direct contribution to eutrophication. Efforts to develop a total nitrogen budget center on addressing contributions of nitrogen in state surface water to protect downstream effects in the Mississippi River basin; however, this effort differs from the need to develop a nitrate toxicity standard in that it does not address the immediate or short-term effects of nitrate in any given lake or stream. In surface water, nitrate is the predominant form of total nitrogen, reported as nitrate-N, in concentrations above about 4 mg/L. (See the River Nutrient technical support document for further discussion). This concentration of nitrate is within the range of concentrations reported for effects to aquatic organisms.

How and why water quality standards are developed

Minnesota's Water Quality Standards (WQSs) are designed to be protective of the beneficial uses of groundwater and surface waters. In surface waters, protection encompasses normal growth and reproduction of aquatic animal and plant populations,

human recreational uses, consumption of aquatic biota, and sources of drinking water in some waters. WQSs consist of three parts: 1) the classification of designated, beneficial uses of water bodies 2) narrative protection goals and numeric criteria that are concentrations of contaminants considered protective of aquatic life or the other designated beneficial uses, and 3) mechanisms designed to avoid degradation [or "promote nondegradation"] (federal anti-degradation) of water quality. This document focuses on the draft water quality standard for protection of the aquatic life community for nitrate.

Development of the draft nitrate standard relies on sound scientific studies that provide the data needed to characterize and quantify how pollutants affect aquatic organisms. Toxicity data used to develop numeric criteria were evaluated based on national EPA guidance (USEPA, 1985), requirements in Minn. R. chs. 7050 and 7052, methods outlined by the American Society for Testing and Materials(ASTM, 2009), and a number of EPA testing methods. The key steps in developing new numeric water quality criteria involved:

1) A thorough search of the scientific literature by using electronic and printed databases. This search was performed for literature published through May 2010.
2) Compiling articles, reports and similar documentation based on their relevance to the issue. In this case, the search terms "nitrate", "toxicity" and "freshwater" served to provide the bulk of literature considered for review.
3) Reviewing these articles to screen out those that were outside of the scope of interest and to determine the usefulness of reported endpoints. For example, articles were found that reported toxicity of silver nitrate or used terrestrial organisms. Neither of these fit the scope of assessing the toxicity of the nitrate ion in freshwater aquatic systems.
4) Tabulating pertinent toxicity endpoints to be used in the calculation of draft acute and chronic standards (see Table 1a).

Articles were reviewed and critiqued based on the information reported. Occasionally, correspondence with the author was needed to clarify issues or obtain additional information. Information from the literature was retrieved from a search of academic databases. Primary literature search databases were MPCA library resources, University of Minnesota library, Scirus (www.scirus.com), Google Scholar (scholar.google.com), U.S. EPA ECOTOX and other sources. Scientific studies were assessed for quality based on guidance provided by the EPA and published ASTM methods of testing protocol (ASTM). Additional information for assessing studies has been summarized in guidance from the MPCA (MPCA 2010). Because WQSs are set to be protective for a specific beneficial use, rounding based on the correct significant figures was done to the preceding digit to maintain a concentration that is below the calculated values.

Aquatic Life Criteria Development

Numeric water quality criteria consist of a Final Acute Value (FAV), a Maximum Standard (MS) and a Final Chronic Value (FCV) (see U.S. EPA (1985) for more details).

These values are interrelated and are calculated on an assumption that allows for protection of 95% of aquatic communities. Much of this assumption is based on the fact that not all aquatic organisms present in the environment can be feasibly tested for their sensitivity to environmental contaminants. Therefore, calculation of numeric water quality criteria relies on toxicity endpoints provided through laboratory tests using organisms that are either cultured for this purpose or collected from the field and tested. These organisms, then, are surrogates or representatives of a variety of different families of organisms, such that they represent an approximation of the assemblage of North American aquatic organisms dependent on adequate water quality for their survival and reproduction. The use of either cultured or field collected organisms must follow consistent methodology that assures for the soundness of outcomes in the tests performed.

Acute effects of nitrate on aquatic organisms include survival endpoints from reported tests. These acute tests are typically of short duration (2 – 4 days). Acute toxicity is described primarily through calculated values of point estimates of lethal or effects concentration affecting 50% of the test population, referred to as LC50 or EC50, respectively. Chronic effects are measured primarily from reports of survival, reproduction, and growth of test organisms. These tests are performed over many days or weeks depending on the organism used and specific protocols for minimum test duration, and are typically referred to as full or partial life cycle tests. Further discussion of chronic endpoints is found in the MPCA guidance (MPCA, 2010).

Toxicity information used for development of the numeric criteria for nitrate was provided through reports from scientific studies published in the open literature. Most studies considered were from work published over the past ten years. Results of acceptable studies were reviewed from 89 references published in the scientific literature. Table 1b lists all the studies considered for use in water quality criteria development, with the acceptable acute studies used to develop the numeric criteria in Table 2. Studies considered for use in numeric criteria development were those performed using sodium nitrate as a toxicant. Other carrier salts reported for the nitrate ion are calcium and potassium. Few studies reported results using calcium nitrate, and based on the recent work by EPA assessing chloride toxicity, the potassium ion exerts its own level of toxicity that would confound effects of toxicity endpoints if used together with nitrate. The literature has much information about the toxicity of ammonium nitrate, which is a common agricultural fertilizer, but these too were not included, because ammonia is a much more toxic chemical. The Minnesota water quality chronic standard for ammonia has already been established at 40 ppb for class 2B surface waters.

Most of the studies reviewed were found to have no useful toxicity information for development of draft criteria because they used species that are not native to North America or the studies were otherwise unsuitable. Nine studies reported endpoints of acute toxicity for ten genera of freshwater animals that were used to calculate the final acute value. Procedures for calculating full (Tier I) aquatic life criteria require acceptable toxicity endpoints for 8 taxonomic family-level categories. This formality provides assurance of calculating a final acute value that is protective of aquatic communities.

During the initial phases of draft standard development, information provided in the published literature was not enough to fulfill this requirement. Discussions with the EPA Region 5 Water Quality Branch resulted in their offer to perform additional toxicity tests to fill this gap. These tests provided toxicity information for seven freshwater species, which served to fulfill the additional taxonomic categories. The endpoints of those tests were provided to the MPCA for use in developing the numeric criteria development. As these endpoints are preliminary, changes to the draft values for water quality standards may be possible. A final report of these tests performed by EPA is anticipated by the end of 2010.

Development of acute water quality criteria

Acute endpoints of nitrate toxicity to aquatic organism ranged from 100.1 milligrams/liter nitrate-N (mg/L) for the aquatic insect *Hydropsyche occidentalis* to 1903 mg/L nitrate-N for the lake whitefish. Overall, invertebrates appeared to be the most sensitive to nitrate toxicity, as this group is represented in the four lowest ranked values in the calculation of the Final Acute Value (FAV) as presented in Table 1a. Invertebrates represent most of the acute toxicity endpoints below the median LC50 of all reported values. Aquatic insects represent the group of invertebrates most commonly reported in the literature, and two caddisfly species were shown to have the lowest acute toxicity values for nitrate (Camargo and Ward, 1995). Study results from the 2010 EPA toxicity tests reported one stonefly (*Amphinemura*) and one midge (*Chironomus*) as being somewhat less sensitive, and mollusks also vary somewhat in their sensitivity to nitrate in tests reported by EPA. Two species of cladoceran, *Ceriodaphnia dubia* and *Daphnia magna* (Scott and Crunkilton, 2000) had the smallest difference in toxicity endpoints reported for any group of related organisms. Overall, invertebrates varied in their toxicity endpoints by just over an order of magnitude. Lowest and highest reported species acute values ranged a little more than two times. In contrast to invertebrates, fish were shown to be the least acutely sensitive of all organisms tested. Toxicity endpoints for amphibians were shown to be more acutely sensitive to nitrate than endpoints reported for fish, but not as sensitive as invertebrates. Supporting data from Smith (Smith et al., 2005), however, reported green frogs as being quite sensitive to nitrate exposure. Nevertheless, these data were not used in the calculation of the draft standard because the tests involved direct feeding of the test organisms during the exposures with nitrate, which is not recommended during acute exposures. Additional testing of amphibian exposures to nitrate is currently underway by EPA.

Development of chronic water quality criteria

The chronic criterion value can be determined either by developing a species sensitivity distribution and following the same methods used to calculate the FAV, or by using an acute to chronic ratio. Data sources provided eighteen acceptable chronic endpoints, but did not provide enough reported endpoints for different species to fulfill the necessary 8 taxonomic categories. Chronic toxicity endpoints (Table 3) for invertebrates were

reported only for two cladoceran species, *Ceriodaphnia dubia* and *Daphnia magna*. For vertebrates, lake trout has the lowest reported chronic endpoints (McGurk et al., 2006). Schuytema and Nebeker (Schuytema and Nebeker, 1999a, Schuytema and Nebeker, 1999c, Schuytema and Nebeker, 1999b) reported 10 day endpoints ([Lowest/No]-Observed-Adverse-Effect-Level, LOAEL/NOAEL respectively) for the amphibian life-stage of the pacific tree frog and the red-legged frog. These endpoints are reported in Table 3 only for purposes of comparison to other chronic data. These tests were of relatively short duration and methods for tests using amphibians vary.

Data were available to compute acute to chronic ratios (ACR) based on acute and chronic toxicity data for three species (Table 1a). An acute to chronic ratio of 17 was calculated for *Ceriodaphnia dubia*, but no ACR could be computed for *Daphnia magna* as its associated chronic test was of short duration. Selecting an appropriate ACR is achieved through examination of the acute toxicity data. EPA guidance recommends calculating the geometric mean ACR for each species for which data are available. The ACR are compared to their corresponding acute toxicity endpoint and examined for any increasing or decreasing trends among the ranks of all acute values. In this dataset, the observed trend was for the ACR values to decrease as the acute values increased. EPA guidance suggests that given this trend, an appropriate ACR can be selected from the species whose acute toxicity value is closest to the FAV, which for the dataset was calculated to be 83.4 mg/L. *Ceriodaphnia dubia*'s acute value of 374 mg/L resulted in an ACR of 17, which among the calculated ACRs is closest in value to 83.4. In lieu of a calculated ACR, Minnesota rules allows for the use of a default ACR of 18. Acute-to-chronic ratios calculated from test data are preferred over use of the default value. Selecting the ACR for an invertebrate is reasonable as invertebrate species account for a number of the most sensitive organisms used for calculating the FAV and invertebrates represent the six lowest acute endpoints used in the numeric criteria calculation. A final chronic value of 4.9 mg/L was calculated as the quotient of the FAV divided by the ACR (Table 1a). This value is considered protective as it falls below most chronic values found in the literature. The exception to this is the chronic toxicity of nitrate to Lake Trout reported by McGurk (McGurk et al., 2006). Effects on fry weight, a critical chronic endpoint, was reported as a NOEC = 1.6 mg/L and a LOEC = 6.25 mg/L Nitrate-N. An acceptable endpoint using the geometric mean of these chronic endpoints was calculated as the Maximum Acceptable Toxicant Concentration (MATC) = 3.16 mg/L nitrate-N. As provided in EPA guidance and in MN R. 7050, selecting a final chronic value for an economically and ecologically important species is appropriate. In Minnesota, cold-water fisheries, designated in MN R. 7050 as class 2A waters, have critical recreational and economic value. This designation provides for a means to protect for cold water species including lake trout. In consideration of this, and using the endpoints reported by McGurk, the draft chronic criterion value for these class 2A waters will be 3.1 mg/L nitrate-N. All other class 2 waters will have a draft chronic criterion value of 4.9 mg/L nitrate-N.

Additional considerations of nitrate toxicity to aquatic organisms

Toxicity testing performed by the EPA included a test using the amphipod *Hyalella azteca*. In March 2010, EPA began efforts to examine existing methods for culturing and testing of *H. azteca* to determine whether common laboratory practices to date may influence undue sensitivity in the organism. As part of this effort, EPA retested *Hyalella* with nitrate using preliminary outcomes from this examination. The retest reported a much less acutely sensitive endpoint for *Hyalella*, recorded as a preliminary value of >800 mg/L. A final report from EPA is anticipated in late 2010. As a result, the place held by *Hyalella* in the species sensitivity ranking was changed (Table 1a). The importance of this retesting is to assure proper assessment of nitrate toxicity to *Hyalella*, and to provide for the amphipod's representation as a key taxonomic position for criteria development.

Another goal in development of this draft standard was to attempt examining whether nitrate toxicity exhibits any trend with water hardness, similar to that shown for some metals. No relationship with hardness was evident based on the review of existing toxicity data.

Conclusion

Nitrate is both a naturally occurring substance and important nutrient in the life-cycle of plants in natural and cultivated settings. It can also be a common toxicant in Minnesota surface waters when present at concentrations exceeding those of reference areas where there is little human impact to the landscape. This document proposes a draft standard for the protection of aquatic life in lakes and streams designated as class 2 waters of the state. This use classification sets specific rules for protecting cold waters (class 2A) uses and cool/warm water (class 2B) uses. The draft water quality standards for nitrate were developed in efforts to protect these uses based on best available scientific information. EPA guidelines provide the means of examining data reported from toxicity tests using aquatic organisms in efforts to calculate concentrations of chemicals that are protective of aquatic life. The draft acute value (maximum standard) calculated is 41 mg/L nitrate-N for a 1-day duration, and the draft chronic value is 4.9 mg/L nitrate-N for a 4-day duration. In addition, a draft chronic value of 3.1 mg/L nitrate- N (4-day duration) was determined for protection of class 2A surface waters.

Table 1a. Ranked endpoints and calculation of draft criteria. Acute and chronic values are mg/L nitrate-N.

Genus/Species	Taxon		GMAV	Endpoint	Reference	Rank	P	Acute endpt./Chronic endpt. = ACR
Coregonus clupeaformis	Fish		1903.00	LC50	McGurk, et al. 20(	17	0.94	
Oncorynchus mykiss	Fish		1658.00	LC50	Buhl, K and J.J. H	16	0.89	
Pimephales promelas	Fish		1426.80	LC50	EPA	15	0.83	1815.9/339.29 = 5.35
Chironomus dilutus	Insecta		1230.50	LC50	EPA	14	0.78	
Salvelinus namaycush	Fish		1121.00	LC50	McGurk, et al. 20(	13	0.72	1121/131.9 (EC50/EC20) = 8.5
Megalonaias nervosa	mussel		937.00	LC50	EPA	12	0.67	
Hyalella			> 800	LC50	EPA	11	0.61	
Rana aurora	Amphibia		636.30	LC50	Schuytema, G.S.	10	0.56	
Pomacea paludosa	Molluska		516.20	LC50	Corrao, N.M. et al	9	0.50	
Amphinemura delosa	Insecta		476.00	LC50	EPA	8	0.44	
Pseudacris regilla	Amphibia		471.12	LC50	Schuytema, G.S.	7	0.39	
Daphnia magna	Crustacea		441.27	LC50	Scott, G., and R.L	6	0.33	
Sphaerium simile	mussel		376.00	LC50	EPA	5	0.28	
Ceriodaphnia dubia	Crustacea		374.00	LC50	Scott, G., and R.L	4	0.22	374/22 = 17
Lampsilis siliquiodea	Molluska		357.00	LC50	EPA	3	0.17	
Cheumatopsyche pettiti	Insecta		138.70	LC50	Carmargo, J. and	2	0.11	
Hydropsyche occidentalis	Insecta		100.10	LC50	Carmargo, J. and	1	0.06	

	GMAV	P	ln GMAV	(ln GMAV)	Sq Rt P
Ceriodaphnia dubia	374.00	0.22	5.9243	35.0968	0.4714
Lampsilis siliquiodea	357.00	0.17	5.8777	34.5478	0.4082
Cheumatopsyche pettiti	138.70	0.11	4.9323	24.3277	0.3333
Hydropsyche occidentalis	100.10	0.06	4.6062	21.2168	0.2357

s2 Num	1.3351			
s2 Denom	0.0309			
S2	43.2348			
Sqrt S2	6.5753	Chronic Criteria:	**83.43/17 =**	**4.91**
L	2.9537			
A	4.4240			
FAV	**83.4300**			

Table 1b. Summary of all nitrate toxicity data considered for use in standard development found in the open literature and provided in preliminary results from EPA toxicity tests.

Species Name/Common name	Native to N.A.?	Taxon	Effect Conc. (mg/L)	Reported Endpoint	Reference
Acipenser beari/ Siberian Sturgeon	No	Fish	1028	LC50	(Hamlin, 2006)
Acipenser beari/ Siberian Sturgeon	No	Fish	601	LC50	(Hamlin, 2006)
Acipenser beari/ Siberian Sturgeon	No	Fish	397	LC50	(Hamlin, 2006)
Amphinemura delosa/ Stonefly	Yes	Insecta	476	LC50	EPA
Catla catla/ Indian major carp	No	Fish	35	LC50	(Tilak, 2006)
Catla catla/ Indian major carp	No	Fish	33	LC50	(Tilak, 2006)
Catla catla/ Indian major carp	No	Fish	1401	LC50	(Tilak, 2006)
Catla catla/ Indian major carp	No	Fish	1251	LC50	(Tilak, 2006)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	374	LC50	(Schuytema and Nebeker, 1999b)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	374	LC50	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	14.1	LOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	113	LOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	14.1	LOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	35.9	LOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	35.9	LOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	7.1	NOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	56.5	NOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	7.1	NOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	17.9	NOEC	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia/ Water flea	Yes	Cladoceran	17.9	NOEC	(Scott and Crunkilton, 2000)
Cheumatopsyche pettiti/ caddisfly	Yes	Insecta	128	LC50	(Camargo and Ward, 1995)
Cheumatopsyche pettiti/ caddisfly	Yes	Insecta	154	LC50	(Camargo and Ward, 1995)
Cheumatopsyche pettiti/ caddisfly	Yes	Insecta	113.5	LC50	(Camargo and Ward, 1992)

Cheumatopsyche pettiti/ caddisfly	Yes	Insecta	165.5	LC50	(Camargo and Ward, 1992)
Chironomus dilutes/ midge	Yes	Insecta	1230.5	LC50	EPA
Cirrhinus mrigala/ Indian major carp	No	Fish	153	LC50	(Tilak, 2006)
Cirrhinus mrigala/ Indian major carp	No	Fish	163	LC50	(Tilak, 2006)
Cirrhinus mrigala/ Indian major carp	No	Fish	1055	LC50	(Tilak, 2006)
Cirrhinus mrigala/ Indian major carp	No	Fish	1023	LC50	(Tilak, 2006)
Coregonus clupeaformis/ Lake whitefish	Yes	Fish	1903	LC50	(Tilak, 2006)
Coregonus clupeaformis/ Lake whitefish	Yes	Fish	64.4	EC50	(Tilak, 2006)
Daphnia magna/ Water flea	Yes	Cladoceran	323	LC50	(Scott and Crunkilton, 2000)
Daphnia magna/ Water flea	Yes	Cladoceran	453	LC50	(Scott and Crunkilton, 2000)
Daphnia magna/ Water flea	Yes	Cladoceran	611	LC50	(Scott and Crunkilton, 2000)
Daphnia magna/ Water flea	Yes	Cladoceran	717	LOEC	(Scott and Crunkilton, 2000)
Daphnia magna/ Water flea	Yes	Cladoceran	717	LOEC	(Scott and Crunkilton, 2000)
Daphnia magna/ Water flea	Yes	Cladoceran	358	NOEC	(Scott and Crunkilton, 2000)
Daphnia magna/ Water flea	Yes	Cladoceran	358	NOEC	(Scott and Crunkilton, 2000)
Eulimnogammarus toletanus/ amphipod	No	Crustacea	85.0	LC50	(Camargo et al., 2005)
Eulimnogammarus toletanus/ amphipod	No	Crustacea	62.5	LC50	(Camargo et al., 2005)
Eulimnogammarus toletanus/ amphipod	No	Crustacea	22.2	LC10	(Camargo et al., 2005)
Eulimnogammarus toletanus/ amphipod	No	Crustacea	9.5	LC10	(Camargo et al., 2005)
Hyalella azteca/ scud	Yes	Crustacea	>800	LC50	EPA
Hydropsyche exacellata/ caddisfly	No	Insecta	269.5	LC50	(Camargo et al., 2005)
Hydropsyche exacellata/ caddisfly	No	Insecta	31.8	LC10	(Camargo et al., 2005)
Hydropsyche exacellata/ caddisfly	Yes	Insecta	90	LC50	(Camargo and Ward, 1995)
Hydropsyche exacellata/ caddisfly	Yes	Insecta	105	LC50	(Camargo and Ward, 1995)
Hydropsyche exacellata/ caddisfly	Yes	Insecta	97.3	LC50	(Camargo and Ward, 1992)
Hydropsyche occidentalis/ caddisfly	Yes	Insecta	109	LC50	(Camargo and Ward, 1992)

Labeo rohia/ Indian major carp	No	Fish	119	LC50	(Tilak, 2006)
Labeo rohia/ Indian major carp	No	Fish	123	LC50	(Tilak, 2006)
Labeo rohia/ Indian major carp	No	Fish	1434	LC50	(Tilak, 2006)
Labeo rohia/ Indian major carp	No	Fish	1351	LC50	(Tilak, 2006)
Lampsilis siliquoidea	Yes	Mussel	357	LC50	EPA
Oncorynchus mykiss/ Rainbow trout	Yes	Fish	1658	LC50	(Buhl and Hamilton, 2000)
Pimephales promelas/ fathead minnow	Yes	Fish	1815.9	LC50	EPA
Pimephales promelas/ fathead minnow	Yes	Fish	339.3		EPA
Pimephales promelas/ Fathead minnow	Yes	Fish	1010	LC50	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	1607	LC50	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	1406	LC50	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	717	NOEC	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	1434	LOEC	(Scott and Crunkilton, 2000)
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Pimephales promelas/ Fathead minnow	Yes	Fish	717	LOEC	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	358	NOEC	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	717	LOEC	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	1435	LOEC	(Scott and Crunkilton, 2000)
Pimephales promelas/ Fathead minnow	Yes	Fish	1435	LOEC	(Scott and Crunkilton, 2000)
Pomacea paludosa/ Florida Apple Snail	Yes	Molluska	1001	LC50	(Corrao et al., 2006)
Pomacea paludosa/ Florida Apple Snail	Yes	Molluska	1001	LC50	(Corrao et al., 2006)
Pomacea paludosa/ Florida Apple Snail	Yes	Molluska	504	EC50	(Corrao et al., 2006)
Pomacea paludosa/ Florida Apple Snail	Yes	Molluska	622	EC50	(Corrao et al., 2006)
Potamopyrgus antipodarum/ aquatic snail	No (Exotic)	Molluska	1042	LC50	(Alonso and Camargo, 2003)

Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	643	LC50	(Schuytema and Nebeker, 1999a)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	578	LC50	(Schuytema and Nebeker, 1999a)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	56.7	NOAEL	(Schuytema and Nebeker, 1999a)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	111	LOAEL	(Schuytema and Nebeker, 1999a)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	1749.8	LC50	(Schuytema and Nebeker, 1999b)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	266.2	LC50	(Schuytema and Nebeker, 1999b)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	259.1	LOAEL	(Schuytema and Nebeker, 1999b)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	126.3	NOAEL	(Schuytema and Nebeker, 1999b)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	30.1	LOAEL	(Schuytema and Nebeker, 1999b)
Pseudacris regilla/ Pacific Treefrog	Yes	Amphibian	30.1	NOAEL	(Schuytema and Nebeker, 1999b)
Rana aurora/ red-legged frog	Yes	Amphibian	636.3	LC50	(Schuytema and Nebeker, 1999c)
Rana aurora/ red-legged frog	Yes	Amphibian	235	LOAEL	(Schuytema and Nebeker, 1999c)
Rana aurora/ red-legged frog	Yes	Amphibian	116.8	NOAEL	(Schuytema and Nebeker, 1999c)
Salvelinus namaycush/ Lake trout	Yes	Fish	1121	LC50	(McGurk et al., 2006)
Salvelinus namaycush/ Lake trout	Yes	Fish	189.6	EC50	(McGurk et al., 2006)
Sphaerium simile/ fingenail clam	Yes	Mussel	376	LC50	EPA
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	438.4	LC50	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	871.6	LC50	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	521.7	EC50	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	56.7	NOAEL	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	111	LOAEL	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	24.8	NOAEL	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	56.7	LOAEL	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	111	NOAEL	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	230.4	LOAEL	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	471	LC50	(Schuytema and Nebeker, 1999a)

Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	471	LC50	(Schuytema and Nebeker, 1999a)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	1,955.8	LC50	(Schuytema and Nebeker, 1999b)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	1236.2	LC50	(Schuytema and Nebeker, 1999b)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	494.4	LOAEL	(Schuytema and Nebeker, 1999b)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	259.1	NOAEL	(Schuytema and Nebeker, 1999b)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	126.3	LOAEL	(Schuytema and Nebeker, 1999b)
Xenopus laevis/ African Clawed Frog	No (Exotic)	Amphibian	65.6	NOAEL	(Schuytema and Nebeker, 1999b)

Table 2. Acute data of nitrate toxicity used for the development of draft water quality criteria.
All test endpoints are for test duration of 96 h unless otherwise noted (*) .

Species Name	Taxon	Effect Conc. (mg/L)	GMAV (mg/L)	Endpoint	Reference
Amphinemura delosa	Insecta	476.0		LC50	EPA
Ceriodaphnia dubia	Crustacea	374	374.0	LC50* (48h)	(Scott and Crunkilton, 2000)
Ceriodaphnia dubia	Crustacea	374		LC50* (48h)	(Schuytema and Nebeker, 1999b)
Cheumatopsyche pettiti	Insecta	154	138.7	LC50	(Camargo and Ward, 1995)
Cheumatopsyche pettiti	Insecta	113.5		LC50	(Camargo and Ward, 1992)
Cheumatopsyche pettiti	Insecta	165.5		LC50	(Camargo and Ward, 1992)
Cheumatopsyche pettiti	Insecta	128		LC50	(Camargo and Ward, 1995)
Chironomus dilutus	Insecta	1230.5		LC50	EPA
Coregonus clupeaformis	Fish	1903		LC50	(McGurk et al., 2006)
Daphnia magna	Crustacea	611.0	447.1	LC50* (48h)	(Scott and Crunkilton, 2000)
Daphnia magna	Crustacea	323		LC50* (48h)	(Scott and Crunkilton, 2000)
Daphnia magna	Crustacea	453.0		LC50* (48h)	(Scott and Crunkilton, 2000)
Hyalella azteca	Crustacea	72.6	72.6	LC50	EPA
Hydropsyche occidentalis	Insecta	105	100.1	LC50	(Camargo and Ward, 1995)
Hydropsyche occidentalis	Insecta	97.3		LC50	(Camargo and Ward, 1992)
Hydropsyche occidentalis	Insecta	109		LC50	(Camargo and Ward, 1992)
Hydropsyche occidentalis	Insecta	90		LC50	(Camargo and Ward, 1995)
Lampsilis siliquiodea	Molluska	357.0	357.0	LC50	EPA
Oncorynchus mykiss	Fish	1658	1658.0	LC50	(Buhl and Hamilton, 2000)
Pimephales promelas	Fish	1815.9	1426.8	LC50	
Pimephales promelas	Fish	1010		LC50	(Scott and Crunkilton, 2000)
Pimephales promelas	Fish	1607		LC50	(Scott and Crunkilton, 2000)

Pimephales promelas	Fish	1406		LC50	(Scott and Crunkilton, 2000)
Pomacea paludosa	Molluska	1001	1001.0	LC50	(Corrao et al., 2006)
Pomacea paludosa	Molluska	1001		LC50	(Corrao et al., 2006)
Pseudacris regilla	Amphibia	266.2	645.0	LC50* (10d)	(Schuytema and Nebeker, 1999b)
Pseudacris regilla	Amphibia	643		LC50	(Schuytema and Nebeker, 1999a)
Pseudacris regilla	Amphibia	578		LC50* (10d)	(Schuytema and Nebeker, 1999a)
Pseudacris regilla	Amphibia	1749.8		LC50	(Schuytema and Nebeker, 1999b)
Rana aurora	Amphibia	636.3	636.3	LC50* (16d)	(Schuytema and Nebeker, 1999c)
Salvelinus namaycush	Fish	1121	1121.0	LC50	(McGurk et al., 2006)
Sphaerium simile	Molluska	376.0	376.0	LC50	EPA

Table 3. Summary of chronic data from nitrate toxicity tests found acceptable in support of water quality standard development

Species Name	Taxon	Effect Conc. (mg/L)	End point	MATC (mg/L)	Test Dur. (d)	ACR Use?	Reference	Notes
Ceriodaphnia dubia	Crustacea	35.9	LOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	17.9	NOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	17.9	NOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	7.1	NOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	7.1	NOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	35.9	LOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	14.1	LOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	113	LOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	14.1	LOEC		7	Y	(Scott and Crunkilton, 2000)	
Ceriodaphnia dubia	Crustacea	56.5	NOEC	22	7	Y; MATC	(Scott and Crunkilton, 2000)	
Coregonus clupeaformis	Fish	64.4	EC50		120	N	(McGurk et al., 2006)	No weight endpoint
Daphnia magna	Crustacea	717	LOEC		7	N	(Scott and Crunkilton, 2000)	Test dur. Short
Daphnia magna	Crustacea	358	NOEC		7	N	(Scott and Crunkilton, 2000)	Test dur. Short
Daphnia magna	Crustacea	358	NOEC		7	N	(Scott and Crunkilton, 2000)	Test dur. Short
Daphnia magna	Crustacea	717	LOEC	507	7	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	358	NOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	1435	LOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	717	NOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	1434	LOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	358	NOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	717	LOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	358	NOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	1435	LOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short

Pimephales promelas	Fish	717	LOEC		18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	717	LOEC	717	18	N	(Scott and Crunkilton, 2000)	Test dur. Short
Pimephales promelas	Fish	339.3	EC50		28	Y	EPA	
Pomacea paludosa	Molluska	504	EC50		14	N	(Corrao et al., 2006)	Beyond Upper limit
Pomacea paludosa	Molluska	622	EC50		14	N	(Corrao et al., 2006)	Beyond Upper limit
Pseudacris regilla	Amphibia	259.1	LOAEL (L)		10	N	(Schuytema and Nebeker, 1999b)	acute test
Pseudacris regilla	Amphibia	126.3	NOAEL (L)	181	10	N	(Schuytema and Nebeker, 1999b)	acute test
Pseudacris regilla	Amphibia	30**	LOAEL (W)		10	N	(Schuytema and Nebeker, 1999b)	acute test
Pseudacris regilla	Amphibia	30**	NOAEL (W)	30	10	N	(Schuytema and Nebeker, 1999b)	acute test
Pseudacris regilla	Amphibia	111	LOAEL		10	N	(Schuytema and Nebeker, 1999a)	acute test
Pseudacris regilla	Amphibia	56.7	NOAEL	79.3	10	N	(Schuytema and Nebeker, 1999a)	Not ELS
Rana aurora	Amphibia	235	LOAEL (W)		16	N	(Schuytema and Nebeker, 1999c)	No acute
Rana aurora	Amphibia	116.8	NOAEL (W)	166	16	N	(Schuytema and Nebeker, 1999c)	No acute
Rana aurora	Amphibia	29**	LOAEL (L)		16	N	(Schuytema and Nebeker, 1999c)	No acute
Rana aurora	Amphibia	29**	NOAEL (L)		16	N	(Schuytema and Nebeker, 1999c)	No acute
Salvelinus namaycush	Fish	189.6	EC50		120	N	(McGurk et al., 2006)	
Salvelinus namaycush	Fish	1.6	NOEC		120	Y	(McGurk et al., 2006)	
Salvelinus namaycush	Fish	6.25	LOEC	3.16	120	Y; MATC	(McGurk et al., 2006)	

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