Surface Water

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Aquatic Life Water Quality Standards Draft Technical Support Document for Nitrate

MINNESOTA POLLUTION CONTROL AGENCY

Authors

Philip Monson

Contributors/acknowledgements

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Editing and graphic design

PIO staff
Graphic design staff
Administrative Staff

Minnesota Pollution Control Agency

520 Lafayette Road North | Saint Paul, MN 55155-4194 |

651-296-6300 | 800-657-3864 | Or use your preferred relay service. | Info.pca@state.mn.us

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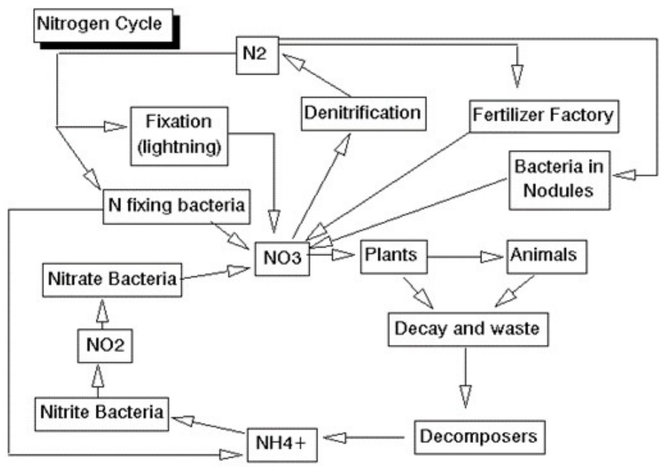
Introduction

Nitrate is a common chemical found in surface waters and groundwater from both natural and anthropogenic sources. 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. These include point sources such as wastewater discharge and non-point sources such as agricultural practices. Forest fires, decay of organic matter, and volcanic discharges are some natural sources that release nitrate to the environment. Nitrogen cycling in the environment results in nitrogenous compounds, such as ammonia, that may covert into the more stable and conservative nitrate ion (NO_3^-).

Figure 1. Illustration of the nitrogen cycle (McShaffrey, n.d.)



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, 2008). In surface water, nitrate is the predominant form of total nitrogen, reported as milligrams (mg) nitrate-nitrogen per liter (L) (alternatively, mg nitrate-N/L or mg N:NO3/L), in concentrations above about 4 mg nitrate-N/L. This concentration of nitrate is within the range of concentrations reported for effects to aquatic organisms.

Concern regarding the toxicity of nitrate to aquatic organisms was brought to the attention of the Minnesota Pollution Control Agency (MPCA) through comments made by the Minnesota Department of Natural Resources and the Minnesota Center for Environmental Advocacy during the 2008 triennial standards review and reported from monitoring studies in 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 aquatic life standard is part of the effort to address these concerns and directives; information on how that path has evolved since 2010 is provided later in this document.

Nitrogen has multiple forms and environmental impacts, which are being addressed in multiple ways.

Nitrate, nitrite, and ammonia all may impact aquatic life. In addition to developing water quality standards (WQS) to protect aquatic life from nitrate, MPCA is also revising the water quality standard (WQS) for ammonia concurrently with the development of this nitrate standard.

Nitrite is another form of nitrogen that has been shown to exert toxicity to aquatic organisms at much lower concentrations compared to nitrate. The nitrite ion, however, is not stable in environments concurrent with the presence of most aquatic organisms considered in the context of natural communities. There may be cases of high nitrite present in places like wastewater ponds, but those are not considered as waters of the state. The ephemeral nature of nitrite under conditions of oxygen, particularly streams and rivers, does not allow it to build up to concentrations known to be toxic to aquatic organisms. Therefore, nitrite is not being considered in development of this aquatic life standard.

Nitrogen can also contribute to nutrient over-enrichment or eutrophication, leading to algae growth and, eventually, oxygen depletion. The MPCA is also engaged in implementing a nutrient reduction strategy for the State that includes goals for total nitrogen in surface waters. This nutrient reduction strategy aims to reduce Minnesota's contribution to eutrophication and "dead zones" in areas such as the Gulf of Mexico. The contribution of nitrogen to eutrophication, either locally or regionally, is not being considered in development of this aquatic life standard. Efforts to develop a total nitrogen budget center on addressing contributions of nitrogen to protect against adverse effects downstream in the Mississippi River basin. However, this effort differs from the need to develop a nitrate toxicity standard to protect aquatic life in any given lake or stream.

Finally, nitrogen (nitrate and nitrite) can also cause human health impacts if present in sufficiently high enough concentrations in drinking water. The surface WQS for Minnesota's Class 1 waters come from the Federal Safe Drinking Water Act, with the Maximum Contaminant Levels set at 10 mg/L for nitrate, and a 1 mg/L for nitrite. The Class 1 WQS are also currently under revision in a separate process.

Still, elevated concentrations of nitrate have been documented in surface waters throughout the state, from both point and non-point sources (Omernik et al, 2016). A comprehensive assessment of these data is beyond the scope of this document, but current trends in the data clearly indicate that increased nitrate concentrations are associated with areas of higher human activity on the landscape.

Currently, there is little guidance for protection of United States waters from the effects 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 ambient environment, the role of nitrate, along with the more toxic forms of nitrogen, ammonia and nitrite, is a subject of greater scrutiny. This document will present the technical discussion of nitrate toxicity to aquatic organisms and will propose draft water quality standards (acute and chronic) necessary for the protection of aquatic life for nitrate.

How and why water quality standards are developed?

Minnesota's WQS are designed to protect the beneficial uses of the state's groundwater and surface waters. In surface waters, protection encompasses normal growth and reproduction of aquatic animal and plant populations (aquatic life), human recreational uses (recreation), consumption of aquatic biota (aquatic consumption), and sources of drinking water (domestic consumption) in some waters.

WQS consist of three parts: 1) the beneficial use classification of the water; 2) narrative and numeric criteria that describe the needed conditions in the water, including concentrations of pollutants, below which are considered protective of the beneficial use;[1] and 3) mechanisms designed to avoid degradation of water quality (antidegradation). This document focuses on numeric standards for protection of the aquatic life community from nitrate toxicity in Class 2 surface waters.

Development of nitrate standards relies on sound scientific studies that provide the data needed to characterize and quantify how nitrate affects aquatic organisms, in this case, freshwater invertebrates and invertebrates. Toxicity data used to develop numeric criteria were evaluated based on national U.S. Environmental Protection Agency (EPA) guidance (EPA, 1985), requirements in Minn. R. chs. 7050 and

[1] *The numeric criteria setting an acceptable level of pollution is usually referred to as "the standard" in Minnesota, while EPA and other states use the word "criteria"*

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 the planned new numeric water quality criteria for nitrate involved:

1. A thorough search of the scientific literature by using electronic and printed databases. This search was performed for literature published through June 2021. In this case, the search terms “nitrate”, “toxicity” and “freshwater” served to provide the bulk of literature considered for review.
2. 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.
3. Tabulating pertinent toxicity endpoints to be used in the calculation of draft acute and chronic standards.

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 included were the (EPA) ECOTOX database, MPCA library resources, University of Minnesota library, Scirus (www.scirus.com), and Google Scholar (scholar.google.com). Other sources and references included scientific papers shared between fellow colleagues or those gleaned from reviews of printed material. Scientific studies were assessed for quality based on guidance provided by the EPA and published ASTM methods of testing protocol (ASTM).

Updates to Technical Support Document

Since the initial effort by MPCA in 2010 to develop nitrate water quality standards for aquatic life, considerable additional aquatic toxicity information has been completed and published in the scientific literature. Appropriate laboratory performance, review and documentation of aquatic toxicity tests sufficient to provide the technical underpinnings for developing WQS takes much time and effort. EPA worked along with the MPCA to garner support for additional toxicity testing to supplement the existing aquatic species evaluated for acute and chronic endpoints. Central to this effort was the addition of new test methods for species like freshwater mussels, a group of macroinvertebrates important to a large area of the United States, including Minnesota. Mayflies are another important group of macroinvertebrates that have been difficult to use in laboratory aquatic toxicity tests. Test methods for a species of mayfly (*Neocloeon triangulifer*) were developed over a number of years and this species is now suitable for toxicity testing. The EPA worked with other federal and academic institutions to develop these new test methods over several years prior to performing the actual toxicity tests. Completion of these test methods and toxicity endpoints reported for these test species fills a critical knowledge gap about the sensitivity of these important taxonomic groups to nitrate in the aquatic environment (EPA, 2010). In addition, the toxicity endpoints derived from these tests fulfilled important requirements of the EPA for developing water quality criteria. The compendium of scientific literature used to develop a water quality standard for nitrate is the result of research studies on nitrate toxicity performed by public, private and academic institutions throughout the United States.

EPA provided support for research and expertise in toxicity test method development and experimental design. Some of these studies were recently completed in 2020 and published in 2021 in the scientific literature. The EPA also manages a large database (ECOTOX) of toxicity test endpoints reported from the published literature. The assemblage of reported toxicity values provides an extensive search of the scientific literature that are used in the development of numeric water quality criteria. There is no one report or publication that provides any cumulative summary of nitrate toxicity testing conducted with the assistance of the EPA. We hope that this technical support document will serve as a source that demonstrates the importance of these investigative endeavors.

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). Methods used to calculate both acute and chronic criteria values follow the EPA document titled "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (EPA, 1985). These values are interrelated and calculated on the assumption that provides for protection of 95% of aquatic communities. Much of this assumption is because 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 observed through laboratory tests exposures using organisms that are either cultured for this purpose or collected from the field and tested. These organisms are used to represent both the specific species and organisms related taxonomically. The EPA guidance requires a minimum dataset representing eight taxonomic categories, referred to in this document as minimum data requirements (MDR). Overall, these MDRs represent an approximation of the assemblage of North American aquatic organisms that depend on adequate water quality for their survival, growth, 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.

Toxicity information used for development of the numeric criteria for nitrate was provided through reports from scientific studies published in the open literature. Results of studies were reviewed from 110 references cited in the scientific literature, and most studies considered were from work published over the past twenty years. All studies considered for use in this criteria development are listed in Table 5 and Table 6, for acute and chronic endpoints, respectively. 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 contains 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 is 40 µg/L for Class 2B surface waters and is being revised concurrently with the development of this nitrate standard.

Based on the recommended EPA guidance (EPA, 1985), procedures for calculating full (Tier I) aquatic life criteria require the utilization of acceptable toxicity endpoints for eight specified taxonomic family-level categories. This method provides assurance of calculating a final acute value that is protective of aquatic communities. During the initial phases of developing this standard, information provided in the published literature was not enough to fulfill this requirement. Since then, additional toxicity tests were performed to fill this gap. These tests provided toxicity information for additional freshwater species, which served to fulfill the eight specified taxonomic categories.

Development of acute water quality criteria

Acute tests are typically of short duration (2 – 4 days), and survival (mortality) is the primary response observed and reported following acute exposures. Acute toxicity endpoints are described primarily through calculated values of point estimates of test concentrations causing lethality or morbidity of 50%

of the test population, referred to as the 50% lethal concentration (LC50) or 50% effective concentration (EC50).

Water quality criteria are calculated based on the Geometric Mean Acute Values (GMAV) for each generic-level taxon having acceptable toxicity information. For many of the nitrate toxicity data, a single species represents the genus. These GMAVs ranged from 103 mg nitrate-N/L for the aquatic insect *Hydropsyche* to 1902 mg nitrate-N/L for the lake whitefish (*Coregonus*) (Table 4; Figure 2). Invertebrates represent the majority of the species with acute toxicity endpoints below the median GMAV of 643 mg nitrate-N/L. Furthermore, invertebrates appeared to exhibit the greatest acute sensitivity to nitrate toxicity, as this group is represented in the four lowest ranked values in the calculation of the Final Acute Value (FAV) = 119.2 mg N:NO3/L (rounded to 120) as presented in Tables 2a-2c. The maximum standard (MS) = 59.6 (rounded to 60) mg N:NO3/L is calculated as half (120 ÷ 2) of the FAV for all Class 2 waters. Aquatic insects represent a group of invertebrates commonly reported in the literature, and who also rank in the four most sensitive taxa. Overall, invertebrate GMAVs varied in their toxicity endpoints by about an order of magnitude with the New Zealand mud snail (*Potamopygrus*) being the least sensitive invertebrate. Vertebrates showed to be the least sensitive group with an amphibian, *Hyla*, being the most sensitive among that group. Fish genera ranked in the top eight of 29 least sensitive taxa.

It is important to point out that three genera are not native to North America but were included in the full list of GMAVs taxa considered for use in developing the acute aquatic life criteria. The previously mentioned New Zealand mud snail is an exotic invasive in many parts of the world, including in North America, and is likely established within the aquatic community where present. In addition, the African Clawed Frog (*Xenopus*) and the Zebrafish (*Danio*) are well documented laboratory test species. Their use in this WQS development, however, is considered supplemental for this technical support document, and the magnitude of their reported endpoints support those from other organisms within the same taxonomic category.

Development of chronic water quality criteria

Methods used for development of chronic criteria follow the same procedures used to develop acute criteria when sufficient toxicity test endpoints are available. For nitrate, sufficient chronic toxicity test endpoints were available to fulfill the eight MDRs needed for calculating chronic water quality criteria. Chronic endpoints are effects of exposure to nitrate measured primarily as lethal endpoints of survival (or mortality), and sublethal endpoints of reproduction and growth of test organisms. These tests are performed over many days or weeks depending on the organism 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).

Endpoints of chronic toxicity effects are often described through hypothesis testing of treatment responses compared to control responses. A No-observed-effect-concentration (NOEC) is the highest concentration with the response not statistically different from that observed in control organisms. A Lowest-observed-effect-concentration (LOEC) is the lowest concentration with a response statistically different from those observed in control organisms. Another important measure of effect uses regression to estimate effect concentrations of the 10th (EC10) and 20th (EC20) percentile test concentration that are observed for chronic endpoints.

Table 5 shows all data used to calculate genus mean chronic values (GMCV). Tables 3 and 4 show the GMCVs and calculation of the Final Chronic Values. GMCVs were reported for seven invertebrate genera and seven vertebrate genera. Invertebrate taxa represented three of four of the most sensitive genera. The remaining invertebrate taxa showed rankings distributed throughout the sensitivity distribution.

Fish and amphibians represented the vertebrate taxonomic categories and neither differed much regarding their sensitivity ranks. The exception to this is the chronic toxicity of nitrate to lake trout reported by McGurk et al (2006). Effects on fry weight, a critical chronic endpoint, were reported as a NOEC = 1.6 mg/L and a LOEC = 6.25 mg/L N:NO3 reported following a 146-day exposure. As provided in EPA guidance and in Minn. R. ch. 7050, more restrictive criteria may be applied when necessary to protect economically and ecologically important species given supporting toxicity information. In Minnesota, coldwater habitats, described in Minn. R. 7050.0420 and designated in Minn. R. 7050.0470 as Class 2A waters, have critical recreational and economic value. This designation provides a means to protect for the coldwater species assemblage, which includes lake trout. For this reason, chronic criteria were developed for both coldwater uses (Class 2A; Table 3 a,b,c) and all other Class 2 water uses (Class 2B and Class 2Bd; Table 4 a,b,c). Toxicity test information for the lake trout serves as a surrogate to the many other aquatic organisms present in coldwater systems. The calculated Final Chronic Value of 5.2 mg/L N:NO3 (adjusted to 5.0 mg/L N:NO3) will provide for that protection. First, the lake trout study's exposure (146 d) was considerably longer than all other chronic test endpoints. The intent of the EPA 1985 guidelines is to provide for a reasonable assurance that a criterion value avoids being too over-protective or under-protective. Given that understanding, the decision to use the LOEC as the chronic endpoint ensures that the observed response (weight) is directly associated with a measured concentration, is significantly different than the control response, and provides better assurance that the selected endpoint will not be overprotective.

Differences in the response of a test species to nitrate can be attributed to the organism age at test start, length of test and endpoint observed. In the case of the lake trout, acute tests were initiated with swim-up fry, whereas chronic tests used newly fertilized eggs at test start. The final observed endpoints for those two different toxicity tests occur at concentrations that are considerably different, but nonetheless relevant. Another example are the tests using the water column crustacean *Daphnia,* where the reported values for both acute exposures (2-d LC50 = 447 mg/L) and chronic (7-d MATC = 506 mg/L) are similar. While acute endpoints reported survival, and chronic endpoints reported offspring produced, the similarity of endpoint values suggests that *Daphnia* are somewhat resistant to nitrate effects. Another water column crustacean, *Ceriodaphnia*, exposed under similar test regimes and reported endpoints, were shown to be much more sensitive to chronic exposures.

In calculating the final chronic value for non-salmonid waters (Class 2B and Class 2Bd), the lake trout endpoint is removed from the genus ranks. This does two things. First, the total number of ranked organisms decreases and a new set of the four most sensitive taxa is established (Table 4b). The Final Chronic Value is recalculated as 8.26 (rounded to 8) mg/L N:NO_3.

Additional considerations of nitrate toxicity to aquatic organisms

A thorough examination of how nitrate exerts toxicity to aquatic organisms is beyond the scope of this document. However, two of the most likely causal actions are nitrate interference with cellular ion exchange, and the endogenous conversion of nitrate to nitrite. The latter action is strongly related to changes in the oxygen-carrying ability of hemoglobin, and may be an important factor in driving effects in fish and other aquatic organisms (Camargo et al. 2005). Examples of other reported effects of nitrate exposure include endocrine disruption in fathead minnows (Kellock et al. 2017) while Moore and Bringolf (2018) observed an impaired ability of a freshwater mussel to attach to their fish host and metamorphose. These reports conclude the need for the additional study of sublethal effects or chronic effects that have ecological relevance.

In addition to observed acute and chronic toxic effects on aquatic organisms, the relative potency of nitrate may vary with different water quality parameters. Potential toxicity effects due to the interaction of ions is well established in the study of water hardness ions, like calcium and magnesium, on the toxicity of certain metals (e.g., zinc, copper and nickel). The toxicity of nitrate has been hypothesized to also be influenced in a similar manner with hardness ions. Perhaps the most thorough study to date on this matter was published by Baker et al. (2017), which documented observed trends of decreasing nitrate toxicity with increased hardness concentration. Though these trends seems suggestive of influence on nitrate toxicity, presence of other water quality ions in the exposures precluded any assurance that hardness ions alone served to mitigate nitrate toxicity.

Why not a nitrate nutrient standard?

Nitrate is the form of nitrogen most available for use by plants. In freshwater systems, nitrogen can be 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. These oxygen-depleted areas are largely the result of nutrient enrichment or eutrophication (excess algal growth and decay) due to nutrients discharged from the Mississippi River. Nitrogen, primarily in the form of nitrate, is the greatest contributor to eutrophication in marine systems.

In 2000, EPA published regional guidance for lakes and reservoirs to help states develop nutrient criteria (EPA, 2000). In Minnesota, WQS have been adopted to protect lakes and rivers from eutrophic conditions (see Minnesota Rules, Chapter 7050.0222). These 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. Though not entirely conclusive, no clear trend was established for the role of nitrogen in the response of these organisms or any direct contribution to eutrophication. The scientific literature has reported some information that describes effects of nitrate and nitrogen on plants ranging from single cellular (algae) to macrophytes. The focus of this research primarily considers the nutritive effects resulting when different ratios of nitrogen and phosphorus are considered within a range of aquatic (mostly lake) systems. These examinations have reported effects on the relative growth and competition of plants that may result in shifts to different plant communities. More recent information has linked excess nitrate in surface water to the production of harmful algal blooms (Wurtsbaugh, 2019). To our knowledge, direct toxic effects of nitrate on plants have not been reported.

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, and excessive nitrate released to surface waters is usually associated with human influence on the landscape. This document proposes draft numeric standards for nitrate to protect 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 WQS for nitrate were developed in efforts to protect these uses based on best available scientific information.

The draft acute value (maximum standard) calculated is 60 mg/L N:NO3 for a one-day duration concentration for all Class 2 waters, and the draft chronic values are 8 mg/L N:NO3 mg/L for Class 2B and 2Bd waters and 5 mg/L N:NO3 for Class 2A waters for concentrations based on a four-day duration (Table 1).

Table 1. Proposed nitrate criteria for the protection of aquatic life

	Acute (all Class 2 waters)	**Chronic (Class 2A)**	**Chronic (2Bd)**
Criteria value	60 mg/L*	5 mg/L^	8 mg/L^

*one day duration

^four day duration

Data

Table 2a. Ranks of genus acute sensitivity for calculating Class 2 value and maximum standard.

Genus	MDR	R	P	GMAV[⬧]
Coregonus	1	28	0.965517	1902.00
Notropis	2,3	26	0.896552	1354.00
Oncorhynchus	1	25	0.862069	1310.59
Micropterus	2,3	24	0.827586	1261.00
Cyprinella	2,3	23	0.793103	1241.48
Pimephales	2,3	22	0.758621	1172.79
Salvelinus	1	21	0.724138	1121.40
Potamopyrgus	7,8	20	0.689655	1042.00
Megalonaias	7,8	19	0.655172	937.00
Allocapnia	6,8	18	0.62069	836.00
Hybognathus	2,3	17	0.586207	760.00
Lithobates	2,3	16	0.551724	694.00
Pseudacris	2,3	15	0.517241	643.00
Acipenser	2,3	14	0.482759	625.97
Hyla	2,3	13	0.448276	601.00
Ceriodaphnia	4	12	0.413793	543.84
Unio	7,8	11	0.37931	504.00
Lampsilis	7,8	10	0.344828	487.24
Amphinemura	6,8	9	0.310345	456.00
Daphnia	4	8	0.275862	447.14
Sphaerium	7,8	7	0.241379	371.00
Anodonta	7,8	6	0.206897	369.00
Hyalella	5	5	0.172414	368.37
Chironomus	6,8	4	0.137931	189.00
Neocloeon	6,8	3	0.103448	179.00
Cheumatopsyche	6,8	2	0.068966	137.06
Hydropsyche	6,8	1	0.034483	102.98

⬧ *mg/L N:NO3*

Table 2b. Four most sensitive genera for calculating Class 2 final acute value

Genus	Rank	GMAV	ln GMAV	(ln GMAV)^2	P = R/(N+1)	SQRT P
Chironomus	4	189.00	5.241747	27.47591	0.137931	0.371391
Neocloeon	3	179.00	5.187386	26.90897	0.103448	0.321634
Cheumatopsyche	2	137.06	4.920387	24.21021	0.068966	0.262613
Hydropsyche	1	102.98	4.634573	21.47927	0.034483	0.185695
	SUM		19.98409	100.0744	0.344828	1.141333

Table 2c. Calculation of Class 2A final acute value

S2 =	12.1751
S =	3.48928
L =	4.00042
A =	4.78064
FAV =	119.181 mg/L
MS =	59.5905 mg/L

Table 3a. Ranks of genus chronic sensitivity for calculating Class 2A final chronic value

Genus	GMCV	R	P
Daphnia	506.64	14	0.933333
Notropis	360.00	13	0.866667
Pimephales	214.13	12	0.8
Ceriodaphnia	65.59	11	0.733333
Potamopyrgus	57.80	10	0.666667
Hyla	47.00	9	0.6
Oncorhynchus	38.00	8	0.533333
Neocloeon	36.00	7	0.466667
Pseudacris	30.10	6	0.4
Rana	29.10	5	0.333333
Hyalella	18.92	4	0.266667
Lampsilis	17.45	3	0.2
Chironomus	9.56	2	0.133333
Salvelinus	6.25	1	0.066667

Table 3b. Four most sensitive genera for calculating Class 2A final chronic value

Genus	Rank	GMCV	ln GMCV	(ln GMCV)2	P = R/(N+1)	SQRT P
Hyalella	4	18.92	2.940	8.646	0.267	0.516
Lampsilis	3	17.45	2.860	8.177	0.200	0.447
Chironomus	2	9.56	2.258	5.097	0.133	0.365
Salvelinus	1	6.25	1.833	3.358	0.067	0.258
	SUM		9.890	25.278	0.667	1.587

Table 3c. Calculation of Class 2A final chronic value

S2 =	22.248
S =	4.717
L =	0.601
A =	1.656
FCV =	5.238 mg/L

Table 4a. Ranks of genus chronic sensitivity for calculating Class 2B final chronic value

Genus	GMCV	R	P
Daphnia	506.64	13	0.928571
Notropis	360.00	12	0.857143
Pimephales	214.13	11	0.785714
Ceriodaphnia	65.59	10	0.714286
Potamopyrgus	57.80	9	0.642857
Hyla	47.00	8	0.571429
Oncorhynchus	38.00	7	0.5
Neocloeon	36.00	6	0.428571
Pseudacris	30.10	5	0.357143
Rana	29.10	4	0.285714
Hyalella	18.92	3	0.214286
Lampsilis	17.45	2	0.142857
Chironomus	9.56	1	0.071429

Table 4b. Four most sensitive genera for calculating Class 2B final chronic value

Genus	Rank	GMCV	ln GMCV	(ln GMCV)2	P=R/(N+1)	SQRT P
Rana	4	29.100	3.371	11.362	0.286	0.535
Hyalella	3	18.923	2.940	8.646	0.214	0.463
Lampsilis	2	17.455	2.860	8.177	0.143	0.378
Chironomus	1	9.560	2.258	5.097	0.071	0.267
SUM			11.428	33.282	0.714	1.643

Table 4c. Calculation of Class 2B final chronic value

S2 =	15.872
S =	3.984
L =	1.221
A =	2.112
FCV =	8.264 mg/L

Figure 2. Distribution of Genus Mean Acute Values by percentile rank of sensitivity to nitrate

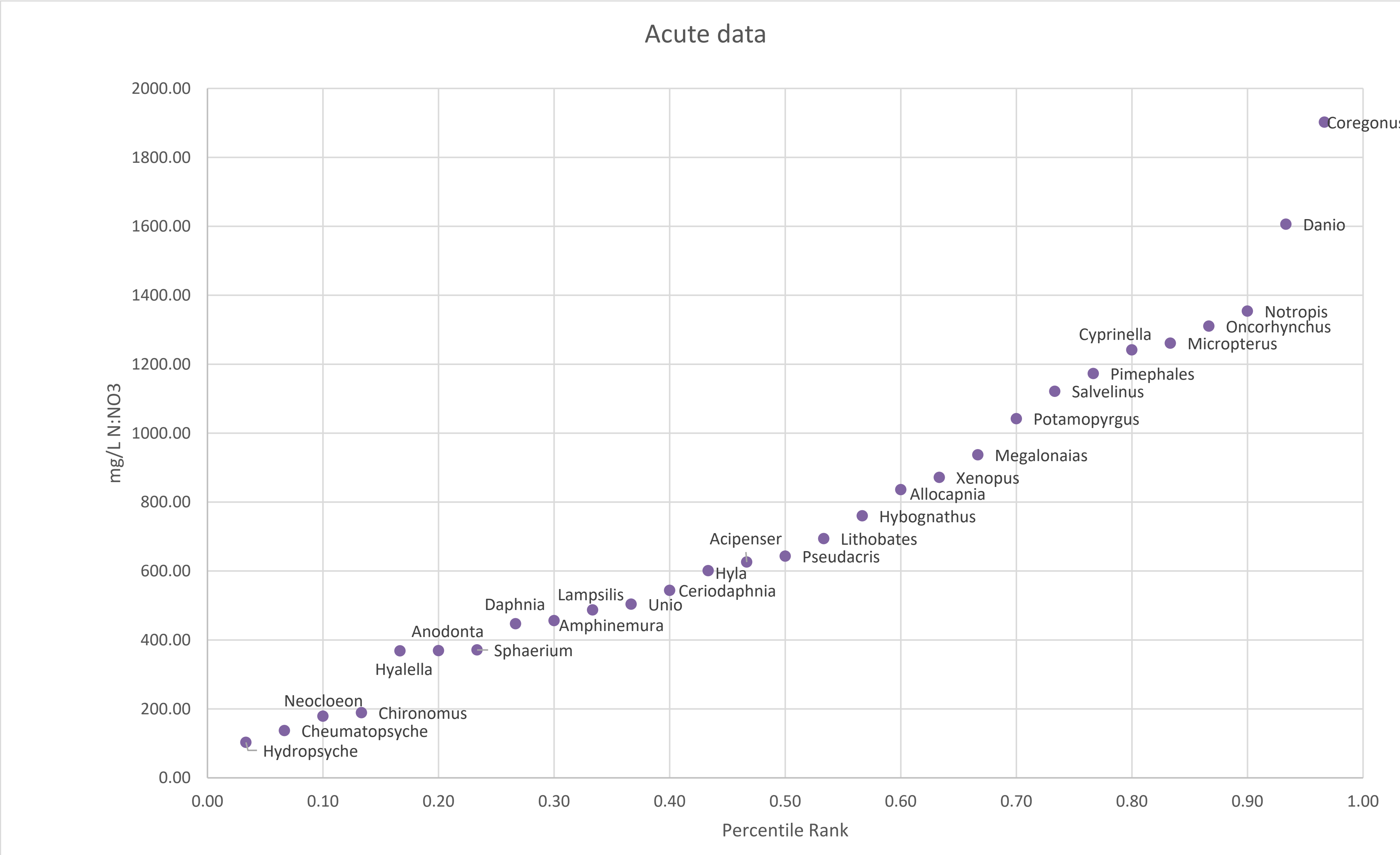


Table 5. All data used for acute criteria development.

Genus	MDR	Endpt Conc. (mg/L N:NO3)	GMAV	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Acipenser	2,3	1028	625.97	Mortality	LC50	4	Hamlin, 2006	OK
Acipenser	2,3	601		Mortality	LC50	4	Hamlin, 2006	OK
Acipenser	2,3	397		Mortality	LC50	4	Hamlin, 2006	OK
Allocapnia	6,8	836	836.00	Mortality	LC50	4	Soucek and Dickinson, 2012	OK
Amphinemura	6,8	456	456.00	Mortality	LC50	4	Soucek and Dickinson, 2012	OK
Anodonta	7,8	369	369.00	Mortality	LC50	4	Douda, 2010	OK; foot movement endpt
Ceriodaphnia	4	799	543.84	Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	780		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	765		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	750		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	716		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	711		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	696		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	685		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	671		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	665		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	619		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	615		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	614		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	566		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	558		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	544		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	509		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	502		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	487		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	478		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt

Genus	MDR	Endpt Conc. (mg/L N:NO3)	GMAV	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Ceriodaphnia	4	453		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	453		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	423		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	417		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	416		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	404		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	399		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	369		Mortality	LC50	2	Soucek and Dickinson, 2016	OK; most sensitive endpt
Ceriodaphnia	4	374		Mortality	LC50	2	Scott and Crunkilton, 2000	OK; most sensitive endpt
Ceriodaphnia	4	374		Mortality	LC50	2	Scott and Crunkilton, 2000	OK; most sensitive endpt
Cheumatopsyche	6,8	165.5	137.06	Mort/Morb	EC50	4	Camargo and Ward, 1992	OK; most sensitive endpt
Cheumatopsyche	6,8	113.5		Mort/Morb	EC50	4	Camargo and Ward, 1992	OK; most sensitive endpt
Chironomus	6,8	189	189.00	Mort/Morb	EC50	2	Wang et al., 2020	OK
Coregonus	1	1902	1902.00	Mortality	LC50	4	McGurk et al., 2006	OK; most sensitive endpt
Cyprinella	2,3	1744	1241.48	Mortality	LC50	4	Moore and Bringolf, 2020	OK; most sensitive endpt
Cyprinella	2,3	1717		Mortality	LC50	4	Moore and Bringolf, 2020	OK; most sensitive endpt
Cyprinella	2,3	639		Mortality	LC50	4	Moore and Bringolf, 2020	OK; most sensitive endpt
Danio	2,3	1606	1606.00	Mortality	LC50	4	Learmonth and Carvalho, 2015	Not used
Daphnia	4	611	447.14	Mortality	LC50	2	Scott and Crunkilton, 2000	OK
Daphnia	4	453		Mortality	LC50	2	Scott and Crunkilton, 2000	OK
Daphnia	4	323		Mortality	LC50	2	Scott and Crunkilton, 2000	OK
Hyalella	4	820	368.37	Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	713		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	682		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	673		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	659		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	641		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt

Genus	MDR	Endpt Conc. (mg/L N:NO3)	GMAV	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Hyalella	4	624		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	526		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	432		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	427		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	421		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	419		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	406		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	384		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	383		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	370		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	340		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	323		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	322		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	259		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	244		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	202		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	177		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	115		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	92		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	86		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	667		Mortality	LC50	4	Soucek et al., 2015	OK; most sensitive endpt
Hyalella	4	921		Mortality	LC50	4	Baker et al., 2017	OK; most sensitive endpt
Hyalella	4	484.9		Mortality	LC50	4	Baker et al., 2017	OK; most sensitive endpt
Hyalella	4	168.1		Mortality	LC50	4	Baker et al., 2017	OK; most sensitive endpt
Hybognathus	2,3	760	760.00	Mort/Morb	EC50	4	Buhl , 2002	OK; most sensitive endpt
Hydropsyche	6,8	109	102.98	Mort/Morb	EC50	4	Camargo and Ward, 1992	OK; most sensitive endpt
Hydropsyche	6,8	97.3		Mort/Morb	EC50	4	Camargo and Ward, 1992	OK; most sensitive endpt

Genus	MDR	Endpt Conc. (mg/L N:NO3)	GMAV	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Hyla	2,3	601	601.00	Mort/Morb	EC50	4	Wang et al., 2020	OK
Lampsilis	7,8	665	487.24	Mort/Morb	EC50	4	Wang et al., 2020	OK
Lampsilis	7,8	357		Mortality	LC50	4	Soucek and Dickinson, 2012	OK
Lithobates	2,3	694	694.00	Mort/Morb	EC50	4	Wang et al., 2020	OK
Megalonaias	7,8	937	937.00	Mortality	LC50	4	Soucek and Dickinson, 2012	OK
Micropterus	2,3	1261	1261.00	Mortality	LC50	4	Tomasso and Carmichael, 1986	OK
Neocloeon	6,8	179	179.00	Mortality	LC50	4	Soucek et al., 2015	OK
Notropis	2,3	1354	1354.00	Mortality	LC50	4	Adelman et al., 2009	OK
Oncorhynchus	1	1958	1310.59	Mortality	LC50	4	Baker et al., 2017	OK
Oncorhynchus	1	883		Mort/Morb	EC50	4	Wang et al., 2020	OK
Oncorhynchus	1	1658		Mortality	LC50	4	Buhl and Hamilton, 2000	OK
Oncorhynchus	1	1913		Mortality	LC50	4	Baker et al., 2017	OK
Oncorhynchus	1	1446		Mortality	LC50	4	Baker et al., 2017	OK
Oncorhynchus	1	808.5		Mortality	LC50	4	Baker et al., 2017	OK
Pimephales	2,3	1607	1172.79	Mortality	LC50	4	Scott and Crunkilton, 2000	OK
Pimephales	2,3	1406		Mortality	LC50	4	Scott and Crunkilton, 2000	OK
Pimephales	2,3	1010		Mortality	LC50	4	Scott and Crunkilton, 2000	OK
Pimephales	2,3	1537		Mortality	LC50	4	Moore and Bringolf, 2020	OK
Pimephales	2,3	1500		Mortality	LC50	4	Moore and Bringolf, 2020	OK
Pimephales	2,3	958		Mortality	LC50	4	Moore and Bringolf, 2020	OK
Pimephales	2,3	1278		Mortality	LC50	4	Buhl,K.J., 2002	OK
Pimephales	2,3	522		Mort/Morb	EC50	4	Buhl,K.J., 2002	OK
Potamopyrgus	7,8	1042	1042.00	Mortality	LC50	4	Alonso and Camargo, 2003	OK
Pseudacris	2,3	643	643.00	Mortality	LC50	4	Schuytema and Nebeker, 1999a	OK
Salvelinus	1	1121.4	1121.40	Mortality	LC50	4	McGurk et al., 2006	OK; most sensitive endpt
Sphaerium	7,8	371	371.00	Mortality	LC50	4	Soucek and Dickinson, 2012	OK
Unio	7,8	504	504.00	Mortality	LC50	4	Douda, 2010	OK; foot movement endpt

Genus	MDR	Endpt Conc. (mg/L N:NO3)	GMAV	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Xenopus	2,3	871.6	871.60	Mortality	LC50	4	Schuytema and Nebeker, 1999a	Not used

Table 6. All data used for chronic criteria development

Genus	Endpt Conc. (mg/L N:NO3)	GMCV (mg/L N:NO3)	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Ceriodaphnia	13.8		Reproduction	IC25	7	Baker et al., 2017	OK; geomean of EC20 and IC25
Ceriodaphnia	23.5		Reproduction	IC25	7	Baker et al., 2017	OK; geomean of EC20 and IC25
Ceriodaphnia	47.5		Reproduction	IC25	7	Baker et al., 2017	OK; geomean of EC20 and IC25
Ceriodaphnia	177	65.59	Reproduction	EC20	7	Soucek and Dickinson, 2016	OK; geomean of EC20 and IC25
Ceriodaphnia	91		Reproduction	EC20	7	Soucek and Dickinson, 2016	OK; geomean of EC20 and IC25
Ceriodaphnia	80		Reproduction	EC20	7	Soucek and Dickinson, 2016	OK; geomean of EC20 and IC25
Ceriodaphnia	263		Reproduction	EC20	7	Soucek and Dickinson, 2016	OK; geomean of EC20 and IC25
Chironomus	9.56	9.56	Biomass	EC20	10	Wang et al., 2020	OK; most sensitive endpt
Daphnia	717	506.64	Reproduction	LOEC	7	Scott and Crunkilton, 2000	OK
Daphnia	717		Reproduction	LOEC	7	Scott and Crunkilton, 2000	OK
Daphnia	358		Reproduction	NOEC	7	Scott and Crunkilton, 2000	OK
Daphnia	358		Reproduction	NOEC	7	Scott and Crunkilton, 2000	OK
Hyalella	11	18.92	Biomass	EC20	42	Soucek and Dickinson, 2016	Geomean of EC20 biomass; most sensitive endpt
Hyalella	22		Biomass	EC20	42	Soucek and Dickinson, 2016	Geomean of EC20 biomass; most sensitive endpt
Hyalella	28		Biomass	EC20	42	Soucek and Dickinson, 2016	Geomean of EC20 biomass; most sensitive endpt
Hyla	47	47.00	Metamorphosis	EC20	52	Wang et al., 2020	OK; most sensitive endpt
Lampsilis	17.39	17.45	Weight	EC20	28	Wang et al., 2020	Geomean of length and weight EC20
Lampsilis	17.52		Biomass	EC20	28	Wang et al., 2020	Geomean of length and weight EC20

Genus	Endpt Conc. (mg/L N:NO3)	GMCV (mg/L N:NO3)	Effect measurement	Endpoint	Test duration (Days)	Author	Status of use for criteria development
Neocloeon	36	36.00	Slowed/ Delayed Development	MATC	22.4	Soucek and Dickinson, 2016	OK; Reported endpoint same MATC for two observed effects (# d to PEN and % PEN WCF)
Notropis	486		Growth rate	LOEC	30	Adelman et al., 2009	OK; MATC
Notropis	268		Growth rate	NOEC	30	Adelman et al., 2009	OK; MATC
Notropis	360	360.00	Growth rate	MATC	30	Adelman et al., 2009	OK; Reported endpoint
Oncorhynchus	38		Biomass	EC20	42	Wang et al., 2020	OK; Endpts acceptable
Oncorhynchus	38	38.00	Weight	EC20	42	Wang et al., 2020	OK; Endpts acceptable
Oncorhynchus	38		Length	EC20	42	Wang et al., 2020	OK; Endpts acceptable
Pimephales	358.3	214.13	Biomass	IC25	7	Baker et al., 2017	Geomean of the four IC25 calcs
Pimephales	358.3		Biomass	IC25	7	Baker et al., 2017	Geomean of the four IC25 calcs
Pimephales	209		Biomass	IC25	7	Baker et al., 2017	Geomean of the four IC25 calcs
Pimephales	69.6		Biomass	IC25	7	Baker et al., 2017	Geomean of the four IC25 calcs
Potamopyrgus	21.4	57.80	Reproduction	LOEC	35	Alonso and Camargo, 2003	OK; MATC
Potamopyrgus	156.1		Reproduction	NOEC	35	Alonso and Camargo, 2003	OK; MATC
Pseudacris	30.1	30.1	Weight	LOEC	10	Schuytema and Nebeker,1999b	OK; most sensitive endpt
Pseudacris	30.1		Weight	NOEC	10	Schuytema and Nebeker, 1999b	OK; most sensitive endpt
Rana	29.1	29.10	Length	LOEL	16	Schuytema and Nebeker, 1999c	OK; most sensitive endpt; MATC of chronic effect (length)
Rana	29.1		Length	NOEL	16	Schuytema and Nebeker, 1999c	OK; most sensitive endpt; MATC of chronic effect (length)
Salvelinus	6.25	3.16	Weight	LOEC	120	McGurk et al., 2006	OK; most sensitive endpt
Salvelinus	1.6		Weight	NOEC	120	McGurk et al., 2006	OK; most sensitive endpt
Xenopus	56.7	37.50	Weight	LOEC	10	Schuytema and Nebeker, 1999a	Not used
Xenopus	24.8		Weight	NOEC	10	Schuytema and Nebeker, 1999a	Not used

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