Canadian Water Quality Guidelines for the Protection of Aquatic Life

NITRATE ION

The nitrate ion (NO_3^-) (CAS No. 14797-55-8, atomic mass 62.0049 $g \cdot mol^{-1}$) is the most oxidized form of nitrogen (N) present in the environment with an oxidation state of +5 (NRC 1978). It is the conjugate base of nitric acid (HNO_3), a strong acid which is completely dissociated in solution (NRC 1978). The nitrate salts of all common metals (e.g. $NaNO_3$, KNO_3, $Ca(NO_3)_2$, $AgNO_3$) are highly soluble in water, while the resulting free nitrate ion is chemically unreactive as it has little tendency to form coordination complexes with other metal ions in solution (NRC 1978).

Units used to report nitrate concentrations in the literature vary considerably. With the exception of the Canadian water quality guideline values presented in Table 2, all nitrate concentrations presented here will be for the ion only (i.e., as mg $NO_3^- \cdot L^{-1}$). Conversion factors for some of the commonly reported units in the literature are provided (Table 1).

Table 1. Conversion factors for various nitrate units to mg $NO_3^- \cdot L^{-1}$.

Base Unit	Multiply by:
mg NO_3^--$N \cdot L^{-1}$	4.43
mg $NaNO_3 \cdot L^{-1}$	0.73
mg $KNO_3 \cdot L^{-1}$	0.61
mg $NH_4NO_3 \cdot L^{-1}$	0.78
$eq \cdot L^{-1}$, *M*, N, or g-at.$\cdot L^{-1}$ *	62.005 x 10^3
ppm NO_3^-	1
ppb NO_3^-	10^{-3}

* note: for these units, the conversion factor is the same whether expressed as NO_3^--N or NO_3^-

Sources to the environment: Natural sources of nitrate to surface waters include wet and dry deposition of HNO_3 or NO_3^-, which are formed through nitrogen cycling in the atmosphere. Atmospheric deposition of dissolved inorganic nitrogen (DIN = NO_3^- + NO_2^- + NH_4^+) in Canada is estimated to contribute 182 kt $N \cdot yr^{-1}$ to surface waters (Chambers et al. 2001). It should be noted, however, that some of this atmospheric nitrogen may have originated anthropogenically. Other natural sources of nitrate include igneous rocks and volcanic activity, mineralization of native soil organic nitrogen and the complete oxidation of vegetable and animal debris (Nordin and Pommen 1986). This latter nitrification process is the principle source of nitrate in terrestrial and aquatic environments (NRC 1978).

Anthropogenic discharges of N include point sources such as municipal and industrial wastewaters, and water discharges from mining (explosives) activity, and non-point sources such as agricultural runoff, feedlot discharges, septic beds, urban runoff, lawn fertilizers, landfill leachate, nitric oxide and nitrogen dioxide from vehicular exhaust, and storm sewer overflow (NRC 1972; NRC 1978). Organic forms of nitrogen (originating from living material, e.g. proteins, amino acids, urea) undergo ammonification and are eventually transformed to ammonia, (NH_3) or ammonium (NH_4^+) by a variety of micro-organisms. All forms of inorganic nitrogen, ammonia, (NH_3) or ammonium (NH_4^+), released into surface waters have the potential to undergo nitrification to nitrate.

Table 2. Canadian water quality guidelines for the nitrate ion for the protection of aquatic life‡.

	Long-Term Exposure [c]	Short-Term Exposure [d]
Freshwater[a]	13 mg $NO_3^- \cdot L^{-1}$	550 mg $NO_3^- \cdot L^{-1}$
	3.0 mg NO_3^--$N \cdot L^{-1}$	124 mg NO_3^--$N \cdot L^{-1}$
Marine[b]	200 mg $NO_3^- \cdot L^{-1}$	1500 mg $NO_3^- \cdot L^{-1}$
	45 mg NO_3^--$N \cdot L^{-1}$	339 mg NO_3^--$N \cdot L^{-1}$

‡ For protection from direct toxic effects; the guidelines do not consider indirect effects due to eutrophication.
[a] = derived from toxicity tests utilizing $NaNO_3$
[b] = derived from toxicity tests utilizing $NaNO_3$ and KNO_3
[c] = derived with mostly no- and some low-effect data and are intended to protect against negative effects to aquatic ecosystem structure and function during indefinite exposures (e.g. abide by the guiding principle as per CCME 2007).
[d] = derived with severe-effects data (such as lethality) and are not intended to protect all components of aquatic ecosystem structure and function but rather to protect most species against lethality during severe but transient events (e.g. inappropriate application or disposal of the substance of concern).

The 2008 National Pollutant Release Inventory calculated a total point source estimate of anthropogenic nitrate ion release from all reporting Canadian sources of 62.8 kt NO_3^- to air, and surface and groundwaters, with a further 4.4 kt NO_3^- transferred off-site for disposal (Environment Canada 2010). Although comprehensive national non-point source release estimates are not available for either nitrate or total nitrogen (Chambers et al. 2001), they are likely to exceed those of point sources; U.S. estimates for non-point source N discharges into receiving waters (9108 kt N·yr^{-1}) greatly outweigh those for point sources (561 kt N·yr^{-1}) (van der Leeden et al. 1990).

Nitrate metal salts such as potassium nitrate, calcium nitrate, silver nitrate and sodium nitrate are used in a variety of industrial applications, including: oxidizing agents in explosives, matches and pyrotechnics; photography; glass making; engraving; textile dyes; food processing (meat preservatives); and as a raw material for manufacturing nitric acid (Nordin and Pommen 1986; WHO 1996). Other industrial processes which are known to result in high nitrate concentrations in their wastestreams include the production of fertilizers, the production of nitroaromatic compounds, the synthesis of nitroorganic compounds in pharmaceuticals, and wastewaters from nuclear fuel processing (Pinar et al. 1997).

Intensive agricultural practices have resulted in a steadily increasing demand for nitrogen in Canada. Estimated annual nitrogen-based fertilizer consumption in Canada in 1975 was approximately 500 kt N; in 2000 this estimate rose to 1700 kt N (CFI 2001). Of the 1600 kt of N sold as fertilizer in Canada over a 12 month period in 1998 and 1999, 90 kt of N were nitrate compounds, with 82% as ammonium nitrate, and the remaining forms including calcium nitrate, calcium ammonium nitrate and potassium nitrate (Korol and Rattray 2000).

Ambient Concentrations: The form of N occurring in surface waters depends primarily on the levels of oxygen present. Systems saturated with dissolved O_2 will promote nitrification by autotrophic bacteria which oxidize reduced forms of inorganic nitrogen (e.g., NH_4^+, NO_2^-) to NO_3^-, while in O_2 deficient waters, auto- and heterotrophic denitrifying bacteria reduce NO_3^- to NO_2^-, and ultimately to gaseous N_2 which is then lost to the atmosphere (Halling-Sorensen and Jorgensen 1993). Further information on the fate and behaviour of nitrate in the environment can be found in the scientific supporting document for the nitrate ion (Environment Canada 2012).

In general, nitrate constitutes two-thirds to four-fifths of the total available nitrogen in surface waters (Crouzet et al. 1999). Naturally occurring nitrate levels in Canadian lakes and rivers rarely exceed 4 mg NO_3^-·L^{-1}. In oligotrophic lakes and streams nitrate concentrations are generally < 0.4 mg NO_3^-·L^{-1} (NRC 1978; Nordin and Pommen 1986). Average 1990 nitrate levels in raw (pre-treated) Canadian municipal drinking water supplies ranged from 0.1 to 3.3 mg NO_3^-·L^{-1} (Government of Canada 1996). In the U.S., concentrations exceeding 2.7 mg NO_3^-·L^{-1} are generally considered the result of anthropogenic inputs, and levels above 4 mg NO_3^-·L^{-1} in fresh waters are often associated with eutrophic conditions (NRC 1978; USGS 1999).

Anthropogenic inputs of inorganic nitrogen may lead to elevated freshwater nitrate levels. Nitrate concentrations downstream from open pit coal mining operations can exceed 44 mg NO_3^-·L^{-1} due to high nitrate levels in the residues from explosives (Nordin and Pommen 1986). Inorganic fertilizer use in rural areas can also result in excessive nitrate loading in localized areas. Mean nitrate concentrations of North American streams in agricultural landscapes generally range between 9 and 180 mg NO_3^-·L^{-1} and levels above 45 mg NO_3^-·L^{-1} can persist for several weeks (Rouse et al. 1999; Castillo et al. 2000). Nitrate concentrations ranging from 19 to 42 mg NO_3^-·L^{-1} were also found in the Cootes Paradise wetland in Dundas, Ontario in 1997, primarily as a result of anthropogenic loading from a sewage treatment plant (Rouse et al. 1999).

Naturally occurring nitrate concentrations in temperate region seawater can reach up to 2.4 mg NO_3^-·L^{-1} (Spencer 1975), the majority of which is due to nitrification processes (Muir et al. 1991). Coastal nitrate levels vary seasonally; for example, off the Canadian Atlantic coast higher concentrations occur during the winter months (up to 0.54 mg NO_3^-·L^{-1}) than in the summer (< 0.03 mg NO_3^-·L^{-1}) when nitrate is depleted in the surface waters due to biological assimilation (Petrie et al. 1999). Concentrations increase with depth in off-shore regions, with levels of up to 1.24 mg NO_3^-·L^{-1} occurring beyond 30 m on the Scotian Shelf (Petrie et al. 1999). Where coastal upwelling events occur, nutrient-rich waters from below can elevate surface water nitrate concentrations (Whitney 2001). Marine nitrate levels are also typically higher closer to shore. For example, on the Canadian Pacific coast, mean (± SD) winter nitrate levels in the upper 100 m were higher in the Strait of Georgia (1.7 ± 0.1 mg NO_3^-·L^{-1}) than in the open ocean along an east-west transect near lower Vancouver Island (0.7 ± 0.2 mg NO_3^-·L^{-1}) (Whitney 2001).

Where there are anthropogenic inputs, nitrate levels in marine and estuarine waters can be much higher. For example, nitrate levels in estuaries of rivers draining agricultural and urbanised areas can exceed 12 mg $NO_3^- \cdot L^{-1}$ (Sharp 1983).

Nitrate serves as the primary source of nitrogen for aquatic plants in well oxygenated systems, and as nitrate levels increase, there is an increasing risk of algal blooms and eutrophication in surface waters (Nordin and Pommen 1986; Meade and Watts 1995). Along with phosphorus, nitrogen plays a major role in eutrophication in both types of waters, depending on the underlying geology and anthropogenic inputs to a given water body (Dodds et al. 1998). Common ecological changes to aquatic systems undergoing nutrient enrichment may include an increase in algal and macrophyte production resulting undesirable blooms, a decrease in water clarity, a loss of cold water fisheries, shortened food chains and changes in species composition (NRC 1978).

Toxicity: The direct toxicity of nitrate ions to aquatic organisms is assessed using either $NaNO_3$, NH_4NO_3 or KNO_3 salts. Studies exposing aquatic test organisms to both $NaNO_3$ and NaCl have demonstrated the observed toxicity is a result of exposure to the NO_3^- anion, rather than the Na^+ cation (Baker and Waights 1994). Therefore, studies using the $NaNO_3$ salt were included in the derivation of both freshwater and marine Canadian Water Quality Guidelines (CWQGs). Due to concerns about confounding toxicity of NH_4^+ ions in both freshwater and marine systems (Schuytema and Nebeker 1999a, 1999b), studies using these salts were excluded from CWQG development. Studies using potassium nitrate as the test compound were also not included in the derivation of the CWQG for freshwater environments due to the confounding toxicity of K^+. Potassium nitrate was shown to be 4.8 times more toxic to freshwater fish than $NaNO_3$ (Trama 1954), and other studies have demonstrated that potassium salts are more toxic to freshwater organisms than the corresponding sodium salt (Dowden and Bennett 1965; Khangarot and Ray 1989; Lilius et al. 1994; Calleja et al. 1994; Mount et al. 1997). As potassium ion concentrations used in marine toxicity studies with KNO_3 are generally within natural ranges of K^+ normally encountered in seawater, KNO_3 studies were not excluded from CWQG development for marine environments.

The mechanisms regulating nitrate uptake in aquatic biota are not fully understood. Limited accumulation of nitrate has been found in bodily fluids and tissues of invertebrates (crayfish and shrimp), and vertebrates (rainbow trout) exposed to high ambient nitrate levels (Jensen 1996; Stormer et al. 1996; Cheng et al. 2002). Mechanisms for nitrate uptake in amphibians have not been investigated, but there is potential for trans-dermal diffusion and uptake through the diet (Hecnar 2001).

Nitrate is considerably less toxic than ammonia or nitrite, with acute median lethal concentrations of NO_3^--N being up to two orders of magnitude higher than for NH_3-N and NO_2^--N (Colt and Armstrong 1981). Nonetheless, nitrate can produce toxic effects. There are two suspected mechanisms for the observed nitrate toxicity in aquatic animals: a) through methaemoglobin formation, resulting in a reduction in the oxygen carrying capacity of blood and b) through the inability of the organisms to maintain proper osmoregulation under high salt contents associated with elevated nitrate levels (Colt and Armstrong 1981).

Toxicity Modifying Factors: Recent work by Elphick (2011) investigated the effect of hardness on the toxicity of nitrate using both short-term and long-term toxicity tests. Short-term exposures were conducted using rainbow trout (*Oncorhynchus mykiss*) and an amphipod (*Hyalella azteca)*. Long-term exposures were conducted using the fathead minnow (*Pimephales promelas*), a water flea (*Ceriodaphnia dubia*), an amphipod (*Hyalella azteca*), and a midge (*Chironomus dilutus* – formerly *Chironomus tentans*). Tests with fish (rainbow trout and fathead minnow) were conducted using four hardness levels (approximately 15, 45, 90 and 160 $mg \cdot L^{-1}$ as $CaCO_3$). Tests with invertebrates (amphipod, water flea and midge) were not tested at the lowest hardness of 15 $mg \cdot L^{-1}$, and only tested at 45, 90 and 160 $mg \cdot L^{-1}$ as $CaCO_3$ hardness. In order to understand the relationship between hardness and nitrate toxicity, data were plotted into a regression of natural logarithmic (ln) of toxicant concentration as the dependent variable against the ln of hardness as the independent variable. Overall, the trend was one of decreasing toxicity with increasing hardness. However, in order to be able to derive a national hardness-adjusted guideline value, the calculated slopes for the hardness-toxicity relationships have to be compared to one other (e.g. comparison of slopes for short-term and long-term exposures separately). If it is concluded that the slopes for all species are not significantly different from one another, a pooled slope can be calculated, one using the short-term data and the second using the long-term data. This single pooled slope (one for short-term and a second for long-term exposures) is then used to derive hardness-adjusting equations for the development of a hardness-adjusted short-term and long-term guideline value. An F-test showed that the slopes for the two species (*O. mykiss* and *H. azteca*) for the short-term exposures were significantly different from one another (p=0.012). The slopes for the four species (*P. promelas, C. dubia, H. azteca* and *C. dilutus*) for the long-term exposures were also found to be significantly different from one another (F-test p value = 0.001). As a result, it was decided that the data would not be combined in order to generate a pooled slope, and there would be no derivation of either a hardness-dependant short-term or long-term equation for use in hardness-dependent short-term or long-term guideline derivation.

A long-term study evaluating the relationship between water hardness and nitrate toxicity was also conducted using a 40-day embryo-alevin-fry test with rainbow trout (*O. mykiss*) (Nautilus Environmental 2011). However, the results did not definitively demonstrate the relationship between increasing hardness and nitrate toxicity. In some cases, sensitivity appeared greater in the moderately hard water (92 $mg \cdot L^{-1}$ as $CaCO_3$) compared to the soft water (50 $mg \cdot L^{-1}$ as $CaCO_3$) and therefore this study was not included in the regression discussed above.

A short-term study conducted by Moore and Poirier (2010) evaluated the response of four species of salmonids to nitrate at three exposure temperatures (5, 10 and 15 deg C): *Oncorhynchus mykiss* (rainbow trout), *Salvelinus alpinus* (arctic charr), *Salvelinus namaycush* (lake trout), and *Coregonus clupeaformis* (lake whitefish). In this study, temperature did appear to have an effect on the 96-h LC_{50} value, but not always in a predictable way. In the case of both *O. mykiss* and *C. clupeaformis*, nitrate was found to be most toxic (96-h LC_{50} of 1690 and 4730 mg $NO_3^- \cdot L^{-1}$, respectively) when tested at the optimal metabolic temperatures for these fish (15 deg C for *O. mykiss* and 10 deg C for *C. clupeaformis*). Nitrate was found to be moderately toxic for *S. alpinus* at optimal metabolic test temperature of 10 deg C (96-h LC_{50} of 6650 mg $NO_3^- \cdot L^{-1}$), and least toxic to *S. namaycush* at optimal metabolic temperature of 10 deg C (96-h LC_{50} of 5230 mg $NO_3^- \cdot L^{-1}$). As for the influence of temperature on nitrate toxicity, species varied in their response, but this is likely due to species tolerance levels of temperature.

Water quality parameters such as pH and dissolved oxygen can influence the conversion of nitrate to other forms of nitrogen, or vice versa. Separate Canadian Water Quality Guidelines exist for some of these other forms of nitrogen (e.g., nitrite and ammonia).

Water Quality Guideline Derivation: Both the freshwater and marine short-term benchmark concentrations and the long-term Canadian water quality guidelines (CWQGs) for the nitrate ion for the protection of aquatic life were developed based on the CCME protocol (CCME 2007) using the statistical (Type A) approach.

Short-term Freshwater Benchmark Concentration: Short-term benchmark concentrations are derived using severe effects data (such as lethality) of defined short-term exposure periods (24 - 96-h). These benchmarks represent a concentration that may result in severe effects to the aquatic ecosystem and are intended to give guidance on the impacts of severe, but transient, situations (e.g., spill events to aquatic receiving environments and infrequent releases of short-lived/non-persistent substances). Short-term benchmark concentrations *do not* provide guidance on protective levels of a substance in the aquatic environment, as short-term benchmark concentrations are levels which *do not* protect against adverse effects.

The minimum data requirements for the Type A short-term benchmark concentration approach were met, and a total of 23 data points (all LC_{50} values) were used in the derivation of the value (Table 3). Each species for which appropriate short-term toxicity data was available was ranked according to sensitivity, and its centralized position on the species sensitivity distribution (SSD) was determined using the Hazen plotting position (estimate of the cumulative probability of a data point). Intra-species variability was accounted for by taking the geometric mean of the studies considered to represent the most sensitive lifestage and endpoint.

The log-Gompertz model provided the best fit of the five models tested (Figure 1). The equation of the Gompertz model is of the form:

$$f(x) = 1 - e^{-e^{\frac{(x-\mu)}{s}}}$$

Where, for the fitted model: x = log (concentration) of nitrate ($mg \cdot L^{-1}$), $f(x)$ is the proportion of species affected, μ = 3.6330 and s = 0.3019. The short-term SSD is shown in Figure 1 and summary statistics are presented in Table 4. The 5th percentile on the short-term SSD is 545 mg $NO_3^- \cdot L^{-1}$. This value is rounded to 2 significant figures to generate the freshwater short-term benchmark concentration of 550 mg $NO_3^- \cdot L^{-1}$ (Table 4). The lower fiducial limit (5%) on the 5th percentile is 456 mg $NO_3^- \cdot L^{-1}$, and the upper fiducial limit (95%) on the 5th percentile is 652 mg $NO_3^- \cdot L^{-1}$. The concentration of 545 mg $NO_3^- \cdot L^{-1}$ is within the range of the data (to which the model was fit). Therefore, the 5th percentile and its confidence limits are interpolations.

Table 3. Endpoints used to determine the freshwater short-term benchmark concentration for the nitrate ion.

Species	Endpoint	Hardness of Exposure water (mg·L^{-1} as $CaCO_3$)	Concentration (mg NO_3^-·L^{-1})	Reference
Fish				
Pimephales promelas Fathead minnow	96h LC_{50}	156-172; 136-140	3304*	Scott and Crunkilton 2000; US EPA 2010
Oncorhynchus mykiss Rainbow trout	96h LC_{50}	106-127; 90	3638*	Moore and Poirier 2010; Elphick 2011
Coregonus clupeaformis Lake whitefish	96h LC_{50}	106-127	4730	Moore and Poirier 2010
Salvelinus namaycush Lake trout	96h LC_{50}	10-16	4968	McGurk et al. 2006
Oncorhynchus tshawytscha Chinook salmon	96h LC_{50}	na	5800	Westin 1974
Notropis topeka Topeka shiner	96h LC_{50}	210-230	5994	Adelman et al 2009
Ictalurus punctatus Channel catfish	96h LC_{50}	102	6200	Colt and Tchobanoglous 1976
Salvelinus alpinus Arctic char	96h LC_{50}	106-127	6650	Moore and Poirier 2010
Lepomis macrochirus Bluegill	96h LC_{50}	45-50	8753	Trama 1954
Amphibians				
Pseudacris regilla Pacific tree frog	96h LC_{50}	70-80	2849	Schuytema and Nebeker 1999a
Xenopus laevis African clawed frog	96h LC_{50}	21	7335	Schuytema and Nebeker 1999c
Invertebrates				
Hydropsyche occidentalis Caddisfly	96h LC_{50}	42.7	431	Camargo and Ward 1992
Cheumatopsyche pettiti Caddisfly	96h LC_{50}	42.7	503	Camargo and Ward 1992
Hyalella azteca Amphipod	96h LC_{50}	80-84; 110-124; 100	774*	US EPA 2010; Soucek and Dickinson 2011; Elphick 2011
Chironomus dilutus Midge	48h LC_{50}	84-136	1582	US EPA 2010
Lampsilis siliquoidea Fatmucket mussel	96h LC_{50}	90-92	1582	US EPA 2010
Sphaerium simile Fingernail clam	96h LC_{50}	90-92	1644	US EPA 2010
Ceriodaphnia dubia Water flea	48h LC_{50}	156-172	1657	Scott and Crunkilton 2000
Amphinemura delosa Stonefly	96h LC_{50}	88-92	2020	US EPA 2010
Daphnia magna Water flea	48h LC_{50}	156-172	2047	US EPA 2010
Allocapnia vivipara Stonefly	96h LC_{50}	98-100	3703	Soucek and Dickinson 2011
Megalonaias nervosa Washboard mussel	96h LC_{50}	90-92	4151	US EPA 2010
Potamopyrgus antipodarum New Zealand mudsnail	96h LC_{50}	90.8	4616	Alonso and Camargo 2003

*Value shown is the geometric mean of comparable values

Table 4. Short-term freshwater benchmark concentration for the nitrate ion resulting from the SSD Method.

	Concentration – as nitrate
SSD 5th percentile	550 mg·L^{-1}
SSD 5th percentile, LFL (5%)	456 mg·L^{-1}
SSD 5th percentile, UFL (95%)	652 mg·L^{-1}

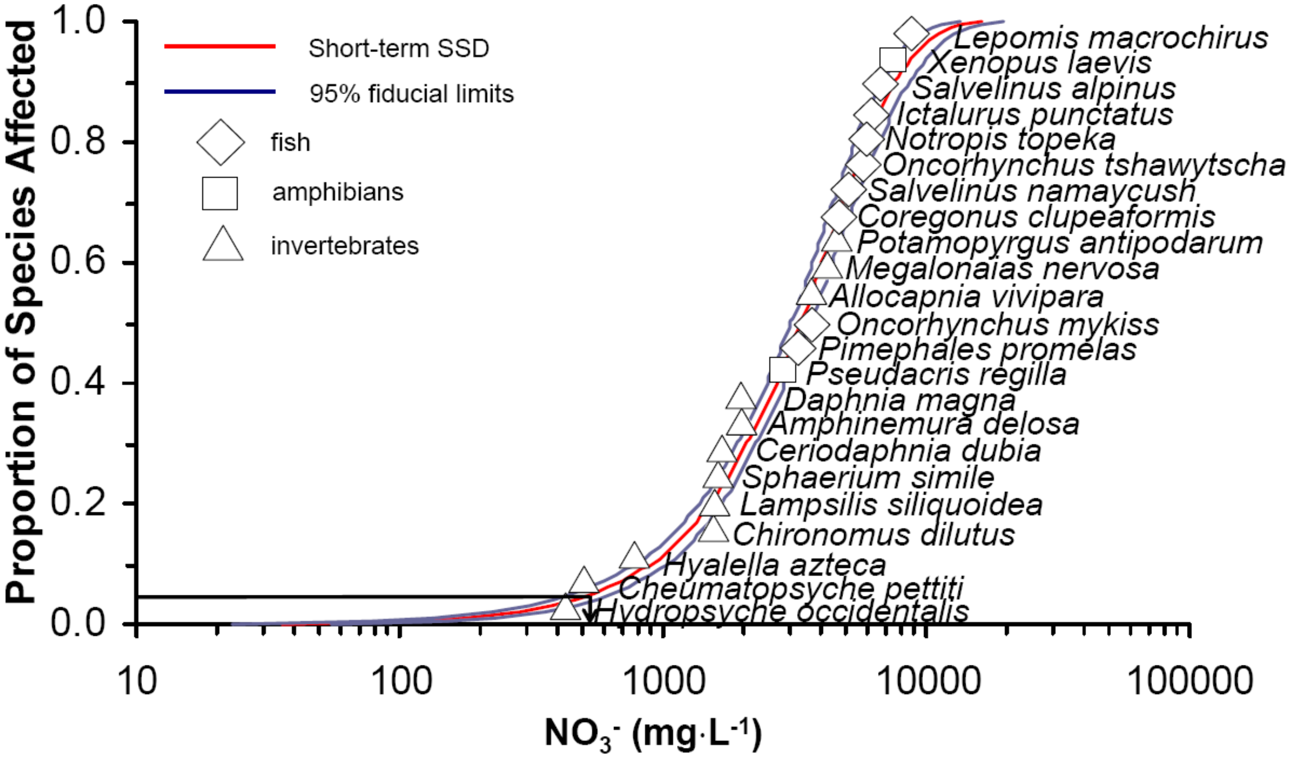

Figure 1. SSD of short-term LC_{50} toxicity data for the nitrate ion in freshwater derived by fitting the Gompertz model to the logarithm of acceptable toxicity data for 23 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding short-term benchmark concentration value.

Two data points fall below the short-term SSD 5th percentile value of 545 mg NO_3^-·L^{-1}. These include the 96-hour LC_{50} of 431 mg NO_3^-·L^{-1} for the caddisfly *Hydropsyche occidentalis (*Camargo and Ward 1992) and the 96-hour LC_{50} of 503 mg NO_3^-·L^{-1} for the caddisfly *Cheumatopsyche pettiti* (Camargo and Ward 1992). From all the invertebrate studies used in deriving the short-term benchmark value, these two caddisfly exposures were conducted in the exposure water of lowest hardness (CCME designated soft water, compared to the other exposures that used CCME moderately hard or CCME hard water). Based on the short-term SSD, short-term exposures to levels of nitrate exceeding the benchmark concentration of 550 mg NO_3^-·L^{-1} *may* pose the greatest hazard to the sensitive caddisflies. Note that meeting the long-term guideline will protect from severe effects.

Therefore, the short-term exposure benchmark concentration indicting the potential for severe effects (e.g. lethality or immobilization) to sensitive freshwater life during transient events is 550 mg NO_3^-·L^{-1}, for the nitrate ion.

Long-term Freshwater Quality Guideline: Long-term exposure guidelines identify a concentration of a parameter in the aquatic ecosystem below which of aquatic life are intended to be protected for indefinite exposure periods. Long-term exposure guidelines are derived using long-term data (≥7d exposures for fish and invertebrates, ≥24h for aquatic plants and algae).

The minimum data requirements for the Type A guideline approach were met, and a total of 12 data points were used in the derivation of the guideline (Table 5). Each species for which appropriate long-term toxicity data was available was ranked according to sensitivity, and its centralized position on the SSD was determined using the Hazen plotting position. Intra-species variability was accounted for by taking the geometric mean of the studies considered to represent the most sensitive lifestage and endpoint.

Table 5. Endpoints used to determine the freshwater long-term CWQG for the nitrate ion.

Species	Endpoint	Hardness of Exposure water ($mg \cdot L^{-1}$ as $CaCO_3$)	Concentration ($mg\ NO_3^- \cdot L^{-1}$)	Reference
Fish				
Salvelinus namaycush Lake trout	146-d MATC (delay to swim-up stage and growth as wet weight)	10-16	14*	McGurk et al. 2006
Oncorhynchus mykiss Rainbow trout	41-d MATC (proportion reaching swim-up)	10	58	Nautilus Environmental 2011
Pimephales promelas Fathead minnow	32-d EC_{10} (survival)	132-180	207	US EPA 2010
Notropis topeka Topeka shiner	30-d MATC (growth)	210-230	1594*	Adelman et al. 2009
Oncorhynchus tshawytscha Chinook salmon	10-d LC_{10}	na	3142	Westin 1974
Amphibians				
Pseudacris regilla Pacific treefrog	10-d LC_{10}	70-80	328	Schuytema and Nebeker 1999c
Xenopus laevis African treefrog	10-d MATC (weight)	21	404*	Schuytema and Nebeker 1999c
Rana aurora Red-legged frog	16-d MATC (weight)	26	734*	Schuytema and Nebeker 1999b
Invertebrates				
Ceriodaphnia dubia Water flea	7-d IC_{25} (reproduction)	44	50	Elphick 2011
Hyalella azteca Amphipod	14-d IC_{25} (growth)	46	57	Elphick 2011
Chironomus dilutus Midge	10-d IC_{25} (growth)	46	217	Elphick 2011
Daphnia magna Water flea	7-d MATC (reproduction)	156-172	2244*	Scott and Crunkilton 2000

*Value shown is the geometric mean of comparable values.

The Normal model provided the best fit of the five models tested (Figure 2). The equation of the Normal model is:

$$f(x) = \frac{1}{2}\left(1 + \operatorname{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right)$$

Where, for the fitted model: x = log (concentration) of nitrate (mg·L^{-1}), $f(x)$ is the proportion of species affected, $\mu = 2.4307$, $\sigma = 0.7992$ and *erf* is the error function (a.k.a. the Gauss error function). The long-term SSD is shown in Figure 2 and summary statistics are presented in Table 6. The 5th percentile on the long-term SSD is 13 mg NO_3^-·L^{-1}. The lower fiducial limit (5%) on the 5th percentile is 7 mg NO_3^-·L^{-1}, and the upper fiducial limit (95%) on the 5th percentile is 24 mg NO_3^-·L^{-1}. The concentration of 13 mg NO_3^-·L^{-1} is outside the range of the data (to which the model was fit). Therefore, the 5th percentile and its confidence limits are extrapolations.

Therefore the long-term exposure CWQG for the protection of freshwater aquatic life is 13 mg NO_3^-·L^{-1} for the nitrate ion.

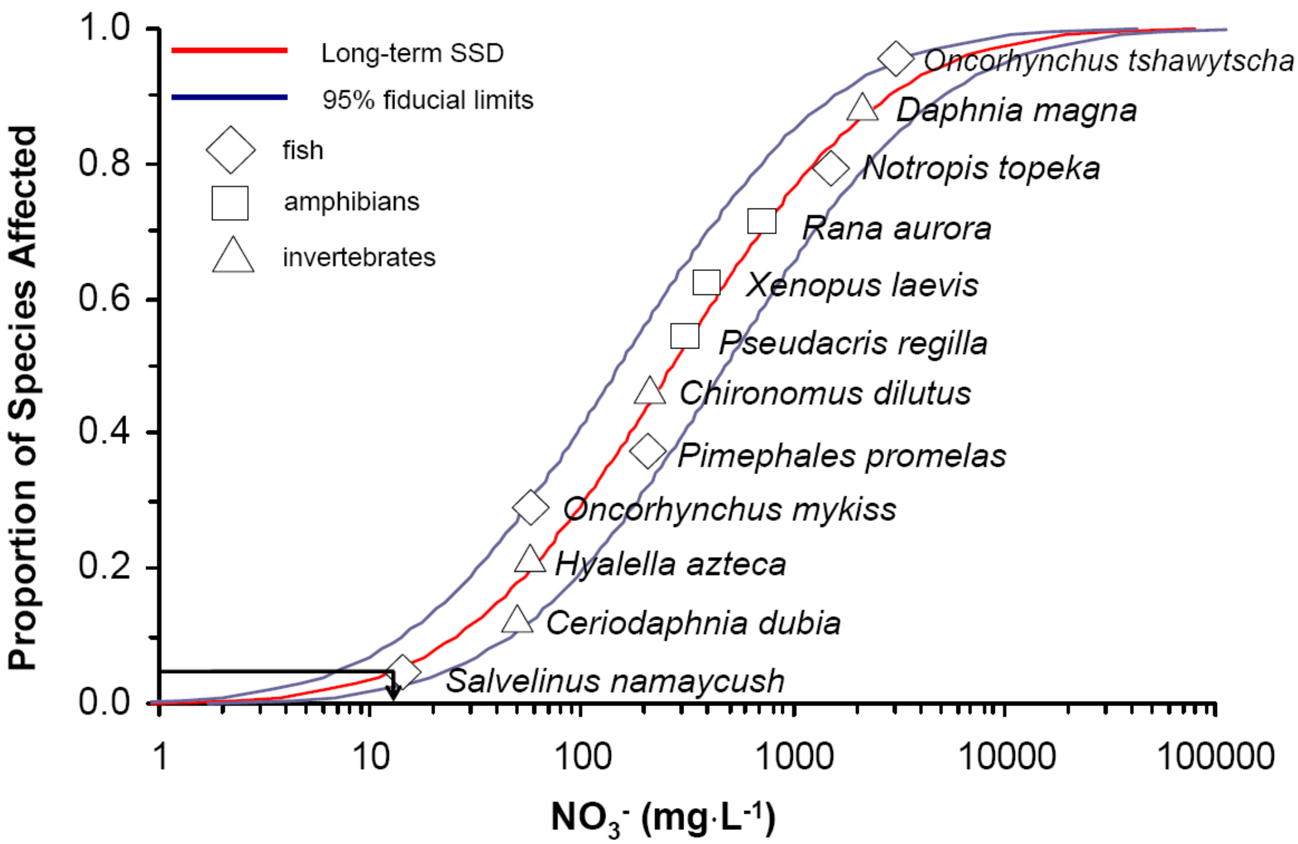

Figure 2. SSD of long-term no- and low-effect endpoint toxicity data for the nitrate ion in freshwater derived by fitting the Normal model to the logarithm of acceptable data for 12 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding long-term Canadian Water Quality Guideline value.

Table 6. Long-term freshwater CWQG for the nitrate ion resulting from the SSD Method.

	Concentration (as nitrate)
SSD 5th percentile	13 $mg{\cdot}L^{-1}$
SSD 5th percentile, LFL (5%)	7 $mg{\cdot}L^{-1}$
SSD 5th percentile, UFL (95%)	24 $mg{\cdot}L^{-1}$

***General Discussion Related to the Freshwater CWQG*:** The use of the 5th percentile of the SSD as the environmental standard is designed to protect at least 95% of aquatic species from low-level effects. It is important to note that one toxicity endpoint lies just above the guideline value (Figure 2), which is the 146-d MATC of 14 mg $NO_3^-{\cdot}L^{-1}$ for the lake trout swim-up fry (*Salvelinus namaycush*) (McGurk et al. 2006). McGurk et al. (2006) observed 2 endpoints that were impacted (or reduced compared to control) at 28 mg $NO_3^-{\cdot}L^{-1}$ (low effect, or LOEC) whereas no effect (NOEC) was observed at 7 mg $NO_3^-{\cdot}L^{-1}$. This was for both 1) growth, measured as wet weight, of lake trout swim-up fry, as well as 2) delay in development of lake trout alevin to the swim-up stage. Because the 2007 CCME protocol indicates that the inclusion of MATC values over LOEC and NOEC values, the geometric mean (MATC of 14 mg $NO_3^-{\cdot}L^{-1}$) was included in the SSD calculations. The equivalent MATC endpoint for delay to swim-up stage for rainbow trout (*O. mykiss*) swim-up fry is 58 mg $NO_3^-{\cdot}L^{-1}$ (Nautilus Environmental 2011) (Table 5). Since the CWQG value of 13 mg $NO_3^-{\cdot}L^{-1}$ is below both the lake trout LOEC (delay to swim-up and growth) of 58 mg $NO_3^-{\cdot}L^{-1}$ and lake trout MATC (mortality) of 886 mg $NO_3^-{\cdot}L^{-1}$ (Environment Canada 2010), no direct effects on development delays, growth or survival are expected. The CCME guideline derivation protocol (CCME 2007) provides the option of implementing the Protection Clause in situations where a data point for a species at risk, a species of commercial or recreational importance, or an ecologically important species falls below the HC5 (CWQG) value on the long-term SSD. In this case, no data-points fell below the HC5 value.

This CWQG of 13 mg $NO_3^-{\cdot}L^{-1}$ calculated using a species sensitivity distribution is the same value as the 2003 freshwater interim guideline of 13 mg $NO_3^-{\cdot}L^{-1}$. In the case of the 2003 freshwater interim guideline, the value was based on a 10-day chronic study examining the toxicity of sodium nitrate to the Pacific treefrog (*Pseudacris regilla*; Schuytema and Nebeker 1999c). Test organisms exposed to 133 mg $NO_3^-{\cdot}L^{-1}$ experienced a mean decrease in weight of 15% when compared to the control group. A safety factor of 0.1 was applied to the LOEC in accordance with CCME (1991) to derive the final interim guideline value. In the case of the 2012 full guideline value, all minimum dataset requirements for the development of a CWQG were fulfilled. It must be noted that the 2003 CWQG was interim, meaning that the required dataset was not fulfilled (one chronic invertebrate study on a non-planktonic organism was missing). A recommendation was also made in the 2003 scientific criteria document to "conduct additional toxicity tests for fish and invertebrate species that are known to be highly sensitive" to nitrate. For the derivation of the 2012 CWQG, additional testing was conducted using the amphipod *Hyalella azteca* (to ensure minimum dataset requirements were fulfilled). Testing was also conducted using the early life stage of the rainbow trout (Stantec 2006) and the lake trout (McGurk et al. 2006). Test results indicated that the CWQG of 13 mg $NO_3^-{\cdot}L^{-1}$ would be protective of the lake trout, the most sensitive of the fish species tested.

Short-term Marine Benchmark Concentration: Short-term benchmark concentrations are derived using severe effects data (such as lethality) of defined short-term exposure periods (24 - 96-h). These benchmarks represent a concentration that may result in severe effects to the aquatic ecosystem and are intended to give guidance on the impacts of severe, but transient, situations (e.g., spill events to aquatic receiving environments and infrequent releases of short-lived/non-persistent substances). Short-term benchmark concentrations *do not* provide guidance on protective levels of a substance in the aquatic environment, as short-term benchmark concentrations are levels which *do not* protect against adverse effects.

The minimum data requirements for the Type A short-term benchmark concentration approach were met, and a total of 10 data points (all LC_{50} values) were used in the derivation of the value (Table 7). Each species for which appropriate short-term toxicity data was available was ranked according to sensitivity, and its centralized position on the species sensitivity distribution (SSD) was determined using the Hazen plotting position (estimate of the cumulative probability of a data point). Intra-species variability was accounted for by taking the geometric mean of the studies considered to represent the most sensitive lifestage and endpoint.

Table 7. Endpoints used to determine the marine short-term benchmark concentration for the nitrate ion.

Species	Endpoint	Concentration ($mg\ NO_3^-\cdot L^{-1}$)	Reference
Fish			
Monacanthus hispidus Planehead filefish	96h LC_{50}	2538	Pierce et al. 1993
Raja eglanteria Clearnose skate	96h LC_{50}	>4253[1]	Pierce et al. 1993
Oncorhynchus tshawytscha Chinook salmon	96h LC_{50}	4400	Westin 1974
Trachinotus carolinus Florida pompano	96h LC_{50}	4430	Pierce et al. 1993
Oncorhynchus mykiss Rainbow trout	96h LC_{50}	4650	Westin 1974
Centropristis striata Gulf black sea bass	96h LC_{50}	10632	Pierce et al. 1993
Pomacentrus leucostictus Beaugregory	96h LC_{50}	>13290[1]	Pierce et al. 1993
Invertebrates			
Strongylocentrotus purpuratus Purple sea urchin	96h EC_{50} (larval development)	1384	Stantec 2006
Penaeus monodon Tiger shrimp	96h LC_{50}	7717*	Tsai and Chen 2002
Penaeus paulensis Prawn	96h LC_{50}	9621	Cavalli et al. 1996

[1] The use of toxicity data from a test where an insufficient concentration range on the higher end has been tested (i.e., where the results are expressed as "toxic concentration is greater than *x*"), are generally acceptable, as they will not result in an under-protective guideline. These studies can be used to fill the minimum data set requirements and in the actual guideline derivation (CCME 2007).
*Value shown is the geometric mean of comparable vlues.

The Logistic model provided the best fit of the five models tested (Figure 3). The equation of the Logistic model is:

$$y = 1/[1+e^{-((x-\mu)/\sigma)}]$$

Where for the fitted model: x = log (concentration) of nitrate ($mg\cdot L^{-1}$), y is the proportion of species affected, $\mu = 3.7290$ and $\sigma = 0.1881$. The short-term SSD is shown in Figure 3 and summary statistics are presented in Table 8. The 5th percentile on the short-term SSD is 1497 mg $NO_3^-\cdot L^{-1}$. This value is rounded to 2 significant figures to generate the marine short-term benchmark concentration of 1500 mg $NO_3^-\cdot L^{-1}$ (Table 8). The lower fiducial limit (5%) on the 5th percentile is 1046 mg $NO_3^-\cdot L^{-1}$, and the upper fiducial limit (95%) on the 5th percentile is 2141 mg $NO_3^-\cdot L^{-1}$. The concentration of 1497 mg $NO_3^-\cdot L^{-1}$ is inside the range of the data (to which the model was fit). Therefore, the 5th percentile and its confidence limits are interpolations.

Table 8. Short-term marine benchmark concentration for the nitrate ion resulting from the SSD Method.

	Concentration (as nitrate)
SSD 5th percentile	1500 $mg\cdot L^{-1}$
SSD 5th percentile, LFL (5%)	1046 $mg\cdot L^{-1}$
SSD 5th percentile, UFL (95%)	2141 $mg\cdot L^{-1}$

One data point falls below the short-term SSD 5th percentile value of 1497 mg $NO_3^-\cdot L^{-1}$, the 96-hour EC_{50} of 1384 mg $NO_3^-\cdot L^{-1}$ for the purple sea urchin *Strongylocentrotus purpuratus* (Stantec 2006). Based on the short-term SSD, short-term exposures to levels of nitrate exceeding the benchmark concentration of 1500 mg $NO_3^-\cdot L^{-1}$ *may* pose the greatest hazard to the sensitive purple sea urchin. Note that meeting the long-term guideline will protect from severe effects.

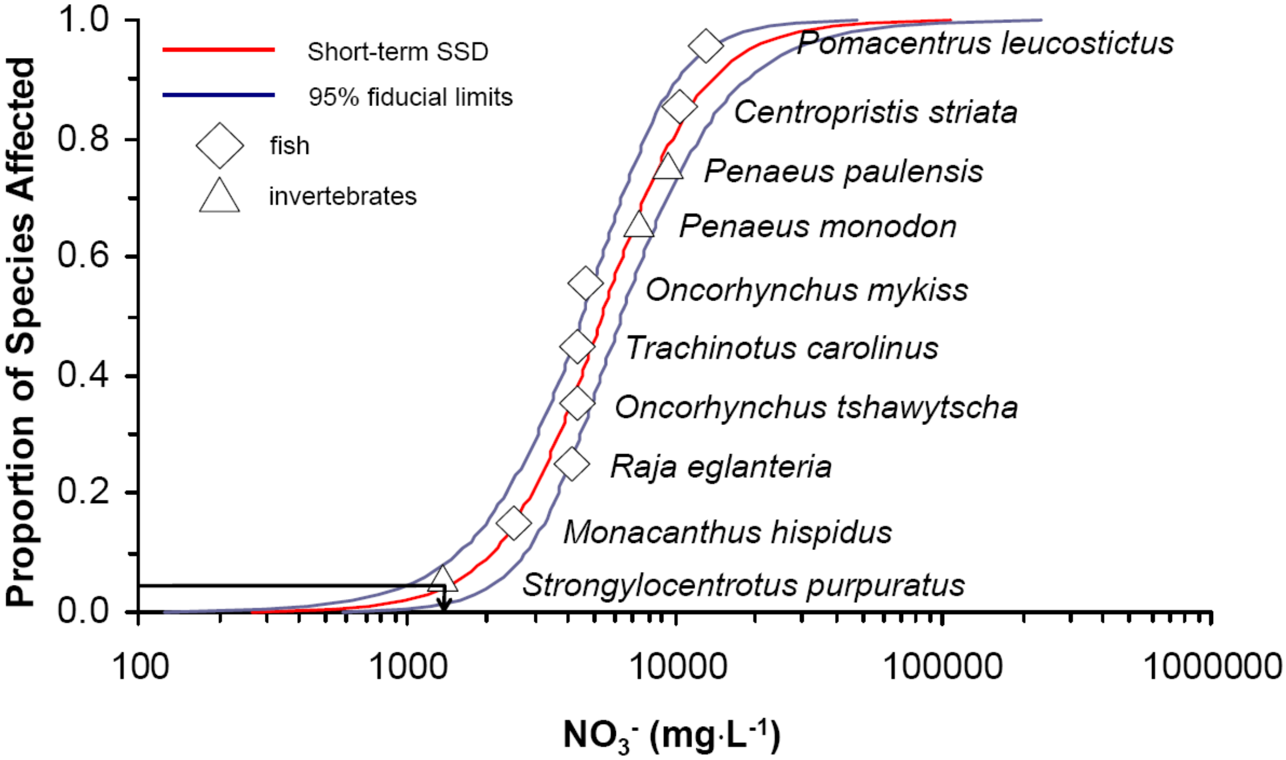

Figure 3. SSD of short-term L/EC_{50} toxicity data for the nitrate ion in saltwater derived by fitting the Logistic model to the logarithm of acceptable toxicity data for 10 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding short-term benchmark concentration value.

Therefore the short-term exposure benchmark concentration indicating the potential for severe effects (e.g. lethality or immobilization) to sensitive marine life during transient events is 1500 mg NO_3^-·L^{-1}.

Long-term Marine Quality Guideline: Long-term exposure guidelines identify a concentration of parameter in the aquatic ecosystem below which all forms of aquatic life are intended to be protected, for indefinite exposure periods. Long-term exposure guidelines are derived using long-term data (≥7d exposures for fish and invertebrates, ≥24h for aquatic plants and algae).

The minimum data requirements for the Type A guideline approach were met, and a total of 12 data points were used in the derivation of the guideline (Table 9). Each species for which appropriate long-term toxicity data was available was ranked according to sensitivity, and its centralized position on the SSD was determined using the Hazen plotting position.

Table 9. Endpoints used to determine the marine long-term CWQG for the nitrate ion.

Species	Endpoint	Concentration ($mg\ NO_3^-\cdot L^{-1}$)	References
Fish			
Amphiprion ocellaris Anemonefish	72-d LOEC (growth, mortality)	443	Frakes and Hoff Jr. 1982
Atherinops affinis Topsmelt	7-d LC_{25}	2554	Stantec 2006
Oncorhynchus mykiss Rainbow trout	7-d LC_{10}	2954	Westin 1974
Oncorhynchus tshawytscha Chinook salmon	7-d LC_{10}	3510	Westin 1974
Invertebrates			
Nereis grubei Polychaete	28-d LC_{10}	214	Reish 1970
Neanthes arenaceodentata Polychaete	28-d LC_{10}	440	Reish 1970
Capitella capitella Polychaete	28-d LC_{10}	660	Reish 1970
Dorvillea articulate Polychaete	28-d LC_{10}	700	Reish 1970
Haliotis tuberculata Abalone	15-d LOEC (growth)	1108	Basuyaux and Mathieu 1999
Paracentrotus lividus Purple Sea Urchin	15-d LOEC (growth / feeding)	1108	Basuyaux and Mathieu 1999
Strongylocentrotus purpuratus Pacific purple sea urchin	4-d IC_{25} (larval development)	1178	Stantec 2006
Cherax quadricarinatus Australian crayfish	5-d LOEC (respiration)	>4430[1]	Meade and Watts 1995

[1] The use of toxicity data from a test where an insufficient concentration range on the higher end has been tested (i.e., where the results are expressed as "toxic concentration is greater than *x*"), are generally acceptable, as they will not result in an under-protective guideline. These studies can be used to fill the minimum data set requirements and in the actual guideline derivation (CCME 2007).

The Normal model provided the best fit of the five models tested (Figure 4). The equation of the Normal model is:

$$f(x) = \frac{1}{2}\left(1 + \mathrm{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right)$$

Where, for the fitted model: *x* = log (concentration) of nitrate ($mg\cdot L^{-1}$), *f(x)* is the proportion of species affected, μ = 3.0385, σ = 0.4539 and *erf* is the error function (a.k.a. the Gauss error function). The long-term SSD is shown in Figure 4 and summary statistics are presented in Table 10. The 5th percentile on the long-term SSD is 196 mg $NO_3^-\cdot L^{-1}$. This value is rounded to 2 significant figures to generate the marine Canadian water quality guideline of 200 mg $NO_3^-\cdot L^{-1}$ (Table 10). The lower fiducial limit (5%) on the 5th percentile is 141 mg $NO_3^-\cdot L^{-1}$, and the upper fiducial limit (95%) on the 5th percentile is 273 mg $NO_3^-\cdot L^{-1}$. The concentration of 196 mg $NO_3^-\cdot L^{-1}$ is outside the range of the data (to which the model was fit). Therefore, the 5th percentile and its confidence limits are extrapolations.

Therefore the long-term exposure CWQG for the protection of marine life is 200 mg $NO_3^-\cdot L^{-1}$.

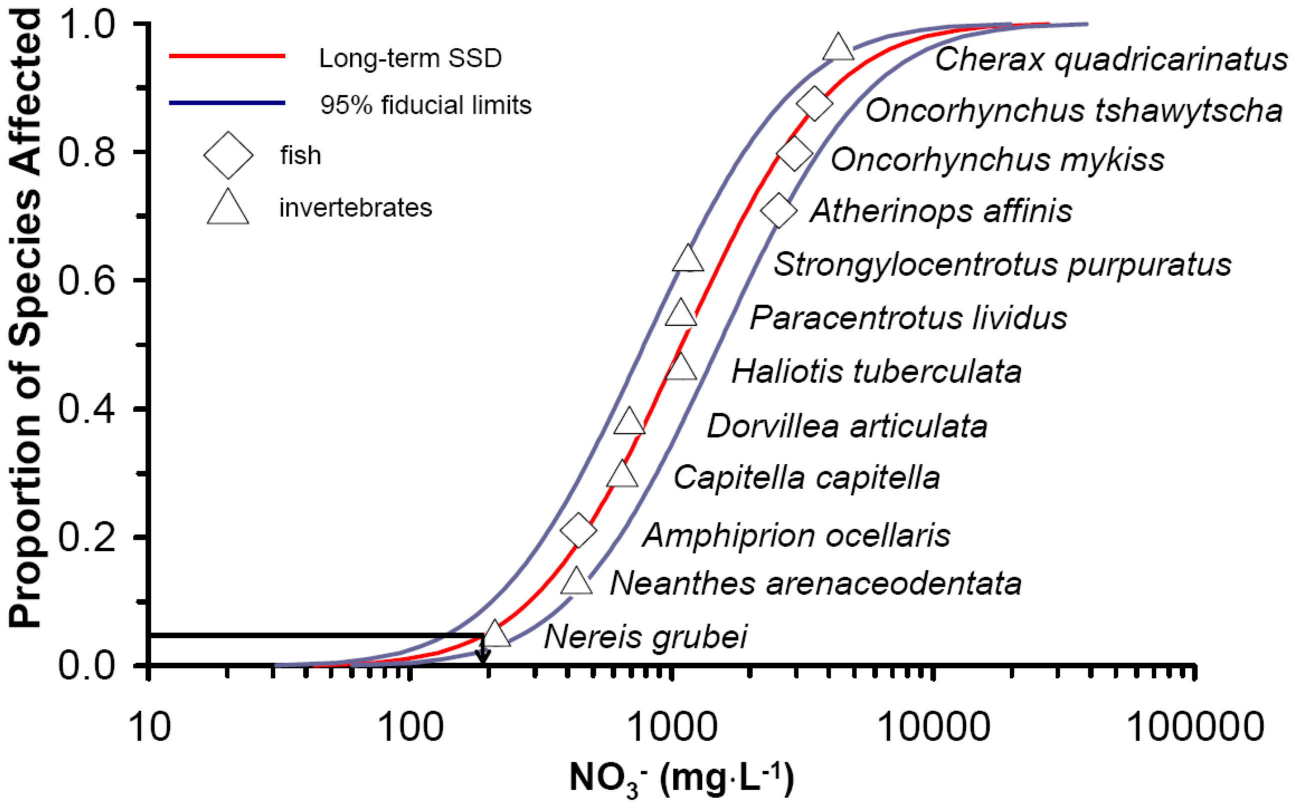

Figure 4. SSD of long-term no- and low-effect endpoint toxicity data for nitrate in saltwater derived by fitting the Normal model to the logarithm of acceptable data for 12 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding long-term Canadian Water Quality Guideline value.

Table 10. Long-term Canadian Water Quality Guideline for the nitrate ion resulting from the SSD Method.

	Concentration
SSD 5th percentile	200 mg·L^{-1}
SSD 5th percentile, LFL (5%)	141 mg·L^{-1}
SSD 5th percentile, UFL (95%)	273 mg·L^{-1}

General Discussion Related to the Marine CWQG: The ionic composition of marine water has resulted in nitrate guideline values much higher than the freshwater numbers. Cations in the water bind to dissolved NO_3^- to offer protection to aquatic species against adverse effects of the nitrate ion (Environment Canada 2003, 2010b). NO_3^- concentrations as high as the CWQG are rarely measured in water quality samples. Caution may be necessary when applying the marine nitrate guideline values in transitional environments such as estuaries and brackish-waters, in which salinity is lower than marine systems.

The newly derived guideline value of 200 mg NO_3^-·L^{-1} (45 mg NO_3^--N·L^{-1}) has increased significantly when compared to the 2003 marine interim guideline of 16 mg NO_3^-·L^{-1} (3.6 mg NO_3^-N·L^{-1}). In the case of the 2003 marine interim guideline, the value was based on 28-d TLm (= LC_{50}) of 329 mg NO_3^-·L^{-1} (74 mg NO_3^--N·L^{-1}) for the temperate marine adult-sized annelid *Nereis grubei*a (Reish, 1970). The guideline value was derived by multiplying the LC_{50} for *N. grubei* by a safety factor of 0.05 (CCME 1991). A conservative safety factor was used for the marine guideline because: the polychaete in the critical study was not tested at its most sensitive life stage; the critical endpoint, although chronic, was based on a median lethal effect rather than a low sublethal effect; and adverse effects have been observed in nonindigenous tropical species exposed to much lower nitrate concentrations. For the derivation of the 2012 CWQG, additional testing was conducted using both the purple sea urchin (*Strongylocentrotus purpuratus)* and the topsmelt (*Atherinops affinis)* by Stantec (2006). A comparison of the marine CWQG of

200 mg NO_3^-·L^{-1} (45 mg NO_3^--N·L^{-1}) to the data for temperate marine species in Appendix B of the scientific criteria document (CCME 2012) indicates that this value is protective. Therefore, even though the marine CWQG value has increased from the 2003 interim value, it is still considered to abide by the guiding principle of protecting all aquatic organisms at all life stages during indefinite exposure periods.

Guidance on the Use of Guidelines: These guidelines for the nitrate ion are intended to protect against direct toxic effects of nitrate; indirect effects resulting from eutrophication may still occur at nitrate concentrations below these guideline values, depending on the total amount of bioavailable nitrogen and other site-specific factors (e.g., phosphorus, light availability). Further guidance on the application of these guidelines is provided in the scientific supporting document (Environment Canada 2010b).

The short-term benchmark concentration and long-term CWQG for nitrate are set to provide protection for short- and long-term exposure periods, respectively. They are based on generic environmental fate and behaviour and toxicity data. The long-term water quality guideline is a conservative value below which all forms of aquatic life, during all life stages and in all Canadian aquatic systems, should be protected. Because the guideline is not corrected for any toxicity modifying factors (e.g. hardness), it is a generic value that does not take into account any site-specific factors. Moreover, since the guideline is mostly based on toxicity tests using naïve (i.e., non-tolerant) laboratory organisms, it is going to be a conservative value, by design. If an exceedence of the guideline is observed, it does not necessarily suggest that toxic effects will be observed, but rather indicates the need to determine whether or not there is a potential for adverse environmental effects. In some situations, such as where an exceedence is observed, it may be necessary or advantageous to derive a site-specific guideline that takes into account local conditions (water chemistry, natural background concentration, genetically-adapted organisms, community structure) (CCME 2007). CCME has outlined several procedures to modify the national water quality guidelines to site-specific water quality guidelines or objectives to account for unique conditions and/or requirements at the site under investigation (CCME 1991; CCME 2003; Intrinsik 2010).

Fiducial limits (FLs) are reported along with the 5th percentile or guideline value, and are considered to be inverse confidence limits (CLs), since they are related to a concentration resulting in a specific effect (FLs are CLs around the independent varialble, as opposed to the dependent variable). FLs can be used to help interpret monitoring data, particularly if the guideline and method detection limit (MDL) are close. In general, when assessing the potential risk of chemical release and where the laboratory MDL is higher than the CWQG, water quality managers could look at the magnitude of the difference between the CWQG and the MDL (fiducial limits can be applied here), the size of the database used to develop the CWQG, and the toxicity of the substance. Note that only the 5th percentile is to be used as the guideline value.

In general, Canadian Water Quality Guidelines (CWQGs) are numerical concentrations or narrative statements that are recommended as levels that should result in negligible risk of adverse effects to aquatic biota. As recommendations, the CWQGs are not legally enforceable limits, though they may form the scientific basis for legislation or regulation at the provincial, territorial, or municipal level. CWQGs may also be used as benchmarks or targets in the assessment and remediation of contaminated sites, as tools to evaluate the effectiveness of point-source controls, or as “alert levels” to identify potential risks.

CWQG values are calculated such that they protect the most sensitive life stage of the most sensitive aquatic life species over the long term. Hence, concentrations of a parameter that are less than the applicable CWQG are not expected to cause any adverse effect on aquatic life. Concentrations that exceed the CWQGs, however, do not necessarily imply that aquatic biota will be adversely affected, or that the water body is impaired; the concentration at which such effects occur may differ depending on site-specific conditions. Where the CWQGs are exceeded, professional advice should be sought in interpreting such results. As with other CWQGs, the guidelines for nitrate are intended to be applied towards concentrations in ambient surface waters, rather than immediately adjacent to point sources such as municipal or industrial effluent outfalls. Various jurisdictions provide guidance on determining the limits of mixing zones when sampling downstream from a point source (see, for example, BC MELP 1986 and MEQ 1991), though Environment Canada and CCME do not necessarily endorse these methods.

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