Scientific Criteria Document for the Development of the

Canadian Water Quality Guidelines for the Protection of Aquatic Life: Nitrate Ion

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NOTE TO READERS

The Canadian Council of Ministers of the Environment (CCME) is the primary minister-led intergovernmental forum for collective action on environmental issues of national and international concern.

This document provides the background information and rationale for the development of the Canadian Water Quality Guidelines for nitrate ion. For additional scientific information regarding these water quality guidelines, please contact:

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PREFACE TO THE REVISED EDITION

The comparison table below provides the 2012 full Canadian Water Quality Guideline (CWQG) and short-term benchmark concentration values for the nitrate ion, as well as the 2003 interim CWQG values, for both freshwater and marine environments. The 2003 interim CWQGs were developed using the 1991 derivation protocol (CCME 1991) using an assessment factor method (lowest effect concentration from an acceptable study divided by a safety factor). The 2003 CWQGs were designated as interim due to data gaps identified during their derivation. The 2012 full CWQGs were developed using the updated 2007 derivation protocol (CCME 2007) using a statistical species sensitivity distribution (SSD) method. In addition to the CWQGs (developed to protect all aquatic species during all life stages for indefinite exposure periods), short-term benchmark concentrations have also been developed. This is new to the 2007 protocol (CCME 2007) where these benchmark concentrations are developed to indicate the level where severe effects are likely to be observed during brief elevated exposure of a substance in water (e.g. spill). They do not provide guidance on protective levels of a substance in the aquatic environment (do not protect against adverse effects). The 2012 CWQGs, described in this document, supersede the 2003 interim CWQGs.

The 2012 full CWQG (freshwater) calculated using a species sensitivity distribution has resulted in the same guideline value as the interim 2003 CWQG value - 13 mg $NO_3^-{\cdot}L^{-1}$ (3.0 mg NO_3^--$N{\cdot}L^{-1}$). 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 full CWQG, additional testing was conducted using the amphipod *Hyalella azteca* (to ensure minimum dataset requirements were fulfilled). Testing was also conducted on the early life stage of the lake trout *Salvelinus namaycush* (McGurk et al. 2006). Test results indicated that the CWQG of 13 mg $NO_3^-{\cdot}L^{-1}$ (3.0 mg NO_3^--$N{\cdot}L^{-1}$) would be protective of this sensitive fish species. In conclusion, this new full CWQG value for the nitrate ion is considered to abide by the guiding principle of protecting all aquatic organisms at all life stages during indefinite exposure periods.

The 2012 CWQG (marine) calculated using a species sensitivity distribution met all minimum dataset requirements for the development of a full guideline. The newly derived guideline value of 200 mg $NO_3^-{\cdot}L^{-1}$ (45 mg NO_3^--$N{\cdot}L^{-1}$) has increased significantly when compared to the 2003 marine interim guideline of 16 $NO_3^-{\cdot}L^{-1}$ (3.6 mg NO_3^--$N{\cdot}L^{-1}$). In the case of the 2003 marine interim guideline, the value was based on 28-d TLm (= LC50) of 329 mg $NO3^-{\cdot}L^{-1}$ (74 mg NO_3^--$N{\cdot}L^{-1}$) for the temperate marine adult-sized annelid *Nereis grubei*a (Reish, 1970). The guideline value was derived by multiplying the LC50 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 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.

Canadian Water Quality Guideline and Benchmark Concentration for Nitrate for the Protection of Aquatic Life[†]

	Long-Term[c] Water Quality Guideline[†]	Short-Term[d] Benchmark Concentration[†]
2012 update		
Freshwater[a]	13 mg NO_3^-·L^{-1}	550 mg NO_3^-·L^{-1}
	3.0 mg NO_3^--N·L^{-1}	124 mg NO_3^--N·L^{-1}
Marine[b]	200 mg NO_3^-·L^{-1}	1500 mg NO_3^-·L^{-1}
	45 mg NO_3^--N·L^{-1}	339 mg NO_3^--N·L^{-1}
2003		
Freshwater	13* mg NO_3^-·L^{-1}	na
	3.0* mg NO_3^--N·L^{-1}	
Marine	16* mg NO_3^-·L^{-1}	na
	3.6* mg NO_3^--N·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).
* interim guideline

PRÉFACE À L'ÉDITION RÉVISÉE

Le tableau comparatif ci-dessous présente les recommandations canadiennes complètes pour la qualité des eaux (RCQE) et les valeurs des concentrations limites concernant l'exposition à court terme visant l'ion nitrate pour 2012, de même que les valeurs provisoires des RCQE de 2003 pour les milieux d'eau douce et les milieux d'eau de mer. Les valeurs provisoires de 2003 ont été élaborées d'après le protocole d'élaboration des RCQE de 1991 (CCME, 1991), selon une méthode fondée sur les facteurs d'évaluation (concentration minimale avec effet observé tirée d'une étude fiable, divisée par un facteur de sécurité donné). Les RCQE de 2003 ont été désignées « provisoires », car on a remarqué, pendant leur élaboration, que des données étaient manquantes. Les RCQE complètes de 2011 ont été élaborées d'après le protocole d'élaboration des RCQE de 2007 (CCME, 2007), à l'aide d'une méthode statistique de distribution de la sensibilité des espèces (DSE). En plus des RCQE (élaborées en vue de protéger toutes les espèces aquatiques à tous les stades vitaux pendant des périodes d'exposition indéterminées), on a établi des concentrations limites concernant l'exposition à court terme. Ces concentrations limites indiquent la concentration à partir de laquelle on peut observer des effets graves lors d'expositions à court terme à de fortes concentrations d'une substance donnée dans l'eau (p. ex., lors d'un déversement), ce qui est nouveau dans le protocole de 2007 (CCME, 2007). Ces valeurs n'assurent pas la protection des organismes aquatiques (c.-à-d., elles ne protègent pas contre les effets nocifs des substances). Les RCQE de 2012, décrites dans le présent document, remplacent les RCQE provisoires établies en 2003.

Le calcul des RCQE (eau douce) complètes de 2012 d'après la DSE a donné lieu à une légère augmentation de la valeur recommandée à 13 mg de $NO_3^-·L^{-1}$ (soit 3,0 mg de NO_3^--N·L^{-1}; la valeur provisoire recommandée en 2003 pour les milieux d'eau douce était de 13 mg de $NO_3^-·L^{-1}$, soit 2,93 mg de NO_3^--N·L^{-1}). Dans le cas de la recommandation provisoire de 2003 pour les milieux d'eau douce, la valeur avait été établie d'après une étude de toxicité chronique d'une durée de 10 jours portant sur la toxicité du nitrate de sodium pour la rainette du Pacifique (*Pseudacris regilla*; Schuytema et Nebeker, 1999c). Le poids des organismes exposés à une concentration de 133 mg de $NO_3^-·L^{-1}$ a diminué en moyenne de 15 % en comparaison avec le groupe témoin. On a appliqué un coefficient de sécurité de 0,1 à la concentration minimale avec effet observé (CMEO), conformément aux exigences du CCME (1991), afin de calculer la valeur finale de la concentration provisoire recommandée. En ce qui concerne la valeur précisée dans les recommandations complètes de 2012, toutes les exigences minimales relatives à l'établissement des RCQE ont été satisfaites. Il est important de noter que la RCQE de 2003 était provisoire, ce qui signifie que les données nécessaires n'avaient pas toutes été fournies (il manquait une étude de toxicité chronique chez les invertébrés portant sur un organisme non planctonique). Un document scientifique à l'appui des RCQE publié en 2003 recommandait également qu'on réalise des essais de toxicité additionnels pour les poissons et les invertébrés qu'on sait sensibles aux nitrates. Pour établir la RCQE de 2012, on a effectué des tests additionnels sur l'espèce d'amphipode *Hyalella azteca* (afin de s'assurer que les exigences minimales en matière de données étaient satisfaites). D'autres essais ont également été réalisés sur des touladis (*Salvelinus namaycush*) aux premiers stades vitaux (McGurk *et al*., 2006). Les résultats de ces tests indiquaient que la RCQE de 13 mg de $NO_3^-·L^{-1}$ (3,0 mg de NO_3^--N·L^{-1}) protégerait cette espèce de poisson sensible. Pour conclure, on considère que cette nouvelle valeur concernant l'ion nitrate suit le principe directeur de protection de tous les organismes aquatiques à tous les stades vitaux pendant des périodes d'exposition indéterminées.

Le calcul des RCQE de 2012 (eau de mer) à l'aide de la distribution de la sensibilité des espèces répondait à toutes les exigences minimales en matière de données pour l'élaboration de recommandations complètes. La nouvelle recommandation établie, soir 200 mg de $NO_3^-·L^{-1}$ (45 14 mg de NO_3^--N·L^{-1}) est significativement plus élevée que la recommandation provisoire établie en 2003 pour les milieux d'eau de mer (16 mg de $NO_3^-·L^{-1}$, soit 3,6 mg NO_3^--N·L^{-1}). La recommandation provisoire de 2003 pour les milieux d'eau de mer était fondée sur la TL_m après 28 j (= CL_{50}) de 329 mg de $NO_3^-·L^{-1}$ (74 mg de NO_3^--N·L^{-1}) établie pour l'annélide d'eau de mer tempérée *Nereis grubeia* adulte (Reish, 1970). La recommandation a été obtenue en multipliant la CL_{50} pour *N. grubei* par un facteur de sécurité de 0,05 (CCME, 1991). Un facteur de sécurité prudent a été utilisé pour fixer la recommandation relative à l'eau de mer pour les raisons suivantes : dans l'étude critique, les polychètes soumis aux essais n'étaient pas à leur stade de vie le plus sensible; le paramètre d'effet critique, même s'il concernait une exposition chronique, était fondé sur un effet létal médian plutôt que sur un faible effet sublétal; des effets nocifs ont été observés chez des espèces tropicales non indigènes exposées à des concentrations de nitrate bien plus faibles. Pour établir la RCQE de 2012, Stantec (2006) a mené d'autres essais portant notamment sur l'oursin violet (*Strongylocentrotus purpuratus)* et la capucette barrée (*Atherinops affinis).* La comparaison de la RCQE pour les milieux d'eau de mer, soit 200 mg de $NO_3^-·L^{-1}$ (45 mg de NO_3^--N·L^{-1}), avec les données relatives aux espèces d'eau de mer tempérée, à l'annexe B, indique que la valeur assure une protection adéquate. Par conséquent, même si la valeur de la RCQE pour les milieux d'eau de mer est supérieure à la recommandation provisoire de 2003, on considère qu'elle demeure conforme au principe directeur consistant à assurer la protection de tous les organismes aquatiques à tous les stades de vie pour des périodes d'exposition indéfinies.

Recommandations canadiennes pour la qualité des eaux et concentrations limites visant l'ion nitrate en vue de la protection de la vie aquatique[‡]

	Recommandation pour une exposition à long terme[c‡]	Valeur de la concentration limite pour une exposition à court terme[d‡]
Mise à jour de 2012		
Eau douce[a]	13 mg $NO_3^-·L^{-1}$	550 mg $NO_3^-·L^{-1}$
	3,0 mg NO_3^--N·L^{-1}	124 mg NO_3^--N·L^{-1}
Eau de mer[b]	200 mg $NO_3^-·L^{-1}$	1 500 mg $NO_3^-·L^{-1}$
	45 mg NO_3^--N·L^{-1}	339 mg NO_3^--N·L^{-1}
2003		
Eau douce	13* mg $NO_3^-·L^{-1}$	n.d.
	3,0* mg NO_3^--N·L^{-1}	
Eau de mer	16* mg $NO_3^-·L^{-1}$	n.d.
	3,6* mg NO_3^--N·L^{-1}	

[‡] protection contre les effets toxiques directs; les recommandations ne tiennent pas compte des effets indirects dus à l'eutrophisation.

[a] valeur dérivée d'essais de toxicité avec du $NaNO_3$.

[b] valeur dérivée d'essais de toxicité avec du $NaNO_3$ et du KNO_3.

[c] valeur dérivée de données sur les concentrations sans effet et sur les concentrations associées à certains effets faibles, destinée à protéger la structure et le fonctionnement de l'écosystème aquatique contre les effets néfastes lors de période d'exposition indéfinies (c'est-à-dire conformément au principe directeur défini dans CCME (2007)).

[d] valeur dérivée de données sur les effets graves (comme la létalité), non destinée à protéger tous les éléments de la structure et du fonctionnement de l'écosystème aquatique, mais plutôt à protéger la plupart des espèces contre les effets létaux lors d'expositions graves, mais transitoires (p. ex., application ou élimination inappropriées de la substance concernée).

* recommandation provisoire.

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ABSTRACT

This scientific supporting document describes the development of Canadian Water Quality Guidelines for the protection of aquatic life for the nitrate ion. It contains a review of technical background information on the chemical and physical properties of nitrate and nitrate salts, a review of sources and releases in Canada, the distribution and behaviour of nitrate in the environment, and the toxicological effects of nitrate on freshwater and marine aquatic life. This information is used to derive ambient water quality guidelines for the nitrate ion, based on direct toxic effects, to protect ecological receptors in Canadian waters. The role of total nitrogen and nitrogen-to-phosphorus ratios in causing indirect toxic effects through eutrophication are discussed in a separate document (CCME 2002; NAESI 2005). The guidelines in this document are based on the best available toxicity data at the time of writing, January, 2012.

Nitrate occurs naturally in the environment and is constantly produced and consumed through the processes of the nitrogen cycle. Nitrate is also produced anthropogenically for uses such as the production of fertilizers, steel, petroleum, pulp and paper, organic and inorganic chemicals, plastics, nitroaromatic compounds, nitroorganic compounds in pharmaceuticals, and explosives. Nitrate salts are used in photography, glass making, engraving, textile dyes, and food processing. The major anthropogenic sources of nitrate to surface waters are agricultural runoff, municipal and industrial wastewaters, urban runoff, landfill leachate, precipitation of nitric oxide and nitrogen dioxide from vehicular exhaust, storm sewer overflow, and septic tanks. Nitrogen from all sources, and in all its forms, can potentially be transformed into nitrate. It is estimated that approximately 600 kt of total nitrogen were released to Canadian surface and groundwaters in 1996 from both natural and anthropogenic sources.

Ambient nitrate levels in Canadian lakes and rivers are typically less than 4 mg $NO_3^-\cdot L^{-1}$ (0.90 mg NO_3^--N$\cdot L^{-1}$). Concentrations less than 0.4 mg $NO_3^-\cdot L^{-1}$ (0.09 mg NO_3^--N$\cdot L^{-1}$) are indicative of oligotrophic lakes and streams. Concentrations exceeding 4 mg $NO_3^-\cdot L^{-1}$ (0.90 mg NO_3^--N$\cdot L^{-1}$) are often associated with eutrophic conditions, and are generally the result of anthropogenic inputs. North American streams in agricultural landscapes typically have elevated levels of nitrate due to fertilizer use, with mean nitrate concentrations ranging between 9 and 180 mg $NO_3^-\cdot L^{-1}$ (2 and 41 mg NO_3^--N$\cdot L^{-1}$). Nitrate levels in marine waters are usually lower than in fresh waters. In Canadian coastal waters, ambient nitrate concentrations rarely exceed 0.5 mg $NO_3^-\cdot L^{-1}$ (0.1 mg NO_3^--N$\cdot L^{-1}$), but in estuaries draining agricultural land, nitrate concentrations can reach 12 mg $NO_3^-\cdot L^{-1}$ (2.7 mg NO_3^--N$\cdot L^{-1}$). Levels of nitrate in Canadian groundwater can range from 1 to 1100 mg $NO_3^-\cdot L^{-1}$ (0.23 to 248 mg NO_3^--N$\cdot L^{-1}$), but in the absence of anthropogenic contamination, levels are generally less than 13 mg $NO_3^-\cdot L^{-1}$ (3.0 mg NO_3^--N$\cdot L^{-1}$).

In water, the fate of nitrate is primarily determined by the biotic processes of assimilation, nitrogen fixation, nitrification, denitrification, ammonification, and decomposition of organic matter. Rates of these processes are affected by pH, temperature, and oxygen availability. Through biotic assimilation, nitrate is taken up by aquatic plants and algae and is used for the synthesis of cellular materials, such as proteins. The mode of nitrate uptake from the water by aquatic animals is unclear. Nitrate's mode of toxicity to aquatic life is also unclear, though two proposed mechanisms are: a) through methaemoglobin formation, with a reduction in oxygen

carrying capacity of the blood, and b) through the inability of the organism to maintain proper osmoregulation under high salt contents associated with elevated nitrate levels.

Nitrate toxicity tests have been conducted through the addition of nitrate salts such as sodium nitrate, potassium nitrate, and ammonium nitrate. Results of tests with ammonium nitrate suggest toxic effects observed are due to the ammonium ion, rather than nitrate. Similarly, in fresh water, the effects of potassium nitrate are likely due to the potassium. In marine waters, however, toxic levels of potassium nitrate occur at potassium concentrations below background levels of potassium in seawater, and therefore the toxicity can be attributed to the nitrate ion. Based on these arguments, nitrate toxicity to freshwater organisms was only evaluated using tests with sodium nitrate, while toxicity data for both sodium nitrate and potassium nitrate were used to evaluate toxicity to marine organisms.

Nitrate has wide-ranging effects in invertebrates, fish and amphibians, with larval stages generally showing greater sensitivity than adults. Adverse effects observed in aquatic organisms include: mortality, growth reduction, reduced feeding rates, reduced fecundity, reduced hatching success, lethargy, behavioural signs of stress, bent spines, and other physical deformities.

For both freshwater and marine environments, the short-term benchmark concentration and the long-term Canadian water quality guideline (CWQG) for the nitrate ion for the protection of aquatic life were developed based on the CCME protocol (CCME 2007) using the statistical (Type A or Species Sensitivity Distribution) approach, as sufficient data were available.

Canadian Water Quality Guideline and Benchmark Concentration for Nitrate for the Protection of Aquatic Life[‡]

	Long-Term[c] Water Quality Guideline	Short-Term[d] Benchmark Concentration
2012 update		
Freshwater[a]	13 mg $NO_3^-·L^{-1}$	550 mg $NO_3^-·L^{-1}$
	3.0 mg NO_3^--N·L^{-1}	124 mg NO_3^--N·L^{-1}
Marine[b]	200 mg $NO_3^-·L^{-1}$	1500 mg $NO_3^-·L^{-1}$
	45 mg NO_3^--N·L^{-1}	339 mg NO_3^--N·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 KN O_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).

These nitrate water quality guidelines are intended to prevent direct toxicity to aquatic organisms and will not necessarily prevent eutrophication. Indirect effects due to excess algal growth may still occur at nitrate concentrations below these guideline levels.

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 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, it is mostly based on toxicity tests using naïve (i.e., non-tolerant) laboratory organisms and is therefore conservative by design. An exceedence of the guideline 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 (e.g. water chemistry such as hardness, natural background concentration, genetically adapted organisms, community structure).

The guideline should be used as a screening and management tool to ensure that nitrate does not lead to the degradation of the aquatic environment. The CWQG for nitrate could, for example, be the basis for the derivation of site-specific guidelines and objectives (derived with site-specific data as well as consideration of technological, site-specific, socioeconomic or management factors).

RÉSUMÉ

Le présent document scientifique complémentaire décrit l'élaboration de recommandations canadiennes pour la qualité des eaux visant la protection de la vie aquatique pour l'ion nitrate. Il présente un examen des données techniques de base sur les propriétés chimiques et physiques de l'ion nitrate et des nitrates ainsi qu'une revue de leurs sources et de leurs rejets au Canada, indique la distribution et le comportement des nitrates dans l'environnement et examine leurs effets toxicologiques sur la vie aquatique d'eau douce et marine. Ces données servent à élaborer des recommandations pour la qualité des eaux concernant l'ion nitrate en se fondant sur les effets toxiques directs afin de protéger les récepteurs écologiques dans les eaux canadiennes. Le rôle joué par l'azote total et les rapports azote/phosphore dans la production d'effets toxiques indirects par eutrophisation est discuté dans un autre document (CCME 2002; NAESI 2005). Les recommandations ici présentées sont fondées sur les meilleures données sur la toxicité disponibles en janvier 2012, au moment où le document fut rédigé.

Les nitrates se retrouvent naturellement dans l'environnement. Ils sont constamment produits et consommés au cours des procédés du cycle de l'azote. Ils peuvent aussi être d'origine anthropique et servir par exemple à la production d'engrais, d'acier, de pétrole, de pâtes et papiers, de composés organiques et inorganiques, de matières plastiques, de composés aromatiques azotés, de composés organiques azotés utilisés dans les produits pharmaceutiques et d'explosifs. Les nitrates sont utilisés en photographie, dans la fabrication du verre, en gravure, dans les teintures pour textile et dans la transformation des aliments. Les principales sources anthropiques des rejets de nitrates dans les eaux de surface sont le ruissellement agricole, les eaux usées municipales et industrielles, le ruissellement urbain, la lixiviation des décharges, les émissions d'oxyde nitrique et de dioxyde d'azote provenant des gaz d'échappement des véhicules, le débordement des égouts pluviaux et les fosses septiques. L'azote provenant de toutes les sources et sous toutes ses formes peut être transformé en nitrate. On estime qu'environ 600 kt d'azote total provenant de sources à la fois naturelles et anthropiques ont été rejetées en 1996 dans les eaux de surface et souterraines au Canada.

En général, la teneur ambiante en nitrates des lacs et des cours d'eau canadiens est inférieure à 4 mg de $NO_3^-\cdot L^{-1}$ (0,90 mg de NO_3^--$N\cdot L^{-1}$). Des concentrations inférieures à 0,4 mg de $NO_3^-\cdot L^{-1}$ (0,09 mg de NO_3^--$N\cdot L^{-1}$) indiquent des lacs et des cours d'eau oligotrophes. Des concentrations supérieures à 4 mg de $NO_3^-\cdot L^{-1}$ (0,90 mg de NO_3^--$N\cdot L^{-1}$) sont souvent associées à des conditions eutrophes et résultent généralement d'apports anthropiques. Dans les cours d'eau nord-américains en milieu rural, les concentrations de nitrates tendent à être élevées en raison de l'utilisation d'engrais, et leur moyenne varie entre 9 et 180 mg de $NO_3^-\cdot L^{-1}$ (2 et 41 mg de NO_3^--$N\cdot L^{-1}$). Dans les eaux marines, les concentrations de nitrates sont ordinairement plus faibles que dans les eaux douces. Dans les eaux côtières canadiennes, la teneur ambiante en nitrates dépasse rarement 0,5 mg de $NO_3^-\cdot L^{-1}$ (0,1 mg de NO_3^--$N\cdot L^{-1}$), mais dans les estuaires qui drainent des terres agricoles, les concentrations peuvent atteindre 12 mg de $NO_3^-\cdot L^{-1}$ (2,7 mg de NO_3^--$N\cdot L^{-1}$). Dans les eaux souterraines du Canada, les concentrations de nitrates peuvent varier de 1 à 1 100 mg de $NO_3^-\cdot L^{-1}$ (0,23 à 248 mg de NO_3^--$N\cdot L^{-1}$), mais en l'absence de contamination anthropique, elles sont généralement inférieures à 13 mg de $NO_3^-\cdot L^{-1}$ (3,0 mg de NO_3^--$N\cdot L^{-1}$).

Dans l'eau, le devenir des nitrates est surtout déterminé par les procédés biotiques d'assimilation, de fixation de l'azote, de nitrification, de dénitrification, d'ammonification et de décomposition

de la matière organique. La vitesse de ces procédés dépend du pH, de la température et de la disponibilité en l'oxygène. L'assimilation biotique fait en sorte que les nitrates sont absorbés par les plantes aquatiques et les algues pour synthétiser des matières cellulaires, comme les protéines. On ne sait pas exactement de quelle façon les animaux aquatiques absorbent les nitrates présents dans l'eau ni quels sont les mécanismes de toxicité des nitrates pour ces organismes, bien que deux aient été proposés: a) la formation de méthémoglobine, accompagnée d'une réduction du pouvoir oxyphorique du sang, et b) l'incapacité de l'organisme d'assurer une osmorégulation convenable à une teneur élevée en sels, conjuguée à de fortes concentrations de nitrates.

Des essais de toxicité des nitrates ont été effectués en ajoutant des sels d'acide nitrique, comme le nitrate de sodium, le nitrate de potassium et le nitrate d'ammonium. Les résultats obtenus portent à croire que, dans les essais utilisant le nitrate d'ammonium, les effets toxiques observés sont dus à l'ion ammonium plutôt qu'à l'ion nitrate. De même, dans l'eau douce, les effets du nitrate de potassium sont probablement dus au potassium. Par contre, dans les eaux marines, les concentrations toxiques de nitrate de potassium correspondent aux teneurs en potassium inférieures aux concentrations de fond de cet élément dans l'eau de mer, ce qui veut dire que la toxicité peut être attribuée à l'ion nitrate. À la lumière de ces arguments, la toxicité des nitrates pour les organismes d'eau douce a été évaluée seulement au moyen d'essais avec du nitrate de sodium, tandis que les données sur la toxicité des nitrates de sodium et de potassium ont été utilisées pour les organismes marins.

Les nitrates produisent des effets importants chez les invertébrés, le poisson et les amphibiens, et les stades larvaires y sont généralement plus sensibles que les adultes. Les effets nocifs observés chez les organismes aquatiques comprennent la mortalité, la réduction de la croissance, la réduction du taux d'alimentation, la diminution de la fécondité, la réduction du succès d'éclosion, la léthargie, des indices de comportement dénotant un stress, le fléchissement de la colonne vertébrale et d'autres malformations.

Ces recommandations canadiennes pour la qualité des eaux visant la protection de la vie aquatique pour l'ion nitrate ont pour but de prévenir la toxicité directe pour les organismes aquatiques, mais elles ne préviendront pas nécessairement l'eutrophisation. Par conséquent, même si les concentrations de nitrates sont inférieures aux valeurs recommandées, il se peut que des effets toxiques indirects dus à la prolifération d'algues se produisent encore.

La concentration limite pour une exposition à court terme et la RCQE à long terme établies pour l'ion nitrate assurent une protection contre l'exposition à court terme et à long terme, respectivement. Elles sont fondées sur des données génériques sur le devenir et le comportement dans l'environnement ainsi que sur la toxicité. La recommandation pour la qualité des eaux à long terme est une valeur prudente, sous laquelle toutes les formes de vie aquatique, à tous les stades de vie et dans tous les milieux aquatiques au Canada, sont protégées. Comme la recommandation n'est corrigée en fonction d'aucun facteur modifiant la toxicité (par exemple, la dureté), il s'agit d'une valeur générique qui ne prend en compte aucun facteur propre au site. De plus, comme la recommandation est fondée principalement sur des essais de toxicité portant sur des sujets de laboratoire naïfs (c'est-à-dire non tolérants), il s'agit d'une valeur prudente en soi. Si la recommandation est dépassée, cela ne signifie pas nécessairement que des effets toxiques seront observés; cela indique plutôt qu'il faut déterminer s'il peut y avoir, oui ou non, des effets

nocifs sur l'environnement. Dans certaines situations, comme dans le cas d'un dépassement de la recommandation, il peut être nécessaire ou avantageux de calculer une recommandation propre au site, prenant en compte les conditions à l'échelle locale (chimie de l'eau, concentration naturelle, organismes génétiquement adaptés, structure des communautés.

Recommandations canadiennes pour la qualité des eaux visant l'ion nitrate en vue de la protection de la vie aquatique[‡]

	Recommandation pour une exposition à long terme[c]	Valeur de la concentration limite pour une exposition à court terme[d]
Mise à jour de 2012		
Eau douce[a]	13 mg NO_3^-·L^{-1}	550 mg NO_3^-·L^{-1}
	3,0 mg NO_3^--N·L^{-1}	124 mg NO_3^--N·L^{-1}
Eau de mer[b]	200 mg NO_3^-·L^{-1}	1 500 mg NO_3^-·L^{-1}
	45 mg NO_3^--N·L^{-1}	339 mg NO_3^--N·L^{-1}

[‡] protection contre les effets toxiques directs; les recommandations ne tiennent pas compte des effets indirects dus à l'eutrophisation.
[a] valeur dérivée d'essais de toxicité avec du $NaNO_3$.
[b] valeur dérivée d'essais de toxicité avec du $NaNO_3$ et du KNO_3.
[c] valeur dérivée de données sur les concentrations sans effet et sur les concentrations associées à certains effets faibles, destinée à protéger la structure et le fonctionnement de l'écosystème aquatique contre les effets néfastes lors de période d'exposition indéfinies (c'est-à-dire conformément au principe directeur défini dans CCME (2007)).
[d] valeur dérivée de données sur les effets graves (comme la létalité), non destinée à protéger tous les éléments de la structure et du fonctionnement de l'écosystème aquatique, mais plutôt à protéger la plupart des espèces contre les effets létaux lors d'expositions graves, mais transitoires (p. ex., application ou élimination inappropriées de la substance concernée).

Les recommandations doivent être considérées comme un outil de dépistage et de gestion visant à s'assurer que la présence de l'ion nitrate n'entraîne pas de dégradation du milieu aquatique. La RCQE relative à l'ion nitrate peut, par exemple, servir de point de départ pour l'élaboration de recommandations et d'objectifs propres à un site donné (en se fondant sur des données propres au site et des facteurs techniques, des facteurs propres au site, des facteurs socioéconomiques ou des facteurs de gestion).

LIST OF ACRONYMS

ADP	adenosine diphosphate
ANC	acid neutralizing capacity
ATP	adenosine triphosphate
CAS	Chemical Abstracts Service
CCME	Canadian Council of Ministers of the Environment
CCREM	Canadian Council of Resource and Environment Ministers
CV	coefficient of variation
[C]WQG	[Canadian] Water Quality Guidelines
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
EC	effects concentration
EC_{50}	median effects concentration
EDTA	ethylenediaminetetraacetic acid
H^+	hydronium ion
H_2SO_4	sulfuric acid
HNO_3	nitric acid
IC	ion chromatography
KNO_3	potassium nitrate
LC_{50}	median lethal concentration
LO[A]EL	lowest observable [adverse] effects level
LOEC	lowest observable effects concentration
MATC	maximum allowable test concentration
MDL	method detection limit
MWWTPs	municipal wastewater treatment plants
N_2	molecular nitrogen
NADH	nicotinamide adenine dinucleotide, reduced form
NAESI	National Agri-Environmental Standards Initiative
$NaNO_3$	sodium nitrate
NH_3	un-ionized ammonia
NH_4^+	ammonium ion
NH_4NO_3	ammonium nitrate
NO	nitric oxide
N_2O	nitrous oxide
NO_2^-	nitrite
NO_3^-	nitrate
NO_3^--N	nitrate-nitrogen
NO[A]EL	no observable [adverse] effects level
NOEC	no observable effects concentration
NPRI	National Pollutant Release Inventory
SCs	safe concentrations
TDS	total dissolved solids
TL_m	median lethal tolerance
TN	total nitrogen
US EPA	United States Environmental Protection Agency
UV	ultraviolet

1 INTRODUCTION

This report describes the development of Canadian Water Quality Guidelines (CWQGs) for nitrate for the protection of freshwater and marine life. CWQGs are numerical limits based on the most current, scientifically-defensible toxicological data. They are nationally consistent benchmarks designed to protect, sustain and enhance the present and potential uses of a water body. CWQGs are used by provincial, territorial, and federal jurisdictions to evaluate water quality issues and manage competing uses of water. The guideline values derived for nitrate are intended to protect all forms of aquatic life and all aspects of aquatic life cycles, including the most sensitive life stage of the most sensitive species over the long term.

This document describes production and uses, sources, and pathways for the entry of the more common nitrate salts into the Canadian environment. Available data on environmental fate and persistence of the nitrate ion are summarised. A comprehensive assessment of the toxicity of the sodium nitrate salt to aquatic life is also presented to evaluate environmental hazards posed by this chemical. Together, this information is used, in accordance with "A Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life 2007" (CCME 2007) to derive numerical water quality guidelines (WQGs) for aquatic organisms.

It should be noted that nitrate concentrations are reported in this document in terms of the nitrate ion rather than as nitrate-nitrogen (i.e., mg $NO_3^-·L^{-1}$, not mg NO_3^--$N·L^{-1}$), with the exception of the concentrations listed in the "Preface to the Revised Edition" and the "Abstract" (where values are presented as both mg $NO_3^-·L^{-1}$ and mg NO_3^--$N·L^{-1}$). Where source publications have used other units, these have been converted for consistency to mg $NO_3^-·L^{-1}$ wherever possible. In a few cases data is presented in this document in terms of nitrogen, rather than nitrate, because we were unable to assume how much of the nitrogen was in the form of nitrate; where this occurs, the information is clearly identified as referring to nitrogen.

2 PHYSICAL AND CHEMICAL PROPERTIES

2.1 Chemistry of the Nitrate Ion

The nitrate ion (NO_3^-), which has a molecular weight of 62 $g·mol^{-1}$, is the most oxidised form of nitrogen (N) present in the environment, with an oxidation state of +5 (NRC 1978). The molecule has a planar and symmetrical structure. The nitrogen atom in the centre forms sigma bonds with the three oxygen (O) atoms using sp^2 hybrid orbitals (NRC 1978). Other *p* orbitals of the nitrogen and oxygen atoms combine to yield a pi bond that is shared among the three sites (Figure 2.1).

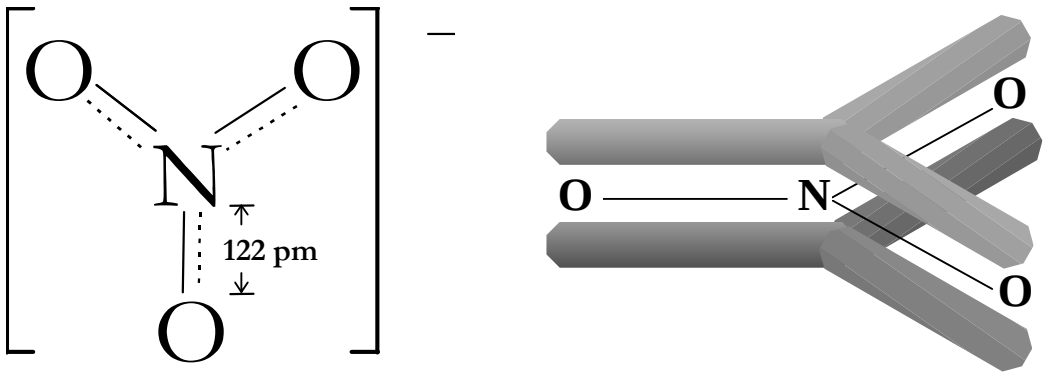


Figure 2.1 Chemical structure of the nitrate ion. The Lewis diagram on the left is adapted from McQuarrie and Rock (1991). The diagram on the right, adapted from Petrucci (1989), depicts the delocalized pi molecular orbital.

The nitrate salts of all common metals (e.g., $NaNO_3$, KNO_3, $Ca(NO_3)_2$, $AgNO_3$) are highly soluble in water, and solutions of these salts are neutral in pH (NRC 1978). While the resulting free nitrate ion has little tendency to form coordination complexes with metal ions in dilute aqueous solutions (NRC 1978), under acidic conditions it can act as a good oxidizing agent, as demonstrated in the reaction below (Petrucci 1989):

$$4\ Zn_{(s)} + 10\ H^{+}_{(aq)} + 2\ NO_3^{-}{}_{(aq)} \rightarrow 4\ Zn^{2+}_{(aq)} + 5\ H_20 + N_2O_{(g)}$$

The nitrate ion also is the conjugate base of nitric acid (HNO_3), a strong acid which is completely dissociated in solution (NRC 1978). Physical and chemical properties of the nitrate ion and selected nitrate salts commonly used in manufacturing are presented in Table 2.1.

Table 2.1. Summary of selected physical and chemical properties for nitrate ion and selected nitrate salts.

Property	Nitrate Ion	Sodium Nitrate	Potassium Nitrate	Ammonium Nitrate	Reference
CAS #	14797-55-8	7631-99-4	7757-79-1	6484-52-2	Merck (1996)
Molecular formula	NO_3^-	$NaNO_3$	KNO_3	NH_4NO_3	CRC (1986)
Physical structure	• chemical structure is trigonal planar	• colourless transparent prisms, white granular or crystal powder • deliquescent in moist air	• colourless transparent prisms, white granular or crystal powder • pungent taste	• odourless, transparent, hygroscopic, deliquescent crystals or white granules	Merck (1996)
Molecular weight ($g \cdot mol^{-1}$)	62.00	84.99	101.10	80.04	CRC (1986)
Melting point (°C)	—	306.8	334	196.6	CRC (1986)
Boiling point (°C)	—	decomposes at 380°	decomposes at 400°	210°	CRC (1986)
Density / Specific gravity	—	2.261	2.109	1.725	CRC (1986)
Solubility in water	—	Very soluble (88 g/100 mL at 20 deg C; 92.1 g/100 mL at 25 deg C)	Soluble (32-35 g/100 mL at 20-25 deg C)	Very soluble (192 g/100 mL at 20 deg C; 187 g/100 g at 20 deg C)	Cheminfo (2011)
pH	—	neutral in aqueous solution	neutral in aqueous solution	5.43 in 0.1 *M* solution	Merck (1996)
Notes on use	—	• manufacture of nitric acid, sodium nitrite, glass and enamels • colour fixative in meats • fertilizer	• fireworks • pickling meat • manufacture of glass • gunpowder • blasting powders • tempering steel	• manufacture of nitrous oxide • freezing mixtures • explosives • matches • pyrotechnics • fertilizers	Merck (1996)

The amount of nitrate present in a solution is often expressed relative to the amount of nitrogen present in the NO_3^- ion, where 1 mg $NO_3^-\cdot L^{-1}$ is equivalent to 0.226 mg NO_3^--$N\cdot L^{-1}$ (WHO 1996). Other, less commonly used base units for nitrate concentration include: g-at$\cdot L^{-1}$ (or g-at $N\cdot L^{-1}$), *M* NO_3^-, eq NO_3^-, and *N* NO_3^-. Conversions between these units are given in Table 2.2. For consistency in this report, unless otherwise specified, all nitrate concentrations will be reported for the ion only (i.e., as mg $NO_3^-\cdot L^{-1}$).

Table 2.2. 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*, or g-at.$\cdot L^{-1}$ *	62.005×10^3
ppm NO_3^-	1
ppb NO_3^-	10^{-3}

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

2.2 Analytical Methods

There are several techniques available for analysing nitrate ions in aqueous solutions. It may be difficult, however, to select the most appropriate technique for a given application due to the limited concentration ranges available with each of the techniques and the potential for interference from other compounds in the sample matrix (APHA 1998). Table 2.3 provides an outline of nitrate ion analytical techniques, their detection ranges and potential sources of interference.

Due to the potential for transformations between nitrate, nitrite, dissolved ammonia, organic nitrogen and ammonia gas, it is important that certain procedures be used in the collection, storage, and preservation of samples for nitrate analysis. Standard methodologies, such as APHA (1998), should be consulted.

In general, nitrate analysis can be divided into three categories: colorimetric analyses (various nitrate reduction processes); potentiometric analysis (ion-selective electrodes); and, direct ion quantification (ion chromatography, capillary electrophoresis).

The automated cadmium reduction method is commonly used for analysing nitrate using colorimetry (NLET 1994; US EPA 2000a). In this method, nitrate present in a sample must first be reduced to nitrite. To do this, the water sample is passed through a glass column packed with cadmium (Cd) granules treated with $CuSO_4$ which completely reduces nitrate to nitrite upon contact. The resulting nitrite is then diazotised with sulfanilamide ($NH_2C_6H_4SO_2NH_2$) and coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a reddish-purple azo dye (NLET 1994). The absorption of the monochromatic radiation by the azo dye is proportional to the nitrite concentration and is measured using a spectrophotometer at 520 nm (NLET 1994). The same procedure without the reduction step is also applied on a subsample to correct for NO_2^-

ions originally present in the sample. It should be noted that this last step of correcting for nitrite is frequently ignored, and some measurements reported in the literature as nitrate concentrations may actually be concentrations of nitrate+nitrite. The amount of nitrite in most water samples, however, is generally quite small, particularly for samples originating from well-oxygenated waters.

The major advantage of the automated cadmium reduction method is greater analytical sensitivity, with nitrate ion detections ranging from 0.004 to 44.3 mg $NO_3^-{\cdot}L^{-1}$ (APHA 1998). Appropriate dilutions are required when analyzing samples with the higher concentrations within this analytical working range (NLET 1994). Potential interferences include: a) suspended matter that can restrict sample flow in the column; b) high metal concentrations (e.g., Fe, Cu, etc. > several mg$\cdot L^{-1}$) that can decrease reduction efficiency (in which case EDTA can be used to chelate metals prior to analysis); c) hydrocarbons such as oil and grease (must be pre-extracted with an organic solvent); and, d) residual chlorine which should also be removed as it can interfere by oxidising the Cd in the column (APHA 1998).

This method is recommended for levels below 0.4 mg $NO_3^-{\cdot}L^{-1}$, where other methods lack adequate sensitivity (APHA 1998). It should be noted, however, that Cd is very toxic and, therefore, care must be taken when handling and disposing of it (US EPA 2000a).

The nitrate electrode method uses a pH meter with a dedicated NO_3^- ion electrode that develops an electric potential across a thin, porous, inert membrane that contains a water-immiscible liquid ion exchanger. The electrode measures ion activity over a potentially wide range between approximately 0.62 to 6200 mg $NO_3^-{\cdot}L^{-1}$ (APHA 1998). Although a complex buffer solution is required to remove potential interferences from unwanted ions (e.g., Cl^-, HCO_3^-, NO_2^-, CN^-, S^{2-}, Br^-, I^-, ClO_3^-, and ClO_4^-), the electrode functions satisfactorily over a pH range of 3 to 9, provided pH and ionic strength in the solution remain constant (APHA 1998). This method cannot be used with samples that have high ionic strength, and therefore may not be appropriate for many brackish or saltwater samples.

Table 2.3. Comparison of available techniques for analysis of nitrate in water.

	Technique	Analytical detector	Detection range (mg NO_3^-·L^{-1})	Sample precision (mean ± CV%) (mg NO_3^-·L^{-1})	Potential sources of interference	Protocol reference
Colorimetry						
	cadmium reduction	spectrophotometer	0.04 to 4.43 [a]	1.8 (12.5) to 4.60 (1.0) [a]	suspended matter; oil and grease; residual chlorine; sample colours in same wavelength	APHA: 4500-NO_3^- E [a] ASTM: D 3867 [b] US EPA: 0353.2 [c]
	automated cadmium reduction	spectrophotometer	0.004 to 44.3 [a] 0.02 to 6.65 [d]	0.4 (0.0) to 9.3 (2.3) [a]	see Cd reduction method	APHA: 4500-NO_3^- F, I [a] ASTM: D3867 [b] US EPA: 0353.2, 0353.6 [c] NLET: 01-1181 [d]
	automated hydrazine reduction	spectrophotometer	0.04 to 44 [a]	1.73 (5.1) to 21.0 (0.6) [a]	sulfide ion concentrations < 10 mg·L^{-1} can cause variations > 10%	US EPA: 0353.1 [c] APHA: 4500-NO_3^- H [a]
	brucine reduction	spectrophotometer	0.44 to 8.8 [c]	5.49 (17.3) [c]	DOM causes colour interference; strong oxidizing and reducing agents	US EPA: 0352.1 [c]
Potentiometry						
	nitrate-specific electrode	pH meter with ion-specific electrode	0.62 to 6200 [a]	± 0.4mV (= ± 2.5%CV) [a]	other anions; inconsistent pH	APHA: 4500-NO_3^- D [a] US EPA: 9210 [e]

Table 2.3 continued:

Direct ion quantification						
	capillary electro-phoresis	capillary electro-pherograph with UV detector	0.0008* [f]	0.031 (2.7) [f]		APHA: 4140 [a]
	ion chroma-tography	ion chromatograph	0.009 to 61.9 [g]	2.7 (33.3) 4.1 (2.17) [d]	any substance with a similar retention time; high concentrations from similar anions may mask anion of interest	APHA: 4110 [a] ASTM: D 4327 [g] US EPA: 0300.0 [e] NLET: 01-1080 [d]

notes:
* - MDL = Method Detection Limit
[a] - APHA 1998
[b] - ASTM 2000a
[c] Keith 1992
[d] - NLET 1994; note: the upper end of this range can be extended with adequate sample dilution
[e] - USEPA 2002
[f] - Bondoux et al. (2000)
[g] - ASTM 2000b

Ion chromatography (IC) is another analytical method for measuring nitrate, with detectable concentrations reported for NO_3^- using IC ranging from 0.009 mg $NO_3^-{\cdot}L^{-1}$ to 62 mg $NO_3^-{\cdot}L^{-1}$ (ASTM 2000c). There are two significant advantages of using IC. First, unlike colorimetric, electrometric, or titrimetric methods for analysing ions, ion chromatography can be used for sequential, rapid analysis of a suite of ions without the need for hazardous reagents. Second, it is also capable of readily distinguishing between NO_2^- and NO_3^- ions (APHA 1998; ASTM 2000c). Anions within a water sample are separated by the ion chromatograph and measured using a conductivity detector. The ion chromatograph consists of a guard column (that protects the separator column from organics or particulates) and an anion separator column and suppressor device (that separates the anions based on their relative affinities for the strongly basic anion exchanger).

Capillary electrophoresis is a relatively new technique for the analysis of ionic analytes. It is similar to IC, in that it can be used to distinguish between several anions or cations simultaneously. Ion separation is based on individual electromigration times and is quantified by direct UV detection (for nitrate and nitrite) and indirect UV detection using a cationic UV chromatophore for the ammonium ion (Padarauskas et al. 2000). The advantages offered by this method for nitrate analysis over IC include short analysis time (~4 min per sample), improved ion resolution (and therefore sensitivity), and more recently, the ability to simultaneously identify various nitrogen anions and cations (e.g., nitrate, nitrite and ammonium) (Padarauskas et al. 2000). Under optimised conditions for anion analysis in pure water samples, Bondoux et al. (2000) report a nitrate detection limit of 0.8 ppb (0.0008 mg $NO_3^-{\cdot}L^{-1}$). Although the innovative simultaneous anion/cation technique allows for precise separation of the three nitrogen ions, further method optimization is required for direct nitrate quantification (Padarauskas et al. 2000).

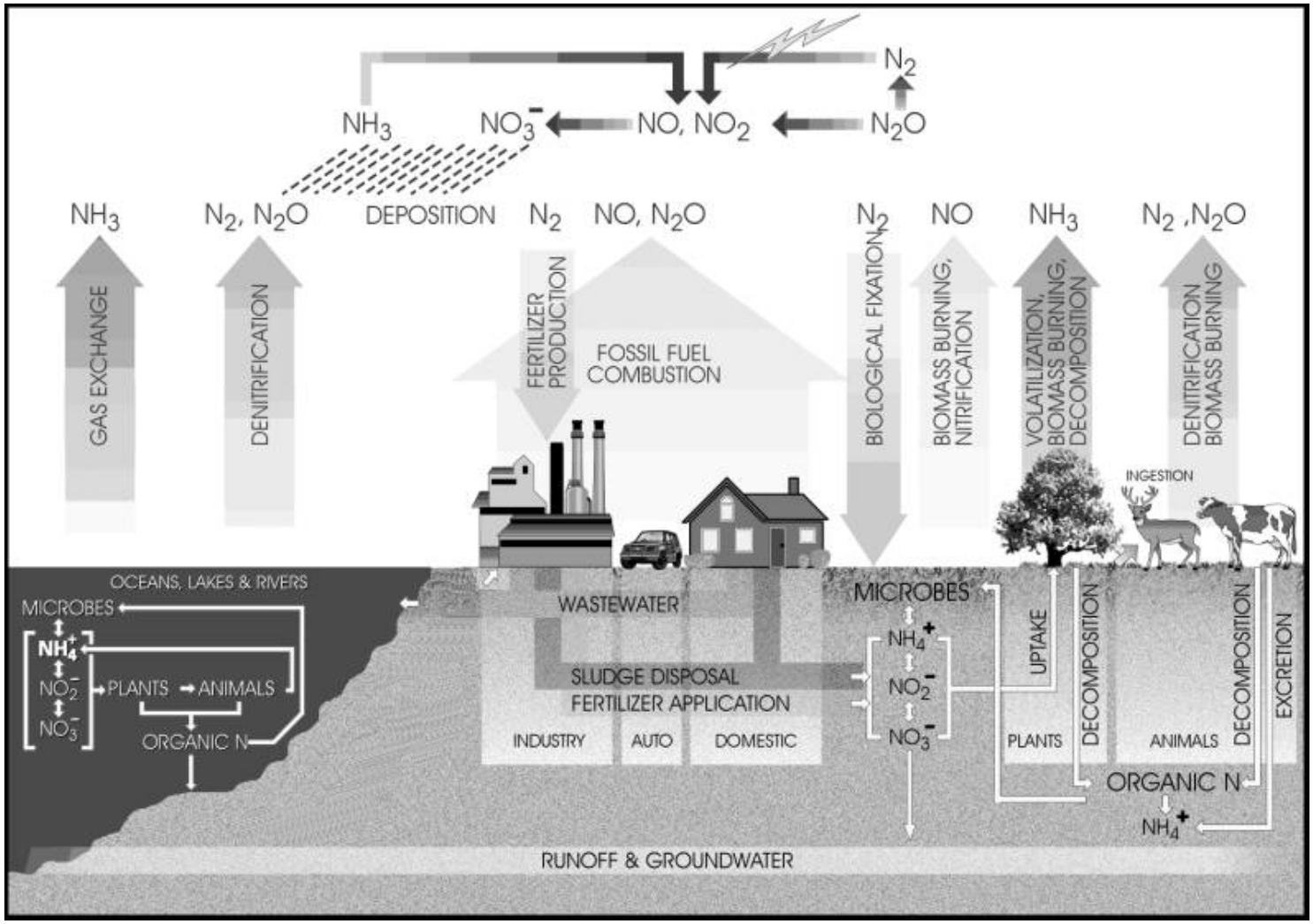
3 NITRATE PRODUCTION AND RELEASE TO THE ENVIRONMENT

3.1 Nitrogen Cycle

Although the Earth's atmosphere is composed of approximately 80% nitrogen, the majority of this nitrogen pool is stored as nitrogen gas (N_2) that is unavailable for use by most organisms. The nitrogen cycle (Figure 3.1) serves to convert this biologically unreactive nitrogen into useable forms for biota that are eventually cycled back to nitrogen gas (Chambers et al. 2001).

Natural processes, such as forest fires and decomposition of organic matter, release un-ionized ammonia (NH_3), nitrous oxide (N_2O), and nitric oxide (NO) into the atmosphere (NRC 1978). In the atmosphere, these gases may undergo various complex reactions (Chambers et al. 2001). The ammonia will react with hydroxyl (OH^-) radicals to produce NO and nitrogen dioxide (NO_2). These two nitrogen oxides (NO_x) are also formed through the reaction of nitrous oxide with an oxygen atom. Nitrous oxide may also dissociate to produce N_2. Nitrogen gas is quite stable, and only through lightning discharges is it converted to NO. Molecules of NO and NO_2 in the atmosphere will cycle back and forth in a complex reaction which involves the formation of ozone. They can also react with water vapour or OH^- radicals to form nitric acid (HNO_3) that can then enter aquatic ecosystems through precipitation (Chambers et al. 2001).

Nitrogen occurs in surface waters in numerous forms, including dissolved molecular nitrogen (N_2), a variety of organic compounds (e.g., amino acids, amines, proteins and refractory humic compounds), un-ionized ammonia (NH_3), ammonium ion (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) (Wetzel 1983). Nitrous oxide (N_2O) may also occur in surface waters, but rarely in appreciable quantities as it is rapidly reduced to N_2 (Wetzel 1983), or outgassed and returned to the atmosphere. All aquatic and terrestrial plants will assimilate nitrogen for protein production as either NO_3^- or NH_4^+, however, the latter form requires less energy to assimilate and is therefore often taken up preferentially (Crouzet et al. 1999). Nitrogen is also incorporated into organic material (typically as amine [NH_2] groups in organic nitrogen-compounds) through biological fixation. In this process, N_2 is reduced to ammonia that is then incorporated into organic nitrogen compounds (NRC 1978). Aquatic nitrogen-fixing species are limited to selected species of cyanobacteria (blue-green algae), and photosynthetic and heterotrophic bacteria (NRC 1978). In terrestrial systems, nitrogen-fixing bacteria in symbiotic association with leguminous plants (e.g., beans, peas, alfalfa, clover, soybeans, lentils, peanuts) are major contributors of nitrogen to the soil (NRC 1978; Chambers et al. 2001).



(from Chambers et al. 2001)

Figure 3.1. The nitrogen cycle.

3.2 Natural Sources

Natural sources of nitrate to surface waters can include wet and dry deposition of HNO_3 or NO_3^-. Atmospheric deposition of nitrate and ammonium in Canada is estimated to contribute 182 kilotonnes (kt) of nitrogen per year to surface waters (Table 3.1) (Chambers et al. 2001). This estimate may be conservative because data on dry deposition is lacking for many locations. Data collected over 1984-1994 show that wet deposition of nitrogen, on an areal basis, is considerably higher in eastern than in western Canada (see Section 5.1). It should be noted wet and dry deposition are not entirely natural sources, as some of the nitrate and ammonia in the atmosphere originates from anthropogenic sources. Other natural sources of nitrate include igneous rocks, volcanic activity, and the complete oxidation of organic nitrogen from vegetable and animal debris in native soil (Nordin and Pommen 1986). This latter nitrification process is the principle source of nitrate in terrestrial and aquatic environments (NRC 1978).

3.3 Anthropogenic Sources

Anthropogenic airborne emissions of nitrogen account for approximately 1.4 million tonnes of nitrogen relesed into the Canadian atmosphere, where manure and fertilizer use contribute the largest releases of ammonia (Schindler et al. 2006). In Canada, off-road vehicles contribute almost as much NO_x as on-road vehicles. The release of both NO_x and NH_3 results in increased concentrations of NO_3^- and NH_4^+ in surface waters, as well as soil acidification and urban smog. Table 3.1 provides the percentage contribution of major emitting Canadian sectors to total nitrogen emissions in Canada (Schindler et al. 2006).

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. Point source discharges of nitrogen include municipal and industrial wastewaters, septic tanks, and water discharges from mining (explosives) activity. On a national scale, point source discharges represent a small fraction of total input of nitrogenous compounds to ground and surface waters (NRC 1978). The National Pollutant Release Inventory (NPRI) total point source estimate of nitrate ion release from all participating Canadian sources for the year of 1999 was 6.8 kt NO_3^- to air, land, and surface and groundwaters (Environment Canada 2001). Reported releases for the year 2008 were much higher at 62.8 kt NO_3^- to air, land, and surface and groundwaters (Environment Canada 2010a). Diffuse sources, however constitute the greatest inputs of anthropogenically-fixed nitrogen and can include agricultural runoff, feedlot discharges, urban runoff, lawn fertilizers, landfill leachate, nitric oxide and nitrogen dioxide from vehicular exhaust, and storm sewer overflow (NRC 1972; NRC 1978). In a review of U.S. nitrogen discharge estimates, van der Leeden et al. (1990) reported that point sources contributed 561 kt $N \cdot a^{-1}$ (1977 data), while non-point sources contributed 9108 kt $N \cdot a^{-1}$ (1980 data). Although point sources account for only a small fraction of the nitrogen released to surface and groundwaters, they can result in higher concentrations because they are released into a small area.

Table 3.1 Nitrogen loading estimates to Canadian surface and ground waters from various sources, 1996.

Nutrient Source	Total Nitrogen (10^3 t·a^{-1})						
	Atlantic	**Québec**	**Ontario**	**Prairies**	**British Columbia**	**Territories**	**Canada**
Municipality							
MWWTPs[1]	4.6	19.9	31.7	13.2	10.6	0.3	80.3
Sewers							11.8
Septic Systems	2.2	3.7	5.0	2.6	1.9	0.05	15.4
Industry	0.1[2]	0.3[3]	9.9	0.6	0.9	0	11.8
Agriculture (residual in the field after crop harvest)	18	46	14	188	29	n/a	294
Aquaculture	0.8	0.04	0.2	0.04	1.2	n/a	2.3
Atmospheric Deposition to Water (NO_3^- N and NH_4^+ N only)	11.9	60.7	54.4	13.9	1.6	39.9	182
Total Loadings	37.6	130.64	115.2	218.34	45.2	40.25	597.6

[1] MWWTPs: municipal wastewater treatment plants
[2] data from Newfoundland only
[3] data for industries discharging to the St. Lawrence River
* (Industrial N loads are based on NO_3^- + NH_3 and not total nitrogen; industrial data are not available for NB, NS and PEI and Québec industries that do not discharge to the St Lawrence River. Agricultural residual is the difference between the amount of nitrogen added to cropland and the amount removed in the harvested crop; data are not available as to the portion of this residual that moves to surface or ground waters.)

(from Chambers et al. 2001)

Table 3.2 Percentage contribution of major emitting sectors to total nitrogen emissions in Canada.

Sector	**NO_x**	**NH_3**
Non-ferrous mining and smelting	-	-
Electrical power generation	11.4	-
Upstream oil and gas	13	-
On-road vehicles	32.6	3.1
Off-road vehicles	26.9	-
Industrial fuel combustion	-	-
Other fuel combustion	-	-
Agriculture (animals)	-	55
Pesticides and fertilizer	-	34.6
Chemicals and products	-	-

(from Schindler et al. 2006)

3.3.1 Municipal Wastewaters

Humans excrete virtually all nitrogen obtained in protein from food sources translating to an average excretion rate of 5.4 kg N per person per annum (NRC 1972). As of 1999, 86% of Canada's population were served by municipal sewer systems; the remaining 14% were served by septic disposal systems and lagoons (Environment Canada 1999). Of those served by sewer systems, 97% were connected to municipal wastewater treatment plants (MWWTPs) employing primary (or better) treatment processes (Environment Canada 1999). The remaining 3% were serviced by sewage collection structures that were not connected to treatment facilities such that untreated wastewater was discharged directly into lakes, rivers or oceans. Canadian loading estimates for nitrogen from wastewater sources for 1996 include 80.3 $kt \cdot a^{-1}$ from municipal water treatment plants, 11.8 $kt \cdot a^{-1}$ from storm sewers and combined stormwater overflows, and 15.4 $kt \cdot a^{-1}$ from septic systems (Table 3.1, Chambers et al. 2001).

Among the facilities in the Canadian NPRI database reporting releases of nitrate, sewage treatment facilities recorded the largest discharges of NO_3^-, due to the nitrification of ammonia wastes (Environment Canada 2001). For example, the Regional Municipality of Ottawa-Carleton released 0.66 kt of NO_3^- directly to receiving waters in 1999; the City of Toronto's Humber and Ashbridges Bay MWWTPs each reported releases of 0.48 kt of NO_3^-; and the City of Medicine Hat MWWTP reported a nitrate release of 0.44 kt (Environment Canada 2001). These four MWWTPs all use secondary treatment or better. Average nitrate concentrations measured between 1987 and 1994 in effluents from selected MWWTPs from across Canada, with varying types of treatment, ranged from 0.05 to 27 mg $NO_3^- \cdot L^{-1}$ (Chambers et al. 1997). The Ontario Ministry of the Environment conducted a characterization study of MWWTPs (MOE 2010a). This survey was conducted in 2004 and 2005 and characterized the influent and effluent of 46 MWWTPs. The measured nitrate concentrations (presented as mg $NO_3^- + NO_2^-$ /L) are presented in Table 3.3 [NB: as stated in Section 2.2, the NO_2^- concentration in a reported measurement of $NO_3^- + NO_2^-$ will generally be quite small, particularly for samples collected from well-oxygenated waters, therefore the measurements below are typically reflective of NO_3^- concentrations].

Table 3.3. Concentration of nitrate (NO_3^-)+ nitrite (NO_2^-) (mg/L) measured in the influent and effluent of 46 Ontario MWWTPs, 2004 and 2005 (MOE, 2010a).

Treatment Type	Total Samples	% of Samples < MDL	Min	Max	Mean	Median	95th %ile	MDL
Primary MWWTP								
Influent	60	82	0.06	1.07	0.05	0.01	0.19	0.05
Effluent	60	27	0.06	2.35	0.32	0.15	1.15	0.05
Secondary MWWTP[1]								
Influent	222	89	0.06	1.35	0.03	0.004	0.12	0.05
Effluent	234	4	0.07	22.5	5.9	3.1	17	0.05
Secondary Nitrifying MWWTP								
Influent	182	80	0.06	72.1	0.32	0.004	0.52	0.05
Effluent	196	1	0.84	31	10.45	9.08	23	0.05
Tertiary MWWTP								
Influent	47	87	0.06	2.21	0.13	0.0002	1.79	0.05
Effluent	48	0	9.6	23.8	18.5	19	21.6	0.05

[1]The proposed *Wastewater System Effluent Regulations* under the *Fisheries Act* list national effluent quality standards that would require secondary wastewater treatment, or equivalent, in wastewater systems across Canada. This level of treatment removes over 95% of the total mass of conventional pollutants in wastewater (i.e. Biological Oxygen Demand matter, suspended solids and nutrients). Significant amounts of non-conventional pollutants and bacteria that may be present are also removed through such treatment (Canada Gazette 2010).

Based on the mean and 95th percentile measurements, nitrate concentrations in effluent from all MWWTPs (primary, secondary, secondary nitrifying and tertiary) are consistently greater than those measured in the influent as a result of nitrification of ammonia waste. Measured concentrations in effluent ranged from a minimum of 0.06 mg NO_3^- + NO_2^- /L, to a maximum of 31 mg NO_3^- + NO_2^- /L.

Examining nitrate levels alone in effluent, however, may only give an indication of the degree of nitrification in the effluent. Concentrations of total inorganic nitrogen in MWWTP effluents give a better indication of nitrate loading, as ammonia and nitrite are readily transformed to nitrate in the receiving waters. Table 3.4 provides the concentrations of total nitrogen (TN) measured in both the influent and effluent of the same 46 Ontario MWWTPs listed in Table 3.3. Total nitrogen concentrations were greater in the influent (1.9 - 138 mg TN/L) when compared with effluent concentrations (0.88 - 61 mg TN/L).

Table 3.4. Concentration of total nitrogen (TN) measured in the influent and effluent of 46 Ontario STPs, 2004 and 2005. TN is the sum of ammonia (NH_3) + ammonium (NH_4^+) + nitrate (NO_3^-) + nitrite (NO_2^-) (mg/L).

Treatment Type	Total Samples	Min	Max	Mean	Median	MDL
Primary STP						
Influent	60	8.8	33	20	19	0.05
Effluent	60	11	26	17	17	0.05
Secondary STP[1]						
Influent	222	3.5	132	33	33	0.05
Effluent	234	0.88	61	22	19	0.05
Secondary Nitrifying STP						
Influent	182	1.9	138	28	28	0.05
Effluent	196	1.2	59	13	11	0.05
Tertiary STP						
Influent	47	10	67	32	29	0.05
Effluent	48	10	28	20	20	0.05

[1]The proposed *Wastewater System Effluent Regulations* under the *Fisheries Act* list national effluent quality standards that would require secondary wastewater treatment, or equivalent, in wastewater systems across Canada. This level of treatment removes over 95% of the total mass of conventional pollutants in wastewater (i.e. Biological Oxygen Demand matter, suspended solids and nutrients). Significant amounts of non-conventional pollutants and bacteria that may be present are also removed through such treatment (Canada Gazette 2010).

Nitrate levels in urban stormwater runoff can be highly variable depending on land use patterns. In a review of 25 years of international runoff data from urban areas, Makepeace et al. (1995) report a range in nitrate concentrations of 0.04 to 53 mg $NO_3^- \cdot L^{-1}$. Mean nitrate concentrations from storm event samples monitored over a one-year period in the Brunette River watershed in British Columbia did not exceed 4.0 mg $NO_3^- \cdot L^{-1}$ (Hall et al. 1999). Airports also contribute nitrate to stormwater runoff through the breakdown of urea-based de-icing agents (DND 1998). A review of nitrate levels between 1992 and 1996 from monitoring stations at federal civil and military airport facilities reported nitrate levels in stormwater runoff of up to 116 and 1465 mg $NO_3^- \cdot L^{-1}$, at respective facilities (DND 1998).

3.3.2 Industrial Sources

Ammonium nitrate production in Canada began during the Second World War for use in explosives. It was not until after the end of the war that large quantities were available for use in

fertilizers (McBeath 1987). Ammonium nitrate is produced by an exothermic reaction between ammonia and nitric acid (McBeath 1987):

$$NH_3 + HNO_3 \rightarrow NH_4NO_3$$

Natural gas is one of the primary raw materials in ammonia synthesis, and as such, the majority of Canadian nitrogen fertilizer production is centred in Western Canada where natural gas reserves are plentiful (SENES 2001). In 1999, there were twelve Canadian facilities producing nitrogen fertilizers, six of which produced either ammonium nitrate (totalling 498 $kt \cdot a^{-1}$) or solutions of urea [$CO(NH_2)_2$] and ammonium nitrate (1273 $kt \cdot a^{-1}$) (SENES 2001). The other six facilities produced ammonia, urea, and/or ammonium sulphate. As urea contains significantly higher levels of fixed nitrogen than ammonium nitrate, on a unit mass basis, this product is displacing traditional ammonium nitrate fertilizer markets, and since 1987, five ammonium nitrate production facilities have ceased operations (McBeath 1987; SENES 2001). Approximately one-half of ammonium nitrate and urea production is used nationally, while the remainder is exported to the U.S. (SENES 2001).

Provincial limits for nitrate in fertilizer plant wastewater in Alberta and British Columbia are 88 and 45 mg $NO_3^- \cdot L^{-1}$, respectively (McBeath 1987). In 1980/81, however, effluent monitoring from selected Canadian fertilizer producers revealed that nitrate levels ranged from 0.13 to 3400 mg $NO_3^- \cdot L^{-1}$ (McBeath 1987). By 1999, six of the twelve Canadian fertilizer plants were "zero discharge" facilities that either directed their effluents to municipal water treatment plants, or used on-site evaporation ponds (SENES 2001). Of the remaining plants for which data exist, nitrate concentrations in effluents discharged directly to receiving waters ranged from 0.4 to 56.2 mg $NO_3^- \cdot L^{-1}$ (SENES 2001).

Nitrate metal salts such as potassium nitrate, calcium nitrate, silver nitrate and sodium nitrate are used in a variety of industrial applications, including oxidising agents in explosives, matches and pyrotechnics, photography, glass making, engraving, textile dyes, food processing (e.g., meat preservatives), and as a raw material for manufacturing nitric acid (Nordin and Pommen 1986; WHO 1996).

Industrial sources with high concentrations of inorganic nitrogen effluents include steel production, petroleum production and refining, pulp and paper, plastics and fertilizer production (Heathwaite et al. 1996). Other industrial processes that are known to result in high nitrate concentrations in their wastestreams include the production of nitroaromatic compounds, the synthesis of nitroorganic compounds in pharmaceuticals, and wastewaters from nuclear fuel processing (Pinar et al. 1997).

Mining activities can also be a source of nitrate to Canadian waters. Nitrate, resulting from the use of explosives containing ammonium nitrate, may enter surface waters through mine drainage from pits and spoil piles, and through seepage from tailing ponds (Pommen 1983). Elevated levels of nitrate have been noted downstream from several Canadian mines (Pommen 1983). For example, on the Fording River in southeastern British Columbia, Nordin (1982) found that nitrate concentrations upstream from a surface coal mine ranged from 0.22 to 0.31 mg $NO_3^- \cdot L^{-1}$, while river concentrations within the minesite were as much as 200 times higher, ranging from 4.4 to 44 mg $NO_3^- \cdot L^{-1}$.

Total Canadian industrial loading of inorganic nitrogen (nitrate and ammonia) to surface waters is estimated at 11.8 kt $N \cdot a^{-1}$ (Table 3.1, Chambers et al. 2001). This value, however, underestimates actual loads as not all industries are monitored nationally, and monitoring data were not available for industries in New Brunswick, Nova Scotia, and Prince Edward Island, nor for industries in Québec which do not discharge directly into the St. Lawrence River Basin (Chambers et al. 2001).

3.3.3 Agricultural Sources

During the last six months of 1998 and the first six months of 1999, a total of 1600 kt of nitrogen as fertilizer were sold (and assumed to be consumed) in Canada (Korol and Rattray 2000). Of this, 90 kt of nitrogen were nitrate compounds, with 82% as ammonium nitrate; remaining forms included calcium nitrate, calcium ammonium nitrate and potassium nitrate (Korol and Rattray 2000). The other 1500 kt of nitrogen sold in Canada were contained in fertilizers such as urea, anhydrous ammonia, and monoammonium phosphate, among others. These levels correspond with 1999 estimates of total nitrogen consumption by plants in Canada of 1626 kt (Korol and Rattray 2000). The amount of nitrogen fertilizer applied to Canadian cropland has increased considerably over the past century, due to both increased fertilizer application rates and increased land usage (Chambers et al. 2001). For example, the amount of nitrogen applied to the western Canadian grain crop in 1986 was four-fold greater than the average amount applied annually between 1883 and 1953 (Chambers et al. 2001). Annual nitrogen fertilizer use in the United States has also increased dramatically from 450 kt to 9980 kt in less than 50 years (Lanyon 1996). Although the total Canadian use of nitrogen fertilizer continues to rise, within the provinces of Ontario and British Columbia sales in recent years have been decreasing, after hitting peaks in 1985 and 1989, respectively (Korol and Rattray 2000).

Among the various regions of Canada, the greatest loadings of nitrogen per unit area of agricultural land in 1996, through the application of fertilizer, occurred in Québec and the Atlantic region, with 89 and 86 kg $N \cdot ha^{-1}$ applied, respectively (Chambers et al. 2001). In Manitoba, British Columbia, Ontario, Alberta, and Saskatchewan, the amounts of nitrogen applied as fertilizer in 1996 were 82, 75, 72, 61, and 52 kg $N \cdot ha^{-1}$, respectively (Chambers et al. 2001).

The practice of spreading animal waste slurries (manure) as organic fertilizer also constitutes a significant source of agricultural-nitrogen loading. In 1994, more than 34 000 kt of manure (containing approximately 141 kt N) were generated in Ontario alone (OMAFRA 1996). Nationally, approximately 384 kt of nitrogen were applied to cropland as manure in 1996 (Chambers et al. 2001).

Nutrient contents of manure vary according to animal source. Solid manure from broiler chicken litter contains 29 kg $N \cdot t^{-1}$, whereas pig and cattle manure contains 6 kg $N \cdot t^{-1}$. For liquid slurries applied directly to fields, pig manure contains 5 kg $N \cdot m^{-3}$ as opposed to cattle slurry with 3 kg $N \cdot m^{-3}$ (Hooda et al. 2000). Within a species, nutrient manure may also vary depending on the diet of the livestock. For example, dairy cattle from Ontario, which are primarily corn-fed, produce manure with a typical nitrogen content of 1.5 kg $N \cdot t^{-1}$, whereas the manure from dairy cattle in Alberta, which are generally grain-fed, typically contains 4.5 kg $N \cdot t^{-1}$ (Hilborn and Brown 1996; Statutes of Alberta 2001). Manure processing also affects nitrate composition. At a

beef cattle feedlot, for example, fresh manure used for crop applications can contain 0.115 kg NO_3^-·t^{-1}, while composted manure allowed to undergo nitrification can contain 5.33 kg NO_3^-·t^{-1} (Eghball and Gilley 1999).

Manure produced on intensive livestock farms often far exceeds the agronomic requirements, resulting in large amounts of unutilised, or surplus, nitrogen. In a study of seven different farming systems in Ontario, Goss and Goorahoo (1995) found larger surpluses of nitrogen were more likely to occur on dairy farms than on swine farms, or farms with crops only. Examination of nitrogen inputs and outputs for a dairy farm in the Waterloo region of Ontario showed a surplus of 77 kg N·ha^{-1} (Millman 1999). Millman noted that the Ontario farm was very efficient compared with other farms from the United States and Europe which reported higher nitrogen surpluses. Hooda et al. (2000) cited an example of 177 Dutch dairy farms showing an average nitrogen surplus of 486 kg N·ha^{-1}. In a study of the effect of fertilizer type on nitrate levels in agricultural runoff, Eghball and Gilley (1999) found NO_3^--N accounted for 21%, 25% and 37% of total nitrogen (TN) found in field runoff waters fertilized with inorganic fertilizers, fresh manure and composted manure, respectively. Several Canadian provinces currently have regulations for manure storage and land application on intensive livestock farms to reduce impacts on aquatic systems.

Canadian soil nitrogen surpluses for 1996, based on national application rates and crop removal from harvesting, are estimated at 294 kt N·a^{-1} (Table 3.1,Chambers et al. 2001). Due to high production levels of nitrogen-intensive crops such as corn and soybeans, Ontario and Québec contained the greatest share (37 and 27%, respectively) of agricultural lands at risk of having > 60 kg N·ha^{-1} residual nitrogen remaining after harvesting (MacDonald 2000a). As soils in these areas also experience water surpluses, they are at the greatest risk of exporting excess nitrogen to the watershed. Using data for soil water-holding capacity and regional 30-year precipitation averages, MacDonald (2000b) determined that 17% and 6% of the agricultural lands in Ontario and Québec, respectively could generate runoff or seepage water with > 14 mg N·L^{-1}. Between 1981 and 1996, the nitrogen content of water moving off agricultural land to surface and groundwater was estimated to increase by at least 1 mg N·L^{-1} on 68% of Ontario's and 77% of Québec's farmlands (MacDonald 2000b). However, it should be noted that MacDonald's estimates are based on modeling, without measurements to evaluate the reliability of the predictions; actual groundwater analyses in rural Ontario have not shown a temporal increase in the proportion of farm wells with nitrate contamination. A survey of domestic well water from Ontario farms in 1992 showed approximately 14% of wells contained nitrate concentrations above the provincial drinking water guideline, the same percentage of exceedances that were observed in a survey conducted in 1950-1954 (Goss et al. 1998a).

Although national estimates quantifying nitrogen loss to surface and groundwaters from agricultural lands are not available (Chambers et al. 2001), NO_3^--N has been shown to account for 97-98% of sub-surface nitrogen in leaching loss studies from Quebec and Georgia (Lowrance 1992; Gangbazo et al. 1995). As such, losses from residual nitrogen from agricultural soils (Table 3.1) can provide a major source of nitrate to surface or groundwaters. In some regions of the United States, up to 54% of nitrogen in surface waters is thought to originate from agricultural runoff or other rural sources (NRC 1972). Mean annual total nitrogen losses to rivers from agricultural subcatchments within the Lake Simcoe, Ontario watershed were highest from low-land cultivated marshes (or polders) used in the production of vegetables, at

25 (± 24) kg $N \cdot ha^{-1} \cdot a^{-1}$, followed by mixed agricultural lands, at 2.2 (± 0.7) to 7.9 (± 3.6) kg $N \cdot ha^{-1} \cdot a^{-1}$ (Winter et al. 2002). By comparison, forested areas in the watershed generally exported the least amount of nitrogen, at 1.7 (± 0.5) to 2.7 (± 0.8) kg $N \cdot ha^{-1} \cdot a^{-1}$ (Winter et al. 2002).

Aquaculture is a $355 million per year industry in Canada, with finfish and shellfish production totalling 53 and 19 kt, respectively in 1996 (DFO 1998). Nutrient loading from animal wastes and decomposition of unused food in semi-closed and open culturing systems are estimated to contribute 1.0 and 1.3 kt $N \cdot a^{-1}$ to inland and coastal surface waters, respectively (Table 3.1, Chambers et al. 2001).

4 ENVIRONMENTAL FATE AND BEHAVIOUR

4.1 Atmospheric Processes

4.1.1 Wet Deposition

Anthropogenic processes such as fossil fuel burning and ore smelting release SO_x and NO_x to the atmosphere where they undergo hydrolysis and oxidation to form the acid rain causing compounds H_2SO_4 and HNO_3 (Galloway and Dillon 1983). Subsequently, nitrate is one of the dominant ions present in precipitation (Fowler et al. 1999). In the early 1980s, nitric acid deposition contributed approximately 35% of the acidity of acid rain in eastern Canada and the northeastern United States (with the other 65% contributed by sulfuric acid) (Galloway and Dillon 1983). However, due to reductions in the emission of sulphur oxides since the early 1980s, nitric acid has accounted for an increasing proportion of the acidity. Between 1976-77 and 1985-86, the ratios of NO_3^- to SO_4^{2-} in atmospheric deposition have increased in central Ontario from 0.43 to 0.68 (Dillon and Molot 1989). A long-term study of atmospheric inputs to Heney Lake, situated in Ontario on the Canadian Precambrian Shield, showed almost no change in the amount of nitrate in precipitation over the period from 1976 to 1987, but by the 1990s, nitrate and sulphate ions were present in precipitation at almost equal amounts (Dillon and Evan 2002). In eastern Canada, where it is common to find lakes of low alkalinity, nitrate can play a role in lowering lake pH. During spring snowmelt, NO_3^- concentrations can exceed 1.24 mg L^{-1} which is enough to contribute to the lowering of both pH and alkalinity in lakes where alkalinity is found to be less than 5 mg L^{-1} as $CaCO_3$ (Schindler et al. 2006).

Wet deposition of ammonium is another major atmospheric source of nitrogen. In some parts of Canada, deposition of ammonia can be as great as, or greater than, deposition of nitrate (Chambers et al. 2001). Once deposited in aquatic or terrestrial ecosystems, some ammonium may be taken up by plants, but the remainder is generally converted to nitrate through nitrification.

In some catchments, atmospheric deposition accounts for the majority of nitrate concentrations in surface waters, with very little export from the terrestrial system (Lovett et al. 2000). There are also areas of Canada where less than 10% of the total deposition of atmospheric nitrate to surface waters occurs through direct deposition, with the majority of the deposition occurring on

land with subsequent transport of the nitrate ion from the terrestrial basin to surface waters (Elder 1984). Most deposited atmospheric nitrogen, however, is likely retained in the terrestrial ecosystem and assimilated into biomass (Jeffries and Semkin 1983). Aquatic systems are most at risk of acidification if the terrestrial system is already saturated with nitrogen, in which case atmospherically deposited nitrate will be released along with an equivalent amount of cations. If the cation is H^+ or Al^{3+}, then acidification of the water will result (Galloway and Dillon 1983). The maximum deposition of nitrogen compounds (NO_x and NH_x) that will not cause eutrophication or acidification is referred to as the critical load of nitrogen (RIVM 1991). Using critical load modelling, extensive mapping has been conducted in Europe to determine which terrestrial and freshwater ecosystems are at risk of acidification and eutrophication due to excess nitrogen deposition (RIVM 2001a). Aquatic critical load models conducted in Canada indicate that approximately 15% of the lakes loacated in eastern Canada (south of 52°N) that historically had pH values of <6, will become acidified based on current acid deposition regimes including both sulfur and nitrogen (Schindler et al., 2006). The number of lakes expected to be affected range from 500,000 to 600,000 (Schindler et al., 2006). Twenty five years of data collected in the Dorset area of Ontario indicate that there has been a significant reduction in sulfur oxide emissions whereas ammonium and nitrate emissions have remained particularly steady (Schindler et al. 2006). This reduction in sulfur oxide emissions has resulted in a decrease in the number of lakes where critical loads are being exceeded (>90% in the late 1970s to <40% in the late 1990s). In the Dorset (central Ontario) area, there have been no detectable trends in stream or lake concentrations of inorganic nitrogen during the last two decades (Schindler et al. 2006). In terms of the Great Lakes, Lake Superior receives small amounts of phosphorus but moderate loading of nitrogen due to air emissions from US industry and agriculture. This has resulted in an increase in the concentration of measured nitrate, from 0.2 mg L^{-1} (1950) to 0.36 mg L^{-1} (2001) (Schindler et al. 2006).

Overall, annual wet nitrate deposition is low (<5 kg NO_3^- ha^{-1} y^{-1}) in eastern Canada (north of 52°N), moderate (10 kg NO_3^- ha^{-1} y^{-1}) in industrial regions of Ontario and Quebec, and as high as 20-25 kg NO_3^- ha^{-1} y^{-1} at the Ontario-US and Quebec-US borders, where the source is industry and automobiles (Schindler et al. 2006). In the case of western Canada, deposition has been fairly low (<2 kg NO_3^- ha^{-1} y^{-1}). There are areas where deposition has been higher, and this includes the lower Fraser Valley in British Columbia (10 kg NO_3^- ha^{-1} y^{-1}), which is an area of concentrated agricultural operations, as well as rapid population and industrial growth. The Athabasca oil sands is an additional area where deposition is on the rise (projected to be 65 kg NO_3^- ha^{-1} y^{-1} (Schindler et al. 2006). Fortunately for now, the highest levels of deposition occur in areas where soil and water are well buffered (Schindler et al. 2006).

4.1.2 Dry Deposition

Dry deposition of oxidised nitrogen generally occurs in the form of nitrogen dioxide (NO_2) or nitric acid (HNO_3) (Fowler et al. 1999). Ammonia may also enter aquatic and terrestrial systems through dry deposition. The nitrate form of nitrogen is only precipitated from the atmosphere in the form of wet deposition. The other nitrogen species that do undergo dry deposition, however, may form nitrate once in the receiving environments.

In eastern Canada, dry deposition ranges from 17 to 41% of total nitrogen deposition and is highest near industrial sites along the US border (Schindler et al. 2006). As of 2000 in western

Canada, NO_x emissions have exceeded those of eastern Canada. Expansion of the Athabasca oil sands operations is expected to cause an increase in deposition by another 5% between 2000 and 2020 (Schindler et al. 2006).

4.2 Terrestrial Processes

4.2.1 Adsorption

The nitrate ion is negatively charged, and therefore does not adsorb to clay minerals or organic matter in soils unless they have a significant anion exchange capacity (Jury and Nielsen 1989). Soils with large anion exchange capacities are very uncommon, except in tropical areas. Therefore, with respect to the Canadian environment, it can be assumed nitrate does not adsorb to soil particles and has a high potential for mobility. Both leaching and surface runoff are major fate processes of nitrate in the terrestrial environment.

4.2.2 Leaching

In soils, the nitrate ion is highly mobile, readily moving with the soil water, and, therefore, can potentially leach below the rooting zone (Hooda et al. 2000). Leaching is the most significant process by which nitrate can enter groundwaters and is dependent on the water supply from precipitation and irrigation, evaporation and drainage rates, tillage practices, the type of fertilizer applied (organic vs. inorganic), the type of ground or crop cover, and the soil structure and porosity (Table 4.1).

Moisture and temperature are major factors affecting the leaching of nitrate in soils. Nitrate is moved downward in the soil with rainfall and irrigation, while upward movement may occur in the very upper layers of the soil through evaporation (NRC 1978). Downward movement of nitrate is reduced at low temperatures because water drains more slowly through cold soils; this effect is only significant, however, when temperatures are below freezing, at which point water completely ceases to drain (NRC 1978). Extreme variations in temperature, such as freezing of soil following by thawing, can lead to greater leaching of nitrate (Mitchell et al. 1996). Saturated soil conditions due to high water tables will enhance denitrification (see Section 4.2.5), while all other processes occur at faster rates when the soil moisture content is below field capacity (Madramatoo et al. 1997).

Table 4.1. Factors affecting nitrate leaching through agricultural soils.

Factor	Less Leaching	More Leaching
Climate	Low rainfall Cold temperatures	High or irregularly distributed rainfall Warm temperatures
Crop	Vigorous crop	Poor crop
Time of Application	At the beginning of the main growing period or during active crop growth Established crop	At the end of the growing season or out of season Seedbed application
Application Rate	Rate appropriate for crop use	Over-application
Soil	Fine soil (e.g., clay) Poor drainage Limited soil tillage	Coarse soil (e.g., sandy) Good drainage Intensive soil tillage

(adapted from Ritter et al. 2001)

Leaching of nitrate from soil into groundwater appears to follow seasonal trends. Through the use of field lysimeters, Roy et al. (2000), in Guelph, Ontario, found very little leaching of nitrate occurred following spring and summer applications of ammonium-nitrate fertilizer to turfgrass, but an average of 16.5% of the applied nitrogen was lost through leaching in late autumn and early winter. Possible reasons for greater nitrate leaching in late autumn include increased precipitation coupled with reduced uptake of water by plants. Roy et al. (2000) speculated washing out of nitrate that has accumulated in soil during the spring and summer could occur as a single autumn pulse to the water table, resulting in high transient concentrations of nitrate in groundwaters. Ezeonu and Okaka (1996) have also observed seasonal trends in the occurrence of nitrate in Nigeria's groundwater. Concentrations of nitrate entering the aquifers are highest at the beginning of the rainy season, decrease throughout the rainy season, and remain at relatively constant low levels during the dry season.

During dry periods, nitrate may accumulate in soil due to decreased transport to streams, decreased uptake by plants, and, with the declining water table, increased capacity for storage of nitrate as the thickness of the unsaturated zone above the water table increases (Lucey and Goolsby 1993). Under wetter conditions, the water table rises, and nitrate stored in what was previously the unsaturated zone becomes mobilised and may be transported by subsurface flow to surface waters. In a test of this nitrate flushing theory, nitrate-nitrogen release from soils was modeled for the forested catchments in the Turkey Lakes Watershed of Ontario (Creed et al. 1996). Two mechanisms were suggested for producing significant concentrations of nitrogen in catchment discharge waters: (1) a rapid flushing of nitrogen from throughwaters entering a previously unsaturated zone high in nitrate from either a period of low biological activity (e.g., spring snowmelt and autumn stormflow), or soils that had previously undergone enhanced

nitrification (e.g., after summer droughts), or (2) through a slow draining of nitrogen from the bioactive soil layers to non-active layers through percolation to be released slowly throughout the year (Creed et al. 1996). Of these two processes, rapid flushing is the dominant mechanism.

In an examination of soils and groundwater beneath an agricultural field receiving nitrate fertilizer applications, nitrate concentrations were generally found to decrease exponentially with soil depth (Schuh et al. 1997). Elevated concentrations at all soil depths occurred temporarily following large rainfall events. During these brief periods of large water influx, concentrations of nitrate in groundwater were observed to increase by an order of magnitude or more (Schuh et al. 1997). In some cases, the downward movement of nitrate during rainfall or flooding events can be quite rapid, due to the vertical hydraulic gradients that are created. For example, stable isotope-labeled ^{15}N sodium nitrate applied to the surface of an Illinois agricultural field was found to travel 4.5 m vertically in the soil horizon within 16 h following an annual flooding event from the nearby Illinois River (Kelly and Wilson 2000).

The type of vegetation or forest cover in a watershed can affect the amount of nitrate retention in the soil. For example, in the Catskill Mountains, New York, Lovett et al. (2000) found forests where red oak and beech trees dominate had higher stream nitrate concentrations than forests dominated by maples. They attributed this difference to the quality of the different leaf litters in terms of lignin-to-nitrogen ratios and potential rates of nitrification.

The type of cropping system used on agricultural lands can have a large influence on the amount of nitrate lost through leaching. Randall et al. (1997) found row-crop systems, such as continuous corn, or annually alternating corn and soybean systems, resulted in nitrate losses about 45 times higher than that in perennial crops, such as alfalfa or mixtures of alfalfa and grasses. Annual crops such as corn and soybeans allow for greater losses of nitrate because they are shallower rooted, have shorter growing seasons, and use water less efficiently (Randall et al. 1997). The water balance in fields planted with annual crops will generally favour drainage rather than evaporation; hence nitrate will also tend to leach downwards.

Certain agricultural practices, such as tilling, fertilizer and manure application, and improved subsurface drainage through tile lines also contribute to greater loss of nitrate through leaching (Randall et al. 1997). A study of rivers in Ireland concluded that the major factor affecting nitrate levels in the rivers was the proportion of ploughed land area in the catchment (Neill 1989). Mean nitrogen loss from ploughed land was estimated at 75.9 $kg \cdot ha^{-1} \cdot a^{-1}$ compared with only 1.9 $kg \cdot ha^{-1} \cdot a^{-1}$ from unploughed land (Neill 1989). In a study of soil plots with a drainage system, the amount of nitrate leached from plots that were ploughed was 21% more than from direct-drilled (untilled) plots (Goss et al. 1993). In plots with subsoil drains, five times more nitrate was lost through leaching than from undrained soils (Goss et al. 1993). Greater nitrate leaching has been observed with grassland that is used for grazing livestock than when the grass is cut, due to the additional nitrogen inputs from the livestock manure (Ryden et al. 1984).

Timing of fertilizer application can have a large effect on nitrate leaching. To reduce the amount of leaching, it is important to synchronize nitrogen additions (through fertilizer or manure applications) with nitrogen mineralization in the soil and nitrogen uptake by the crop (Izaurralde et al. 1995). Application methods for organic fertilizers may also affect the amount of leaching that occurs. Leaching of nitrate is more likely when the injection method for manure application

is used than when it is broadcast on the soil surface (Sutton et al. 1982). The injection method, however, is better for reducing volatilization. Differences in leaching have also been noted among different forms of fertilizers. For example, Sutton et al. (1978) observed greater downward movement of nitrate through soil that had received inorganic fertilizer than soil that had received swine manure, despite the higher nitrogen content of the manure. The original form of nitrogen in the fertilizer was urea, while the manure contained both ammonium and organic forms of nitrogen. Therefore, less inorganic nitrogen may have been available for leaching from the swine manure due to the slower decomposition of the organic matter (Sutton et al. 1978). The higher carbon content in manure than in inorganic fertilizers may also promote increased denitrification in the soil profile, reducing the potential for nitrate contamination of groundwater (Burton et al. 1994).

Winter crop covers can also aid in reducing nitrate runoff from agricultural fields. During a three year study on winter soil nitrate leaching under sweet corn (*Zea mays* L.) or broccoli (*Brassica oleracea* var. *italica* Plenck) crops in Oregon, Brandi-Dohrn et al. (1997) found recommended crop-specific nitrogen application rates (up to 280 kg $N \cdot ha^{-1} \cdot a^{-1}$) resulted in flow-weighted mean nitrate levels in winter leachate under fallow fields of 77 mg $NO_3^- \cdot L^{-1}$. Planting a winter cereal rye (*Secale cereale* L.) cover crop, however, significantly ($p < 0.05$) reduced nitrate levels in soil leachate by 34 to 39% (Brandi-Dohrn et al. 1997). On the Western Canadian prairies, continuous cropping has been found to result in less nitrate leaching than that observed for crop rotations including a fallow season (Campbell et al. 1984). Goss et al. (1998b) also found, compared with leaving fields fallow, winter cover crops decreased nitrate leaching by 36% in the periods in which they were growing. However, they also found over the long term, growth of winter cover crops could result in greater net levels of nitrate leaching due to nitrogen releases from the cover crop residues in the following autumn (Goss et al. 1998b).

A reduction of vegetative cover through forest fires, logging, or insect defoliation can result in increased inputs of nitrate to surface waters. For example, a study of peatlands in northern Alberta razed by fire showed the water of lakes from burnt catchments contained three-fold higher nitrate concentrations than reference lakes (McEachern et al. 2000). Clear-cutting of a watershed in the Hubbard Brook Experimental Forest increased streamwater nitrate concentrations by approximately 50-fold (Likens et al. 1970). Increased stream export of nitrate was observed in Appalachian hardwood forests during periods of intense defoliation by cankerworms and gypsy moth (Swank et al. 1981; Webb et al. 1995).

Soil type is another factor affecting the amount of nitrate leaching. Coarse-textured soils generally support greater leaching, or infiltration, and, therefore, favour transport of nitrate to groundwater (Druliner 1989; Spalding and Exner 1991). The largest nitrate losses occur in sandy and peat soils, moderate nitrate leaching occurs in loamy soils, and smaller losses occur in clay soils (Bergstrom and Johansson 1991). Although there may be less leaching of nitrate from fine-textured, less permeable, or poorly-drained soils, these soils may lose more nitrate to streams through surface runoff (Hooda et al. 2000). In agricultural fields comprised of fine textured soils, significant amounts of nitrate may also be transported to surface waters through tile drain systems.

Geochemical characteristics of the soil may also affect the degree of nitrate leaching. Robertson et al. (1996) found that, where reduced sulphur compounds were present at higher concentrations

in the soil, greater attenuation of nitrate leaching occurred. They reasoned that the sulphur provided an electron donor for autotrophic denitrification of the nitrate. Again, less leaching of nitrate would be expected in silt and clay-rich soils as these typically have higher sulphur contents than sandy soils (Robertson et al. 1996).

4.2.3 Water-driven Erosion or Runoff

During heavy precipitation and snowmelt episodes, when soils are water-saturated, or where the ground is impermeable, surface runoff will occur. Runoff may transport dissolved nitrate to surface waters, or, where soils are unstable, it may result in the erosion of soil containing nitrate into surface waters.

Lamontagne et al. (2000) examined the fate of ^{15}N-labelled nitrate applied to a Boreal Shield catchment at the Experimental Lakes Area in northwestern Ontario. $NaNO_3$ was applied to the test area at a rate of 40 kg $N \cdot ha^{-1} \cdot a^{-1}$ over a two year time period. The fate of the nitrate was then determined by measuring the amount of ^{15}N stored in the biomass of trees, ground vegetation, litter and soil, and by estimating ^{15}N loss through runoff. Elevated levels of nitrate in runoff were associated with snowmelt and small rain events following a dry period. Approximately 16% of the ^{15}N added to the experimental area was lost through runoff (Lamontagne et al. 2000). Estimates from similar temperate forest experiments suggest approximately 10% of elevated nitrogen inputs are lost through leaching or volatilisation (Nadelhoffer et al. 1999). Large-scale manipulations of forests in Europe have indicated there is a critical threshold for nitrogen loading (Dise and Wright 1995; Bredemeier et al. 1998). At inputs below the threshold (of approximately 10 kg $N \cdot ha^{-1} \cdot year^{-1}$), the forest ecosystems were capable of retaining most of the N, but when the threshold was exceeded, saturation occured and the ecosystems responded rapidly with high N outputs in runoff (Dise and Wright 1995). In saturated forests, it was possible for N exports to equal, or even exceed, inputs (Bredemeier et al. 1998).

Short-term increases in acidity of lakes may occur during periods of heavy surface runoff, for example, during the snow-melt period (Elder 1984). Analyses of the snowpack in Algoma, Ontario showed nitrate concentrations in the snow gradually increase throughout the winter months, in tandem with an increase in water content, reaching a maximum in March (Jeffries and Semkin 1983). Both the water content and nitrate concentration of the snow plummet in April as the snow melts. The nitrate content decreases more rapidly than the water content with the result that the discharge of the early meltwater has a much higher nitrate content, and lower pH, than the snowpack or the later snowmelt. With a brief, but intense pulse of nitrate in the watershed, an associated pH depression can occur. In some Adirondack lakes of Vermont with low baseline acid neutralising capacity throughout the year, nitrate pulses are more likely to reduce pH than a concomitant increase in the dilution of base cations (Stoddard and Kellog 1993). Although these acidification episodes are generally short-lived, the timing is cause for concern because many aquatic organisms are at sensitive life stages during the spring (Harvey et al. 1981).

Mueller et al. (1997) found land use and hydrologic basin characteristics can be used to predict areas where high nitrate concentrations are likely to occur in streams. Logistic regression modelling was used with the predictive variables being streamflow, the amount of surrounding land area in corn production (or, alternatively, the amount of fertilizer application), soil texture and water drainage characteristics, and population density. In a study of the Duffin Creek

drainage basin (just east of Toronto, Ontario), nitrate losses from soils were highly correlated with the amount of land area used in crop production, and to a lesser extent, with the area of imperfectly drained soils, sandy loam soils, main stream channel gradient and drainage basin relief ratio (Hill 1978). The factor most highly correlated with mean annual nitrate concentrations in the stream water was crop area (Hill 1978). Examination of a watershed in Massachusetts showed nitrate concentrations were positively correlated ($R^2 = 0.68$) with the percentage of the catchment area classified for human use, i.e., agricultural, residential, commercial, industrial, urban open, and transportation areas (Rhodes et al. 2001). Through studies in Maryland and Pennsylvania, Correll et al. (1995) also found a strong relationship between nitrate concentrations in streams and the dominant land use of the watershed. Streams surrounded by cropland and pasture had consistently higher concentrations of nitrate than streams in forested watersheds (Correll et al. 1995). Similar observations have been made in Alberta where the amount of inorganic nitrogen exported from agricultural watersheds was more than an order of magnitude higher than that in forested watersheds (Cooke and Prepas 1998). The speciation of inorganic nitrogen also differed with land use. Nitrate was the predominant nitrogen species in runoff from cropland, comprising 98% of the total inorganic nitrogen pool (Cooke and Prepas 1998). In forested watersheds, approximately half of the inorganic nitrogen was nitrate, and in a mixed agricultural watershed (comprising cropland and two cattle operations), 94% of the nitrogen in runoff was NH_4^+ (Cooke and Prepas 1998). In this case, the authors speculated that the large nitrate inputs from cropland could be attributed to excessive inorganic fertilizer use, whereas the large ammonium inputs from the mixed agricultural land were likely due to poor manure management.

The amount of nitrate loss from agricultural land can be reduced by certain cropping practices. On sensitive landscapes, reduced or zero tillage and the planting of perennial forages can help to alleviate erosion. Vegetative buffer strips along the edges of water courses can also help to reduce the amount of nitrate entering the water through erosion and runoff.

4.2.4 Biotic Uptake and Assimilation

There are several forms of inorganic nitrogen (e.g., nitrate, ammonium, dinitrogen) and organic nitrogen (e.g., urea, amino acids) available to plants in soils (Crawford and Glass 1998). Under typical aerobic conditions found in agricultural soils, nitrate is far more prevalent, as shown in a review of 35 agricultural soils where nitrate levels (6.0 mM NO_3^-) greatly exceeded those of ammonium (0.77 mM NH_4^+) (Crawford and Glass 1998, and references therein). Nitrate in soil is rapidly absorbed by plant roots for assimilation into proteins (Viets, Jr. 1965; Jury and Nielson 1989). The rate of absorption will depend somewhat on the rate of water uptake by the plant due to transpiration; however, it is not entirely a passive process as plants are also able to regulate nitrate uptake rates (Viets, Jr. 1965). To compensate for large seasonal and regional variations in soil nitrate concentrations, plants have evolved genetically regulated transport systems that take up nitrate from the soil against an electrochemical gradient (Crawford and Glass 1998). The energy required by the plant (even when external concentrations are relatively high), is provided from proton gradients (or proton motive forces), which facilitates the transport of the nitrate ion and two accompanying protons from the external medium into the cell (Crawford and Glass 1998).

Because terrestrial plants can absorb nitrate against a concentration gradient, bioaccumulation can occur. Nitrate levels can fluctuatte rapidly in plants, where accumulation occurs only in the vegetative parts of plants, not in the grain or fruit. Highest levels are found in the lowest part of the stalk. Nitrate concentrations in the roots or stems of plants may become hundreds of times higher than that in the surrounding soil or culture solution (Viets, Jr. 1965). For example, cytoplasmic nitrate levels in barley seedlings, which were below detection limits in nitrate-deprived conditions, increased from 620 to 2170 mg $NO_3^-{\cdot}L^{-1}$ when available nitrate levels were increased from 0.62 to 62 mg $NO_3^-{\cdot}L^{-1}$ (Siddiqi et al. 1991). This accumulation of nitrate is not of concern for the plant, but rather for livestock that consume plants that may have high nitrate content (Government of Saskatchewan, 2008).

Riparian zones, or buffer strips, between agricultural fields and streams can help to reduce nitrate loadings from shallow groundwaters to the stream (Cook 1999). Hunt et al. (1995) found a riparian zone removed substantial amounts of nitrate from the shallow groundwater of a swine wastewater disposal site. Nitrate levels of up to 97 mg $NO_3^-{\cdot}L^{-1}$ in subsurface water, passing through either grassland or woodland buffer zones, are consistently reduced to less than 9 mg $NO_3^-{\cdot}L^{-1}$ (Muscutt et al. 1993). Subsurface nitrate removal below buffer zones appears to occur over short distances, as the majority of nitrate removal in studies by Cooper (1990), and Haycock and Burt (1993) occurred within the first 5 m and 8 m of the zones, respectively (Muscutt et al. 1993). In a study of woody and grassy riparian zones separating agricultural fields from both Carroll Creek and the Speed River, in southern Ontario, nitrate concentrations in shallow groundwater were essentially 100% depleted, with most of the decrease occurring within the first 20 to 30 m of the riparian zone (Martin et al. 1999). Woody riparian zones appear to be slightly more effective than grassy ones at removing nitrate from groundwater (Martin et al. 1999).

Some of the nitrate removal occuring beneath buffer strips is due to root uptake of the nitrate by vegetation. The vegetation also increases nitrate removal indirectly by providing a carbon source for anaerobic microbial denitrification in the root zone (Gold et al. 1999). In geologically recent groundwater reserves, Spruill (2000) found that there was a 95% reduction in nitrate levels in young groundwater beneath vegetative buffer strips relative to groundwater in areas without buffer strips. Spruill (2000) attributed approximately 70% of this difference to denitrification processes that are facilitated by the higher levels of dissolved organic carbon (DOC) provided by the decaying vegetation from the buffer strips. Using isotopic tracers, Mengis et al. (1999) also confirmed that denitrification, as opposed to plant uptake, was the major route for nitrate removal from groundwater flowing through a grassed buffer strip in an agricultural watershed.

In locations where leaching of nitrate to deep groundwaters occurs, or where artificial underdrainage has been constructed, buffer strips may be “underpassed” and thus ineffective at preventing nitrate loss to streams (Cook 1999).

4.2.5 Microbial Transformation

In soils, nitrate is relatively stable except when biologically transformed by denitrification. Denitrification, in which NO_3^- is converted by bacteria to gaseous nitrogen, occurs under low oxygen or anaerobic conditions, and in the presence of a carbon source. As such, it is most likely to occur in very wet soils, inside of soil aggregates at high moisture content, or in other

anaerobic microsites within the soil (Jury and Nielsen 1989). It is an important soil process primarily in wetlands or after spring snowmelt and heavy rainstorm events (Melillo et al. 1983; Post et al. 1985). Unlike aquatic ecosystems, the role of denitrification in the nitrogen dynamics of terrestrial ecosystems is relatively minor (Stoddard 1994).

4.3 Aquatic Processes

4.3.1 Physico-chemical Factors and Nitrogen Speciation

The predominant form of nitrogen present in a water body (Figure 4.1) is dependent on a number of factors, including pH, temperature, oxygen availability, plant uptake, and mineralisation rates of organic nitrogen (Johnes and Burt 1993). Because many of these factors are largely a function of season, it can be said season indirectly controls the speciation balance of nitrogen in waters (Johnes and Burt 1993).

Dvir et al. (1999) examined the influence of pH on nitrogen speciation in a marine model ecosystem. Variations in pH affected the rates of oxidation of ammonia to nitrite and nitrate by nitrifying bacteria in the test vessels. Nitrate production rates were similar at pH 7 and pH 8, but lower at pH 9. Overall, nitrification was optimal at pH 8, resulting in greater nitrate+nitrite production rates (Dvir et al. 1999).

Season not only influences nitrogen speciation, but also the total concentrations of nitrogen present in surface waters. In fresh and marine waters, seasonal variations in nitrate concentrations occur. Numerous researchers in northern temperate climates have found nitrate concentrations in fresh surface waters are highest in the fall and winter months, particularly when there is greater precipitation (Hill 1978; Neill 1989; Haycock and Burt 1993; Johnes and Burt 1993). In marine waters, nitrate concentrations are also highest in the late fall and winter, largely due to the breakdown of offshore stratification that results in the entrainment and mixing of deep nutrient-rich waters into the surface layer (Louanchi and Najjar 2000). In some nearshore coastal waters, runoff of nutrient-rich water from the land can also contribute to higher nitrate concentrations in the fall and winter (Louanchi and Najjar 2000). Nitrate concentrations in marine waters are lowest in the spring and summer, reflecting the greater biological uptake (Louanchi and Najjar 2000).

Schindler et al. (1971) demonstrated the influence of seasonal biological uptake on nitrate concentrations in a whole-lake enrichment study in the Experimental Lakes Area of northwestern Ontario. Weekly additions of 0.66 mg $NO_3^-{\cdot}L^{-1}$ resulted in water column nitrate concentrations of up to 0.88 mg $NO_3^-{\cdot}L^{-1}$ in the early spring, > 1.3 mg $NO_3^-{\cdot}L^{-1}$ in the fall and winter, but generally only 0.04 to 0.22 mg $NO_3^-{\cdot}L^{-1}$ were present in the productive late spring and summer months (Schindler et al. 1971).

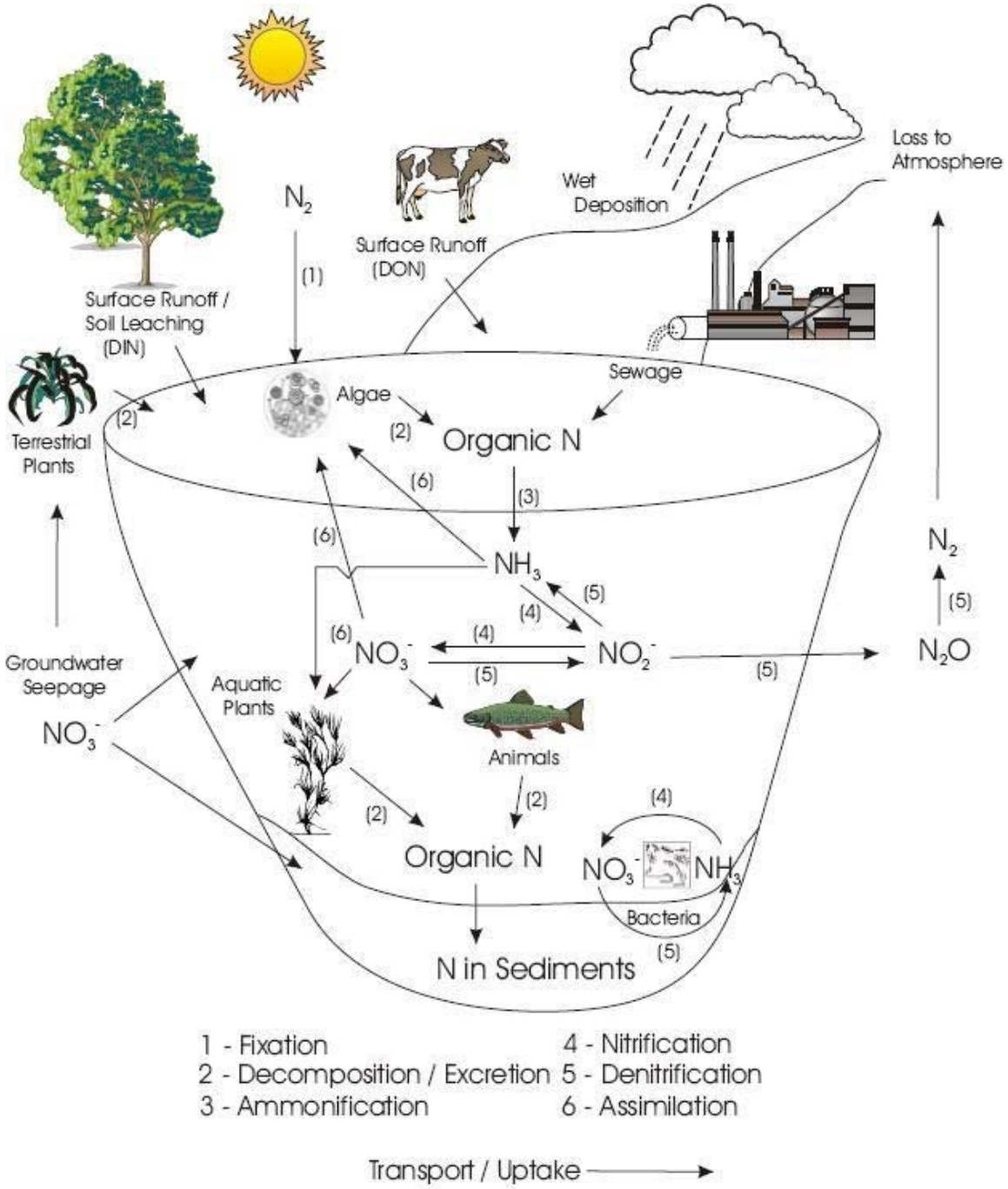


Figure 4.1 Schematic representation of the nitrogen cycle emphasizing aquatic transformations (Adapted from NRC 1978).

4.3.2 Advective and Diffusional Movement within a Water Body

In marine waters, nitrate concentrations are typically very low in the upper euphotic zone due to rapid assimilation by phytoplankton. Movement of nitrate from deeper waters to the surface may occur seasonally, or sporadically through upwelling and mixing caused by surface cooling, wind, or other processes affecting thermal stratification (Ryther and Dunstan 1971).

In examining lake-wide responses to manipulated nutrient levels, Levine and Schindler (1989) found nitrate levels within in-lake enclosures were about 0.3 mg $NO_3^-{\cdot}L^{-1}$, compared to enclosures with solid plastic bottoms (< 0.02 mg $NO_3^-{\cdot}L^{-1}$), showing that in this shallow study lake with a mean depth 1.5 m, nitrate levels in the water column were directly affected by mobilisation from the sediments.

Using ^{15}N tracers, Peterson et al. (2001) determined nitrate regeneration from sediments of small (< 10 m wide) headwater streams contributed significantly to inorganic nitrogen concentrations in the overlying water. Once in the stream, nitrate molecules travelled approximately 5 to 10 times as far as ammonium molecules before being assimilated by biota, or undergoing denitrification (Peterson et al. 2001).

4.3.3 Microbial Nitrification

Nitrification is a two-step microbial process by which ammonium is oxidised to nitrite and then nitrate (Figure 4.1). This oxidation is primarily conducted by autotrophic bacteria under aerobic conditions. Certain heterotrophic bacteria are capable of carrying out nitrification, but at a much slower rate than autotrophic nitrification (Verstraete and Alexander 1973; Brock 1978; Killham 1986; Wolfe et al. 1988). Fungi are also known to carry out nitrification (Stoddard 1994). Other than nitrate formed from nitrogen oxides in the atmosphere, nitrification is the sole natural source of nitrate in the biosphere (NRC 1978).

In the first step of nitrification, ammonium is oxidised to nitrite (Wolfe et al. 1988):

$$NH_4^+ + 3/2\ O_2 \prod NO_2^- + H_2O + 2\ H^+ (\Delta G° = -272\ kJ{\cdot}mol^{-1})$$

The genera of bacteria most frequently associated with this step are *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus*, *Nitrosovibrio*, and *Nitrospira* (Watson et al. 1981). During the production of NO_2^- from NH_4^+, several intermediate products are formed, including hydroxylamine (NH_2OH), pyruvic oxime ($N_2H_2O_2$) and nitrous acid (HNO_2) (Wetzel 2001). Nitrous oxide (N_2O) can subsequently be produced from the breakdown of NH_2OH (Kaplan1983).

The second step involves the oxidation of nitrite to nitrate (Wetzel 2001):

$$NO_2^- + ½\ O_2 \prod NO_3^- \qquad (\Delta G° = -75\ kJ{\cdot}mol^{-1})$$

This process is carried out primarily by members of the genus *Nitrobacter*.

As there is more free energy liberated per mole of NH_4^+ than NO_2^- during the nitrification process, *Nitrosomonas* obtains more energy per mole of nitrogen oxidised than *Nitrobacter*. Maximum growth rates, however, for *Nitrobacter* (0.8 day^{-1}) are much greater than

Nitrosomonas at 20°C (0.5 day^{-1}), and therefore the intermediate nitrite form will not accumulate in large amounts as it is generally oxidised as rapidly as it is formed (NRC 1978; Halling-Sorensen and Jorgensen 1993). Nitrification is a strongly acidifying process, producing two moles of hydrogen ions for each mole of ammonium that is nitrified. This oxidation process can also be costly to oxygen budgets in surface waters, as 4.57 mg O_2 are consumed per mg NH_4^+-N oxidized to NO_3^--N.

Temperature, dissolved oxygen, and pH have all been found to affect rates of nitrification (Dvir et al. 1999). Most strains of nitrifying bacteria grow optimally at a pH of 7.5-8.0, warm temperatures of 25-30°C, and in darkness (Watson et al. 1981; Alleman et al. 1987; Wolfe et al. 1990). Submersed macrophytes can enhance rates of nitrification in the water column by providing a substrate for epiphytic communities of microbial nitrifiers (Eriksson and Weisner 1999). Nitrification in epiphytic communities is greater in light than in dark, presumably due to increased oxygen concentrations at macrophyte surfaces produced during photosynthesis (Eriksson and Weisner 1999). The presence of macrophytes may also stimulate nitrification in sediments through the release of oxygen from their roots into sediments that might otherwise be anoxic (Iizumi et al. 1980). Bioturbation by benthic invertebrates may also enhance nitrification rates in sediments (Seitzinger 1988). Nitrifying organisms are typically slow-growing.

The rates of nitrification per unit volume occurring in sediments are typically at least an order of magnitude greater than nitrification rates in the water column (Seitzinger 1988). For example, Kaplan (1983) found typical nitrification rates in coastal sediments were 0.28 mg $N \cdot L^{-1} \cdot h^{-1}$, whereas in coastal waters nitrification rates were generally less than 0.014 mg $N \cdot L^{-1} \cdot h^{-1}$.

Half-saturation constants for *Nitrosomonas* range from 0.2 to 8.0 mg NH_4^+-$N \cdot L^{-1}$, whereas phytoplankton range from 1.4 to 140 µg NH_4^+-$N \cdot L^{-1}$ (NRC 1978). As growth rates between the two types of organisms are similar (i.e., 1 to 3 doublings per day), and because ammonia concentrations in the euphotic zone of lakes and oceans are typically less than 100 $\mu g \cdot L^{-1}$, phytoplankton can outcompete the nitrifying bacteria for ammonia (NRC 1978).

During the early summer, following stratification, nitrification in the hypolimnion of lakes can consume a significant amount of oxygen, and the resulting nitrate produced is denitrified as the water becomes anoxic. This process of nitrification-denitrification provides an important pathway for the ultimate removal of fixed nitrogen from surface waters (NRC 1978).

4.3.4 Microbial Denitrification

Denitrification (also known as dissimilatory reduction) occurs in the presence of facultative heterotrophic bacteria under extremely low oxygen conditions (Kapoor and Viraraghavan 1997; Dvir et al. 1999). In the absence of oxygen, anaerobic bacteria use oxidized forms of nitrogen (e.g., NO_3^-, NO_2^-) as a terminal electron acceptor during the oxidation of an organic substrate (e.g., methanol in MWWTPs or DOC in surface and groundwaters) to produce gaseous forms of nitrogen, such as N_2, that are then lost to the atmosphere (Seitzinger 1988). Denitrification occurs along the following pathway:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The two-step dissimilatory reduction process can be illustrated using methanol as the electron provider (Halling-Sorensen and Jorgensen 1993):

1) $NO_3^- + 1/3\ CH_3OH \rightarrow NO_2^- + 1/3\ CO_2 + 2/3\ H_2O$

2) $NO_2^- + 1/2\ CH_3OH \rightarrow 1/2\ N_2 + 1/2\ CO_2 + 1/2\ H_2O + OH^-$

Certain genera of bacteria such as *Pseudomonas*, *Micrococcus* and *Bacillus* first reduce nitrate to nitrite and then subsequently to two intermediates (NO and N_2O) before being lost to the atmosphere as N_2 gas (Halling-Sorensen and Jorgensen 1993). This denitrification process provides an important pathway for nitrogen removal. In almost all inland and coastal ecosystems, more nitrogen is lost via denitrification than is gained through direct N_2 fixation (Seitzinger 1988).

In both freshwater and marine systems, an oxygen concentration of about 0.2 $mg \cdot L^{-1}$ or less, or an electron activity level (pE) of ~ 10 - 14 is required for denitrification in water or sediment (Seitzinger 1988; Hemond and Fechner 1994). Open-water denitrification may occur, but bottom sediments are the main site for denitrification in aquatic systems (Keeney et al. 1971; Seitzinger 1988).

Both heterotrophic bacteria (e.g., *Pseudomonas nitrificans*) and autotrophic bacteria (e.g., *Thiobacillus denitrificans, Micrococcus denitrificans*) are capable of denitrification by using nitrate as a terminal electron acceptor in place of oxygen in the respiratory process (Halling-Sorensen and Jorgensen 1993; Kapoor and Viraraghavan 1997). Heterotrophic bacteria require a carbon source from organic substrates such as methanol, ethanol or acetic acid to provide an electron donor for the reduction process; however, autotrophic bacteria can use hydrogen or reduced sulfur compounds (Kapoor and Viraraghavan 1997). In shallow groundwater, dissolved organic carbon (DOC) provides the energy source for bacteria, and elevated levels of DOC are associated with increased denitrification (Spruill 2000).

Anaerobic bacteria in sediments may also reduce nitrate to ammonium under the appropriate conditions, utilizing a portion of that ammonium as a nitrogen source for growth (Hattori 1983). The most efficient denitrification pathway for the bacteria which are reducing nitrate in the presence of H_2 is dependent on limiting amounts of substrates. When organic matter (the electron donor in the reaction) is limiting, the ultimate production of N_2 is more energetically favorable (as amount of free energy). However, when nitrate levels are limiting and organic matter is abundant, the reduction of nitrate to ammonium would be more advantageous (Hattori 1983). Although dissimilatory reduction is a possible pathway for ammonium production, the primary source of ammonium in aquatic systems is via waste products from the breakdown of organic matter (i.e., the deamination of proteins, urea, amino acids, etc.) by heterotrophic bacteria (Wetzel 2001).

Numerous researchers have found denitrification rates increase with increasing temperature (Cavari and Phelps 1977; Messer and Brezonik 1984). Other factors affecting rates of denitrification in aquatic systems include oxygen concentration, and the supply of nitrate and organic matter (Seitzinger 1988). Denitrification rates reported for freshwater lake and river sediments range from 0 to 4.8 mg N·m-2·h-1 (Seitzinger 1988). The reported range of

denitrification rates for coastal marine sediments is greater, ranging from 0 to 14.9 mg N·m-2·h-1, but are most commonly between 0.7 and 3.5 mg N·m-2·h-1 (Seitzinger 1988).

Christensen et al. (2000) found denitrification in marine waters occurred in late autumn when NO_3^- levels in the water column were high. During the summer months, little denitrification occurred based on water column NO_3^-; however, denitrification did occur during this time in the sediments based on nitrate produced through nitrification in the sediments (Christensen et al. 2000).

Through the use of wetland mesocosm studies, Crumpton et al. (1993) found nitrate concentrations decreased rapidly in water overlying wetland sediments, even under highly aerobic conditions. With initial NO_3^- application rates of approximately 12 and 33 mg $NO_3^-·L^{-1}$, the nitrate was completely removed from the water in 3 and 5 days, respectively. It is likely that the nitrate removal was due, in part, to both microbial denitrification and assimilation by macrophytes growing in the mesocosms.

4.3.5 Biotic Assimilation

Assimilatory nitrate reduction is the process by which plants (including phytoplankton) and a number of aerobic bacteria and fungi endogenously reduce nitrate to ammonium that then provides the nitrogen source for the synthesis of cellular materials (Hattori 1983). Aquatic plants will preferentially take up NH_4^+ because it is more energetically favourable than NO_3^- (Stoddard 1994). Nonetheless, large quantities of nitrate may be removed from surface waters through assimilation by algae and macrophytes (Johnes and Burt 1993). Diatoms, for example, have been observed to actively accumulate nitrate so that the internal concentration within their cells is more than 100 times higher than concentrations in the surrounding medium (Cresswell and Syrett 1981). Large nitrate removals from a stream in central Ontario, as a result of assimilation, were observed by Devito and Dillon (1993). Their study of a beaver pond located along the stream showed annual inputs of NO_3^- to the pond exceeded outputs, whereas annual outputs of organic nitrogen from the pond exceeded inputs, suggesting transformation through biotic assimilation (Devito and Dillon 1993).

In temperate zones, assimilation rates vary with season, and consequently nitrate levels will also vary seasonally. Hunt et al. (1995) found that establishment of an in-stream wetland was effective at the removal of nitrogen from the stream water in warmer months. Summer concentrations of nitrate immediately downstream from the wetland site dropped from 5.5 mg $NO_3^-·L^{-1}$, prior to establishment of the wetland, to 1 mg $NO_3^-·L^{-1}$ or less (Hunt et al. 1995). The wetland was less effective at nitrate removal during cooler months, presumably due to slower denitrification and less plant growth.

Diurnal patterns have also been observed for rates of assimilatory nitrate reduction. In a marine tank system containing seaweed, nitrate levels in the water were found to increase during the day, but decrease at night (Dvir et al. 1999). Diurnal nitrate fluctuations have also been observed in the Neversink River, New York, but with an opposite trend. Nitrate concentrations decreased during the day due to uptake by photoautotrophs that were actively photosynthesizing; nitrate concentrations in the water increased during the night, peaking in the early morning before

sunrise (Burns 1998). These results are supported by a study in which a lack of nitrate uptake by diatoms was observed when the culture was incubated in darkness (Cresswell and Syrett 1981). Also, when the diatoms were exposed to light but aerated with CO_2-free air, nitrate uptake was inhibited (Cresswell and Syrett 1981). The authors speculated that nitrate uptake requires a supply of ATP from either photophosphorylation or oxidative phosphorylation.

According to Stumm and Morgan (1981), the form of nitrogen assimilated by aquatic autotrophs will strongly influence the chemistry of surrounding waters. When nitrate is used as the nitrogen substrate, more oxygen is produced in the surrounding water than with the ammonium ion, which can result in super-saturated conditions (Crouzet et al. 1999). Similarly, alkalinity will also increase with nitrate assimilation due to the consumption of H^+ (Crouzet et al. 1999). This is demonstrated in the following equations:

$$106\ CO_2 + 16\ NO_3^- + HPO_4^{2-} + 122\ H_2O + 18\ H^+ \underset{\text{respiration}}{\overset{\text{photosynthesis}}{\rightleftharpoons}} (C_{106}H_{263}O_{110}N_{16}P_1) + 138\ O_2$$

"algae"

$$106\ CO_2 + 16\ NH_4^+ + HPO_4^{2-} + 108\ H_2O \underset{\text{respiration}}{\overset{\text{photosynthesis}}{\rightleftharpoons}} (C_{106}H_{263}O_{110}N_{16}P_1) + 107\ O_2 + 14\ H^+$$

"algae"

(after Crouzet et al. 1999)

Yamaguchi and Itakura (1999) found that out of 26 different forms, or sources of inorganic and organic nitrogen, the dinoflagellate *Gymnodium mikimotoi* showed the greatest yield and growth rates when supplied with nitrate or nitrite. The authors speculated that the high concentrations of ammonia and urea used in the assays (250 µM) may have inhibited the dinoflagellates, whereas these nitrogen species might be used more in the natural environment where they would occur at lower concentrations. The diatom *Phaeodactylum tricornutum* was observed to actively take up nitrate, but this uptake was inhibited in the presence of ammonium (Cresswell and Syrett 1981).

Eventual decomposition of biota will release organically bound nitrogen to the water again where it will be mineralised to ammonium, and if the waters are sufficiently oxic, will be oxidised to nitrate (Johnes and Burt 1993).

Microbial assimilation also occurs, in which nitrate is reduced to ammonia and incorporated into organic compounds, such as amino acids, that may subsequently be used in the production of nucleic acids and proteins (Brezonik 1975). The general pathway for bacterially mediated assimilatory nitrate reduction is:

$$NO_3^- \rightarrow NO_2^- \rightarrow X\ (\text{unknown}) \rightarrow NH_2OH \rightarrow \text{Organic N}$$

(Halling-Sorensen and Jorgensen 1993)

4.3.6 Movement from Water to Sediments

Most of the nitrate found in sediments is produced *in situ* through the biodegradation of organic matter to NH_4^+ that is then oxidized to NO_3^- (Seitzinger 1988). Smaller quantities of nitrate, however, may enter the sediments from the water column.

Christensen et al. (2000) examined the flux of nitrate across the marine sediment-water interface at locations below fish farm cages and at reference sites. No significant differences were observed between the two types of sites, and generally there was only a minor influx to the sediments of < 62 mg $NO_3^- \cdot m^{-2} \cdot d^{-1}$.

Stammers et al. (1978) found that sediment with a high organic matter content was very effective as an agent for the removal of nitrate from stream water, with removal occurring through denitrification. Within reduced sediments, anaerobic bacteria, such as sulfate-reducing bacteria, may reduce nitrate to ammonium (Christensen et al. 2000).

4.3.7 Exchanges between Surface Waters and Groundwater

Nitrate in surface waters can move downwards through sediments and the hyporheic zone into groundwater. The hyporheic zone is a biologically active subsurface ecotone between the surficial streambed and groundwater, where surface and subsurface waters may mix. The downward movement of surface water into the hyporheic zone occurs where the altitude of the water table is lower than the stream or lake water surface; within streams this is typically at the head of riffles (Winter et al. 1998; Biksey and Brown 2001). Downward movement of nitrate to groundwater is largely controlled by hydraulic recharge/discharge processes. Therefore, factors that affect groundwater recharge rates, such as the permeability of surface water sediments, can also influence the movement of nitrate. For example, Grimaldi and Chaplot (2000) observed downstream decreases in nitrate concentrations, with loss to the underlying groundwater, for a stream flowing on granite, but not on schist. On granite, exchanges with the hyporheic zone were favoured by coarse-grained sediments with a high permeability, whereas on schist the grain-size distribution is much finer and permeability is reduced, thus preventing exchanges between surface and subsurface waters (Grimaldi and Chaplot 2000). Downwelling zones are characterized by high oxygen levels and aerobic processes (Biksey and Brown 2001); therefore the production of nitrate through nitrification is likely to occur in these areas.

Movement of nitrate can also occur in the opposite direction, with seepage of groundwater up into surface water bodies. Discharge and upwelling of groundwater occurs where the altitude of the water table is higher than the stream or lake water surface, such as at the base of pools within streams (Winter et al. 1998; Biksey and Brown 2001). Nitrate present in groundwater may be advected through freshwater sediments (Keeney et al. 1971), or coastal marine sediments (Slater and Capone 1987). In temperate regions, the greatest flux of nitrate from groundwater to surface waters occurs in the spring. For example, the spring that feeds Swifts Brook, a small headwater stream within the Grand River Watershed of Southern Ontario, has its highest concentrations of nitrate during peak flow rates in March and April, and lowest nitrate concentrations in October or November following the periods of lowest flow (August or September) (Stammers et al. 1978). The movement of chemical constituents, such as nitrate, between groundwater and surface water is affected by biogeochemical processes in the hyporheic zone (Winter et al. 1998). Upwelling

zones are characterized by anoxic conditions and anaerobic processes (Biksey and Brown 2001); therefore, much of the nitrate present in discharged groundwater will likely undergo denitrification within this zone. Tobias et al. (2001) tracked the fate of ^{15}N-labelled nitrate that had been introduced into a groundwater plume upgradient of a salt marsh in Virginia. Up to 90% of the groundwater nitrate load discharging into the marsh was reduced rapidly in the upper 10 cm of sediment. Denitrification (primarily to N_20) accounted for 70% of the total nitrate loss rate, and the other 30% was due to dissimilatory nitrate reduction to ammonium (Tobias et al. 2001). Another study using nitrogen isotope tracers compared the fate of groundwater nitrate in two different drainage basins in Maryland (Böhlke and Denver 1995). The groundwater nitrate concentrations in the two basins were similar when recharged, but the basins differed in terms of the depths at which reducing sediments occurred. Lower nitrate concentrations were observed in groundwater discharges to the stream where the reducing sediments were shallower because a larger fraction of the groundwater was able to pass through those sediments, and therefore more denitrification took place (Böhlke and Denver 1995).

4.3.8 Anthropogenic Nitrate Removal from Ground and Surface Waters

Nonpoint sources of nitrate (such as leaching and surface runoff from agricultural land, and urban stormwater runoff) pose the greatest source of contamination to surface waters (NRC 1978). Nitrate reaching surface waters can subsequently be consumed by vegetative uptake (algae and macrophytes), denitrification, and assimilation by microorganisms (Laposata and Dunson 1998). Efforts to remove nitrate before entering receiving waters in agricultural areas can include the use of vegetative buffer strips to assimilate nitrate from shallow groundwaters and runoff (see Section 4.2.2), reducing field slopes to slow runoff and facilitate greater biological uptake, and by collecting and treating runoff from feedlots and crop fields in holding ponds (NRC 1978). Other measures for reducing nitrate export from agricultural land include the use of zero tillage to reduce erosion and runoff, planting of perennial forages in marginal areas, and encouraging grassed waterways. Fencing off access for livestock to waterways assists in the regeneration of plant growth, and increases habitat availability for littoral aquatic species (Magilligan and McDowell 1997).

There are several biological, physical, and chemical processes available for the removal of nitrogen from point source discharges such as MWWTPs (Table 4.2). Biological denitrification is the most commonly used technique to remove nitrate from municipal and industrial wastewaters before they are released into receiving waters (NRC 1978; Kapoor and Viraraghavan 1997). This involves a two-step process that can be carried out in conjunction with secondary or tertiary waste treatment, whereby wastewater is first oxygenated to convert any ammonia-nitrogen present to nitrate using nitrifying bacteria, followed by denitrification with heterotrophic bacteria under anoxic conditions and a readily usable carbon energy source (e.g., methanol) to reduce nitrate to nitrogen gas (N_2) (Halling-Sorensen and Jorgensen 1993). Nitrate removal efficiency using this process ranges from 80-90%; however, the second step involving denitrification is less efficient at ambient temperatures < 6°C and in the presence of dissolved oxygen (Kapoor and Viraraghavan 1997). In a study on the removal of nitrate from dairy wastewaters, Zayed and Winter (1998) found that a mixed bacterial culture was able to completely denitrify loads of $4000\ mg\ NO_3^- \cdot L^{-1} \cdot d^{-1}$ for 15 days using existing organic compounds as electron donors, suggesting that more costly methanol-addition operations may not be necessary for all applications. Reactive barriers have been investigated as a low-cost,

low-maintenance method for *in situ* removal of nitrate from septic systems or farm field drainage (Robertson et al. 2000). These barriers, which consist of waste cellulose solids such as wood mulch, sawdust and leaf compost, reduce nitrate levels by providing a carbon source for heterotrophic denitrification. Under varying conditions, the reactive barriers can result in nitrate removal rates ranging from 3 to 142 mg $NO_3^-{\cdot}L^{-1}{\cdot}d^{-1}$ (Robertson et al. 2000).

Non-biologically mediated denitrification techniques include ion exchange, reverse osmosis and electrodialysis (Table 4.2). Ion exchange resin beds substitute nitrate ions from contaminated water with chloride or bicarbonate ions until the resin's exchange capacity is exhausted, at which point the resin must be regenerated (Kapoor and Viraraghavan 1997). Ion exchange has been shown to be effective for the removal of nitrate from groundwater, drinking water, agricultural subsurface drainage, and activated sludge plant effluent; however, the ion exchange efficiency is reduced from the presence of organic matter and by competition with SO_4^{2-} (Eliassen et al. 1965; Magette et al. 1990; Halling-Sorensen and Jorgensen 1993; Kapoor and Viraraghavan 1997). Close to 100% nitrate removal is possible through ion exchange (Clifford and Liu 1993). Ion exchange can also be used in combination with biological denitrification of the spent brine to reduce the salt consumption and waste discharge (van der Hoek et al. 1988; Clifford and Liu 1993).

The reverse osmosis process excludes ions by forcing water across a semipermeable membrane at pressures exceeding the ionic species' osmotic pressure. Water is forced through cellulose acetate or polyamide membranes at pressures ranging from 2070 to 10 350 kPa (Kapoor and Viraraghavan 1997). Such high pressures require a greater expenditure of energy, resulting in much larger operating costs than ion exchange (Kapoor and Viraraghavan 1997).

Electrodialysis is another membrane separation technique that uses a direct electric current to transfer ions from a less concentrated to a more concentrated solution through a semipermeable membrane. This process is not very widely used for nitrate removal as it is also costly, works only for soft waters, and requires considerable pretreatment of the influent to remove organics (Kapoor and Viraraghavan 1997).

Although NO_3^- stripping through resin columns is widely available, global drinking water treatment processes are generally not equipped to remove nitrate, and as such, drinking water concentrations frequently contain nitrate levels similar to that of source waters (Heathwaite et al. 1996).

Table 4.2. Selected wastewater treatment processes for nitrate removal.

Treatment process	% Removal of nitrogen form			Process advantages	Process disadvantages
	Organic N	NH_3/NH_4^+	NO_3^-		
Biological					
Denitrification using methanol (following nitrification stage)	-	-	80 - 90	• rapid denitrification • high degree of nitrogen removal possible	• up to 3 weeks for start-up • methanol required • high operational space requirements
Physical/Chemical					
Ion exchange	slight	slight	75-90	• immediate start-up • not influenced by climatic conditions (i.e., low temperatures) • low TDS in effluent • ease of product quality control	• pre-treatment by filtration required • organic matter and other anions reduce efficiency • disposal of regeneration material (brine) • higher capital costs • requires highly skilled operator
Reverse osmosis	60 - 90	60 - 90	60 - 90	• simultaneously removes all forms of nitrogen • large amounts of nitrogen removed • not affected by lower temperatures	• membrane elements easily fouled by colloidal material • pre-treatment of secondary effluent required • high maintenance
Electrodialysis	100 (suspended organic nitrogen)	30 - 50	30 - 50	• simultaneously removes all forms of nitrogen	• precipitation of salts on membrane surface • clogging of membrane from residual colloidal organic matter • ~10% of feed volume required to continuously wash membrane

5 ENVIRONMENTAL CONCENTRATIONS

5.1 Nitrate Levels in Precipitation

Atmospheric deposition can provide a substantial route for nitrate contamination of surface and groundwaters, especially in urban areas with little ground cover or natural vegetation to take up the deposited nitrate and ammonium that can then accumulate in groundwater (via leaching processes), or in surface waters as a result of runoff (Rouse et al. 1999). Areal estimates of nitrate deposition vary widely across Canada. Annual total deposition (dry + wet) of nitrate at the Abbotsford Aquifer, British Columbia is estimated at 192 mg NO_3^-·m^{-2}·a^{-1} (= 1.92 kg NO_3^-·ha^{-1}·a^{-1}) (McGreer and Belzer 1999). Meteorological sampling between 1995 and 1998 suggested that 9.2 (± 1.6) kg N·ha^{-1}·a^{-1} were being deposited in Lake Simcoe, Ontario (Winter et al. 2002). In general, atmospheric deposition of NO_3^- and NH_4^+ is greater in Eastern Canada, with a ten-year average for 1984-1994 of 3.44 kg N·ha^{-1}·a^{-1} occurring east of the Manitoba-Ontario border, compared to 0.80 kg N·ha^{-1}·a^{-1} west of the border (Chambers et al. 2001). However, atmospheric deposition of nitrogen may be on the rise west of the Manitoba-Ontario border, where increased livestock and poultry operations, as well as other agricultural activities, are expected to cause a 57% increase in ammonia emissions between 2000 and 2020 (Schindler et al. 2006). Increased mining activities will also result in increased nitrogen emissions. For example, expansion of the Athabasca oil sands will be one driver in increasing atmospheric nitrogen deposition in western Canada. An increase in the number of trucks utilized to carry extremely heavy loads (hundreds of tonnes) will cause nitrogen emissions to increase by 359% (compared to 1998 values) in the very near future (Schindler et al. 2006). In the Athabasca oil sands region, mid-1990 nitrate deposition was approximately 2 kg ha^{-1} h^{-1}. This value is expected to increase to 65 kg N ha^{-1} y^{-1} near the centre of oil sands activity (Schindler et al. 2006). Note that the Schindler (2006) study focused on oil sands related issues, and similar increases in atmosphereic nitrogen deposition could be expected wherever extensive minining operations occur.

Heidorn (1979) showed a link between days with high nitrate deposition in suspended particulate matter (> 9.9 µg·m^{-3}) in the Southern Ontario corridor and high-pressure systems originating from south of the lower Great Lakes area. These periods of higher nitrate concentrations were biased towards colder months when greater quantities of NO_x gases are released due to larger energy demands (e.g., space heating). Nitrate is then formed from the nitrogen oxides collected in air masses over the Great Lakes, and is precipitated out of the atmosphere, with deposition decreasing as distance from the Great Lakes increases (Heidorn 1979). Nitrate levels in precipitation around the heavily populated Great Lakes often exceeds 2 mg NO_3^-·L^{-1}, resulting in loading estimates in excess of 20 kg NO_3^-·ha^{-1}·a^{-1} for this region, compared to less than 1 kg NO_3^-·ha^{-1}·a^{-1} for more remote locations such as Snare Rapids in the Northwest Territories (Ro et al. 1995; CNACD 2001).

Volume-weighted concentrations of nitrate in precipitation of the Muskoka-Haliburton region from 1976 to 1986 ranged from ~1.9 to 2.5 mg NO_3^-·L^{-1} (Dillon et al. 1988). In 2000, annual weighted-mean nitrate concentrations from selected Canadian Air and Precipitation Monitoring Network (CAPMoN) locations ranged from 0.25 mg NO_3^-·L^{-1} in Snare Rapids, Northwest Territories to 2.23 mg NO_3^-·L^{-1} in Longwoods, near Lake Erie, Ontario (CNACD 2001).

5.2 Environmental Levels in Surface Waters

5.2.1 Freshwater

Inorganic nitrogen is the predominant form of nitrogen in surface waters, of which nitrate is the most abundant form in well-oxygenated systems (Wetzel 1983). In general, nitrate-nitrogen constitutes two-thirds to four-fifths of the total available nitrogen in surface waters (Crouzet et al. 1999).

Nitrate levels in Canadian lakes and rivers rarely exceed 4 mg $NO_3^-{\cdot}L^{-1}$ (Table 5.1). In oligotrophic lakes and streams nitrate concentrations are generally < 0.4 mg $NO_3^-{\cdot}L^{-1}$ and primary productivity is low (NRC 1978; Nordin and Pommen 1986). High nitrate concentrations (i.e., exceeding 4 mg $NO_3^-{\cdot}L^{-1}$) tend to be associated with eutrophic conditions and algal blooms are more common (NRC 1978). In the U.S., stream nitrate concentrations above the national background level of 2.7 mg $NO_3^-{\cdot}L^{-1}$ are considered to have been affected by human activities (USGS 1999). In a 1996 study of streams in agricultural regions of Alberta, flow-weighted mean nitrate concentrations were 5.3 mg $NO_3^-{\cdot}L^{-1}$ in regions of high agricultural intensity compared to 0.10 mg $NO_3^-{\cdot}L^{-1}$ in low intensity regions (Anderson et al. 1998). Reference values reported by the European Environment Agency for nitrate in non-impacted European rivers range from 0.4 to 4.4 mg $NO_3^-{\cdot}L^{-1}$ (Crouzet et al. 1999). In Canada, average 1990 nitrate levels in raw (pre-treated) municipal water supplies ranged from 0.1 to 3.3 mg $NO_3^-{\cdot}L^{-1}$ (Government of Canada 1996).

Correlations often exist between nitrate concentrations in a waterbody and factors such as human population growth, or the percentage of a catchment altered by anthropogenic land uses (Rhodes et al. 2001). High nitrate levels have been noted in surface waters as a result of various human activities. In areas downstream of open pit coal mining operations, explosives residues result in elevated nitrate concentrations (Nordin and Pommen 1986). Inorganic fertilizer use in rural areas can also result in excessive localized nitrate levels. Mean nitrate concentrations of North American streams in agricultural landscapes generally range between 9 and 180 mg $NO_3^-{\cdot}L^{-1}$, and levels above 45 mg $NO_3^-{\cdot}L^{-1}$ can persist for several weeks (Rouse et al. 1999; Castillo et al. 2000). Irrigation water used in crop fertilization studies carried out on a Nebraska farm contained nitrate concentrations of 93 mg $NO_3^-{\cdot}L^{-1}$ (Eghball and Gilley 1999). Sewage treatment plants may also contribute to elevated nitrate levels; concentrations ranging from 19 to 42 mg $NO_3^-{\cdot}L^{-1}$ found in the Cootes Paradise wetland in Dundas, Ontario in 1997, were primarily attributed to anthropogenic loading from a local sewage treatment plant (Rouse et al. 1999).

Table 5.1. Representative nitrate concentrations in Canadian ambient surface waters.

Province/Territory	Water Body	$[NO_3^-]$ (mg $NO_3^-·L^{-1}$)‡	Reference
FRESHWATER			
British Columbia	Arrow Lake	0.69	NLET (2000)*
	Thompson River	1.37	NLET (2000)*
	Flathead River	0.09 (< DL - 0.487)	McDonald et al. (1987)*
		0.04 (0.001 – 1.34)	Environment Canada (2010b)*
Alberta	Athabasca River	0.22 - 0.27	NLET (2010, 2000)*
	various Boreal Plains headwater lakes (wetland dominated)	0.05 (0.005 - 0.44)	Prepas et al. (2001)*
	various Boreal Plains headwater lakes (upland dominated)	0.02 (0.005 - 0.06)	Prepas et al. (2001)*
Saskatchewan	Battle River	0.190 - 0.602	NLET (2000)*
Manitoba	Assiniboine River	1.27 (< DL - 14.2)	Manitoba Conservation (2000)*
	Lake Winnipeg	0.24 (< DL - 0.93)	Manitoba Conservation (2000)*
	Red River	0.59 (< DL - 21.5)	Manitoba Conservation (2000)*
Ontario	Ausable River	28.5 (4.7 - 86.4)	OMOE (2001)*
		23.5 (0.02 [trace] - 105)	OMOE (2010b)*
	Georgian Bay	0.89 (0.22 – 1.99)	OMOE (2010c)
	Grand River	13.4 (1.22 - 29.0)	OMOE (2001)*
		11.5 (0.02 [trace] - 54)	OMOE (2010b)*
	Lake Erie	1.44 (0.18 – 10.4)	OMOE (2010c)
	Lake Ontario	1.46 - 2.04	NLET (2000)*
	Hamilton Harbour	8.7 (5.8 – 10.0)	OMOE (2010c)
	Lake St. Clair	3.92 (0.79 – 20.5)	OMOE (2010c)
	Lake Superior	1.38	Bennett (1982)
		1.32 (0.39 – 1.59)	OMOE (2010c)

Province/Territory	Water Body	$[NO_3^-]$ (mg $NO_3^- \cdot L^{-1}$)‡	Reference
	Mississagi River	0.56 (0.02 [trace] - 1.57)	OMOE (2001)*
		0.35 (0.13 – 0.88)	OMOE (2010b)*
	Turkey Lakes	0.252 - 61.0	NLET (2000)*
Quebec	Richelieu River	4.8 - 13.7	NLET (2010, 2000)*
	St. Lawrence River	1.13 (0.22 - 7.93)	Hudon and Sylvestre (1998)*
	Trois-Rivières	0.31 - 0.66	NLET (2000)*
New Brunswick	Miramichi River	0.920	NLET (2000)*
Nova Scotia	Gold River	0.02 (< DL - 0.09)	Dalziel et al. (1998)*
	Annapolis River	2.3 (0.67 - 6.49)	Dalziel et al. (1998)*
	various lakes	0.04 (< DL - 2.22)	NSDEL (2001)*
Northwest Territories	Great Slave Lake (western basin)	0.46 (0.41 - 0.85)	Evans (1997)*
MARINE			
Nova Scotia	coastal waters	< DL to 0.37	Keizer et al. 1996
	Bay of Fundy (depth: 0 - 5 m)	0.41 (0.01 to 1.12)	Petrie et al. 1999
	Bay of Fundy (depth: 100 - 275 m)	0.76 (0.26 to 1.34)	Petrie et al. 1999)
British Columbia	coastal waters - summer	0.11 (~0 to 0.31)	Ahn et al. 1998)
	coastal waters - winter	1.1 - 1.7	Whitney 2001
	off-shore waters (depth: 0 - 100 m)	0.9 (0.5 to 1.7)	Whitney 2001)

note: NLET (2000) samples represent median values from a large, interlaboratory quality assurance study (n = 20 - 50); OMOE (2001) data from 1996-2000; OMOE (2010b) data from 2003-2007; OMOE (2010c) nearshore data from 2004-2009; Environment Canada (2010b) data from 1994-2004; Manitoba Conservation (2000) data from 1980-2000; < DL = below detection limit, i.e, < 0.02 mg $NO_3^- \cdot L^{-1}$ for OMOE (2001), < 0.04 mg $NO_3^- \cdot L^{-1}$ for Manitoba Conservation (2000), and < 0.006 mg $NO_3^- \cdot L^{-1}$ for Dalziel et al. (1998); ‡ concentrations are means, with ranges indicated in brackets; * - concentrations are reported as $NO_2^- + NO_3^-$, but are considered to consist entirely of NO_3^- as NO_2^- concentrations were not detected in surface water samples (Alkema 2000).

N/A = not available (i.e., a description of the watershed use was not provided in the reference document)

5.2.1.1 Seasonal Variation

Nitrate concentrations are seasonally variable, with increased biological uptake in warmer productive months reducing ambient surface water concentrations. In 1983, nitrate levels in the

tributaries of the inner bay of Rondeau Provincial Park, on the north shore of Lake Erie, declined between winter and spring (range: 31 to 58 mg $NO_3^-·L^{-1}$) and summer (18 mg $NO_3^-·L^{-1}$) (OMOE 1983). On the St. Lawrence River downstream of the Montreal Archipeligo, higher nitrate concentrations in the winter and spring (1.90 and 1.55 mg $NO_3^-·L^{-1}$, respectively), declined in the summer and autumn months to 0.84 and 1.06 mg $NO_3^-·L^{-1}$, respectively, due to greater biological productivity and nitrate uptake (Hudon and Sylvestre 1998).

5.2.1.2 Temporal Trends

Contrary to decreasing trends in other nutrients, such as phosphorus, which have been specifically targeted for removal from municipal sewage treatment plants, there has been a general increasing trend in nitrate levels in the surface waters of the Great Lakes. Comparison of mean spring and summer nitrate levels in the western basin of Lake Erie between 1983-87 and 1989-93 showed significant increases from 2.53 to 3.54 mg $NO_3^-·L^{-1}$ (Makarewicz et al. 2000). Ranges in spring and summer nitrate concentrations in the Grand River, Ontario, which empties into Lake Erie have also increased from 0.22 - 2.8 mg $NO_3^-·L^{-1}$ in 1966 to 0.04 - 18.0 mg $NO_3^-·L^{-1}$ in 1994 (Rott et al. 1998). Caution should be used in interpreting trends from this data for the Grand River, however, as the authors only compared two years; nitrate levels can vary considerably on a year-to-year basis. Nitrate monitoring data from Ontario's Provincial Water Quality Monitoring Network was retrieved for these same Grand River sites for the years 2003 to 2007 (MOE 2010b). Nitrate concentrations are indeed on the rise, with nitrate concentrations ranging from 0.10 to 37.4 mg $NO_3^-·L^{-1}$ with a median of 10.4 mg $NO_3^-·L^{-1}$ (MOE 2010b). Mean lake-wide spring surface nitrate + nitrite concentrations in Lake Superior have been increasing from ~1.17 mg $NO_3^-·L^{-1}$ in 1970 to ~1.56 mg $NO_3^-·L^{-1}$ in 1992, with a predicted increase of 0.014 mg $NO_3^-·L^{-1}·a^{-1}$ (Williams and Kuntz 1999). The mean near-shore nitrate concentration measured in Lake Superior (away from any immediate influence of tributaries or effluent pipes) in 2005 was 1.32 mg $NO_3^-·L^{-1}$, with concentrations ranging from 0.40 to 1.59 mg $NO_3^-·L^{-1}$ (OMOE 2010c). Mean annual spring nitrate + nitrite concentrations in Lake Ontario have also been steadily increasing from 0.95 (± 0.075) mg $NO_3^-·L^{-1}$ in 1968 to 1.74 (± 0.035) mg $NO_3^-·L^{-1}$ in 1993 (Williams et al. 1998a). Nitrate concentrations measured in 2006 and 2009 in the middle of Hamilton Harbour ranged from 5.76 to 10.0 mg $NO_3^-·L^{-1}$, with a mean concentration of 8.71 mg $NO_3^-·L^{-1}$ (MOE 2010c). This area of the harbour is influenced by 4 STPs which ultimately discharge into the water body. Nitrogen-to-phosphorus ratios (N:P) are increasing in Lake Ontario (ratios, expressed on a molecular weight basis, currently range from 36 to 40) due to decreasing phosphorus and increasing nitrogen concentrations. This increase could result in changes to Lake Ontario's algal species composition as the prevalence of cyanobacterial dominance tends to decrease at N:P ratios > 29 (by weight), and are replaced by diatoms and chlorophytes (Williams et al. 1998a [cf Smith 1983]; CCME 2002). In contrast with observations from the Great Lakes, mostly downward trends have been observed for nitrate concentrations in Québec rivers and streams for the period from 1988 to 1998 (MENV 2000).

International estimates for nitrate concentrations in surface waters are generally consistent with Canadian levels. Increasing trends have also been observed in lakes surrounded by intensive agricultural production in the English Lake District, with mean nitrate concentrations increasing from approximately 1.8 mg $NO_3^-·L^{-1}$ in 1945 to 6.2 mg $NO_3^-·L^{-1}$ in 1980 (Heathwaite et al. 1996). Results from a European-wide survey revealed that approximately 15% of rivers exceeded an annual average concentration of 33 mg $NO_3^-·L^{-1}$ between 1992 and 1996 (Crouzet et

al. 1999). Surface water samples from the Netherlands have been shown to range from 2.7 to 24.3 mg $NO_3^-·L^{-1}$ (Brinkhoff 1978), and the average nitrate concentrations from 584 Norwegian lakes is 0.48 (± 0.46) mg $NO_3^-·L^{-1}$ (maximum = 3.08 mg $NO_3^-·L^{-1}$) (Bulger et al. 1993). The much higher nitrate concentrations in the Netherlands' waters, compared to Norway, may be due in part to the higher population density and higher percentage of agricultural land that is intensively farmed. For comparison, the population densities of the Netherlands, Norway, and Canada are approximately 378, 14, and 3 persons per square km, respectively (based on data from Times Books 1999). The land base proportions that are used for agriculture in the Netherlands, Norway, and Canada are approximately 53%, 3%, and 8%, respectively (World Atlas 2002).

In a review of water quality in U.S. rivers between 1974 and 1981, Smith et al. (1987) reported trends of increasing nitrate concentrations at 116 monitoring stations versus 27 stations which showed decreasing nitrate trends. The majority of stations showing nitrate increases were located in the eastern half of the country and were strongly associated with agricultural activities. Total nitrogen loads delivered to the Gulf of Mexico from intensive agricultural areas of the Mississippi Basin have increased three-fold since the 1970's with a mean annual nitrogen-flux for 1980 to 1996 being 1600 $kt·a^{-1}$ (Goolsby et al. 2001). Nitrate-nitrogen accounted for 61% of the total nitrogen, with the remainder being comprised of organic N (37%) and ammonium-N (2%) (Goolsby et al. 2001).

5.2.1.3 Spatial Trends

Longitudinal trends in nitrate concentrations for lotic waters are highly variable and depend on site-specific factors such as catchment basin size (Johnes and Burt 1993), land-use activities (Rott et al. 1998; Van Herpe and Troch 2000), floodplain lithology (Grimaldi and Chaplot 2000), and stream size, substrate composition and geochemistry (Devito et al. 2000; Peterson et al. 2001). For example, longitudinal gradients have been noted for Québec rivers flowing from the Appalachians and the Laurentians through the St. Lawrence lowlands. Nitrate levels in headwaters were in the range of 0.09 to 0.4 mg $NO_3^-·L^{-1}$, whereas in the lowlands concentrations ranged from 0.4 to 22 mg $NO_3^-·L^{-1}$ (MENV 2000).

During biologically productive seasons, standing waters consistently show lower nitrate levels in the upper euphotic zones where the nitrate is readily assimilated by phytoplankton and heterotrophic bacteria. Evans (1997) suggested elevated nitrate levels can also occur at greater depths due to nutrient regeneration; in study sites > 60 m deep in the western basin of Great Slave Lake, NT, nitrate + nitrite concentrations observed near the lake floor (up to 0.19 mg $NO_3^-·L^{-1}$) were greater relative to surface waters (~0.10 mg $NO_3^-·L^{-1}$). Photoinhibition in surface waters of light-sensitive nitrifying bacteria such as *Nitrosomonas* may also explain observed increases in nitrification rates with depth (Hall 1986).

5.2.2 Marine

Although gaseous N_2 is the most abundant species of nitrogen in ocean waters, nitrate is the most abundant biologically-reactive form (Sharp 1983). Nitrogen budgets for coastal marine waters indicate more biologically available nitrogen is lost through denitrification than is gained through N_2 fixation resulting in an overall ecosystem-level nitrogen deficiency (Paerl 1993). In contrast,

microorganisms and benthic invertebrates living within the sediments effectively recycle phosphorus in coastal marine sediments and overlying water, which results in a nitrogen-limited environment that is often reliant on external nitrogen inputs to maintain ecosystem productivity (Paerl 1993).

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). Nitrate levels in European and North American estuaries of rivers draining agricultural and urbanised areas can exceed 12 mg $NO_3^-·L^{-1}$ (Sharp 1983). These concentrations tend to decrease when there is increased mixing with more saline waters (Sharp 1983).

Nitrate levels from the central Scotian Shelf off the Canadian Atlantic coast follow seasonal trends with the highest surface water concentrations (up to 0.535 mg $NO_3^-·L^{-1}$) being found in the winter months (Petrie et al. 1999). By mid-spring, nitrate is largely depleted in the surface waters (~0.038 mg $NO_3^-·L^{-1}$) due to biological assimilation, with increasing concentrations (up to ~1.24 mg $NO_3^-·L^{-1}$), occurring beyond 30 m depth. Nitrate levels remain low throughout the summer (< 0.031 mg $NO_3^-·L^{-1}$) and do not increase again until the late fall (Petrie et al. 1999). Nitrate levels from two near-shore sampling locations in Nova Scotia from 1992 to 1994 ranged from below detection limits to 0.37 mg $NO_3^-·L^{-1}$ (Keizer et al. 1996). On the Canadian Pacific coast, nitrate levels tend to be higher in the winter months than in the summer, and they also typically increase with depth (Whitney 2001). Nitrate concentrations measured at various depths in February 2001, in the Strait of Georgia (between Vancouver Island and mainland British Columbia) and in a transect running west from the southwestern end of Vancouver Island, ranged from 0.18 to 2.9 mg $NO_3^-·L^{-1}$ (Whitney 2001). Ambient nitrate levels in seawater near a salmon farm in British Columbia were typically less than 0.31 mg $NO_3^-·L^{-1}$ between May and July (Ahn et al. 1998) (Table 5.1)..

Nitrate levels in European marine waters are also generally below 1 mg $NO_3^-·L^{-1}$ with average concentrations reported for the U.K. at 0.44 to 0.88 mg $NO_3^-·L^{-1}$, and from the North Sea coast at ~0.4 to 1.0 mg $NO_3^-·L^{-1}$ (Wickins 1976; van Duijvenbooden and Matthijsen 1989).

5.3 Environmental Levels in Groundwater

Nitrate levels in groundwater are primarily a human health concern as well water systems with elevated nitrate concentrations could pose a risk of methaemoglobinaemia (see Section 6.2.2.1) to infants that do not have sufficient gastric acids to control nitrate-reducing bacteria in their guts (Hill 1999). Groundwater can, however, impact aquatic biota through discharge into streams and other surface waters. Nitrate concentrations in groundwater tend to exceed those of surface waters due to increased accumulation of nitrate leaching through soils under intense agricultural and livestock production. Generally, up to 13 mg $NO_3^-·L^{-1}$ can be found naturally in groundwaters; levels above this indicate anthropogenic contamination (Rouse et al. 1999).

Nitrate concentrations in well water in Canada can often exceed the guideline for Canadian drinking water quality of 45 mg $NO_3^-·L^{-1}$. In a summary of nitrate levels in rural wells from each of the provinces, 1.5% to 64% of wells surveyed had greater than 45 mg $NO_3^-·L^{-1}$ (Chambers et al. 2001). Nitrate levels up to 1100 mg $NO_3^-·L^{-1}$ have been reported in semi-arid regions of western Canada and the United States (Rodvang et al. 1998). In 1991-1992, 14% of 1292 wells

sampled in Ontario had nitrate levels greater than 45 mg $NO_3^-·L^{-1}$; this trend appears to have remained relatively consistent from the 1950s (Goss et al. 1998a). During the 1980s and 1990s, mean groundwater concentrations in the Maritime provinces ranged from 8.9 to 132.9 mg $NO_3^-·L^{-1}$, with up to 44% of dairy farm wells in Prince Edward Island exceeding 45 mg $NO_3^-·L^{-1}$ (AAFC 2000). Nitrate concentrations measured in groundwater samples from Nova Scotia range from approximately 1 to 204 mg $NO_3^-·L^{-1}$, with a mean that is likely less than 20 mg $NO_3^-·L^{-1}$ (Moerman and Briggins 1994). In western Canada, the Abbotsford aquifer, which spans southern British Columbia and northern Washington State, is also dominated by agricultural activity. Here, 54% of 117 domestic, municipal, and monitoring wells exceeded 45 mg $NO_3^-·L^{-1}$ in 1993, and it is estimated 80% of all groundwater exceeds 40 mg $NO_3^-·L^{-1}$ Wassenaar 1994).

Reported groundwater nitrate concentrations from other international jurisdictions are comparable to Canadian levels. In 53% of shallow groundwater studies in U.S. agricultural and urban areas, median nitrate concentrations exceeded the U.S. national background concentration estimate of 8.9 mg $NO_3^-·L^{-1}$, and median concentrations in 13 of 36 agricultural areas were > 22 mg $NO_3^-·L^{-1}$ (USGS 1999). These elevated nitrate levels in groundwater were strongly related to agricultural land use and the widespread application of fertilizers in excess of crop uptake. Of thirty-three U.S. aquifers tested, the four that exceed the US EPA drinking water standard of 10 mg NO_3^--N·L^{-1} (approximately 45 mg $NO_3^-·L^{-1}$) were all shallow, composed of sand and gravel and situated beneath agricultural areas (USGS 1999).

Similar inorganic fertilizer contamination of the shallow Sparta aquifer in Greece resulted in 65% of samples exceeding the 50 mg $NO_3^-·L^{-1}$ European drinking water standards, with mean and maximum nitrate concentrations of 63 and 177 mg $NO_3^-·L^{-1}$, respectively (Antonakos and Lambrakis 2000). High nitrate levels persist in this aquifer due to the influx of large quantities of oxygenated water and the presence of carbonate formations that resulted in strong oxidising conditions that inhibit denitrification (Antonakos and Lambrakis 2000).

A province-wide survey of Ontario farmstead domestic wells illustrated nitrate concentrations in groundwater typically decrease exponentially with depth (Rudolph et al. 1998). Likewise, in a review of factors influencing aquifer nitrate levels, Kolpin et al. (1994) found a consistent decrease in the percentage of samples with nitrate concentrations > 13 mg $NO_3^-·L^{-1}$ with increasing aquifer depth (> 40 m below the earth's surface). Aquifers from areas of unconsolidated materials (i.e., glacially deposited sand and gravel, or alluvium deposits) also had significantly higher nitrate levels ($p < 0.001$) than those on sandstone, limestone or dolomite bedrock (Kolpin et al. 1994). Kolpin et al. (1994) explain this difference as resulting from less low-permeability material overlying the wells of the unconsolidated aquifers such that contamination from the surface occurs more readily; also, in the unconsolidated aquifers, the groundwater flow paths for recharge of the wells is shorter than in bedrock, resulting in faster recharge rates.

6 TOXICITY OF NITRATE TO AQUATIC ORGANISMS

Nitrate is considerably less toxic to aquatic organisms 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). Nitrate is generally considered to be of low toxicity to aquatic organisms due to its limited uptake (lower branchial permeability when compared to ammonia and nitrite) and absence of major physiological effects (Russo 1985; Jensen 1996; Camargo et al. 2005).

There is a wide response in aquatic biota to nitrate exposure, both between taxonomic groups, and between life stages. In general, based on acute median lethal concentrations, invertebrates and amphibians are typically more sensitive than fish (though there are broad ranges in tolerance among species within each taxonomic group). One to fifteen-day LC_{50} values for the nitrate ion in freshwater range from 24 to 3070 mg $NO_3^-{\cdot}L^{-1}$ for invertebrates, from 73 to 7752 mg $NO_3^-{\cdot}L^{-1}$ for amphibians, and from 847 to 9344 mg $NO_3^-{\cdot}L^{-1}$ for fish (Appendix A). For marine species, LC_{50} values for invertebrates range from 496 to > 19 840 mg $NO_3^-{\cdot}L^{-1}$, while those for fish range from 2538 to 22 372 mg $NO_3^-{\cdot}L^{-1}$ (Appendix B). Freshwater organisms tend to be generally more sensitive to nitrate when compared to marine species, where increased water sailinity may play a role in ameliorating the effects of nitrate in marine environments (Camargo et al. 2006). Nitrate concentration ranges at which chronic effects occur are comparable for these three taxonomic groups.

Early life stages are, for the most part, more sensitive than juvenile or adult stages. While Westin (1974) reported median nitrate lethal concentrations of 5800 and 6000 mg $NO_3^-{\cdot}L^{-1}$ for chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhyncus mykiss*), respectively, Kincheloe et al. (1979) found concentrations as low as 10 and 20 mg $NO_3^-{\cdot}L^{-1}$ could significantly increase egg and fry mortality in these species. An important item to note with the Kincheloe et al. (1979) study is that although this study demonstrated sensitivity of eggs and early salmonid life stages to nitrate, additional egg mortalities caused by *Saprolegnia* fungal infestations could not be segregated from the data by the authors, therefore the results of this study are considered unreliable. In addition, early instars of two Nearctic net-spinning caddisfly species had consistently lower LC_{50}s when exposed to $NaNO_3$ relative to late instar stages (Camargo and Ward 1992). One study by Adelman et al. (2009) provided a lower effect concentration for the juvenile (7-months) fathead minnow (*Pimephales promelas*) when compared to the embryo-larval life stage, with respective 30-d MATCs of 372 and 952 mg $NO_3^-{\cdot}L^{-1}$.

6.1 Effects of Water Quality Parameters on Toxicity

There is little information related to the influence of water chemistry on nitrate toxicity to aquatic organisms. No studies to date have evaluated the influence of factors such as pH and DO. Some recent work however, has looked at the influence of hardness (Elphick 2011; Nautilus Environmental 2011) and temperature (Moore and Poirier 2010).

Water hardness refers to the concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in water and comes mainly from the dissolution of $CaCO_3$ in calcareous soils and sediments. Alkalinity refers to the buffering capacity of water (ability to neutralize acid) (Welsh, 1996). It is primarily a measure of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) concentrations in exposure water (Welsh, 1996). It is well known that both water hardness and alkalinity ameliorate the toxicity of metals to aquatic organisms. With respect to hardness, the mechanism behind metal

toxicity mitigation involves competition between the hardness cations and metal cations for binding sites at cellular surfaces (e.g. fish gills) (Paquin *et al*., 2002). Of the two hardness cations, Ca^{2+} has been identified as the primary cation involved in protecting against metal uptake and toxicity in both fish (Part *et al*., 1985; Carrol *et al*., 1979) and invertebrates (Heijerick *et al*., 2002; Jackson *et al*., 2000; Wright 1980). The reason Ca^{2+} may exert a more protective effect is because the molar concentration of Ca^{2+} is typically twice that of Mg^{2+} in surface waters (Everall *et al*., 1989). The mechanism behind the amelioration of any observed nitrate toxicity at higher hardness levels has not been definitively described (Nautilus Environmental 2011). Any observed effects may be may be related to Ca^{2+} and/or Mg^{2+} effecting membrane permeability, or due to competitive exclusion at passive uptake sites by Ca^{2+} and Mg^{2+} (or other ions) (Nautilus Environmental 2011).

Alkalinity reduces metal toxicity by decreasing the number of free metal ions by forming metal-CO_3^{2-} or metal-HCO_3^- complexes (Welsh, 1996). In order to be able to determine whether or not hardness alone has the ability to ameliorate toxicity, one would need to isolate for true hardness, for example, by adding Ca^{2+} in the form of $CaSO_4$ or $CaCl_2$ to exposure water. Tests that add in $CaCO_3$ salts to the exposure solutions will actually confound the effects of hardness with alkalinity (Charles *et al*., 2002).

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 (*O. mykiss*) and an amphipod (*Hyalella azteca)*. Long-term exposures were conducted using rainbow trout (*O. mykiss*), the fathead minnow (*P. promelas*), a water flea (*C. dubia*), an amphipod (*H. azteca*), and a midge (*C. dilutus* – formerly *C. tentans*). Tests with fish (rainbow trout and fathead minnow) were conducted using four hardness levels (approximately 15, 45, 90 and 160 mg/L as $CaCO_3$). Tests with invertebrates (amphipod, water flea and midge) were not tested at the lowest hardness of 15 mg/L, and only tested at 45, 90 and 160 mg/L as $CaCO_3$ hardness.

6.1.1 Evaluating the Hardness-Toxicity Relationship for Nitrate – Short-Term Exposures

When evaluating studies that examine the ameliorating effect of hardness on the toxicity of a substance, guidance provided by US EPA (2001) on the selection of appropriate tests is used. In order for a species to be included, definitive acute values had to be available over a range of hardness such that the highest hardness was at least three times the lowest, and such that the highest was at least 100 mg/L higher than the lowest (US EPA 2001). Two species met these criteria: *Oncorhynchus mykiss* (rainbow trout) and *Hyalella azteca* (amphipod) (Table 6.1). The selected 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 (Figure 6.1). A slope of the hardness-toxicity relationship was calculated for the rainbow trout (0.3734) and the amphipod (1.2933) (Figure 6.1).

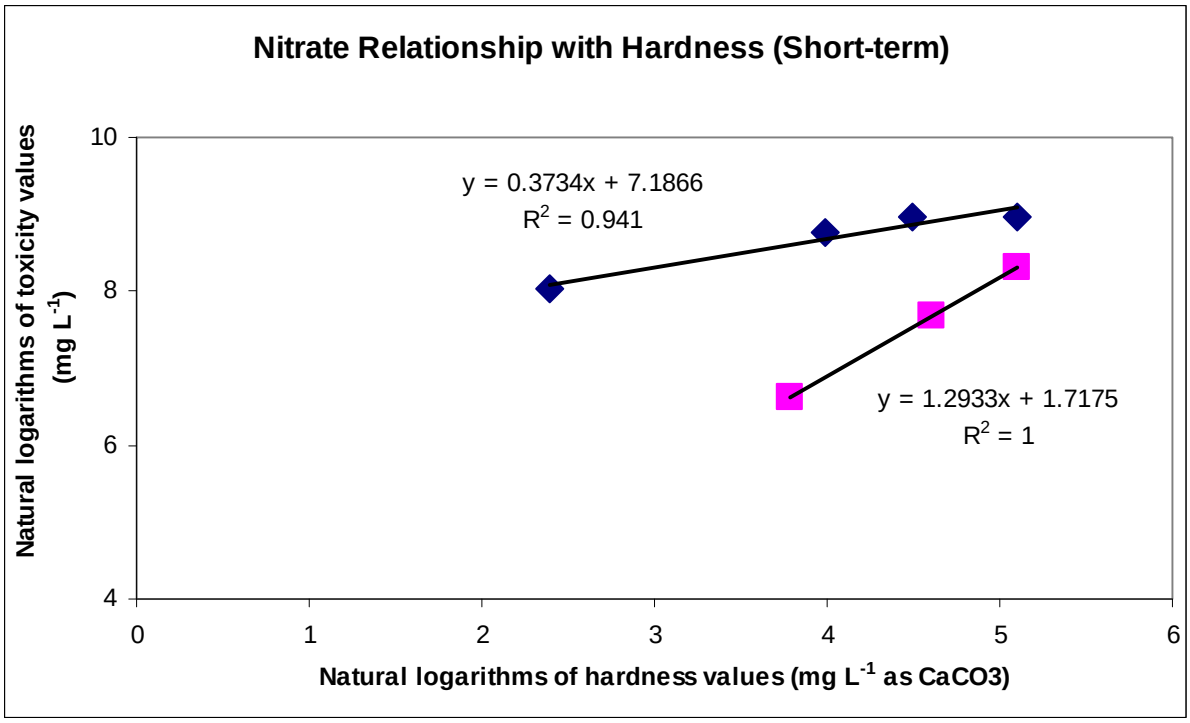


Figure 6.1 Hardness-toxicity relationships for short-term data. Diamonds represent endpoints for the rainbow trout (*O. mykiss*) and squares represent endpoints for the amphipod (*H. azteca*). The LC_{50} data plotted in this graph is listed in Table 6.1.

The regressions in Figure 6.1 were able to explain a large portion of the variability because the coefficients of determination (R^2) varied from 0.941 to 1.00. However, an F-test showed that the slopes for the two species were significantly different from each other ($p = 0.012$). As a result, it was decided that the data could not be combined in order to generate a pooled slope, and there would be no derivation of a hardness-dependant short-term equation for use in hardness-dependent short-term guideline derivation.

6.1.2 Evaluating the Hardness-Toxicity Relationship for Nitrate – Long-Term Exposures

As per the US EPA (2001) guidance on the evaluation of studies examining hardness-toxicity relationships, only long-term studies utilizing a range of hardness such that the highest hardness was at least three times the lowest, and such that the highest was at least 100 mg/L higher than the lowest, were reviewed. Five species met these criteria: rainbow trout (*O. mykiss*), fathead minnow (*P. promelas*), water flea (*C. dubia*), amphipod (*H. azteca*), and midge (*C. dilutus* – formerly *C. tentans*) (Table 6.1). All of the selected data (with the exception of the 40-day rainbow trout embryo-alevin-fry 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 (Figure 6.2). The 40-day rainbow trout study was not included as the study 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/L as CaCO3)

compared to the soft water (50 mg/L as CaCO3) and therefore this study was not included in the regression discussed above (Table 6.1). For the remainder of the data, a slope of the hardness-toxicity relationship was calculated for each species: the fathead minnow (0.7058), the water flea (1.0072), the amphipod (1.984) and the midge (0.9582) (Figure 6.2).

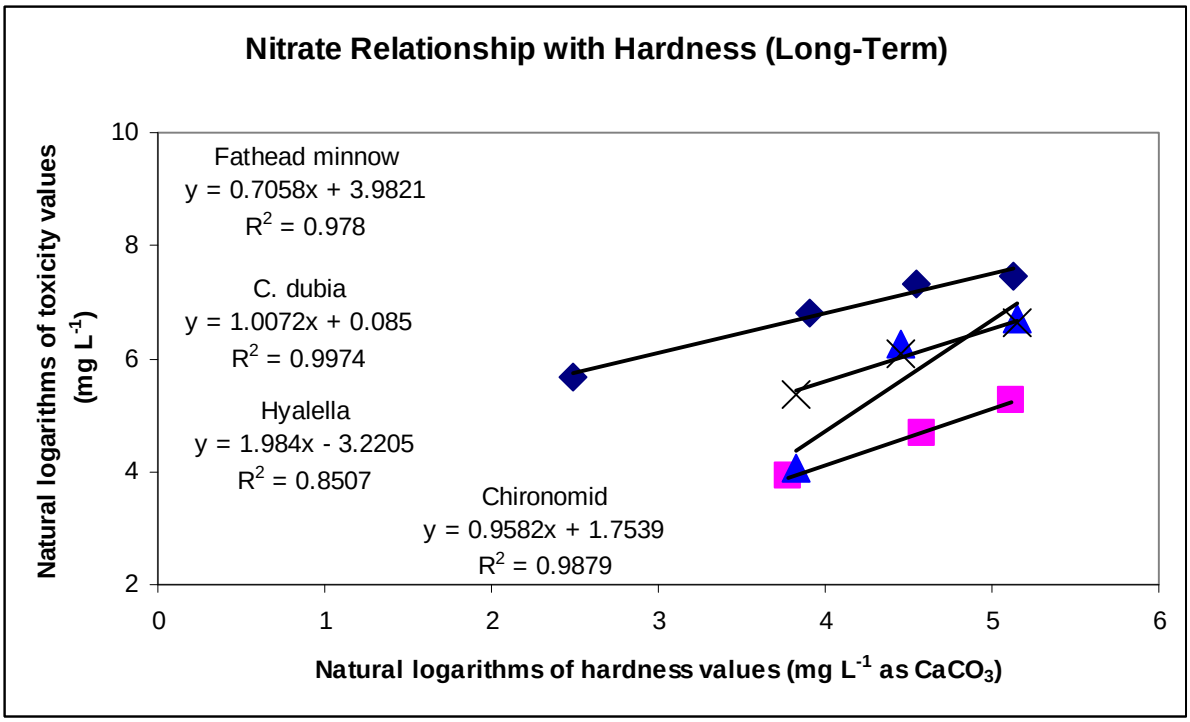


Figure 6.2 Hardness-toxicity relationships for long-term data. Diamonds represent endpoints for the fathead minnow (*P. promelas*), squares represent endpoints for the water flea (*C. dubia*), triangles represent endpoints for the amphipod (*H. azteca*), and cross-hairs represent endpoints for the chironomid (*C. dilutus*). The IC_{25} data plotted in this graph is listed in Table 6.1.

The regressions in Figure 6.2 were able to explain a large portion of the variability because the coefficients of determination (R^2) varied from 0.8507 to 0.9974. However, an F-test showed that the slopes for the four species were significantly different from each other ($p = 0.001$). As a result, it was decided that the data could not be combined in order to generate a pooled slope, and there would be no derivation of a hardness-dependant long-term equation for use in hardness-dependent long-term guideline derivation.

6.1.3 Evaluating the Temperature-Toxicity Relationship for Nitrate – Short-Term Exposures Only

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). The data is presented in Table 6.6. In

this study, temperature did appear to have an effect on the 96-h LC50 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 LC50 of 1690 and 4730 mg NO_3^-/L, 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 LC50 of 6650 mg NO_3^-/L), and least toxic to *S. namaycush* at optimal metabolic temperature of 10 deg C (96-h LC50 of 5230 mg NO_3^-/L). 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.

Yet other studies have indicated that temperature does not appear to affect the toxicity of nitrate to freshwater fish. Colt and Tchobanoglous (1976) concluded that median lethal concentrations observed for channel catfish (*Ictalurus punctatus*) exposed to nitrate were independent of temperatures at 22, 26 and 30ºC. It should be noted, however, that this experiment used a small range of temperatures and catfish are fairly robust.

Table 6.1. Summary of studies that investigated hardness as a toxicity modifying factor.

Taxa/organism	Short-term or long-term	Tox. Endpoint	Effective Concentration (NO_3^- mg/L)	Hardness (as mg/L $CaCO_3$)	Effect of hardness on toxicity[1]	Comments	Reference
Fish (short-term and long-term)							
Rainbow trout *Oncorhynchus mykiss*	Short-term (96h)	LC50	3061 6361 7832 7832	11 54 90 164	Minor effect of hardness on toxicity. An ~ 15-fold increase in hardness results in an ~ 2.6 fold decrease in toxicity (survival).		Elphick 2011[2]
Rainbow trout *Oncorhynchus mykiss*	Long-term (40d)	LC10	651 >1794 >1794 >1794	10 (very soft) 50 (soft) 92 (mod hard) 176 (hard)	Substantial effect of hardness on toxicity. A 5-fold increase in hardness results in an ~ 2.8 fold decrease in toxicity (survival).	Hardness effect is observed when increasing from a hardness of 10 to 50 mg/L as $CaCO_3$. Beyond that, no observed effect of hardness.	Nautilus 2011[2]
		LC25	815 >1794 >1794 >1794	10 (very soft) 50 (soft) 92 (mod hard) 176 (hard)	Substantial effect of hardness on toxicity. A 5-fold increase in hardness results in an ~ 2.2 fold decrease in toxicity (survival).	Hardness effect is observed when increasing from a hardness of 10 to 50 mg/L as $CaCO_3$. Beyond that, no observed effect of hardness.	
		IC10 (weight)	421 780 585	10 (very soft) 50 (soft) 92 (mod hard)	Minor effect of hardness on toxicity (growth). A 17.6-fold increase in hardness resulted in an ~ 3.5 fold decrease in	Sensitivity appears greater in the moderately hard water (92 mg/L as CaCO3) compared to the soft water (50 mg/L as CaCO3).	

Taxa/organism	Short-term or long-term	Tox. Endpoint	Effective Concentration (NO_3^- mg/L)	Hardness (as mg/L $CaCO_3$)	Effect of hardness on toxicity[1]	Comments	Reference
			1484	176 (hard)	toxicity (growth).		
		IC25 (weight)	>1794 >1794 >1794 >1794	10 (very soft) 50 (soft) 92 (mod hard) 176 (hard)	No observed effect of hardness on toxicity (growth).		
		IC10 (length)	492 >1794 1085 >1794	10 (very soft) 50 (soft) 92 (mod hard) 176 (hard)	These results do not definitively demonstrate the relationship between increasing hardness and nitrate toxicity.	Sensitivity appears greater in the moderately hard water (92 mg/L as CaCO3) compared to the soft water (50 mg/L as CaCO3).	
		IC25 (length)	>1794 >1794 >1794 >1794	10 (very soft) 50 (soft) 92 (mod hard) 176 (hard)	No observed effect of hardness on growth.		
		EC10 (proportion reaching	58 >1794 235	10 (very soft) 50 (soft) 92 (mod hard)	These results do not definitively demonstrate the relationship between increasing hardness and nitrate toxicity.	Sensitivity appears greater in the moderately hard water (92 mg/L as CaCO3) compared to the soft water (50 mg/L as CaCO3).	

Taxa/organism	Short-term or long-term	Tox. Endpoint	Effective Concentration (NO_3^- mg/L)	Hardness (as mg/L $CaCO_3$)	Effect of hardness on toxicity[1]	Comments	Reference
		swim-up)	>1794	176 (hard)			
		EC25 (proportion reaching swim-up)	142 >1794 306 >1794	10 (very soft) 50 (soft) 92 (mod hard) 176 (hard)	These results do not definitively demonstrate the relationship between increasing hardness and nitrate toxicity.	Sensitivity appears greater in the moderately hard water (92 mg/L as CaCO3) compared to the soft water (50 mg/L as CaCO3).	
Fathead minnow *Pimephales promelas*	Long-term (7d)	LC50	501 1014 1772 2011	12 50 94 168	Substantial effect of hardness on toxicity. A 14-fold increase in hardness results in a 4-fold decrease in toxicity (survival).		Elphick 2011[2]
Fathead minnow *Pimephales promelas*	Long-term (7d)	IC25 (growth)	292 908 1506 1741	12 50 94 168	Substantial effect of hardness on toxicity. A 14-fold increase in hardness resulted in an ~ 6-fold decrease in toxicity (growth).		Elphick 2011[2]
Invertebrates (short-term and long-term)							
Amphipod *Hyalella azteca*	Short-term	LC50	744 2149	44 100	Substantial effect of hardness on toxicity. A 3.7-fold increase in hardness		Elphick 2011[2]

Taxa/organism	Short-term or long-term	Tox. Endpoint	Effective Concentration (NO_3^- mg/L)	Hardness (as mg/L $CaCO_3$)	Effect of hardness on toxicity[1]	Comments	Reference
	(96h)		4080	164	resulted in a 5.5-fold decrease in toxicity.		
Amphipod *Hyalella azteca*	Long-term (14d)	LC50	558 1271 >2835	46 86 172	Substantial effect of hardness on toxicity. A 3.7-fold increase in hardness resulted in an ~ 5-fold decrease in hardness.	Test run with sediment.	Elphick 2011[2]
Amphipod *Hyalella azteca*	Long-term (14d)	IC25 (growth)	57 518 806	46 86 172	Substantial effect of hardness on toxicity. A 3.7-fold increase in hardness resulted in an ~ 14-fold decrease in toxicity.	Test run with sediment.	Elphick 2011[2]
Water flea *Ceriodaphnia dubia*	Long-term (7d)	LC50	196 523 536	44 98 166	Substantial effect of hardness on toxicity. A 3.7-fold increase in toxicity resulted in a 2.7-fold decrease in toxicity.		Elphick 2011[2]
Water flea *Ceriodaphnia dubia*	Long-term (7d)	IC25 (repro-duction)	50 106 192	44 98 166	Substantial effect of hardness on toxicity. A 3.7-fold increase in toxicity resulted in a 3.8-fold decrease in toxicity.		Elphick 2011[2]
Midge	Long-term	LC50	505	46	Substantial effect of hardness on toxicity.	Test run with sediment.	Elphick 2011[2]

Taxa/organism	Short-term or long-term	Tox. Endpoint	Effective Concentration (NO_3^- mg/L)	Hardness (as mg/L $CaCO_3$)	Effect of hardness on toxicity[1]	Comments	Reference
Chironomus dilutus	(10d)		975 1493	86 172	A 3.7-fold increase in toxicity resulted in a 3-fold decrease in toxicity.		
Midge *Chironomus dilutus*	Long-term (10d)	IC25 (growth)	217 447 771	46 86 172	Substantial effect of hardness on toxicity. A 3.7-fold increase in toxicity resulted in a 3.6-fold decrease in toxicity.	Test run with sediment.	Elphick 2011[2]
Plants, including algae							
NA							

[1]For the purposes of a simple trend analysis, results were compared on a mg/L basis. The qualitative terms of "no apparent effect", "minor effect" and "substantial effect" are subjectively assigned, but consistent among studies. "No apparent effect" was assigned if there was no consistent decrease in toxicity with increasing hardness. "Substantial effect" was assigned if the ratio of decrease in toxicity to increase in hardness was greater than or equal to 0.21. For example, in the fourth entry under invertebrates (*Ceriodaphnia dubia*), this ratio is 2.6/2.1 = 1.2; hence, this would be classified as substantial effect. The 0.21 cut-off is derived from the subjective estimate of the reasonable extremes of water hardness values (5 mg/L to 240 mg/L as $CaCO_3$, or 48-fold (NRCAN, 1978), and an arbitrary decrease in toxicity (10-fold decrease, a common safety factor used). Hence, 10-fold/48-fold = 0.21. "Minor effect" was assigned if the ratio was less than 0.21.

[2]Salt additions followed the ratios of salts specified in USEPA (2002). $CaSO_4$ and $MgSO_4$ salts were used so as not to confound true hardness with alkalinity.

Anecdotal evidence suggests nitrate uptake may be pH-limited. While Jensen (1996) reported the freshwater crayfish *Astacus astacus* exhibited limited nitrate uptake at pH ≈ 8.3 (e.g., nitrate concentrations in the haemolymph were below ambient water values), the authors refer to McMahon and Stuart (1989) who found extracellular NO_3^- concentrations higher than ambient water values in the crayfish *Procambarus clarki* held in water acidified to pH 4 with nitric acid.

Higher chloride concentrations tend to reduce nitrite toxicity to fishes, as the chloride ion will bind competitively with chloride cells (the primary site of nitrite uptake), thereby limiting the amount of nitrate entering the blood stream (Wedemeyer and Yasutake 1978; Russo et al. 1981; Lewis and Morris 1986). These same chloride interactions however, do not appear to reduce the toxicity of nitrate to salmonids. For chinook salmon and rainbow trout exposed to nitrate in both freshwater and 15‰ salinity salt water, nitrate was more toxic ($p < 0.05$) in saltwater by a factor of up to 1.4 (Westin 1974; for comparisons, see Appendices A, B). No explanation however, was provided for the increased toxicity in trials with greater salinity.

6.2 Influence of Various Nitrate Salts on Toxicity

The toxicity of nitrate ions to aquatic organisms is assessed using either $NaNO_3$, KNO_3, or NH_4NO_3 salts. As there are differing responses among organisms in response to the type of salt used (Dowden and Bennett 1965; Schuytema and Nebeker 1999c), it was necessary to screen toxicity assays based on salt type. Ammonium nitrate is often used in amphibian toxicity assays due to its potential to collect in the runoff from fertilizer applications in agricultural regions and, therefore, provides a potentially concentrated source of nitrate to sensitive developing amphibian embryos and larvae (Hecnar 1995; Oldham et al. 1997; Schuytema and Nebeker 1999a). Acute lethality values (96-h LC_{50}s), however, for amphibian larvae exposed to ammonium nitrate can be an order of magnitude lower than for larvae exposed to sodium nitrate (Schuytema and Nebeker 1999a). As the ammonium ion can cause adverse effects on larval survival or growth at lower concentrations than required for adverse effects from nitrate ions, this result suggests the toxicity of ammonium nitrate compounds are due to the influence of the ammonium ion rather than the nitrate ion (Schuytema and Nebeker 1999c). Therefore, toxicity studies using ammonium nitrate as the test compound were excluded from the data set used for the development of the CWQGs for nitrate. Canadian Water Quality Guidelines for ammonia already exist (CCME 2000).

Sodium salts are generally used in the study of the physiological effects of anions, due to their high degree of solubility and low toxicity from the cation relative to the anion (Jones 1941). For freshwater benthic insect larvae (*Hydropsyche occidentalis* and *Cheumatopsyche pettiti*), Camargo and Ward (1992) demonstrated toxicity from exposure to $NaNO_3$ was due to NO_3^- rather than Na^+ ions. No mortality was observed in test organisms exposed to NaCl at 1000 mg $NaCl \cdot L^{-1}$ (= 393 mg $Na^+ \cdot L^{-1}$), whereas the most sensitive LC_{50} with $NaNO_3$ was 290 mg $NO_3^- \cdot L^{-1}$, which represents a sodium concentration 3.7 times lower (= 108 mg $Na^+ \cdot L^{-1}$). Similarly, Baker and Waights (1994) found no statistically significant effect on the growth or survival of tree frog (*Litoria caerulea*) tadpoles exposed to NaCl at the same Na^+ concentrations as those required to produce an effect using $NaNO_3$. Therefore, toxic effects from exposure to $NaNO_3$ are likely due to the nitrate ion, and studies using $NaNO_3$ were included in the dataset for the derivation of the nitrate WQGs.

Potassium nitrate (sometimes used in inorganic fertilizers) has also been used to assess the toxicity of the nitrate ion to aquatic organisms. In freshwater studies exposing animals to nitrate of both potassium and sodium salts, the former are often found to be more toxic than the latter (Table 6.2). The only exception was for the freshwater hydra (*Hydra attenuata*), for which sodium nitrate was more toxic (Tesh et al. 1990). As animals in the Tesh et al. (1990) study were kept in distilled water, possible disruptions in normal osmoregulatory functions may have contributed to the observed differences in toxicity.

Table 6.2. Relative toxicity of sodium and potassium nitrate salts to freshwater organisms.

Organism	Duration (h)	Endpoint	$[NO_3^-]$ (mg $NO_3^- \cdot L^{-1}$)		Reference
			K+ Salt	Na+ Salt	
Lepomis macrochirus (bluegill)	96	LC_{50}	1840	8753	Trama (1954)
	24	LC_{50}	3373	9338	Dowden and Bennett (1965)
Daphnia magna (water flea)	96	TL_m	552	3069	Dowden and Bennett (1965)
Polycelis nigra (planaria)	48	survival	555	2696	Jones (1940)
Gasterosteus aculeatus (stickleback)	240	lethal concen-tration limit	79	1348	Jones (1939)
Hydra attenuata (hydra)	288	NOEL	150 - 250	< 50	Tesh et al. (1990)

A review of the relative toxicity of K^+ and Na^+ ions from chloride salts to freshwater organisms also indicates that potassium salts are between 1.6 and 8.7 times more toxic than the corresponding sodium salt (Table 6.3). Using sulfate as the associated anion, the potassium salt was 4.7 to 11.7 times more toxic than the sodium salt for *Ceriodaphnia dubia*, *D. magna* and *Pimephales promelas* (Mount et al. 1997). Using a stepwise logistic regression model, Mount et al. (1997) found that the K^+ ion contributed significantly to observed mortality in both invertebrate and vertebrate organisms, while the Na^+ did not. Although Mount et al. (1997) found the toxicity of K^+ decreased with the addition of other cations to the test solution, it is not known whether a threshold exists for physiological effects from the K^+ ion.

Table 6.3. Relative toxicity of sodium and potassium chloride salts to freshwater invertebrates.

Organism	Duration (h)	Endpoint	Salt concentration NaCl ($mg \cdot L^{-1}$)	Salt concentration KCl ($mg \cdot L^{-1}$)	[NaCl]/[KCl]	Reference
Daphnia magna (water flea)	24	EC_{50}	2184	1127	1.9	Lilius et al. 1994
	24	EC_{50}	1023	625	1.6	Khangarot and Ray 1989
	24	EC_{50}	3606	548	6.6	Calleja et al. 1994
	24	LC_{50}	6380	740	8.6	Mount et al. 1997
	48	EC_{50}	1023	271	3.8	Khangarot and Ray 1989
	48	LC_{50}	4770	660	7.2	Mount et al. 1997
Ceriodaphnia dubia (water flea)	24	LC_{50}	3380	630	5.4	Mount et al. 1997
	48	LC_{50}	1960	630	3.1	Mount et al. 1997
Pimephales promelas (fathead minnow)	24	LC_{50}	8280	950	8.7	Mount et al. 1997
	48	LC_{50}	6510	910	7.2	Mount et al. 1997
	96	LC_{50}	6390	880	7.3	Mount et al. 1997

These various lines of evidence suggest the concentrations at which toxic effects are observed in freshwater organisms exposed to KNO_3 are primarily a function of the potassium ion. This finding is supported by Demaël et al. (1980) who stated the metabolic and hormonal effects, indicative of osmoregulatory stress, observed when the freshwater fish *Tinca tinca* (tench) was exposed to potassium nitrate at 8.5 mg $K^+ \cdot L^{-1}$, were due to K^+, not NO_3^-. Therefore toxicity data from studies using KNO_3 were not considered in the development of the freshwater nitrate guideline.

The salinity of the world's seawater largely ranges from 33 to 37‰, while most fresh inland waters have salinities ranging from 0.1 to 0.5‰ (Stumm and Morgan 1981; Wetzel 1983). The ionic salinity of water is largely determined by the concentrations of four cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and four anions (HCO_3^-, CO_3^{2-}, SO_4^{2-}, Cl^-) (Wetzel 1983). Therefore, additions of sodium nitrate or potassium nitrate in toxicity tests can affect the overall salinity of the test solution. Mean naturally occurring seawater concentrations of Na^+ and K^+ are 10 770 and 399 $mg \cdot L^{-1}$ (or 10.8 and 0.40‰), respectively (Stumm and Morgan 1981). In contrast, fresh North American river water contains mean concentrations of Na^+ and K^+ of 9 and 1.4 $mg \cdot L^{-1}$ (or 0.009 and 0.0014‰), respectively (Wetzel 1983). Mean ambient levels of potassium found in the Great Lakes and a variety of rivers from the Canadian maritimes are generally less than 2 mg $K^+ \cdot L^{-1}$ (Dalziel et al. 1998; Williams et al. 1998a,b; Williams and Kuntz 1999).

At concentrations of nitrate salts that elicit toxic responses in aquatic organisms, sodium ion levels tend not to greatly exceed ambient sodium concentrations in fresh water. For example, at the LOEC for growth reduction in frog embryos of 129 mg $NO_3^-·L^{-1}$ (Schuytema and Nebeker 1999b), the corresponding concentration of Na^+ was 48 mg $Na^+·L^{-1}$, which is less than 5 times greater than ambient Na^+ levels (Wetzel 1983). Alternatively, at the lowest LOEC for a primary study using potassium nitrate (55 mg $NO_3^-·L^{-1}$; Marco et al. 1999), the corresponding potassium concentration of 35 mg $K^+·L^{-1}$ is approximately 25 times higher than ambient levels.

The only available study exposing both Na^+ and K^+ ions to a marine species found a significant increase (~20%) in larval shrimp mortality for both cations at the lowest treatment concentration of 1 mg $NO_3^-·L^{-1}$ (Muir et al. 1991). As there is no available data to suggest that K^+ ions are more toxic in saline environments, and because ion fluxes in marine fish are an order of magnitude higher than in freshwater fish (Heath 1995), KNO_3 studies were included in CWQG development for marine environments.

Unless otherwise specified, discussions of toxic responses by organisms in this report are a result of exposure to the sodium nitrate salt.

6.3 Modes of Action

6.3.1 Uptake Mechanisms

The mechanisms regulating nitrate uptake in aquatic vertebrates and invertebrates are not fully understood; however, elevated levels of nitrate have been found in bodily fluids and tissues of invertebrates (crayfish and shrimp), and fish (rainbow trout) exposed to high ambient nitrate levels (Jensen 1996; Stormer et al. 1996; Cheng et al. 2002). Nitrate uptake was minor in crayfish (*Astacus astacus*) and rainbow trout, each exposed to 62.0 mg $NO_3^-·L^{-1}$ (Jensen 1996 and Stormer et al. 1996, respectively). Crayfish had significantly increased nitrate concentrations ($p < 0.01$) in haemolymph relative to control animals when exposed to sodium nitrate for seven days; however, these levels were still far below exposure concentrations (Jensen 1996). Similarly, Stormer et al. (1996) found that the nitrate concentrations in rainbow trout plasma increased significantly from less than 1.9 mg $NO_3^-·L^{-1}$ in control fish to 12.4 mg $NO_3^-·L^{-1}$ in exposed fish, and remained constant over an eight-day exposure period. As per the crayfish studied by Jensen (1996), the amount accumulated accounts for only a fraction of the ambient concentration, suggesting only a weak uptake route. This limited NO_3^- uptake did not measurably influence the electrolyte balance or haematology in the rainbow trout (Jensen 1996). In addition to increases in haemolymph nitrate levels, Cheng et al. (2002) found significant relationships ($p \leq 0.001$) between increasing ambient nitrate levels (48 to 2237 mg $NO_3^-·L^{-1}$) and tissue concentrations in the tropical marine prawn *Penaeus monodon*. At lower exposure levels (i.e., 48 and 226 mg $NO_3^-·L^{-1}$), the majority of nitrate accumulation in *P. monodon* occurred within the first 12 h, and tissue levels were still increasing after 24 h at higher nitrate levels (i.e., 1317 and 2237 mg $NO_3^-·L^{-1}$) (Cheng et al. 2002). At the lowest exposure level of 48 mg $NO_3^-·L^{-1}$, nitrate levels in tissues (muscle, hepatopancreas, foregut, midgut, heart, gill) were 20 to 80% ambient levels, while concentrations in eyestalks were 1.2 times greater than ambient levels (Cheng et al. 2002).

Although nitrite is actively transported into tissues via branchial chloride cells, nitrate ion uptake through this route is either severely limited, or absent (Stormer et al. 1996; Jensen 1996; Cheng et al. 2002). As plasma Cl^- concentrations in rainbow trout were shown to decrease under nitrite exposure (due to competitive exclusion at chloride cell uptake sites), an associated decrease of plasma Cl^- would also be expected if nitrate shared the same uptake mechanism (Stormer et al. 1996). A lack of change in plasma Cl^- concentrations under nitrate exposure therefore suggests that uptake is not likely to occur via chloride cells (Stormer et al. 1996). Another possible route of nitrate influx may be via the diffusion of nitric acid (HNO_3). However, due to the readily dissociable properties of the nitrate ion, the proportion of nitrate as nitric acid is negligible, and the accumulation of nitrate in tissues is thought to be attributed to some type of active uptake mechanism (Cheng et al. 2002).

Mechanisms for nitrate uptake in amphibians have not been investigated. Due to the permeability of amphibian skin, however, it is likely that dissolved nitrate could readily enter trans-dermally (Hecnar 2001). There is also the potential for nitrate uptake through the diet if tadpoles are feeding on algae or macrophytes that have accumulated nitrate (Hecnar 2001).

There is little information on nitrate excretion rates in aquatic animals. In mammals however, kidneys have been found to accumulate ~60% of ^{15}N-labelled nitrate doses (Packer 1995), and as such the majority of nitrate in animals is lost via urine within 24 hours (WHO 1986). Nitrate concentrations in crayfish haemolymph remained high over the 7-d exposure period despite a very large osmotic gradient relative to the surrounding water, suggesting a slow rate of depuration, most likely through urine (Jensen 1996). In rainbow trout, nitrate is most likely excreted through bile and urine (Doblander and Lackner 1997). Stormer et al. (1996) suggest that urinary loss plays a larger role in trout than in crayfish, with nitrate levels reaching a quasi-steady balance between passive branchial influx and removal.

6.3.2 Direct Toxicity

6.3.2.1 Methaemoglobin formation

In animals, uptake of nitrate can ultimately inhibit the ability of haemoglobin, a pigment in the blood, to carry oxygen to the various tissues of the body (WHO 1986). This inhibition occurs through several steps. First, nitrate is reduced to nitrite within the alimentary canal and guts of animals via bacteria such as *Nitrobacter* which use NADH as an electron donor for the oxidative phosphorylation of ADP to ATP:

$$NO_3^- + NADH_2 + 2\ ADP + 2\ Pi \rightarrow NO_2^- + NAD^+ + 2\ ATP + H_2O$$

(deSaint-Blanquat 1980)

Nitrite produced from this reaction is then free to be taken up into the blood stream where it will react with the haem iron (as Fe^{2+}) in oxyhaemoglobin (HbO_2), oxidizing it to Fe^{3+}, and thereby creating methaemoglobin (Hb^+):

$$4HbO_2 + 4NO_2^- + 4H^+ \rightarrow\ 4Hb^+ + 4NO_3^- + O_2 + 2H_2O$$

(Stormer et al. 1996)

As methaemoglobin binds irreversibly with oxygen molecules, transfer of oxygen from the blood to cells in the body is inhibited, and appreciable levels of Hb^+ can result in hypoxia.

Background levels of methaemoglobin in fish blood, and the response in methaemoglobin levels when fish are exposed to nitrate, can vary, and may be related to exposure conditions, or the duration of exposure. Salmon blood normally contains between 3.3 to 17.5% methaemoglobin in the absence of nitrite (Lewis and Morris 1986; Brauner et al. 1993). Grabda et al. (1974) found exposure to potassium nitrate at 31 mg $NO_3^-{\cdot}L^{-1}$ for up to eleven weeks increased methaemoglobin levels in rainbow trout to approximately 28%, relative to 1% in controls. In contrast, methaemoglobin levels in rainbow trout exposed to 62 mg $NO_3^-{\cdot}L^{-1}$ (as sodium nitrate) for eight days, remained below 3% of total haemoglobin (Stormer et al. 1996).

At blood levels of 20-25% methaemoglobin, hepatic tissue respiration rates decrease, potentially leading to serious liver damage (Grabda et al. 1974). Methaemoglobin levels above 50% inhibit "the cough response", thereby preventing salmon from purging sediment collected in the buccal cavity. At levels above 70% the fish becomes torpid which can lead to anoxic death if the fish suddenly has increased oxygen demands (Lewis and Morris 1986). Other effects observed due to increased methaemoglobin include serious damage to the peripheral blood, and hematopoietic (blood production) centres of the kidney (Grabda et al. 1974). Low haemoglobin levels in fish could reduce survival, as Jones (1971) has demonstrated induced hemolytic anaemia (abnormally low haemoglobin levels) resulted in a 34 to 40% reduction in maximum sustained swimming speeds for rainbow trout.

Long-term sublethal toxicity from elevated methaemoglobin levels are unlikely as fish possess defense mechanisms, such as the NADH-reductase system, which will reduce methaemoglobin back to haemoglobin (Kamstra et al. 1996). Methaemoglobin levels in rainbow trout exposed to 0.32 mg $NO_2^-{\cdot}L^{-1}$ increased from approximately 3% in control fish to 27% after 14 days, however, then declined to near control-levels after 48 days (Doblander and Lackner 1997). Huey and Beitinger (1982) demonstrated the NADH-methaemoglobin reductase enzyme in catfish (*I. punctatus*) provides a rapid detoxification mechanism, with a 5-fold decrease in catfish methaemoglobin levels occurring within 24-h of placing the animals in a nitrite-free medium. Doblander and Lackner (1997) also determined nitrite present in blood plasma can be taken up by erythrocytes and oxidized to nitrate under oxic conditions, thereby preventing the nitrite from oxidizing the haemoglobin to methaemoglobin. It is estimated that erythrocytes, and other cells such as hepatocytes, could detoxify almost 20% of nitrite taken up (Doblander and Lackner 1997). Enhanced activation of these defence mechanisms however, have an associated metabolic cost for the fish that may redirect energies obtained from food sources and, therefore, limit growth rates (Kamstra et al. 1996).

It is not known whether fish possess the same capability as mammals for endogenous nitrate reduction, in which the bacterial flora within the animal reduce nitrate to nitrite, or whether nitrate must first be converted to nitrite in the surrounding water prior to uptake. In a review of the Grabda et al. (1974) study, Colt and Armstrong (1981) suggested, because nitrite levels were not monitored in the water, it was possible that bacteria in the water surrounding the fish were reducing nitrate to nitrite (Colt and Armstrong 1981). Supporting evidence by Anuradha and Subburam (1995) showed that for carp (*Cyprinus carpio*) exposed to 36 mg $NO_3^-{\cdot}L^{-1}$ (as $NaNO_3$), methaemoglobin levels were significantly higher (43.7%, $p = 0.01$) when held in water

containing nitrate reducing sewage bacteria, than in water without bacteria (10.0%), or control water without nitrate (6.5%). Nitrate reducing bacteria present in sewage, such as *Pseudomonas* (Anuradha and Subburam 1995), are numerous in all natural surface waters (McCoy 1972).

Another potential link between nitrate and methaemoglobin formation has been shown in the physiological response of freshwater mosquito fish (*Gambusia affinis*) exposed to sodium nitrate (Nagaraju and Ramana Rao 1983, 1985). Nagaraju and Ramana Rao (1983) found that exposure to 29 mg NO_3^-·L^{-1} resulted in an increase of succinic dehydrogenase activity, and a decrease in lactate dehydrogenase activity. These changes indicate the fish were likely using an enhanced glycolysis process to produce the H^+ required to reduce methaemoglobin (formed due to nitrate exposure) back to haemoglobin. At this level of nitrate exposure, fish were also found to have significantly elevated enzyme levels which would aid in the conversion of methaemoglobin back to haemoglobin (Nagaraju and Ramana Rao 1985). These results suggest a biochemical response by the fish to counteract stresses induced by nitrate toxicity (Nagaraju and Ramana Rao 1985).

As with fish species, the main toxic mode of action of nitrate in crayfish is the conversion of oxygen-carrying pigments (haemoglobin, haemocyanin) to forms incapable of oxygen carrying capacity (methaemoglobin, methaemocyanin) (Camargo et al. 2006). The entry of nitrite into the blood plasma of crayfish results in the oxidation of copper atoms (Cu^{1+} to Cu^{2+}) which converts haemocyanin to the non-oxygen releasing methaemocyanin (Camargo et al. 2006). A similar event occurs in fish, whereby entry of nitrite into red blood cells results in the oxidation of iron atoms (Fe^{2+} to Fe^{3+}) converting haemoglobin into methaemoglobin, a form unable to release oxygen to body tissues (Camargo et al. 2006).

Methaemoglobinemia is also a likely mode of toxicity in amphibians (Huey and Beitinger 1980a,b). In studies with bullfrog larvae (*Rana catesbiana*) and channel catfish (*Ictalurus punctatus*), Huey and Beitinger (1980b) observed increased blood levels of methaemoglobin in both species when exposed to nitrite; however, they noted the tadpoles were more resistant than the fish to nitrite-induced methaemoglobin formation. The authors speculated that there may be less nitrite uptake in tadpoles, and/or tadpoles may have a more efficient methaemoglobin reductase system than fish.

The mechanism of nitrate toxicity in invertebrates has yet to be determined, but evidence suggests that, similar to vertebrates, nitrate may affect the oxygen carrying pigments (Muir et al. 1991). For example, histological examination of penaeid larvae has shown that exposure to 10 mg NO_3^-·L^{-1} elicited vacuolative change and tissue damage to the midgut and hypodermis that are thought to be the sites of haemocyanin synthesis and uptake/removal, respectively, in decapods (as per Senkbeil and Wriston 1981a,b). Such sublethal histopathological changes may affect the survival of larval forms in the environment (Muir et al. 1991).

6.3.2.2 Osmoregulation Disruption

Although the physiological mechanisms are not fully known, it appears that the lethal toxicity of nitrate may be related, in part, to the inability of the animal to maintain adequate osmoregulation under waters with high salt contents (Brownell 1980; Colt and Armstrong 1981). Acute mortality estimates for freshwater fish exposed to $NaNO_3$ range from 1300 to 9300 mg NO_3-·L^{-1} (Appendix A). At these concentrations, it may be difficult to determine whether the toxic

response is due to the cation or anion, as lethal $NaNO_3$ levels at this magnitude are comparable to lethal NaCl levels (Colt and Armstrong 1981). For example, 24-h LC_{50}s for bluegills exposed to $NaNO_3$ (3200 and 3500 mg $Na^+ \cdot L^{-1}$), are similar to those for NaCl (5100 and 5600 mg $Na^+ \cdot L^{-1}$) (Trama 1954; Dowden and Bennett 1965). Similarly, Brownell (1980) found acutely toxic levels of $NaNO_3$ for marine fish (24-h LC_{50}s > 15 283 mg NO3-·L-1) raised the salinity of the test waters from 35‰ to 59 - 83‰. When seawater salinity was increased to 50 and 70‰ using NaCl, 15% and 100% of test fish (n = 20 each) died, respectively (Brownell 1980).

Sodium ions are normally passively taken up through the guts of marine fish, and actively pumped out of the body via chloride cells in the gills, while freshwater fish actively take up Na^+ across the gill surface via chloride cells in exchange for other monovalent waste products in the blood (e.g., ammonium, hydrogen ions) (Heath 1995). Fish tend to maintain plasma Na^+ concentrations of approximately 150 to 160 mM in fresh- and marine waters, while ambient concentrations range from approximately 0.3 mM in fresh waters to 520 mM in marine waters (Bone and Marshall 1986). Marine fish generally have a greater number of chloride cells than freshwater fish to help accommodate these greater ionic fluxes (Heath 1995). Fish subjected to a higher osmotic gradient from the surrounding water than normal may undergo cellular stress from loss of water.
At high concentrations nitrate is also able to remove proteins from cell membranes (Manzano et al. 1976). No information was available on osmoregulatory disruption in amphibians or invertebrates due to nitrate exposure.

6.3.3 Indirect Toxicity

6.3.3.1 Role of Nitrate in Nutrient Enrichment

Nitrate serves as the primary source of nitrogen for aquatic plants in well oxygenated systems, and excessive concentrations have been shown to result in algal blooms and eutrophication in ponds (Nordin and Pommen 1986; Meade and Watts 1995). While it is generally acknowledged phosphorus is the nutrient that limits primary production in freshwater systems, and nitrogen is limiting in marine systems (Paerl 1993; Crouzet et al. 1999; US EPA 2000b), the role of nitrogen in eutrophication may vary considerably in both types of systems. The dependence of the relative contributions of both nutrients (i.e., N:P ratios) are examined in a separate CCME and NAESI discussion paper (CCME 2002; NAESI 2005). In general, for freshwater environments with low N:P loading ratios, nitrogen can play a significant role in net primary production (Camargo et al. 2006). In the case of marine environments, as the N:P loading ratio increases, P can become more limiting with respect to primary production (Camargo et al. 2006). In fact, due to increasing evidence between increased nitrogen levels and eutrophication, benchmark concentrations for both phosphorus and nitrogen have been developed to prevent eutrophication. In the case of freshwater systems, Dodds et al. (1998) proposed upper limits for both total phosphorus and total nitrogen for eutrophic temperate lakes (71 µg TP/L and 1260 µg TN/L) and eutrophic temperate streams (75 µg TP/L and 1500 µg TN/L). The US EPA (2002) takes into consideration two causal variables (TN and TP) and two response variables (algal biomass and water clarity) for both freshwater and marine systems as nutrient criteria guidance. The Swedish Protection Agency (2000), in order to prevent eutrophication of coastal marine ecosystems, has set not-to-exceed values of 440 µg TN/L and 30 µg TP/L.

Adverse ecological effects associated with eutrophication include a loss of water clarity, changes in plankton and fish species composition, physical obstructions in waterways which can impede fish migration or rearing, and potentially fatal oxygen depletion (Environment Australia 2000b). Increased phytoplankton, and/or aquatic plant biomass can lead to increased biological oxygen demands (BOD) on a system for two main reasons: a) plants and algae consume oxygen when not undergoing photosynthesis, which results in greater diurnal respiration rates, and b) after senescense, or death, greater populations of bacteria are required to break down the additional organic matter from excess plants/algae, which requires greater oxygen consumption. Therefore, the risks of low oxygen (hypoxia), or complete lack of oxygen (anoxia) events can increase, and fish kills may result if critical oxygen levels are not maintained.

Over-stimulation of phytoplankton production in the pelagic zone can reduce the amount of light penetrating the water column, and as a result, primary production of benthic algae (periphyton) and rooted plants (macrophytes) can be adversely affected. Nutrient enrichment studies on small ($\leq$ 3.4 ha), relatively shallow (mean depth $\leq$ 5.7 m) lakes in Michigan demonstrated increased phytoplankton production accompanied reductions in periphyton production (Vadeboncoeur et al. 2001). In nutrient-enriched coastal waters where light penetration is adequate, over-stimulation of epiphytic algae has been linked to the widespread loss of seagrass communities, as epiphytes can also limit the photosynthetic capabilites of the underlying macrophytes (Coleman and Burkholder 1994). In mesocosm experiments, nitrate supply levels were found to have a controlling influence on the community structure and species dominance of epiphytes on the eelgrass (*Zostera marina* L.). Additions of 0.2 and 0.4 mg $NO_3^- \cdot L^{-1}$ stimulated total epiphyte productivity (primarily as blue-green algae and diatoms) over a period of 6 weeks (170 ± 47 and 157 ± 10 mg $C \cdot m^{-2} \cdot d^{-1}$, respectively, versus 102 ± 9 mg $C \cdot m^{-2} \cdot d^{-1}$ in controls; $p < 0.05$) (Coleman and Burkholder 1994).

Nutrient enrichment can lead to the proliferation of algae and photosynthetic bacteria that produce toxic metabolites. Ingestion of these algal toxins can impair the health of aquatic organisms and they may accumulate in shellfish to levels toxic to consumers, including humans (Smith et al. 1999). Of the toxin-producing algae, cyanobacteria, or blue-green algae (Cyanophyta), are of primary importance in fresh waters, and diatoms and dinoflagellates are important sources in marine waters (Chambers et al. 2001). Cyanobacteria are unique in that all species will assimilate fixed inorganic nitrogen (i.e., nitrate, nitrite and ammonia), but some species are also capable of directly fixing atmospheric nitrogen (N_2) into organic nitrogen (Environment Australia 2000b). This ability provides a competitive advantage over other primary producers in low nitrogen environments, and as such, cyanobacteria tend to dominate the algal species assemblage when N:P ratios (by weight) fall below 29:1 (Smith 1983). Cyanobacteria known to produce toxins in Canadian inland surface waters include *Anabaena*, *Aphanizomenon*, *Microcystis* and *Phormidium* (Chambers et al. 2001). Although passive ingestion of cyanobacterial toxins have not been known to be fatal to humans, severe skin irritations can occur, and their neurotoxic and hepatotoxic properties have been responsible for liver damage and death of livestock (Environment Australia 2000b; Health Canada 1998).

Diatoms (Bacillariophyceae) are a very large, diverse group of primarily sessile marine and freshwater phytoplankton occuring in both unicellular and colonial forms (Wetzel 1983). The diatom *Nitzschia pungens* produces domoic acid, a toxin that can cause amnesiac shellfish poisoning in humans consuming mussels from contaminated waters (Chambers et al. 2001). In

1987, 108 cases of acute poisoning (including three deaths), were reported in Prince Edward Island after people ingested blue mussels (*Mytilus edulis* L.) contaminated with domoic acid (Bates et al. 1989). The cause of the bloom of *N. pungens* responsible for the elevated toxin levels was thought to be related to inorganic nitrogen enrichment. *Nitzschia pungens* population levels, and domoic acid production have been shown to respond postively to both nitrate and ammonium in *in situ* experiments (Bates et al. 1993), and blooms of *N. pungens* in eastern Prince Edward Island occurred only when ambient nitrate levels exceeded 1.1 µg $NO_3^-{\cdot}L^{-1}$ (Smith et al. 1990). As a result, the massive bloom of *N. pungens* which led to the accumulation of domoic acid in in 1987, was attributed to a long dry summer followed by heavy nitrate runoff during an intensely wet autumn (Chambers et al. 2001).

Dinoflagellates (Dinophyceae) are unicellular flagellated algae and most have a conspicuous armoured cell wall with large spines (Wetzel 1983). Large colonies of dinoflagellates can produce 'red tides' in coastal marine waters, leading to widespread fouling of waterways and the production of shellfish toxins (Chambers et al. 2001). Isolated outbreaks of shellfish toxicity from dinoflagellate blooms such as *Gonyualax acatenalla* have been documented along the coast of British Columbia, however, causal links to nutrient additions were difficult to demonstrate (Chambers et al. 2001). In a review of factors influencing global red tide occurrences, Hodgkiss and Ho (1997) reported decreasing N:P ratios in Tolo Harbour, Hong Kong, were associated with an increase in red tide events, and occurrences were highly probable when dissolved nitrogen and phosphorus levels exceeded 0.1 mg $N{\cdot}L^{-1}$ and 0.02 mg $P{\cdot}L^{-1}$, respectively. An increase in dinoflagellate abundance, however, does not always result in increased toxic effects. Isolated population increases of the toxin-producing dinoflagellate *Alexandrium catenella* in Hong Kong were not followed by paralytic shellfish poison contamination of the resident shellfish (Siu et al. 1997).

The relationship between increasing nitrogen concentrations in both marine and fresh waters and eutrophication are not clearly defined. For example, there is a wide range in nitrate concentrations that produce optimal growth of the marine dinoflagellate *Alexandrium catenella*, from 14 to 548 mg $NO_3^-{\cdot}L^{-1}$ (Siu et al. 1997), which would make predicting a population response based on nitrate exposure levels alone extremely difficult. Total nitrogen levels are also poor predictors of algal biomass (measured as Chlorophyll *a*, or Chl *a*) in lakes and coastal regions; algal biomass can be predicted better from either total phosphorus, or a combination of the two nutrients (Mazumder and Havens 1998; Meeuwig et al. 2000). It should also be noted that other factors can affect plant and algal growth, so in some cases a relationship between nutrient levels and primary productivity may not exist. For example, where there is light limitation due to very high turbidity, added nutrients might not necessarily stimulate growth. In a study of the potential for eutrophication in coastal inlets in Nova Scotia, Strain and Yeats (1999), found that eutrophic inlets were associated with poor flushing characteristics, and tended to have more than 50% of the water trapped behind the inlet sill, while non-eutrophic inlets were at, or near 0% entrainment. To better predict how altering water column nitrogen and phosphorus levels will influence eutrophication processes, further research is required in understanding factors regulating internal nutrient cycling, and in the complex interactions between nutrients and food webs (Smith et al. 1999).

In many aquatic ecosystems, eutrophication-related effects will occur at nitrate concentrations lower than those required to cause direct toxicity. Total nitrogen levels associated with highly

eutrophied lakes, rivers, and coastal waters around the world are often below 1 mg $N \cdot L^{-1}$ (Table 6.4). If all nitrogen were in the form of nitrate, this would correspond to a level of 4.4 mg $NO_3^- \cdot L^{-1}$, well below levels at which the majority of direct toxic effects have been documented (Appendix A). As part of the whole-lake fertilization program of the Experimental Lakes Area, northwestern Ontario, one-half of Lake 226 was fertilized with carbon and nitrogen (as nitrate) over an eight year period (Findlay and Kasian 1987). The increase in ambient total nitrogen concentrations in the nitrate-fertilized portion of Lake 226 (= 0.46 ± 0.09 mg $TN \cdot L^{-1}$, compared to 0.31 ± 0.04 mg $TN \cdot L^{-1}$ in an unfertilized control lake), resulted in overall phytoplankton biomass increasing by a factor of 2 to 4 over unfertilized years (Findlay and Kasian 1987). Mean phytoplankton biomass levels (3070 ± 1210 mg $\cdot m^{-3}$) were also substantially higher than those found in the control lake not undergoing nitrate fertilization (720 ± 200 mg $\cdot m^{-3}$) (Findlay and Kasian 1987). Similarly, in enclosure experiments in a eutrophic Hungarian reservoir, phytoplankton production responded quickly to nitrate-nitrogen additions. Within one week of nitrate additions (bringing the mesocosm nitrate level to 13 mg $NO_3^- \cdot L^{-1}$), total phytoplankton biomass increased from 24 to 59 mg $\cdot L^{-1}$ (Présing et al. 1997). By the end of the week, all nitrate-nitrogen supplied to the mesocosm had been used in algal production (primarily by diatoms and cryptomonads), and levels had returned to those seen in controls (Présing et al. 1997).

Table 6.4. Average total nitrogen levels in global lakes, streams and coastal marine waters of varying trophic status.

	TN (mg $N \cdot L^{-1}$)		
Trophic State	**Lakes[a]**	**Streams[b]**	**Marine[c]**
Oligotrophic	< 0.35	< 0.7	< 0.26
Mesotrophic	0.35-0.65	0.7-1.5	0.26-0.35
Eutrophic	0.65-1.2	> 1.5	0.35-0.40
Hypereutrophic	> 1.2		> 0.40

[a]Nürnberg 1996 [North American, European and Asian lakes];
[b]Dodds et al. 1998 [North American and New Zealand Streams];
[c]Håkanson 1994 [source waters not known]; from Smith et al. 1999).

Increasing nitrate levels in surface waters may also lead to changes in algal species compositions. The Grand River in southern Ontario is situated in a lowland area dominated by heavy urban and agricultural development, and is subject to increasingly high nitrate loads (e.g., up to 18 mg $NO_3^- \cdot L^{-1}$ in 1994) (Rott et al. 1998). Multivariate analyses of benthic diatom species assemblages along the river showed *Surirella brébissonii* and *Navicular lanceolata* were associated with higher nitrate values, while *N. gregaria* and *N. tripunctata* were associated with moderate nitrate levels (Rott et al. 1998). From factorial enrichment experiments exposing natural Lake Huron phytoplankton assemblages to nitrate (0.27 to 4.3 mg $NO_3^- \cdot L^{-1}$) and phosphorus (4 to 16 µg $P \cdot L^{-1}$), Pappas and Stoermer (1995) determined populations of cyanophytes, flagellates, and the diatom *Cyclotella commensis*, responded positively to increasing nitrate additions, while other species were either not affected by, or as in the case of *Cyclotella pseudostelligera*, were inhibited by higher nitrate levels (Pappas and Stoermer 1995).

The authors suggest increasing nitrate levels in the Great Lakes would therefore affect algal species composition in these waters (Pappas and Stoermer 1995).

In coastal regions, phytoplankton have been shown to readily respond to nitrate enrichment. In nutrient limitation studies using mesocosms in coastal lagoons in Narragansett Bay, Rhode Island, additions of high levels of nitrate (514 mg NO_3^-·L^{-1}) resulted in substantial phytoplankton blooms, with Chl *a* levels 12 times greater than in controls, and 3 times greater than in mesocosms enriched with phosphorus alone (22 mg P·L^{-1}) (Fisher's LSD test, $p < 0.010$) (Taylor et al. 1995). Enrichment experiments performed on waters collected from a variety of salinity levels (0 - 30‰) in Waquoit Bay, Massachusetts, showed that addition of 6.2 mg NO_3^-·L^{-1} in highly saline waters (23 - 30‰) increased Chl *a* levels from ~5 µg·L^{-1} in controls to ~18 µg·L^{-1} (Tomasky et al. 1999). However, in fresh (0‰), and brackish waters (10 - 19‰), phytoplankton growth responded to phosphorus additions only (Tomasky et al. 1999).

6.3.3.2 Role of Nitrate in Acidification

Acid neutralising capacity (ANC) is a measure of surface water's capacity to consume H^+ and therefore buffer against acidification (Laudon et al. 2000). Increased inputs of HNO_3 to surface waters from precipitation could potentially decrease the neutralising capacity of the water body through H^+ inputs. Driscoll and Van Dreason (1993) linked an increasing trend in nitrate levels of 0.1 mg NO_3^-·L^{-1}·a^{-1} between 1982 and 1990 in Constable Pond (Adirondack mountains, New York) with a simultaneous decrease in the ANC of the system. Decreases in the ANC of the pond corresponded with spring snowmelt when high concentrations of nitrate were released from the snowpack (Heathwaite et al. 1996). The nitrate itself would not have contributed any acidity to the system, as it is the conjugate base to a strong acid, and therefore would act as a pH neutral ion. This finding suggests HNO_3 precipitated in snow contributed the H^+ responsible for lowering the ANC. In contrast, a review of studies on the Muskoka-Haliburton lakes in Ontario between 1976 and 1980 showed no relationship between H^+ concentrations and NO_3^- (Elder 1984). This result suggests either the nitrate in these lakes was primarily due to sources other than atmospheric HNO_3 deposition, or the H^+ primarily originated from some other source (such as atmospheric deposition of H_2SO_4). Similarly, a study quantifying the sources to pH reductions in spring melt waters of 12 Swedish streams found no correlation between nitrate levels and pH decline; in this case, organic acids were the primary contributors to the acidity of the streams (Laudon et al. 2000).

6.4 Toxicity to Freshwater Life

Nitrate is toxic to sensitive early life-stages of freshwater invertebrates, amphibians, and fish. For members of each group, nitrate can affect embryonic or larval survival, growth, or behaviour. Invertebrates and amphibian larvae tend to be more susceptible to nitrate during short-term exposures, when compared to larval fish. With respect to long-term nitrate exposures, fish (early life stages) are among the most sensitive (Appendix A).

Key studies (primary and secondary classification) used in guideline derivation with environmentally relevant endpoints included mortality, growth, physical deformities and reproduction.

6.4.1 Short-Term Freshwater Toxicity Data

6.4.1.1 Invertebrates

A search of the primary literature for short-term nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation (Environment Canada, 2003). Web of Science was searched using keywords including: nitrate, NO_3^-, toxicity, lake, river, freshwater, and aquatic. Three new published studies with 8 invertebrates (stonefly *Amphinemura delosa*, stonefly *Allocapnia vivipara*, midge *Chironomus dilutus*, amphipod *Hyalella azteca*, fatmucket mussel *Lampsilis siliquoidea*, washboard mussel *Megalonaias nervosa*, snail *Potamopyrgus antipodarum*, and fingernail clam *Sphaerium simile*) were identified containing toxicity data appropriate for guideline derivation (Alonso and Camargo 2003; US EPA 2010b; Soucek and Dickinson 2011) (Table 6.5). A third published study was obtained (Camargo et al., 2005), and is listed in Table 6.5, but was not included in the short-term guideline dataset since the two amphipods tested (*Echinogammarus echinosetosus* and *Eulimnogammarus toletanus)* and the one caddisfly (*Hydropsyche exocellata)* are all tropical species. One additional unpublished study with one invertebrate (amphipod *Hyalella azteca*,) was obtained and was determined to be appropriate for inclusion in guideline derivation (Elphick 2011) (Table 6.5). The Elphick (2011) study utilized standardized toxicity testing methods. The complete list of nitrate toxicity data can be found in Appendix A.

Alonso and Camargo (2003) conducted short-term (96-h) nitrate toxicity studies on the New Zealand mudsnail, *Potamopyrgus antipodarum*, an invasive snail species found in Europe and North America. These snails were found to be quite tolerant of nitrate toxicity with a 96-hr LC_{50} of 4616 mg $NO_3^-\cdot L^{-1}$ (Table 6.5). Soucek and Dickinson (2011) also conducted short-term (96-h) nitrate toxicity studies on five invertebrates, where the order of sensitivity (lowest to highest LC_{50}) was found to be: *L. siliquoidea* > *S. simile* > *A. delosa* > *H. azteca* > *A. vivipara* > *M. nervosa*. The corresponding measured 96-h LC_{50} values ranged from 1582 $NO_3^-\cdot L^{-1}$ for *L. siliquoidea* to 4151 $NO_3^-\cdot L^{-1}$ for *M. nervosa*. Camargo et al. (2005) included short-term nitrate toxicity data for adults of tropical amphipods *Eulimnogammarus toletanus* and *Echinogammarus echinosetosus* and the tropical caddisfly *Hydropsyche exocellata* in a review of nitrate toxicity to aquatic animals. Four separate experiments run for 48-, 72-, 96- and 120-h found the LC_{50} for each species decreased by approximately 50% as exposure durations increased (Table 6.5). Of the three species, *E. echinosetosus* was the most sensitive to nitrate. Following their review, Camargo et al. (2005) concluded a safe concentration of NO_3^- in water should be 8.9 mg $NO_3^-\cdot L^{-1}$ (2 mg NO_3^--N$\cdot L^{-1}$). Elphick (2011) tested the toxicity of nitrate to the amphipod *Hyalella azteca* in exposure water of varying hardness (soft water, moderately hard water and hard water) to derive the three 96-h LC_{50} effect concentrations listed in Table 6.5. The observed trend was an increase in effect concentration with increasing hardness (see Appendix A for full dataset). The US EPA (2010b) study with *H. azteca* produced the lowest 96-h LC_{50} for this species (73 mg $NO_3^-\cdot L^{-1}$, tested at a hardness of 80-84 mg$\cdot L^{-1}$ as $CaCO_3$) (Table 6.5). The US EPA (2010b) study also reported a 48-h LC_{50} of 1582 mg$\cdot L^{-1}$ for the midge *C. dilutus* (Table 6.5).

With respect to short-term nitrate benchmark concentration derivation, all invertebrate studies were classified as either primary or secondary as per CCME (2007). Exposure durations of 48- and 96-h were reported. In general, invertebrates were found to be more sensitive to acute nitrate

exposures when compared to fish and amphibians (see Table 7.3 in Section 7.1.3.1). With respect to the data presented in Table 7.3, the first nine most sensitive organisms in the entire short-term dataset of 23 aquatic species are all invertebrate species. The range in short-term sensitivity to nitrate for invertebrates ranges from the most sensitive caddisfly *Hydropsyche occidentalis* (96-h LC_{50} of 431 mg $NO_3^-{\cdot}L^{-1}$) to the most tolerant New Zealand mudsnail *Potamopyrgus antipodarum* (96-h LC_{50} of 4616 mg $NO_3^-{\cdot}L^{-1}$). Two of the invertebrate exposures (*H. occidentalis, C. pettiti*) were conducted using CCME soft water exposure water (0-60 mg·L^{-1},as $CaCO_3$), eight (*H. azteca, C. dilutus, L. siliquoidea, S. simile, A. delosa, A. vivipara, M. nervosa* and *P. antipodarum*) were conducted using CCME moderately hard exposure water (61-120 mg·L^{-1} as $CaCO_3$), and two were conducted using CCME hard water (121-180 mg·L^{-1} as $CaCO_3$). As is discussed in Section 6.1.1 - Evaluating the Hardness-Toxicity Relationship for Nitrate – Short-Term Exposures – the decision was made to not adjust the national nitrate short-term guideline for water hardness. The hardness of the water utilized in the short-term tests is listed in Table 7.3 to help with interpretation of the ranking of species sensivities to nitrate. The short-term hardness toxicity relationships presented in Section 6.1.1 for the two organisms for which data existed (*O. mykiss, H. azteca*) did show a trend in decreasing nitrate toxicity with increasing water hardness, but the relationship between organisms was not the same, and this is the basis for not deriving a national hardness-adjusted nitrate guideline.

Table 6.5. Freshwater invertebrate short-term nitrate toxicity data published since 2001.

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-{\cdot}L^{-1}$)	Reference
INVERTEBRATES				
Amphinemura delosa (Eastern forestfly)	Field-collected nymphs	96-h LC_{50}	2020	Soucek and Dickinson 2011
Allocapnia vivipara (stonefly)	Field-collected nymphs	96-h LC_{50}	3703	Soucek and Dickinson 2011
Chironomus dilutus (midge)	10 days old	48-h LC_{50}	1582	US EPA 2010b
Echinogammarus echinosetosus[a] (amphipod)	Adult	48-h LC_{50}	473	Camargo et al., 2005
	Adult	72-h LC_{50}	331	Camargo et al., 2005
	Adult	96-h LC_{50}	277	Camargoet al., 2005
	Adult	120-h LC_{50}	249	Camargo et al., 2005
Eulimnogammarus toletanus[a] (amphipod)	Adult	48-h LC_{50}	798	Camargo et al., 2005
	Adult	72-h LC_{50}	483	Camargo et al., 2005
	Adult	96-h LC_{50}	377	Camargo et al., 2005
	Adult	120-h LC_{50}	324	Camargo et al., 2005

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
Hyalella azteca (amphipod)	Juvenile	96-h LC_{50}	744 (tested at hardness 44 mg·L^{-1} as $CaCO_3$)	Elphick 2011
	Juvenile	96-h LC_{50}	2149 (tested at hardness 100 mg·L^{-1} as $CaCO_3$)	Elphick 2011
	Juvenile	96-h LC_{50}	4080 (tested at hardness 164 mg·L^{-1} as $CaCO_3$)	Elphick 2011
	10 days old	96-h LC_{50}	73 (tested at hardness 80-84 mg·L^{-1} as $CaCO_3$)	US EPA 2010b
	7-14 days old	96-h LC_{50}	2955 (tested at hardness 117 mg·L^{-1} as $CaCO_3$)	Soucek and Dickinson 2011
Hydropsyche exocellata[a] (caddisfly)	Adult	48-h LC_{50}	2622	Camargo et al., 2005
	Adult	72-h LC_{50}	1551	Camargo et al., 2005
	Adult	96-h LC_{50}	1194	Camargo et al., 2005
	Adult	120-h LC_{50}	1019	Camargo et al., 2005
Lampsilis siliquoidea (fatmucket mussel)	<5 day old juveniles	96-h LC_{50}	1582	Soucek and Dickinson 2011
Megalonaias nervosa (washboard mussel)	<5 day old juveniles	96-h LC_{50}	4151	Soucek and Dickinson 2011
Potamopyrgus antipodarum (New Zealand mudsnail)	Adult	96-h LC_{50}	4616	Alonso and Camargo, 2003
Sphaerium simile (fingernail clam)	juveniles	96-h LC_{50}	1644	Soucek and Dickinson 2011

[a]Tropical species (not included in short-term guideline dataset)

The following provides an overview of some additional short-term studies listed in Appendix A, but with effect concentrations that were not selected for short-term benchmark concentration derivation (e.g. a more favourable effect concentration was selected from the same study for benchmark derivation). Similar to the findings of the study conducted by Camargo et al. (2005), two caddisfly species tested by Camargo and Ward (1992), *H. occidentalis* and *C. pettiti,* had acute LC_{50} values decreasing with increasing exposure time (72- to 120-h) and from last to early

instar stage. For early instars of *H. occidentalis*, and *C. pettiti*, the 120-h LC_{50}s were 290 and 472 mg $NO_3^-·L^{-1}$, respectively, suggesting a differential response to toxicity between species (Camargo and Ward 1992). The caddisflies were also exposed to high NaCl levels (up to 1100 mg $Na^+·L^{-1}$). As no mortality was observed, it is likely the toxic effects seen in the study were fundamentally due to the nitrate ion (Camargo and Ward 1992). Using mortality data from the above study, Camargo and Ward (1995) determined safe concentrations (SCs = 8760-h $LC_{0.01}$s) for the two caddisfly species. These values are analogous to NOECs and are intended to be protective of animals throughout their entire larval stage (approximately 1 year or 8760 h). Calculated SCs for early instars of *H. occidentalis* and *C. pettiti* are 6.2 and 10.6 mg $NO_3^-·L^{-1}$, respectively (Camargo and Ward 1995). These values are lower than estimated safe concentrations for salmonid fish at 25 to 35 mg $NO_3^-·L^{-1}$ (see Westin 1974, Section 6.4.1.1).

Jones (1940) determined the toxicity of a variety of anions to the freshwater planaria, *Polycelis nigra*, using distilled water in the test media. When exposed to $NaNO_3$ at pH 6.4, the planaria in both studies responded in a very similar fashion; the concentrations corresponding to a median survival time of 48 hours were 2666 mg $NO_3^-·L^{-1}$ (Jones 1941) and 2697 mg $NO_3^-·L^{-1}$ (Jones 1940).

Sodium and potassium salts were used to determine the impact of nitrate on the survival of *Lymnea* snails (Dowden and Bennett 1965). Snails exhibited a differential response to sodium and potassium salts, with median lethal tolerance limits (TL_ms; 50% hatching success of eggs) of 2373 and 671 mg $NO_3^-·L^{-1}$, respectively (Dowden and Bennett 1965). Dowden and Bennett (1965) speculate that the firm gelatin-like covering of the egg masses for these snails may afford extra protection to the developing embryos.

Acute toxicity (96-hr LC_{50}) values for *D. magna* exposed to KNO_3 and $NaNO_3$ in standard reference water were 549 and 3070 mg $NO_3^-·L^{-1}$, respectively (Dowden and Bennett 1965). In studies where *Daphnia magna* were exposed to sodium nitrate in centrifuged Lake Erie water, concentrations required to produce a threshold limit that would just fail to immobilise *D. magna* (analogous to a NOEC) after 16 and 48-h exposures were 6205 and 3650 mg $NO_3^-·L^{-1}$ (Anderson 1944, 1946, respectively).

6.4.1.2 Fish

A search of the primary literature for nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation (Environment Canada, 2003). Four new published studies with 5 different fish species (Indian major carp *Catla catla,* lake whitefish *Coregonus clupeaformis,* lake trout *Salvelinus namaycush,* topeka shiner *Notropis Topeka,* and fathead minnow *Pimephales promelas*) were identified (Table 6.6). Two unpublished studies with 4 different fish species (lake whitefish *Coregonus clupeaformis,* lake trout *Salvelinus namaycush,* rainbow trout *Oncorhynchus mykiss,* and arctic char *Salvelinus alpinus*) were also identified and considered appropriate for consideration for guideline derivation since the studies utilized standardized toxicity testing methods (Table 6.6). Short-term data was also obtained for the fathead minnow (*Pimephales promelas*) from Nautilus Environmental (2010). This data was not used because it was obtained from a slide deck presented at a workshop (37th Annual Aquatic Toxicity

Workshop in Toronto – October 2010). In addition, short-term data from a published study (US EPA 2010b) was already available for this fish species. The complete list of short-term nitrate toxicity data for fish can be found in Appendix A.

Table 6.6. Freshwater fish short-term nitrate toxicity data published since 2001.

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1}) (test temp)	Reference
FISH				
Catla catla (Indian major carp)	Juvenile	24-h-LC_{50}	6935	Tilak et al., 2002
	Juvenile	24-h-LC_{50}	2144	Tilak et al., 2002
Coregonus clupeaformis (Lake whitefish)	Alevin	96-h LC_{50}	9683 (7.5°C)	McGurk et al., 2006
	Fry	96-h LC_{50}	8429 (7.5°C)	McGurk et al., 2006
	Fry	24-h-LC_{50}	4730 (10°C)	Moore and Poirier 2010
	Fry	24-h-LC_{50}	9840 (15°C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	4730 (10°C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	8440 (15°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	4730 (10°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	5110 (15°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	6400 (5°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	4730 (10°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	5110 (15°C)	Moore and Poirier 2010
Salvelinus namaycush (Lake trout)	Alevin	96-hr LC_{50}	10 377 (7.5°C)	McGurk et al., 2006
	Fry	96-hr LC_{50}	4968 (7.5°C)	McGurk et al., 2006
	Fry	24-h-LC_{50}	5230 (5°C)	Moore and Poirier 2010
	Fry	24-h-LC_{50}	5230 (10°C)	Moore and Poirier 2010
	Fry	24-h-LC_{50}	4550 (15°C C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	5230 (5°C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	5230 (10°C)	Moore and Poirier 2010

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$) (test temp)	Reference
	Fry	48-h LC_{50}	4550 (15°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	5230 (5°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	5230 (10°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	4550 (15°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	5230 (5°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	5230 (10°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	4550 (15°C)	Moore and Poirier 2010
Notropis topeka (Topeka shiner)	Adult	96-h LC_{50}	6902	Adelman et al. 2009
	Juvenile	96-h LC_{50}	5994	Adelman et al. 2009
Oncorhynchus mykiss (rainbow trout)	Juvenile	96-h LC_{50}	3061 (14°C, hardness 15 mg $CaCO_3·L^{-1}$)	Elphick 2011
	Juvenile	96-h LC_{50}	6361 (14°C, hardness 45 mg $CaCO_3·L^{-1}$)	Elphick 2011
	Juvenile	96-h LC_{50}	7832 (14°C, hardness 90 mg $CaCO_3·L^{-1}$)	Elphick 2011
	Juvenile	96-h LC_{50}	7832 (14°C, hardness 160 mg $CaCO_3·L^{-1}$)	Elphick 2011
	Fry	24-h-LC_{50}	8010 (5°C)	Moore and Poirier 2010
	Fry	24-h-LC_{50}	7710 (10°C)	Moore and Poirier 2010
	Fry	24-h-LC_{50}	2640 (15°C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	5710 (5°C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	5720 (10°C)	Moore and Poirier 2010
	Fry	48-h LC_{50}	2020 (15°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	3980 (5°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	5720 (10°C)	Moore and Poirier 2010
	Fry	72-h LC_{50}	1690 (15°C)	Moore and

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$) (test temp)	Reference
				Poirier 2010
	Fry	96-h LC_{50}	2790 (5°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	3580 (10°C)	Moore and Poirier 2010
	Fry	96-h LC_{50}	1690 (15°C)	Moore and Poirier 2010
Salvelinus alpinus (arctic char)		24-h-LC_{50}	6650 (5°C)	Moore and Poirier 2010
		24-h-LC_{50}	14490 (10°C)	Moore and Poirier 2010
		24-h-LC_{50}	16120 (15°C)	Moore and Poirier 2010
		48-h LC_{50}	6680 (5°C)	Moore and Poirier 2010
		48-h LC_{50}	6200 (10°C)	Moore and Poirier 2010
		48-h LC_{50}	10620 (15°C)	Moore and Poirier 2010
		72-h LC_{50}	5320 (5°C)	Moore and Poirier 2010
		72-h LC_{50}	6650 (10°C)	Moore and Poirier 2010
		72-h LC_{50}	9570 (15°C)	Moore and Poirier 2010
		96-h LC_{50}	5320 (5°C)	Moore and Poirier 2010
		96-h LC_{50}	6650 (10°C)	Moore and Poirier 2010
		96-h LC_{50}	9570 (15°C)	Moore and Poirier 2010
Pimephales promelas (fathead minnow)	Larvae	96-h LC_{50}	655 (hardness 5-15 mg $CaCO_3$/L)	Nautilus Environmental 2010
	Larvae	96-h LC_{50}	1505 (hardness 40-60 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2010
	Larvae	96-h LC_{50}	2391 (hardness 80-110 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2010
	Larvae	96-h LC_{50}	2594 (hardness 160-190 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2010
	weight 0.11 g; length 16 mm	96-h LC_{50}	1838 (hardness 136-140 mg $CaCO_3·L^{-1}$)	US EPA 2010b

Tilak et al. 2002 compared static and flow-through designs on nitrate toxicity to Indian major carp (*Catla catla*). They noted median toxicity concentrations in short-term (24-h) nitrate toxicity conducted in static experimental environments were higher (6935 mg $NO_3^-·L^{-1}$) than LC_{50} values obtained in flow through tests 2144 mg $NO_3^-·L^-$ (Table 6.6). This study was not included in short-term benchmark concentration derivation because this is a non-resident species and is not an appropriate surrogate species.

McGurk et al. (2006) looked at short-term NO_3^- toxicity to alevins and swim-up fry of Lake whitefish (*Coregonus clupeaformis*) collected from Great Slave Lake, NWT and Lake trout (*Salvelinus namaycush*) from Lake Simcoe, ON following the same experimental design used for the Rainbow trout tests by Stantec (2006). They observed that the swim-up fry life-stage was more sensitive to acute nitrate exposures, when compared to the alevin life-stage, for both species. The short-term (96-h) toxicity for Lake trout swim-up fry occurred at 4968 mg $NO_3^-·L^{-1}$ whereas short-term toxicity for Lake whitefish swim-up fry occurred at 8429 mg $NO_3^-·L^{-1}$ (Table 6.6).

Moore and Poirier (2010) tested the effect of varying temperature (5, 10 and 15°C) on the short-term toxicity of NO_3^- to swim-up fry of lake whitefish (*Coregonus clupeaformis*), lake trout (*Salvelinus namaycush*), rainbow trout (*Oncorhynchus mykiss*) and arctic char (*Salvelinus alpinus*). LC_{50} values were recorded at 24-, 48-, 72- and 96-h time periods. 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^-/L, 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^-/L), and least toxic to *S. namaycush* at optimal metabolic temperature of 10 deg C (96-h LC_{50} of 5230 mg NO_3^-/L). 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. With respect to lake whitefish, the 96-h LC_{50} of 4730 mg $NO_3^-·L^{-1}$ at 10°C was lower than the 96-h LC_{50} generated by McGurk et al. (2006) of 8429 mg $NO_3^-·L^{-1}$, tested at 7.5°C. In the case of lake trout, the 96-h LC_{50} of 5230 mg $NO_3^-·L^{-1}$ tested at 10°C was found to be similar to the 96-h LC_{50} derived by McGurk et al. (2006) of 4968 mg $NO_3^-·L^{-1}$, tested at 7.5°C.

Adelman et al. (2009) determined the lethal effects of nitrate on the Topeka shiner (*Notropis topeka*), which was listed as an endangered species by the U.S. Fish and Wildlife Service in 1998. This species was added to the short-term dataset because it is considered to be a close relative of the COSEWIC (Committee on the Status of Endangered Wildlife in Canada) endangered Pugnose shiner (*Notropis anogenus*) (COSEWIC 2002), for which no toxicity data was available. The Topeka shiner 96-h LC_{50} values for nitrate were 5,994 and 6,902 mg $NO_3^-·L^{-1}$ for the juvenile (19 months) and adult (32 months) life stage. As a comparison, Camargo et al. (2005) reported 96-h LC_{50} values ranging from 4,471 to 8,743 mg $NO_3^-·L^{-1}$ for warmwater freshwater fish species.

Elphick (2011) tested the effects of short-term nitrate exposures of rainbow trout over a range of

water hardness, where the trend was one of decreasing nitrate toxicity with increasing hardness when tested at hardness levels of 15, 45 and 90 mg $CaCO_3 \cdot L^{-1}$. No additional protection was afforded by water hardness when tested at the highest hardness of 160 mg $CaCO_3 \cdot L^{-1}$.

Nautilus Environmental (2010) reported fathead minnow survival at 96-h which was obtained during a 7-day survival and growth toxicity test and is reported in both Table 6.6 and Appendix A. The same relationship between water hardness and nitrate toxicity that was observed for rainbow trout by Elphick (2011) was observed for fathead minnows. Nitrate toxicity decreased with increasing hardness when tested at a water hardness of 5-15, 40-60, and 80-110 mg $CaCO_3 \cdot L^{-1}$, with no observed reduction in toxicity when tested at the highest hardness of 160-190 mg $CaCO_3 \cdot L^{-1}$. This data was preliminary and therefore was not considered appropriate for consideration for inclusion in the short-term dataset. A published study by US EPA (2010b) also provided short-term toxicity data for the fathead minnow and was considered for inclusion in the short-term dataset for guideline derivation. The 96-h LC_{50} of 1838 mg $NO_3^- \cdot L^{-1}$ tested in dechlorinated Lake Michigan water (hardness 136-140 mg $CaCO_3 \cdot L^{-1}$) was slightly lower (but in general agreement with) than the 96-h LC_{50} of 2594 mg $NO_3^- \cdot L^{-1}$ tested using a water hardness of 160-190 mg $CaCO_3 \cdot L^{-1}$ (Nautilus Environmental 2010).

With respect to short-term nitrate benchmark concentration derivation, a total of 9 fish species were included in the dataset from studies classified as either primary or secondary as per CCME (2007). Exposure durations of 96-h were reported. In general, fish were more tolerant of acute nitrate exposures when compared to invertebrates and one amphibian species (the Pacific tree frog, *P. regilla*), but some fish were found to be quite sensitive to nitrate (e.g. fathead minnow and rainbow trout) (see Table 7.3 in Section 7.1.3.1). The most sensitive fish species was the fathead minnow (*P. promelas*) tested at the larval life-stage with a 96-h LC_{50} of 3304 mg $NO_3^- \cdot L^{-1}$ (Scott and Crunkilton 2000; US EPA 2010b). The most tolerant fish species was the bluegill sunfish (*L. macrochirus*), tested at the juvenile life-stage, with a 96-h LC_{50} of 8753 mg $NO_3^- \cdot L^{-1}$ (Trama 1954). It is of interest to note that the more sensitive fathead minnow (*P. promelas*) was tested in harder water (CCME hard 121-180 mg$\cdot L^{-1}$ as $CaCO_3$) when compared to the most tolerant bluegill (*L. macrochirus*), tested is softer water (CCME soft 0-60 mg$\cdot L^{-1}$ as $CaCO_3$).

The following studies suggest juvenile stages of these fish are not acutely susceptible to nitrate levels commonly found in the environment. Goldfish (*Carassius carassius*) and bluegills exposed to $NaNO_3$ had very similar 24-hour median tolerance limits (TL_m) in standard reference water at 8870 and 9344 mg $NO_3^- \cdot L^{-1}$, respectively (Dowden and Bennett 1965). Juvenile Guadalupe bass (*Micropterus treculi*), a species native to streams and rivers of central Texas, USA, exhibited acute toxicity (96-h LC_{50}) at 5586 mg $NO_3^- \cdot L^{-1}$ in hard water (310 mg $NO_3^- \cdot L^{-1}$) (Tomasso and Carmichael 1986). Exposing guppies (*Poecilia reticulatus*) to KNO_3, Rubin and Elmaraghy (1977) determined that acute mortality increased with exposure time. The median lethal concentration estimates of nitrate for the guppy fry reared in tap water for 24 and 96 hours were 1181 and 847 mg $NO_3^- \cdot L^{-1}$, respectively (Rubin and Elmaraghy 1977).

The 96-h LC_{50} for fingerling channel catfish (50 to 76 mm total length) exposed to sodium nitrate using static bioassays at 30°C was 6200 mg $NO_3^- \cdot L^{-1}$ (Colt and Tchobanoglous 1976). Although survival times of catfish exposed to nitrate generally decreased with increasing temperatures, the incipient LC_{50} values were independent of experimental temperatures (22°, 26° and 30°) (Colt and Tchobanoglous 1976).

Concentrations of nitrate which affect larval stages of common bluegill (*Lepomis macrochirus*) are comparable to those that are toxic to channel catfish. Trama (1954) determined the acute toxicity (96-h LC_{50}) of sodium nitrate to juveniles (5 to 9 cm total length) of the common bluegill in relatively soft water (up to 50 mg $CaCO_3 \cdot L^{-1}$; pH 7.4 to 8.8) to be 8753 mg $NO_3^- \cdot L^{-1}$.

Mosquito fish (*Gambusia affinis*) exposed to 29 mg $NO_3^- \cdot L^{-1}$ were shown to significantly increase enzyme activity levels which may have been indicative of a physiological response to combat nitrate and nitrite stress (Nagaraju and Ramana Rao 1983, 1985). Results were presented as a 96-h LOEC rather than a 96-h LC/EC_{50}, which is required for short-term benchmark concentration derivation (CCME 2007). As well, the results presented were physiological endpoints (not lethality or immobility). These studies did not provide adequate information on experimental conditions or control mortalities, and, being tropical freshwater fish, may not be applicable to Canadian fish physiology. As a result, this study was not considered for short-term benchmark concentration derivation.

6.4.1.3 Amphibians

A search of the primary literature for short-term amphibian nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation (Environment Canada, 2003). No new data was found.

Amphibians are susceptible to water pollution as they have permeable skin and rely on aquatic habitats for reproduction, larval development and hibernation (Hecnar 1995). Amphibians are particularly sensitive ecological receptors because they often inhabit surface waters that collect agricultural drainage. As breeding season in the spring tends to coincide with fertilizer application, developing eggs and embryos are placed in contact with potentially elevated nitrate pulses (Hecnar 1995).

Very few studies were found testing the short-term toxicity of nitrate on amphibian species. One study, classified as primary and used in short-term benchmark concentration derivation, was that by Schuytema and Nebeker (1999a). The Pacific treefrog (*P. regilla*) was exposed to nitrate for 96-h at both the embryo and tadpole lifestage. The embryo was found to be more sensitive, with respective LC_{50} concentrations of 2849 and 7752 mg $NO_3^- \cdot L^{-1}$. A second study by Schuytema and Nebeker (1999c), also classified as primary, provided data for both 96- and 120-h exposure durations. Embryos of the African clawed frog (*Xenopus laevis*) were exposed for 120-h to assess impacts on weight, length and resulting deformities, with weight being the most sensitive endpoint. The 120-h LOECs for *X. laevis* embryos were 251 (weight), 492 (length) and 1021 (deformities) mg $NO_3^- \cdot L^{-1}$. Physical deformities noted for *X. laevis* and *P. regilla* at concentrations from 492 to 4338 mg $NO_3^- \cdot L^{-1}$ included cardiac and abdominal edemas and lordosis (curvature of the spine) (Schuytema and Nebeker 1999a). The 120-h LC_{50} for the embryo life-stage of *X. laevis* was 1942 mg $NO_3^- \cdot L^{-1}$, compared to the 96-h LC_{50} of 7335 mg $NO_3^- \cdot L^{-1}$ for the tadpole life-stage. A third study that provided short-term toxicity data was that for the European common frog (*Rana temporaria*) (Johansson et al. 2001). A 72-h LOEC of >4425 mg $NO_3^- \cdot L^{-1}$ was provided for the larval lifestage. This study was found to be unacceptable for use in guideline derivation since water quality data was not reported. As well, the 120-h exposure data discussed here is neither considered short-term nor long-term as per the

CCME (2007) protocol for guideline derivation. Short-term exposures for amphibians are designated as being 96 hours or less, whereas long-term exposures for amphibians are ≥ 7 days. Shorter exposure periods (e.g. 120-h or 5-d) may be classified on a case-by-case basis by best scientific judgement as long-term exposures, and used in the derivation of the long-term exposure guidelines. In this case, the 120-h data was considered short-term. Following a review of all the available data, this study was not used for guideline derivation.

With respect to short-term nitrate benchmark concentration derivation, a total of 2 amphibian species were included in the dataset from studies classified as primary as per CCME (2007). The most sensitive amphibian species listed in Table 7.3 was the Pacific tree frog (*Pseudacris regilla*) with a 96-h LC_{50} of 2849 mg $NO_3^- \cdot L^{-1}$, which was more sensitive to short-term nitrate exposure than any of the 9 fish species listed in Table 7.3 (short-term guideline dataset). The only other amphibian for which short-term toxicity data was utilized for short-term guideline derivation was for the African clawed frog (*Xenopus laevis*) with a 96-h LC_{50} of 7335 mg $NO_3^- \cdot L^{-1}$. Interestingly, the *P. regilla* exposure was conducted in harder water (70-80 $mg \cdot L^{-1}$ as $CaCO_3$) compared to the less sensitive *X. laevis* (21 $mg \cdot L^{-1}$ as $CaCO_3$).

6.4.1.4 Algae and Plants

Nitrate is a required element for plant growth, and due to its greater abundance in surface waters relative to other fixed nitrogen species (e.g., ammonium), it is the most widely used form of nitrogen by vascular plants and algae (Pinar et al. 1997; Crouzet et al. 1999). As nitrate is actively taken up by aquatic primary producers, its uptake is generally not limited by low environmental concentrations (Cresswell and Syrett 1981; Pinar et al. 1997). More information related to algae and plants can be found in Section 6.3.2.4, where long-term freshwater toxicity data is presented.

Plant toxicity data were not included in the development of the short-term nitrate benchmark concentration value as nitrate is a plant nutrient.

6.4.2 Long-Term Freshwater Toxicity Data

6.4.2.1 Invertebrates

To address the data gaps identified during the derivation of the 2003 interim NO_3^- CWQG for the protection of freshwater life, an additional long-term toxicity test was commissioned for a non-planktonic invertebrate. Full results are reported elsewhere in Stantec (2006). Briefly, juvenile *Hyalella azteca* (1-d old at test initiation) were exposed to a nitrate concentration range over a 10-d period under static-renewal test conditions (Borgmann et al., 2005; Stantec, 2006) and were run using sodium nitrate ($NaNO_3$). All tests satisfied the minimum requirements for test validity as outlined in the specific test methods. Results of the definitive test with *Hyalella azteca* revealed growth to be the most sensitive endpoint (Table 6.7). The effect concentrations observed were typical of other studies on similar species (Environment Canada, 2003; Appendix A).

Table 6.7. The results of the nitrate (as $NaNO_3$) toxicity tests to *Hyalella azteca*. Table values are expressed in mg $NO_3^-·L^{-1}$ and include 95% confidence limits in parentheses.

	Life Stage	Endpoint	Effect Concentration (mg $NO_3^-·L^{-1}$)	Reference
Hyalella azteca (amphipod)	Juvenile	10-d LC_{50} (Survival)	2725 (hardness 310 mg $CaCO_3·L^{-1}$)	Stantec 2006
	Juvenile	10-d IC_{25} (Growth)	830 (hardness 310 mg $CaCO_3·L^{-1}$)	Stantec 2006
	Juvenile	10-d NOEC (survival)	1018 (hardness 310 mg $CaCO_3·L^{-1}$)	Stantec 2006
	Juvenile	10-d LOEC (survival)	2083 (hardness 310 mg $CaCO_3·L^{-1}$)	Stantec 2006
	Juvenile	10-d NOEC (growth)	2083 (hardness 310 mg $CaCO_3·L^{-1}$)	Stantec 2006
	Juvenile	10-d LOEC (growth)	4274 (hardness 310 mg $CaCO_3·L^{-1}$)	Stantec 2006

A search of the primary literature for invertebrate long-term nitrate toxicity studies published after 2001 was conducted. No published studies were located. One unpublished study (Elphick 2011) with 3 different invertebrate species (water flea *Ceriodaphnia dubia*, midge *Chironomus dilutus*, and amphipod *Hyalella azteca*) was identified and considered appropriate for consideration for guideline derivation since the study utilized standardized toxicity testing methods and met CCME (2007) protocol requirements for a primary study Table 6.8). The complete list of long-term nitrate toxicity data for invertebrates can be found in Appendix A.

Table 6.8. Freshwater invertebrate long-term nitrate toxicity data published since 2001.

	Life Stage	Endpoint	Effect Concentration (mg NO_3^-·L^{-1})	Reference
Ceriodaphnia dubia (water flea)	Neonate (<24h old)	7-d LC50	196 (hardness 44 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d LC50	523 (hardness 98 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d LC50	536 (hardness 166 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d IC25 (reproduction)	50 (hardness 44 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d IC25 (reproduction)	106 (hardness 98 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d IC25 (reproduction)	192 (hardness 166 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d NOEC (survival)	177 (hardness 44 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d NOEC (survival)	354 (hardness 98 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d NOEC (survival)	354 (hardness 166 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d LOEC (survival)	354 (hardness 44 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d LOEC (survival)	709 (hardness 98 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d LOEC (survival)	709 (hardness 166 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d NOEC (reproduction)	44 (hardness 44 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d NOEC (reproduction)	89 (hardness 98 mg $CaCO_3$·L^{-1})	Elphick 2011
	Neonate (<24h old)	7-d NOEC (reproduction)	177 (hardness 166 mg	Elphick 2011

			$CaCO_3 \cdot L^{-1}$)	
	Neonate (<24h old)	7-d LOEC (reproduction)	89 (hardness 44 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	Neonate (<24h old)	7-d LOEC (reproduction)	177 (hardness 98 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	Neonate (<24h old)	7-d LOEC (reproduction)	354 (hardness 166 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
Chironomus dilutus (midge)	3rd instar	10-d LC50	505 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LC50	975 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LC50	1493 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d IC25 (growth)	217 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d IC25 (growth)	447 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d IC25 (growth)	771 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d NOEC (survival)	177 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d NOEC (survival)	709 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d NOEC (survival)	709 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LOEC (survival)	354 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LOEC (survival)	1418 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LOEC (survival)	1418 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d NOEC (growth)	177 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011

	3rd instar	10-d NOEC (growth)	354 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d NOEC (growth)	709 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LOEC (growth)	354 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LOEC (growth)	709 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	3rd instar	10-d LOEC (growth)	1418 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
Hyalella azteca (amphipod)	6-8 day old	14-d LC50	558 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d LC50	1271 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d LC50	>2835 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d IC25 (growth)	57 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d IC25 (growth)	518 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d IC25 (growth)	806 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d NOEC (survival)	354 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d NOEC (survival)	709 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d NOEC (survival)	2835 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d LOEC (survival)	709 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d LOEC (survival)	1418 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
	6-8 day old	14-d LOEC (survival)	>2835 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011

6-8 day old	14-d NOEC (growth)	44 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
6-8 day old	14-d NOEC (growth)	354 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
6-8 day old	14-d NOEC (growth)	709 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
6-8 day old	14-d LOEC (growth)	89 (hardness 46 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
6-8 day old	14-d LOEC (growth)	709 (hardness 86 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011
6-8 day old	14-d LOEC (growth)	1418 (hardness 172 mg $CaCO_3 \cdot L^{-1}$)	Elphick 2011

Elphick (2011) tested the effects of long-term nitrate exposures to three invertebrates (*C. dubia, C. dilutus, H. azteca*) over a range of water hardness levels of 44-46, 86-98 and 166-172 mg $CaCO_3 \cdot L^{-1}$. In the case of *C. dubia*, the trend was one of decreasing toxicity with increasing hardness, going from a water hardness of 44 to 98 mg $CaCO_3 \cdot L^{-1}$. No additional protection was afforded when tested at the highest hardness of 166 mg $CaCO_3 \cdot L^{-1}$. For example, the 7-d LC_{50} for *C. dubia* when tested in water hardness of 44, 98 and 166 mg $CaCO_3 \cdot L^{-1}$ was 196, 523 and 536 mg $NO_3^- \cdot L^{-1}$, respectively. The 7-d IC_{25} (reproduction) values displayed a similar trend, with resulting effect concentrations of 50, 106 and 192 mg $NO_3^- \cdot L^{-1}$, when tested in water hardness of 44, 98 and 166 mg $CaCO_3 \cdot L^{-1}$, respectively (Appendix A). With both *C. dilutus* and *H. azteca*, the highest hardness water did appear to continue to offer greater protection against toxicity than either the low or medium hardness waters. For *C. dilutus*, the 10-d LC_{50} and 10-d IC_{25} (growth) were 505, 975 and 1493 mg $NO_3^- \cdot L^{-1}$, and 217, 447 and 771 mg $NO_3^- \cdot L^{-1}$, tested at a water hardness of 46, 86, 172 mg $CaCO_3 \cdot L^{-1}$. For *H. azteca*, the 14-d LC_{50} and 14-d IC_{25} (growth) were 558, 1271 and >2835 mg $NO_3^- \cdot L^{-1}$, and 57, 518 and 806 mg $NO_3^- \cdot L^{-1}$, tested at a water hardness of 46, 86, 172 mg $CaCO_3 \cdot L^{-1}$. See Section 6.1 – Effects of water quality parameters on toxicity – for more information on the evaluation of toxicity-hardness relationships, and the decision to not derive a hardness-adjusted long-term CWQG for the nitrate ion.

With respect to long-term nitrate guideline derivation, four invertebrates were represented from two studies (Elphick 2011; Scott and Crunkilton 2000) classified as primary as per CCME (2007) (Table 7.7). Exposure durations of 7- , 10- and 14-d were reported. In general, freshwater invertebrates display a similar wide-ranging sensitivity to long-term nitrate exposures when compared to fish (Table 7.8; Appendix A). However, the most sensitive invertebreate (*C. dubia*, 7-d IC_{25} [reproduction] of 50 mg $NO_3^- \cdot L^{-1}$) was found to be less sensitive than the most sensitive fish (*S. namaycush*, 146-d MATC [delay to swim-up stage and growth as wet weight] of 14 mg $NO_3^- \cdot L^{-1}$). It is of interest to note that both the *C. dubia* and the *S. namaycush* exposures were conducted in soft water (44 mg $CaCO_3 \cdot L^{-1}$ and 10-16 mg $CaCO_3 \cdot L^{-1}$, respectively). Toxic responses for invertebrates include mortality, reduction in fecundity and growth as well as

immobilisation. The most tolerant invertebrtate was the daphnid *D. magna*, with a 7-d MATC (reproduction) of 2244 mg $NO_3^-·L^{-1}$ (tested in hard water, 156-172 mg $CaCO_3·L^{-1}$). The effect concentration for *D. magna* is comparable to that of the most tolerant fish species (Chinook salmon, *O. tshawytscha*), where the 10-d LC_{10} is 3142 mg $NO_3^-·L^{-1}$ (water hardness for this fish exposure was not provided). The daphnid *D. magna* was also more tolerant of long-term nitrate exposures when compared to the 3 species of amphibians listed in the long-term dataset (see Table 7.8 in Section 7.1.3.1).As is discussed in Section 6.1.2 - Evaluating the Hardness-Toxicity Relationship for Nitrate – Long-Term Exposures – the decision was made to not adjust the national nitrate long-term guideline for water hardness. The hardness of the water utilized in the long-term tests is listed in Table 7.8 to help with interpretation of the ranking of species sensivities to nitrate. The long-term hardness toxicity relationships presented in Section 6.1.2 for the four organisms for which data existed (*P. promelas, C. dubia, H. azteca, C. dilutus*) did show a trend in decreasing nitrate toxicity with increasing water hardness, but the relationship between organisms was not the same, and this is the basis for not deriving a national hardness-adjusted nitrate guideline.

The following study was not considered for guideline derivation. Tesh et al. (1990) used sodium and potassium salts to determine the toxicity of nitrate to the growth of hydra (*Hydra attenuata*) populations. The no-effect level of the nitrate ion on hydra population growth when exposed to KNO_3 was > 150 mg $NO_3^-·L^{-1}$, based on NOECs of between 150 and 250 mg $NO_3^-·L^{-1}$; with $NaNO_3$, the NOEC for population growth was less than 50 mg $NO_3^-·L^{-1}$ (Tesh et al. 1990). Population levels of the freshwater hydra (*Hydra attenuata*) declined with increasing nitrate concentrations (up to 150 mg $NO_3^-·L^{-1}$) after 5 days exposure, and individuals in the highest concentration exhibited clubbed tentacles and rapid mortality (Tesh et al. 1990). This study was not selected for guideline development as no water quality conditions were reported for the hydra-specific growth media used to test the organisms, and no statistical interpretations were made on differences in survival between treatments.

6.4.2.2 Fish

To address the data gaps identified during the derivation of the 2003 interim NO_3^- CWQG for the protection of freshwater life, additional toxicity tests were commissioned for an early life stage test for a salmonid. Full results are reported elsewhere in Stantec (2006). The assessment of nitrate toxicity to Rainbow trout (*Oncorhynchus mykiss*) was based on Environment Canada's Embryo-Alevin-Fry (EAF) test (Environment Canada, 1998; Stantec, 2006). Toxicity tests were conducted using sodium nitrate ($NaNO_3$). All tests satisfied the minimum requirements for test validity as outlined in the specific test methods. Results of the definitive test with rainbow trout revealed growth to be the most sensitive endpoint (Table 6.9).

Table 6.9. The results of the nitrate (as $NaNO_3$) toxicity tests to Rainbow trout (*Oncorhynchus mykiss*). Table values are expressed in mg $NO_3^-·L^{-1}$ and include 95% confidence limits in parentheses.

	Life Stage	Endpoint	Effect Concentration (mg $NO_3^-·L^{-1}$)	Reference
Oncorhynchus mykiss (Rainbow trout)	Egg	32-d EC_{25} (Egg Mortality)	2168 (1919-2499)	Stantec 2006[1]
	Fry	64-d LC_{50} (Swim-up Fry Mortality)	2023 (1616-2702)	Stantec 2006
	Egg, Alevin, Fry	64-d IC_{25} (Growth)	718 (563-899)	Stantec 2006
	Egg, Alevin, Fry	64-d NOEC (Growth)	511	Stantec 2006
	Egg, Alevin, Fry	64-d LOEC (Growth)	1062	Stantec 2006

[1]Tested at water hardness of 310 $mg·L^{-1}$ as $CaCO_3$

A search of the primary literature for long-term nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation (Environment Canada, 2003). Four new published studies with 5 different fish species (lake whitefish *Coregonus clupeaformis*, topeka shiner *Notropis topeka*, medaka *Oryzias latipes*, fathead minnow *Pimephales promelas* and lake trout *Salvelinus namaycush*) were identified (Table 6.10). Two unpublished studies (Nautilus Environmental 2011; Elphick 2011) with 2 different fish species (rainbow trout *Oncorhynchus mykiss* and fathead minnow *Pimephales promelas*) were also identified and considered appropriate for consideration for guideline derivation since the studies utilized standardized toxicity testing methods (Table 6.10). Long-term data was also obtained for the Indian major carp (*Catla catl*a) (Tilak et al., 2002), but was classified as ancillary and not considered for guideline derivation (not listed in Table 6.10 but can be located in Appendix A). The complete list of long-term nitrate toxicity data for fish can be found in Appendix A.

Table 6.10. Freshwater long-term nitrate toxicity data for fish published since 2001.

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$)	Reference
FISH				
Coregonus clupeaformis (Lake whitefish)	Egg to Embryo	90-d LOEC (survival)	1772	McGurk et al., 2006
	Egg to Embryo	90-d NOEC (survival)	443	McGurk et al., 2006
	Embryo to Alevin	90-d LOEC (survival)	443	McGurk et al., 2006
	Embryo to	90-d NOEC	111	McGurk et al.,

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	Alevin	(survival)		2006
	Eyed-Embryo to Alevin	90-d LOEC (survival)	443	McGurk et al., 2006
	Eyed-Embryo to Alevin	90-d NOEC (survival)	111	McGurk et al., 2006
	Embryo to Fry	120-d LOEC (survival)	>443	McGurk et al., 2006
	Embryo to Fry	120-d NOEC (survival)	111	McGurk et al., 2006
	Eyed-Embryo to Fry	120-d LOEC (survival)	>443	McGurk et al., 2006
	Eyed-Embryo to Fry	120-d NOEC (survival)	443	McGurk et al., 2006
	Embryo to Alevin	90-d LOEC (hatching)	111	McGurk et al., 2006
	Embryo to Alevin	90-d NOEC (hatching)	28	McGurk et al., 2006
	Embryo to Fry	120-d LOEC (development)	111	McGurk et al., 2006
	Embryo toFry	120-d NOEC (development)	28	McGurk et al., 2006
	Alevin	120-d LOEC (behaviour)	>443	McGurk et al., 2006
	Alevin	120-d NOEC (behaviour)	443	McGurk et al., 2006
	Alevin	120-d LOEC (deformation)	>443	McGurk et al., 2006
	Alevin	120-d NOEC (deformation)	443	McGurk et al., 2006
	Fry	120-d LOEC (behaviour)	>443	McGurk et al., 2006
	Fry	120-d NOEC (behaviour)	443	McGurk et al., 2006
	Fry	120-d LOEC (deformation)	>443	McGurk et al., 2006
	Fry	120-d NOEC (deformation)	443	McGurk et al., 2006
Notropis topeka (Topeka shiner)	Juvenile	30-d NOEC (growth)	1186	Adelman et al. 2009
	Juvenile	30-d LOEC (growth)	2152	Adelman et al. 2009
	Juvenile	30-d MATC (growth)	1594	Adelman et al. 2009

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$)	Reference
Oncorhynchus mykiss (rainbow trout)	Fry (40d EAF test)	40-d LC_{10}	651 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{10}	>1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{10}	>1794 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{10}	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{25}	815 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{25}	>1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{25}	>1794 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{25}	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{50}	1041 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{50}	>1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{50}	>1794 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LC_{50}	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (survival)	199 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (survival)	598 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (survival)	1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF	40-d LOEC (survival)	>1794 (hardness 30-40 mg	Nautilus Environmental

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	test)		$CaCO_3$·L^{-1})	2011
	Fry (40d EAF test)	40-d NOEC (survival)	1794 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (survival)	>1794 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (survival)	1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (survival)	>1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (weight, wet wt)	421 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (weight, wet wt)	780 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (weight, wet wt)	585 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (weight, wet wt)	1484	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (weight, wet wt)	>1794 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (weight, wet wt)	>1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (weight, wet wt)	>1794 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (weight, wet wt)	>1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (weight, wet wt)	>1794 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (weight, wet wt)	>1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (weight, wet wt)	>1794 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry	40-d IC_{50}	>1794	Nautilus

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	(40d EAF test)	(weight, wet wt)	(hardness 110-120 mg $CaCO_3$·L^{-1})	Environmental 2011
	Fry (40d EAF test)	40-d NOEC (weight, wet wt)	199 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (weight, wet wt)	598 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (weight, wet wt)	598 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (weight, wet wt)	1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (weight, wet wt)	199 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (weight, wet wt)	598 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (weight, wet wt)	199 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (weight, wet wt)	598 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (length)	492 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (length)	>1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (length)	1085 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{10} (length)	>1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (length)	>1794 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (length)	>1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{25} (length)	>1794 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	Fry (40d EAF test)	40-d IC_{25} (length)	>1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (length)	>1794 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (length)	>1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (length)	>1794 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d IC_{50} (length)	>1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (length)	66 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (length)	199 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (length)	66 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (length)	199 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (length)	199 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (length)	598 (hardness 60-70 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (length)	1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (length)	>1794 (hardness 110-120 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{10} (proportion reaching swim-up)	58 (hardness 5-15 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{10} (proportion reaching swim-up)	>1794 (hardness 30-40 mg $CaCO_3$·L^{-1})	Nautilus Environmental 2011

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$)	Reference
	Fry (40d EAF test)	40-d EC_{10} (proportion reaching swim-up)	235 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{10} (proportion reaching swim-up)	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{25} (proportion reaching swim-up)	142 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{25} (proportion reaching swim-up)	>1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{25} (proportion reaching swim-up)	306 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{25} (proportion reaching swim-up)	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{50} (proportion reaching swim-up)	315 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{50} (proportion reaching swim-up)	>1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{50} (proportion reaching swim-up)	474 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d EC_{50} (proportion reaching swim-up)	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (proportion reaching swim-up)	66 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (proportion reaching swim-	199 (hardness 5-15 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$)	Reference
		up)		
	Fry (40d EAF test)	40-d NOEC (proportion reaching swim-up)	1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (proportion reaching swim-up)	>1794 (hardness 30-40 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (proportion reaching swim-up)	199 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (proportion reaching swim-up)	598 (hardness 60-70 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d NOEC (proportion reaching swim-up)	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
	Fry (40d EAF test)	40-d LOEC (proportion reaching swim-up)	>1794 (hardness 110-120 mg $CaCO_3·L^{-1}$)	Nautilus Environmental 2011
Oryzias latipes (Medaka)	Egg	124-d LOEC (hatching)	332	Shimura et al., 2002
	Adult	298-d NOEC (survival, growth, feeding)	111	Shimura et al., 2002
	Adult	298-d LOEC(survival)	443	Shimura et al., 2002
	Adult	298-d LOEC (growth)	332	Shimura et al., 2002
	Juvenile	298-d LOEC (feeding)	222	Shimura et al., 2002
Pimephales promelas (fathead minnow)	Juvenile	30-d NOEC (survival)	257	Adelman et al. 2009
	Juvenile	30-d LOEC (survival)	536	Adelman et al. 2009
	Juvenile	30-d MATC (survival)	372	Adelman et al. 2009
	Embryo-larval	30-d NOEC (growth)	695	Adelman et al. 2009
	Embryo-	30-d LOEC		Adelman et al.

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	larval	(growth)	1302	2009
	Embryo-larval	30-d MATC (growth)	952	Adelman et al. 2009
	Larvae (<24-h post-hatch)	7-d LC_{50}	501 (hardness 12 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LC_{50}	1,014 (hardness 50 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LC_{50}	1,772 (hardness 94 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LC_{50}	2,011 (hardness 168 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d IC_{25} (growth)	292 (hardness 12 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d IC_{25} (growth)	908 (hardness 50 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d IC_{25} (growth)	1506 (hardness 94 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d IC_{25} (growth)	1741 (hardness 168 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (survival)	222 (hardness 12 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (survival)	443 (hardness 50 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (survival)	886 (hardness 94 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (survival)	886 (hardness 168 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LOEC (survival)	443 (hardness 12 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LOEC (survival)	886 (hardness 50 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-	7-d LOEC (survival)	1772 (hardness 94 mg	Elphick 2011

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	hatch)		$CaCO_3$/L)	
	Larvae (<24-h post-hatch)	7-d LOEC (survival)	1772 (hardness 168 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (growth)	222 (hardness 12 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (growth)	886 (hardness 50 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (growth)	886 (hardness 94 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d NOEC (growth)	1772 (hardness 168 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LOEC (growth)	443 (hardness 12 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LOEC (growth)	1772 (hardness 50 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LOEC (growth)	1772 (hardness 94 mg $CaCO_3$/L)	Elphick 2011
	Larvae (<24-h post-hatch)	7-d LOEC (growth)	3544 (hardness 168 mg $CaCO_3$/L)	Elphick 2011
	<24 hour fertilized embryos	Embryo percent hatch NOEC	1954	US EPA 2010b
	<24 hour fertilized embryos	32-d LC_{50}	340	US EPA 2010b
	<24 hour fertilized embryos	32-d NOEC (survival)	217	US EPA 2010b
	<24 hour fertilized embryos	32-d LOEC (survival)	483	US EPA 2010b
	<24 hour fertilized embryos	32-d NOEC (growth)	217	US EPA 2010b
	<24 hour fertilized embryos	32-d LOEC (growth)	483	US EPA 2010b
	<24 hour	32-d LC_{25}	302	US EPA 2010b

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Reference
	fertilized embryos			
	<24 hour fertilized embryos	32-d LC_{20}	286	US EPA 2010b
	<24 hour fertilized embryos	32-d LC_{10}	246	US EPA 2010b
	<24 hour fertilized embryos	32-d EC_{50} (growth)	404	US EPA 2010b
	<24 hour fertilized embryos	32-d EC_{25} (growth)	289	US EPA 2010b
	<24 hour fertilized embryos	32-d EC_{20} (growth)	265	US EPA 2010b
	<24 hour fertilized embryos	32-d EC_{10} (growth)	207	US EPA 2010b
Salvelinus namaycush (Lake trout)	Egg to Embryo	120-d LOEC (survival)	>1772	McGurk et al., 2006
	Egg to Embryo	120-d NOEC (survival)	1772	McGurk et al., 2006
	Embryo to Alevin	90-d LOEC (survival)	>1772	McGurk et al., 2006
	Embryo to Alevin	90-d NOEC (survival)	1772	McGurk et al., 2006
	Eyed-Embryo to Alevin	90-d LOEC (survival)	>1772	McGurk et al., 2006
	Eyed-Embryo to Alevin	90-d NOEC (survival)	1772	McGurk et al., 2006
	Embryo to Fry	146-d LOEC (survival)	1772	McGurk et al., 2006
	Embryo to Fry	146-d NOEC (survival)	443	McGurk et al., 2006
	Eyed-Embryo to Fry	146-d LOEC (survival)	1772	McGurk et al., 2006
	Eyed-Embryo to Fry	146-d NOEC (survival)	443	McGurk et al., 2006
	Embryo to Alevin	90-d LOEC (hatching)	>1772	McGurk et al., 2006

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$)	Reference
	Embryo to Alevin	90-d NOEC (hatching)	1772	McGurk et al., 2006
	Embryo to Fry	146-d LOEC (developmental delay)	28	McGurk et al., 2006
	Embryo to Fry	146-d NOEC (developmental delay)	7	McGurk et al., 2006
	Alevin	120-d LOEC (deformation)	>1772	McGurk et al., 2006
	Alevin	120-d NOEC (deformation)	1772	McGurk et al., 2006
	Alevin	120-d LOEC (behaviour)	>1772	McGurk et al., 2006
	Alevin	120-d NOEC (behaviour)	1772	McGurk et al., 2006
	Fry	146-d LOEC (deformation)	>443	McGurk et al., 2006
	Fry	146-d NOEC (deformation)	443	McGurk et al., 2006
	Fry	146-d LOEC (behaviour)	>443	McGurk et al., 2006
	Fry	146-d NOEC (behaviour)	443	McGurk et al., 2006
	Fry	146-d LOEC (length)	443	McGurk et al., 2006
	Fry	146-d NOEC (length)	111	McGurk et al., 2006
	Fry	146-d LOEC (wet weight)	28	McGurk et al., 2006
	Fry	146-d NOEC (wet weight)	7	McGurk et al., 2006

McGurk et al. (2006) looked at long-term NO_3^- toxicity to embryos, alevins and swim-up fry of Lake whitefish (*Coregonus clupeaformis*) collected from Great Slave Lake, NWT and Lake trout (*Salvelinus namaycush*) from Lake Simcoe, ON following the same experimental design used for the Rainbow trout tests by Stantec (2006). In the long-term (130-150-d) egg-alevin-fry tests on Lake trout, fry were smaller and developed later at nitrate concentrations as low as 28 mg $NO_3^-·L^{-1}$. Results for Lake whitefish were similar; however the study had unacceptably high mortality in the controls. Based on Environment Canada's protocol for early life stage (egg-alevein-fry) toxicity testing of salmonid fish (rainbow trout), control survival should be ≥60% when 50% of the embryos reach the swim-up stage (Environment Canada 1998). In the McGurk et al. (2006) study, lake whitefish control survival was only 37.2% when embryos reached the swim-up stage, compared to lake trout control survival of 69.2% at the swim-up stage. The source of this high lake whitefish control mortality is thought to be due to the toxicity tests being run at too high a

temperature. McGurk et al. (2006) conducted the lake whitefish experiments at 7.5 ± 0.1°C. Price (1940) found 80% of whitefish eggs died before hatching at 8°C and recommended lake whitefish be kept at temperatures below 2°C to avoid temperature related mortality. Indeed, whitefish eggs are maintained at less than 2°C at Ontario's whitefish hatchery (Glenn Hooper, Ontario Ministry of Natural Resources, personal communication). It is likely temperature that influenced the results of the McGurk et al. (2006) toxicity results and therefore the data for lake whitefish was not considered for inclusion in the derivation of the CWQG. The results for the lake trout were found to be acceptable for inclusion in the long-term dataset.

Adelman et al. (2009) determined the sublethal effects of nitrate on the Topeka shiner (*Notropis topeka*), which was listed as an endangered species by the U.S. Fish and Wildlife Service in 1998. Data for the Topeka shiner was added to the short-term dataset because it is considered to be a close relative of the COSEWIC (Committee on the Status of Endangered Wildlife in Canada) endangered Pugnose shiner (*Notropis anogenus*) (COSEWIC 2002), for which no toxicity data was available. In the 30-d growth tests with juvenile Topeka shiners, the corresponding nitrate LOEC and NOEC values were 1,186 and 2,152 mg $NO_3^-{\cdot}L^{-1}$, with a MATC of 1,594 mg $NO_3^-{\cdot}L^{-1}$. Interestingly, Topeka shiners were found to be more tolerant of nitrate in the 30-d growth tests when compared to fathead minnows. Adelman et al. (2009) conducted 30-d growth tests with fathead minnows at the embryo-larval life stage, as well as with juveniles (7 months). Surprisingly, the juvenile life stage was more sensitive to nitrate when compared with the embryo-larval stage. The NOEC, LOEC and MATC for the juveniles was 257, 536 and 372 mg $NO_3^-{\cdot}L^{-1}$, respectively, whereas the NOEC, LOEC and MATC for the embryo-larval stage was 695, 1,302 and 952 mg $NO_3^-{\cdot}L^{-1}$, respectively. Two other studies also tested the effects of long-term exposures to nitrate on the fathead minnow. US EPA (2010b) ran an exposure of similar duration to that of Adelman et al. (2009), using the early life stage in a flow-through system for 32 days. The most sensitive endpoint was the 32-d EC_{10} (growth) of 207 mg $NO_3^-{\cdot}L^{-1}$. High quality data from an unpublished study by Elphick (2011) was also considered for long-term guideline derivation. The exposure duration in this case was 7 days, using exposure water of 4 different hardness levels (12, 50, 94, and 168 mg$\cdot L^{-1}$ as $CaCO_3$). The 7d IC_{25} (growth) ranged from 292 mg $NO_3^-{\cdot}L^{-1}$ (tested at a hardness of 12 mg$\cdot L^{-1}$ as $CaCO_3$) to 1741 mg $NO_3^-{\cdot}L^{-1}$ (tested at a hardness of 168 mg$\cdot L^{-1}$ as $CaCO_3$). The results from US EPA (2010b) was selected for inclusion in the long-term dataset for long-term guideline derivation.

Nautilus Environmental (2011) tested the toxicity of nitrate to the rainbow trout (*O. mykiss*) in exposure water of varying hardness (soft water, moderately hard water and hard water) to derive the respective 41-d L/IC_x and NOEC/LOEC values listed in Table 6.10. The results did not definitively demonstrate the relationship between increasing hardness and nitrate toxicity. For example, the 40-d LC_{25} was 815 mg $NO_3^-{\cdot}L^{-1}$ at a water hardness of 10 mg$\cdot L^{-1}$ as $CaCO_3$, whereas the 40-d LC_{25} was >1794 mg $NO_3^-{\cdot}L^{-1}$ at water hardnesses of 50, 92 and 176 mg$\cdot L^{-1}$ as $CaCO_3$, respectively. At times, sensitivity actually 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 $CaCO_3$). For example, 40-d EC_{10} (proportion reaching swim up) was >1794 mg $NO_3^-{\cdot}L^{-1}$ at a water hardnesses of 50 mg$\cdot L^{-1}$ as $CaCO_3$, whereas the 40-d EC_{10} (proportion reaching swim up) was 235 mg $NO_3^-{\cdot}L^{-1}$ at a water hardnesses of 92 mg$\cdot L^{-1}$ as $CaCO_3$.

Shimura et al. (2002) investigated the effects of accumulated nitrate on spawning, hatching and development of Medaka (*Oryzias latipes*) in ten-month, life-cycle experiments studying the efficiency of simple nitrifying and denitrifying filters. They noted that decreased feeding occurred at a LOEC of 222 mg $NO_3^-·L^{-1}$. Delayed hatching time and reduced hatching, as well as decreased growth, began at 332 mg $NO_3^-·L^{-1}$. Increased mortality occurred at a LOEC of 443 mg $NO_3^-·L^{-1}$ (Table 6.10). This data was not added to the data-set for guideline derivation because the Medaka is not resident, the study provided insufficient test details / water quality information, and there was a lack of statistical support.

With respect to long-term guideline value derivation, a total of 5 fish species were represented in the dataset. The most sensitive of the fish species was the lake trout (*Salvelinus namaycush*) with a 146-d MATC (wet weight) of 14 mg $NO_3^-·L^{-1}$ (McGurk et al. 2006). The next most sensitive fish species was the rainbow trout (*Oncorhynchus mykiss*) with a 41-d EC_{10} (proportion reaching swim-up) of 58 mg $NO_3^-·L^{-1}$ (Nautilus Environmental 2011). Interestingly, the 2 most sensitive invertebrates (*Ceriodaphnia dubia* and *Hyalella azteca*) had effect concentrations similar to that of the rainbow trout (7-d IC_{25} [reproduction] of 50 mg $NO_3^-·L^{-1}$ and a 14-d IC_{25} [growth] of 57 mg $NO_3^-·L^{-1}$, respectively). Both the rainbow trout and invertebrate exposures were conducted using what is considered CCME soft water, with a hardness ranging from 0-60 $mg·L^{-1}$ as $CaCO_3$. The most tolerant fish species in the long-term dataset were the Topeka shiner (*Notropis topeka*) and the chinook salmon (*Oncorhynchus tshawytscha*), with a 30-d MATC (growth) of 1594 mg $NO_3^-·L^{-1}$ (Adelman et al. 2009) and a 10-d LC_{10} of 3142 mg $NO_3^-·L^{-1}$ (Westin 1974), respectively (see Table 7.8 in Section 7.1.3.2).

In general, early life stages of freshwater fish were found to be the most sensitive to nitrate exposure (the following studies were not included in the dataset for long-term guideline derivation). Eggs and fry of two salmon and three trout species were exposed to $NaNO_3$ concentrations ranging from 3 to 30 mg $NO_3^-·L^{-1}$ in flow-through systems with low water hardness (25 to 39 mg $CaCO_3·L^{-1}$) for a period lasting from egg fertilization to 30 days past yolk absorption (first feeding stage) (Kincheloe et al. 1979). Significant increases ($p \leq 0.05$) in total mortality for anadramous steelhead and freshwater rainbow trout (both *Oncorhynchus mykiss*) were found at nitrate concentrations of 5 and 10 mg $NO_3^-·L^{-1}$, respectively. Significant mortality was also found for chinook salmon fry at 20 mg $NO_3^-·L^{-1}$ and Lahontan cutthroat trout (*Salmo clarki*) eggs and fry at 20 and 30 mg $NO_3^-·L^{-1}$, respectively (Kincheloe et al. 1979). The authors also found morphological abnormalities in some surviving fry, however details were not provided. Although this study demonstrated sensitivity of eggs and early salmonid life stages to nitrate, additional egg mortalities caused by *Saprolegnia* fungal infestations could not be segregated from the data by the authors, therefore the results of this study were not considered useable for CWQG development. In a study looking at the effects of eutrophication on carp reproduction, Bieniarz et al. (1996) exposed fertilized eggs to sodium nitrate concentrations of 15, 150 and 500 mg $NO_3^-·L^{-1}$. The percentage of eggs hatching was significantly lower ($p < 0.01$) than that in the control at all experimental concentrations, suggesting that levels of nitrate normally found in the environment may lower the reproductive effort in carp (Bieniarz et al. 1996). It should be noted, however, that even within the control group there was a very low hatch rate of approximately 48%.

Fingerling and juvenile stages of fish are significantly more sensitive to nitrate exposure than egg stages. This is likely related to oxygen carrying capacity in the blood (which is a non-factor in

the egg stage). For example, this difference in sensitivity can be seen in the Stantec (2006) data for *O. mykiss*, where the 34-d EC25 for the egg lif stage is 2168 mg $NO_3^-·L^{-1}$, and in comparison, the 64-d IC25 growth for the fry life stage is 718 mg $NO_3^-·L^{-1}$ (Appendix A).

Fingerlings of chinook salmon and rainbow trout were exposed in fresh water to $NaNO_3$ for 10 days to a maximum concentration of 6500 mg $NO_3^-·L^{-1}$, with renewal of the test solutions after 4 days (Westin 1974). Median lethal tolerance limits (7-d TL_m) for these older salmonids are 4800 and 4700 mg $NO_3^-·L^{-1}$, respectively (Westin 1974). Behavioural responses to nitrate exposure for the fish in this study included an inability to swim upright, laboured respiration, reduced movement with erratic swimming, yawning, and accelerated opercular movements. For all exposure concentrations, no abnormalities were found in tissues examined histopathologically (Westin 1974). [Note: Westin also conducted toxicity tests with these two fish species in saline water. Those results are discussed in Section 6.4.2.2].

Channel catfish (*Ictalurus punctatus*) juveniles are similarly tolerant to nitrate. In an observational study on increasing catfish populations in a closed, recirculating system, Knepp and Arkin (1973) found ambient nitrate concentrations allowed to reach 400 mg $NO_3^-·L^{-1}$ over 170 days did not have an impact on individual growth or behaviour (e.g., lethargy). In a ten-week study of the humoral immune response of channel catfish exposed to low (558 mg $NO_3^-·L^{-1}$) and high (1280 mg $NO_3^-·L^{-1}$) nitrate levels, Collins et al. (1976) did not find a consistent effect on antibody levels of the fish, suggesting that those levels of nitrate stress did not significantly increase immunosuppression in *I. punctatus*.

In contrast to Kincheloe et al. (1979), Scott and Crunkilton (2000) found significant failures of hatching for fertilized *P. promelas* eggs only at 6353 mg $NO_3^-·L^{-1}$. The difference in susceptibility of the fertilized eggs could be species-specific, as *P. promelas* incubation time is only 4 days, compared to over 30 days for the salmonids (Scott and Crunkilton 2000). Chronic nitrate exposure to fathead minnows produced 7-d larval and 11-d embryo-larval LOECs (with growth as the endpoint) of 3176 mg $NO_3^-·L^{-1}$ (Scott and Crunkilton 2000). At this exposure level, larvae were lethargic and exhibited bent spines before death (Scott and Crunkilton 2000).

The lethal concentration limits for sticklebacks (*Gasterosteus aculeatus*) 30 - 50 mm in length were 1348 mg $NO_3^-·L^{-1}$ for exposure to $NaNO_3$ for 10 days, and 79 mg $NO_3^-·L^{-1}$ for exposure to KNO_3 (Jones 1939).

Sub-lethal, physiological endpoints in the perch (*Perca fluviatilis*) and the Crusian carp (*Cyprinus carassius*), were also not significantly altered at environmental nitrate concentrations. Lahti et al. (1985) found no clear relationship between nitrate levels up to 11.0 mg $NO_3^-·L^{-1}$ and radioiodine accumulation in organs, suggesting uptake of iodide (a trace element required for normal physiological functioning in fish) (Heath 1995), is not affected at environmental levels of nitrate.

Methaemoglobin in the blood of rainbow trout, which occurred at 1% in control treatments, reached elevated levels of 21 and 27% when the fish were exposed for 11 weeks to 26 mg $NO_3^-·L^{-1}$ [as $Ca(NO_3)_2$] and 31 mg $NO_3^-·L^{-1}$ (as KNO_3), respectively (Grabda et al. 1974). These increased rates of methaemoglobin formation corresponded to a dramatic decline in hepatic tissue respiration rates (up to 48%) which, according to the authors, would result in

extreme physiological stress (Grabda et al. 1974). This study, however, was not used to derive guideline values as there was a large range in water oxygen levels among the test aquaria (3.1 to 7.8 $mg \cdot L^{-1}$), which may have promoted the reduction of nitrate to nitrite by anaerobic bacteria in the surrounding water for some treatments. All 20 experimental fish for each nitrate salt treatment were held in the same aquarium, resulting in insufficient replication. As only one test concentration was administered for each salt, it was also not possible to determine a dose-response relationship for methaemoglobin formation in the trout.

In a study looking at the effects of eutrophication on carp reproduction, Bieniarz et al. (1996) exposed fertilized eggs to sodium nitrate concentrations of 15, 150 and 500 mg $NO_3^- \cdot L^{-1}$. The percentage of eggs hatching was significantly lower ($p < 0.01$) than that in the control at all experimental concentrations, suggesting that levels normally found in the environment may reduce the reproductive effort in carp (Bieniarz et al. 1996). This study was not used for deriving the freshwater guideline, however, for two reasons. First, only nominal concentrations were reported, with no analytical confirmation of the nitrate levels in the test vessels. Second, the hatching success in the control group was quite low, at approximately 48%. Various other authors report hatch rates of greater than 90% for carp eggs under control conditions (Huckabee and Griffith 1974; Mattice et al. 1981; Oyen et al. 1991; Kaur et al. 1993). This suggests that there may have been some problem with the experimental conditions or the condition of the test organisms in the study by Bieniarz et al. (1996).

Lahti et al. (1985) also found that nitrate levels between 0.88 and 1.5 mg $NO_3^- \cdot L^{-1}$ were sufficient to inhibit iodine uptake in the thyroids of Crusian carp (*Carassius carassius*), rainbow trout, and perch (*Perca fluviatilis*). However, when these fish were subjected to higher nitrate levels (up to 11 mg $NO_3^- \cdot L^{-1}$), iodine uptake in the thyroid appeared to be activated; therefore a clear dose-response relationship was not established.

6.4.2.3 Amphibians

A search of the primary literature for long-term amphibian nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation (Environment Canada, 2003). No new data from either primary or secondary studies was found for inclusion in the long-term dataset for guideline derivation.

Observed toxic responses to nitrate exposure for amphibian species include reductions in egg hatching success, increases in embryo and larval (tadpole) mortality and developmental impacts including decreased length and weight and the appearance of deformities (Appendix A). Amphibians are particularly relevant ecological receptors because they often inhabit surface waters that collect agricultural drainage. As breeding season in the spring tends to coincide with fertilizer application, developing eggs and embryos are placed in contact with potentially elevated nitrate pulses (Hecnar 1995).

Of the primary studies available, the red-legged frog embryos (*Rana aurora*) collected from the Cascade mountains of western Oregon, USA, were the most susceptible to nitrate with a LOEC of 129 mg $NO_3^- \cdot L^{-1}$ significantly reducing overall length after 16 days exposure in soft well water (Schuytema and Nebeker 1999b). The 16-d MATC (weight) of 734 mg $NO_3^- \cdot L^{-1}$ was used

for guideline derivation. Growth of the common northern leopard frog (*Rana pipiens*) was also significantly reduced ($F_{2,213} = 4.04$, $p = 0.019$) by ~2 mm over a 9 week period from exposure to 133 mg $NO_3^-·L^{-1}$ in hard water (324 mg $CaCO_3·L^{-1}$) (Allran and Karasov 2000). Impacts on growth could have a significant detrimental impact on the frog's size at maturity, rate of sexual maturation, mate selection, rate of locomotion for predator evasion and overall probability of survival (Allran and Karasov 2000). In this case however, even though the observed growth inhibition was statistically significant, Allran and Karasov (2000) state that this finding does not imply ecological significance. There are many other natural environmental variables, both biotic and abiotic, that can affect growth of anuran larvae to a greater degree than that caused by nitrate exposure (e.g. food availability, temperature, density of larvae, reduction in water volume, and presence of predators or competitors). Therefore the results for the northern leopard frog (*R. pipiens)* were not included in the data-set for long-term guideline derivation.

Schuytema and Nebeker (1999a,c) demonstrated that younger (embryonic) amphibian life stages can be more sensitive to nitrates than more developed larval forms. The length of developing embryos of *P. regilla* was also restricted at lower nitrate levels (10-d LOEC = 492 mg $NO_3^-·L^{-1}$) as compared to nitrate levels for tadpoles (10-d LOEC = 1148 mg $NO_3^-·L^{-1}$) (Schuytema and Nebeker 1999a,c; Appendix A).

The African clawed frog (*Xenopus laevis*), a common laboratory test organism showed toxic responses in a similar range to native North American frog species. Five-day LOECs for *X. laevis* embryos were 251, 492 and 1021 mg $NO_3^-·L^{-1}$ for changes in weight, length and deformities, respectively (Schuytema and Nebeker 1999c). The datapoint used for guideline derivation was the 10-d MATC (weight) of 404 mg $NO_3^-·L^{-1}$ (Schuytema and Nebeker 1999c). Physical deformities noted for *X. laevis* and *P. regilla* at concentrations from 492 to 4338 mg $NO_3^-·L^{-1}$ included cardiac and abdominal edemas and lordosis (curvature of the spine) (Schuytema and Nebeker 1999a). The chronic mortality estimate for *P. regilla* larvae (10-d LC_{50} = 1179 mg $NO_3^-·L^{-1}$) was ~15% of the acute value (96-h LC_{50} = 7752 mg $NO_3^-·L^{-1}$) (Schuytema and Nebeker 1999c). Exposure period had a substantial effect on mortality for the pacific tree frog (*Pseudacris regilla*), with a 10-d LC_{50} value of 1180 mg $NO_3^-·L^{-1}$, compared to a 4-d LC_{50} value of 7752 mg $NO_3^-·L^{-1}$ (Schuytema and Nebeker 1999c). The calculated 10-d LC_{10} of 328 mg $NO_3^-·L^{-1}$ (see Table 7.6) was used as an endpoint in guideline derivation (Schuytema and Nebeker 1999a) , since regression-based no effect concentrations (e.g. LC_{10}) are preferred for inclusion in guideline datasets (CCME 2007).

Larvae of tree frogs (*Litoria caerulea*), and the common toad (*Bufo bufo*) were highly sensitive to $NaNO_3$ exposure in distilled water. Data from these studies was excluded for guideline derivation due to the use of distilled water as exposure water. Following 13 days of exposure to 40 mg $NO_3^-·L^{-1}$, the mean length of exposed larvae was significantly reduced ($p < 0.05$) from approximately 25 to 17 mm and survival was reduced from 92 to 15% ($p < 0.05$) (Baker and Waights 1993). Baker and Waights (1994) found no difference in tadpole growth between 40 and 100 mg $NO_3^-·L^{-1}$ treatments, but growth in these treatment groups was reduced relative to controls, from approximately 43 to 20 mm ($p < 0.05$). Survival was also significantly reduced from 77% in controls to 46% in treatments ($p < 0.05$), and of the remaining larvae, significantly fewer had attained the developmental Gosner stage 27 (9%) than in controls (76%; $p < 0.001$). Underdeveloped larvae can be more susceptible to predation, be less able to escape unfavourable environmental conditions, or have reduced adult body size; all of which can ultimately reduce

survival (Baker and Waights 1994).

In contrast to the studies of Baker and Waights (1993, 1994), there were no significant effects on the proportion of eggs hatching or of deformed larvae in two species of salamander (*Ambystoma jeffersonianum* and *A. maculatum*), the American toad (*B. americanus*) or the wood frog (*Rana sylvatica*) when exposed to 40 mg NO_3^-·L^{-1} for a maximum of 44 days in irrigated pond water (Laposata and Dunson 1998). A deformity involving substantial curling of the spines to a crescent shape, resulting in reduced swimming speeds and swimming in helical patterns, was observed in the wood frog larvae. However, there was no statistically significant difference in the frequency at which this physical deformity occurred among the control and treatment groups.

Synergistic effects from other environmental stressors on amphibian egg survival (also applicable to invertebrates and fish) are possible, and potential interactive effects with nitrate should not be ruled out (Laposata and Dunson 1998). Survival and activity levels in larval Cascades frogs (*Rana cascadae*) from Oregon have been shown to be significantly reduced in the presence of high levels of nitrate (20 mg NO_3^-·L^{-1}), ultraviolet radiation (UV-B; 280 - 315 nm) and low pH (pH 5), while not being significantly affected by high nitrate levels alone (Hatch and Blaustein 2000). Romansic et al. (2006) looked at the possible interaction between the pathogenic water mold *Saprolegnia* and nitrate (0, 5, 20 mg NO_3^-·L^{-1}) to three species of amphibians. These included the northwestern salamander (*Ambystoma gracile*), the Pacific treefrog (*Hyla regilla*) and the red-legged frog (*Rana aurora*). Survival of *H. regilla* was not affected. Survival of *R. aurora* was affected by a less-than-additive interaction between *Saprolegnia* and nitrate, where increased nitrate prevented *Saprolegnia* from causing mortality to *R. aurora*. Survival of *A. gracile* followed a similar pattern to that of *R. aurora*. One point to note about this study is that the authors added water conditioners (Novaqua® and Amquel®) to the exposure waters, which coats aquarium animals and prevents the uptake of nitrite, which may have confounded the results.

Amphibian responses to exposure from KNO_3 resulted in acute toxicity at lower concentrations than $NaNO_3$, with 15-d LC_{50} estimates of 73 mg NO_3^-·L^{-1} for the Oregon spotted frog (*Rana pretiosa*) and 104 mg NO_3^-·L^{-1} for the northwestern salamander (*Ambystoma gracile*) (Marco et al. 1999). At higher nitrate exposures (up to 111 mg NO_3^-·L^{-1}), Marco et al. (1999) found evidence of reduced feeding activity and swimming vigor, disequilibrium, physical abnormalities (mainly edemas and bent tails), paralysis, and death in *R. pretiosa* and *A. gracile*. In contrast, other species tested, namely the Western toad (*Bufo boreas*) and Pacific tree frog experienced very little mortality or sub-lethal effects at all concentrations, suggesting differential responses to nitrate exposure between amphibian species (Marco et al. 1999).

Hecnar (1995) found chorus frogs (*Pseudacris triseriata*) and leopard frogs (*Rana pipiens*) had significantly lower survivorship at nitrate concentrations as low as 44.3 mg NO_3^-·L^{-1} (10 mg NO_3^--N·L^{-1}) after 100-d of exposure. Hecnar (1995) used ammonium nitrate as his nitrate source because of interest in the effects of ammonium nitrate fertilizer on amphibian populations. Evidence suggests that in tests with ammonium nitrate, toxic effects observed may be due to the ammonium ion, rather than the nitrate ion; hence, the results of this study were not considered useable for CWQG development.

In an evaluation of how environmentally-relevant NO_3^- concentrations influence time to

metamorphosis for Southern toad (*Bufo terrestris*), Edwards et al. (2006) compared the toxic effects of nitrate added to reverse-osmosis water with electrolytes added back (RO_e) to effects when nitrate is added to natural spring water. They found toads in 133 mg $NO_3^-{\cdot}L^{-1}$ in RO_e water displayed nitrate stress responses typical of other laboratory toxicity tests and metamorphosed 5 days earlier than control animals in RO_e water without nitrate. Animals tested in spring water delayed metamorphosis by 7 days compared to animals in spring water with no added nitrate. These animals were also larger suggesting additional chemical stressors in spring water combined with nitrate toxicity to effect growth rates in toads (Edwards et al., 2006).

Effects on growth rate, as well as size and age at metamorphosis for larvae of the European common frog (*Rana temporaria*) were observed at a concentration of 22 mg $NO_3^-{\cdot}L^{-1}$ (Johansson et al. 2001). These effects were marginal, however, and were observed with frogs from one region, but not from another. A clear dose-response relationship was not demonstrated, as effects were only observed at the highest concentration tested. Also, this species is not native to Canada. Due to these various factors, the data could not be used in deriving the guideline.

Tadpoles of the common toad (*B. bufo*) and the tree frog (*L. caerulea*) showed significant reductions ($p < 0.05$) in growth when exposed to 40 mg $NO_3^-{\cdot}L^{-1}$ for 16 days (Baker and Waights 1993, 1994 respectively). At this concentration, significantly fewer ($p < 0.05$) of the surviving *L. caerulea* reached the Gosner developmental stage 27 than those in controls (Baker and Waights 1994). However, these studies were not considered for guideline development because: neither of these species is native to Canada; distilled water was used as the test medium, which may have placed the tadpoles under additional ionoregulatory stress; and nitrate levels in some chambers of the 1994 study decreased by as much as 50%.

6.4.2.4 Plants and Algae

Nitrate is a required element for plant growth, and due to its greater abundance in surface waters relative to other fixed nitrogen species (e.g., ammonium), it is the most widely used form of nitrogen by vascular plants and algae (Pinar et al. 1997; Crouzet et al. 1999). As nitrate is actively taken up by aquatic primary producers, its uptake is generally not limited by low environmental concentrations (Cresswell and Syrett 1981; Pinar et al. 1997).

Results from the tissue analysis of half a dozen macrophyte species suggest a minimum of 1.3% nitrogen per dry weight of plant tissue is necessary for macrophyte growth (Gerloff and Krombholz 1966, as cited in Forsberg 1975). No effect on the yield occurred when tissue nitrogen content was above this critical concentration. The critical nitrogen concentration for the blue-green algae *Microcystis aeruginosa* was determined to be 4% (Gerloff and Skoog 1954, as cited in Forsberg 1975).

Only one study was located that directly tested nitrate toxicity to aquatic primary producers. A 72-h IC_{25} (algal cell growth inhibition) of 3061 mg $NO_3^-{\cdot}L^{-1}$ for the green algae *Pseudokirchneriella subcapitata* was obtained by Elphick (2011). Incubation studies using the alga *Scenedesmus subspicatus* showed all levels of sodium nitrate that were added to the test medium (from 4 to 285 mg $NO_3^-{\cdot}L^{-1}$) increased algal growth, with maximum growth occurring at 55 mg $NO_3^-{\cdot}L^{-1}$ (Hund 1997).

Although not directly toxic to the plants, nitrate taken up by aquatic plants could prove to be an environmental hazard to herbivorous consumers. From agricultural studies, it is known that an excess of nitrate in fodder can be toxic to livestock. A nitrate-nitrogen content of around 0.2% dry wt. is generally accepted as the upper limit for forage crops used for livestock feeds; however, toxic effects may occur at nitrate concentrations as low as 0.07% if that crop is the sole food source (Tucker and Debusk 1983). Aquatic plants can sequester nitrate to levels above the safe level for livestock. For example, Tucker and Debusk (1983) examined NO_3^--N uptake in water hyacinth (*Eichhornia crassipes*) cultured for one year in a flow-through system with an ambient concentration of 1.4 mg $N \cdot L^{-1}$. Plant tissue nitrate-nitrogen content ranged from 0.05 to 0.21% dw (= 3.2% total nitrogen dw, assuming NO_3^--N accounts for 6.6% of the total nitrogen), with the greatest concentrations accumulating in the plant during the slow growing fall and winter months. For ten of the twelve study months (April and May excluded) *E. crassipes* grown in water with 1.4 mg NO_3^--$N \cdot L^{-1}$ had NO_3^--N contents $\geq$ 0.07% dry wt. Unfortunately, no information is available on the effects of elevated nitrate levels in aquatic plants to aquatic and terrestrial consumers of those plants. Nonetheless, the possibility exists that secondary poisoning through elevated plant nitrate levels could occur even though ambient water levels of nitrate are not directly toxic to aquatic life.

Plant toxicity data were not included in the development of the long-term nitrate guideline value as nitrate is a plant nutrient.

6.5 Toxicity to Marine Life

There are relatively few studies available on nitrate toxicity to marine fish. With the exception of Westin (1974), those which do exist are on tropical or subtropical species (Brownell 1980; Frakes and Hoff Jr. 1982; Pierce et al. 1993). There appears to be a greater body of information on nitrate toxicity responses from commercially important marine invertebrates in aquaculture operations, such as prawns, crayfish and bivalves (Epifanio and Srna 1975; Wickins 1976; Muir et al. 1991; Meade and Watts 1995). Toxic responses to marine organisms include mortality, reductions in feeding and growth, and physiological responses such as respiration and cellular changes. As with freshwater animals, invertebrates, especially during larval stages, tend to be more sensitive to nitrate than fish (Appendix B).

6.5.1 Short-Term Marine Toxicity Data

6.5.1.1 Invertebrates

A search of the primary literature for nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation. Web of Science was searched using keywords including: nitrate, toxicity, marine, saltwater, salinity and aquatic.

One new study was identified to contain toxicity data for juvenile tiger shrimp (*Penaeus monodon*) (Table 6.11). Tsai and Chen (2002) investigated the short-term toxicity of different nitrate concentrations at varying salinities. They determined safe concentrations in which to rear juvenile tiger shrimp as 642, 700 and 1029 mg $NO_3^- \cdot L^{-1}$ at salinities of 15, 25 and 35‰ respectively. They also derived an equation to express the relationships among nitrate, salinity

and exposure time. Essentially, Tsai and Chen (2002) found that both salinity and exposure time had a significant effect on the resulting LC50 value. Statisitcal analysis also indicated that there was a significant interaction between salinity and exposure time on the LC50.

Only two invertebrate species were included in the short-term dataset for nitrate benchmark concentration derivation. This included the tiger shrimp (*Penaeus monodon*) and the prawn (*Penaeus paulensis*).

The tiger shrimp *Penaeus monodon* was the marine species most sensitive to nitrate exposure (Appendix B). Tsai and Chen (2002) exposed juvenile tiger shrimp (*P. monodon*) to nitrate for 96-h exposure periods. The geometric mean (7717 mg $NO_3^-{\cdot}L^{-1}$) of three 96-h LC_{50} effect concentrations (6419, 6977 and 10260 mg $NO_3^-{\cdot}L^{-1}$) was input into the short-term dataset.

Muir et al. (1991) also exposed penaeid larvae to potassium and sodium nitrate salts at 1, 10 and 100 mg $NO_3^-{\cdot}L^{-1}$ in 40-h static tests. Significant mortality ($p < 0.01$) was observed at 1 mg $NO_3^-{\cdot}L^{-1}$ for both potassium (37% mortality) and sodium (31% mortality) salts. Histological examination of surviving larvae revealed vacuolation and shrinkage of the ganglionic neuropiles, and minor muscle fragmentation and shrinkage. At 10 and 100 mg $NO_3^-{\cdot}L^{-1}$, effects also included the splitting of the hypodermis from the cuticle and cytoplasmic vacuolation of cells in the midgut and proventriculus (Muir et al. 1991).

Table 6.11. Marine invertebrate nitrate toxicity data published since 2001.

Organism	Life Stage	Endpoint	Effect concentration (mg $NO_3^-·L^{-1}$)	Salinity (‰)	Reference
INVERTEBRATES					
Penaeus monodon (Tiger shrimp)	Juvenile	48-h LC_{50}	12 741	15	Tsai and Chen, 2002
	Juvenile	48-h LC_{50}	17 250	25	Tsai and Chen, 2002
	Juvenile	48-h LC_{50}	10 260	35	Tsai and Chen, 2002
	Juvenile	72h LC_{50}	7633	15	Tsai and Chen, 2002
	Juvenile	72-h LC_{50}	11 102	25	Tsai and Chen, 2002
	Juvenile	72-h LC_{50}	15 616	35	Tsai and Chen, 2002
	Juvenile	96-h LC_{50}	6419	15	Tsai and Chen, 2002
	Juvenile	96-h LC_{50}	6977	25	Tsai and Chen, 2002
	Juvenile	96-h LC_{50}	10 260	35	Tsai and Chen, 2002

The prawn larvae in the Muir et al. (1991) study moulted from Protozoea I to Protozoea II stage during the trials. As crustaceans are reportedly more susceptible to toxins during the sensitive ecdysis stage (moulting), the increased susceptibility to nitrate found by Muir et al. (1991) is likely due to developmental sensitivity. This level of sensitivity to nitrate exposure is not seen in older penaeid shrimp. Wickins (1976), found that the 48-h LC_{50} for five species of penaeids (pooled) was 15 062 mg $NO_3^-·L^{-1}$. Adult penaeid shrimp (*Penaeus paulensis*) were similarly tolerant to high nitrate exposure, with a 96-h LC_{50} of 9621 mg $NO_3^-·L^{-1}$ (Cavalli et al. 1996).

Juvenile and adult hard clams (*Mercenaria mercenaria*) and American oysters (*Crassostrea virginica*) from the U.S. east coast (Delaware) were found to be extremely tolerant to nitrate (Epifanio and Srna 1975). For both species, sublethal responses (20-h ECs for reduced feeding) and acute 96-h LC_{50}s ranged from 2480 to > 19 840 mg $NO_3^-·L^{-1}$ as $NaNO_3$, suggesting that these species are insensitive to acute exposures of environmentally relevant levels (Epifanio and Srna 1975).

6.5.1.2 Fish

A search of the primary literature for nitrate toxicity studies published after 2001 was conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation. Web of Science was searched using keywords including: nitrate, toxicity, marine, saltwater, salinity and aquatic. No additional short-term toxicity data was found for marine fish.

There are few studies available on nitrate toxicity to marine fish. Pierce et al. (1993) tested the responses of five tropical and sub-tropical marine fish to increasing sodium nitrate levels in response to concern over elevated nitrate levels in recirculating aquarium systems. All five species were tolerant to nitrate in 32‰ salinity seawater with 96-h LC_{50} values ranging from 2538 mg $NO_3^-·L^{-1}$ for the planehead filefish (*Monacanthus hispidus*) to > 13 290 mg $NO_3^-·L^{-1}$ for beaugregory (*Pomacentrus leucostictus*) (Pierce et al. 1993). Effect concentrations for these 5 fish species were included in the dataset for short-term benchmark concentration derivation (Table 7.10).

The only data located on nitrate toxicity to temperate marine fish species were for chinook salmon and rainbow trout reared in 15‰ salinity reconstituted seawater (Westin 1974). The salmonids were exposed to $NaNO_3$ for 7 days, with renewal of the test solution after 4 days, to a maximum concentration of 6500 mg $NO_3^-·L^{-1}$, resulting in a 7-d TL_m of 4000 mg $NO_3^-·L^{-1}$, for both species. All trout exhibited acute signs of toxic stress after 2 days of exposure; however, chinook exposed at ≤ 4400 mg $NO_3^-·L^{-1}$ did not exhibit toxic stress symptoms until after 5 to 8 days. Symptoms included an inability to swim upright, laboured respiration, and reduced movement with erratic swimming. Other behavioural signs of stress included yawning, or gulping, and accelerated opercular movements, with some fish breaking the surface of the water (Westin 1974). None of these behavioural modifications were observed in fish from control tanks. Westin (1974) also proposed safe concentrations of 25 to 35 mg $NO_3^-·L^{-1}$ for hatchery-reared salmonids based on $1/100^{th}$ of the 7-d LC_{10} at 15‰ salinity (not reported in Appendix B).

The nitrate concentration required to reduce first-feeding incidence by 50% after a 24-h exposure (24-h first feeding EC_{50}) in marine fish larvae was assessed for four species of sub-tropical fish from South Africa (Brownell 1980). Again, all four species were found to be very tolerant to nitrate, with EC_{50} values ranging from 2658 to 4582 mg $NO_3^-·L^{-1}$. A shorter exposure time (24-h) to nitrate was used in this study to avoid potential complications with the sensitive timing to first feeding event, as prolonged toxicant exposure to marine teleost eggs and larvae can delay development (Brownell 1980). Acute mortality (24-h LC_{50}) values of up to 22 372 mg $NO_3^-·L^{-1}$ were observed (Appendix B), but Brownell (1980) demonstrated that mortality at these high levels of $NaNO_3$ were just as likely due to the elevated salinity of the treatment waters.

Mortality responses in marine fish tend to be similar to that of freshwater fish, with acute and chronic LC50 values ranging from approximately 2500 to 10 600 mg $NO_3^-·L^{-1}$ (Appendix B). In a direct comparison between freshwater and marine conditions, Westin (1974), exposed rainbow trout and Chinook salmon to sodium nitrate at concentrations ranging between 3500 and 6500 mg $NO_3^-·L^{-1}$ in freshwater and 15% salinity reconstituted seawater for 96 hours. It was found that nitrate was 1.3 times more toxic in saltwater for both species; however, no explanation was given for the increase in toxicity with increasing salinity.

If data for species native to Canadian marine waters is not available, and minimum data set requirements can be set by including data for tropical or subtropical species, so long as the test exposure temperatures are relevant to Canadian temperate waters.

6.5.2 Long-Term Marine Toxicity Data

6.5.2.1 Invertebrates

To address the data gaps identified during the derivation of the 2003 interim NO_3^- CWQG for the protection of marine aquatic life, a toxicity test was commissioned for a long-term fish study on a marine invertebrate endemic to Canadian coastal waters. Full results are reported elsewhere in Stantec (2006). Toxicity of nitrate to the Pacific purple sea urchin (*Strongylocentrotus purpuratus*) was assessed according to the echinoid embryo development test in which newly fertilized eggs were exposed to nitrate under static test conditions and ambient light levels for four days (Environment Canada, 1992; ASTM, 1995; Stantec, 2006). Nitrate toxicity tests were conducted following standard toxicological laboratory methods involving either static or static renewal exposure conditions, and were run using sodium nitrate ($NaNO_3$). As well, all tests satisfied the minimum requirements for test validity as outlined in the specific test methods. Discussion on why the four-day test was included in the long-term dataset can be found in Section 7.2.3.2.

Results of the definitive test with Pacific purple sea urchin are provided in Table 6.12. The result values were typical of other studies on similar species (Environment Canada, 2003; Appendix B).

Table 6.12. Results of the nitrate (as $NaNO_3$) toxicity test to the Pacific purple sea urchin (*Strongylocentrotus purpuratus*). Table values are expressed in mg $NO_3^-\cdot L^{-1}$ and include 95% confidence limits in parentheses.

	Life Stage	**Endpoint**	**Effect Concentration (mg $NO_3^-\cdot L^{-1}$)**	**Reference**
Stronglyocentrotus purpuratus (Pacific purple sea urchin)	Embryo	4-d IC_{25} (Larval Development)	1178 (1162 – 1192)	Stantec, 2006

A search of the primary literature for nitrate toxicity studies published after 2001 was also conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation. No additional long-term toxicity data for marine invertebrates was found. The complete list of nitrate toxicity data can be found in Appendix B.

A total of 8 effect concentrations for 8 marine invertebrate species were included in the long-term dataset. Four species of polychaetes were found to be the most sensitive to nitrate exposures, with effect concentrations ranging from a 28-d LC_{10} of 214 mg $NO_3^-\cdot L^{-1}$ for *Nereis grubei* (Reish 1970) to a 28-d LC_{10} of 700 mg $NO_3^-\cdot L^{-1}$ for *Dorvillea articulata* (Reish 1970). The least sensitive of the 8 species was the Australian crayfish (*Cherax quadricarinatus*) with a 5-d LOEC (respiration) of 4430 mg $NO_3^-\cdot L^{-1}$ (Meade and Watts 1995) (Table 7.14).

Polychaetous annelids collected from the vicinity of a domestic sewage outfall in California were exposed to KNO_3 in a static 28-day test (Reish 1970). Median lethal mortalities (28-d TL_m) for

the semi-healthy zone indicator species *Neanthes arenaceodentata* and *Dorvillea articulata* were 496 and 880 mg $NO_3^-·L^{-1}$, respectively, and 329 mg $NO_3^-·L^{-1}$ for *Nereis grubei* which are found in healthy zones surrounding the outfalls.

Basuyaux and Mathieu (1999) tested growth (as daily % increase in mass) and feeding rate ($g·kg^{-1}·d^{-1}$) in sea urchin (*Paracentrotus lividus*) and abalone (*Haliotis tuberculata*) in response to increasing nitrate concentrations (0 to 1108 mg $NO_3^-·L^{-1}$). Safe levels resulting in 1% mortality, were determined to be around 443 mg $NO_3^-·L^{-1}$ for *P. lividus*, and between 443 and 1108 mg $NO_3^-·L^{-1}$ for *H. tuberculata*. At 1108 mg $NO_3^-·L^{-1}$, statistically significant decreases in growth relative to controls of 76% and 71% were seen for sea urchins and abalone, respectively ($p < 0.001$). A concentration of 1108 mg $NO_3^-·L^{-1}$ also resulted in a statistically significant decrease in feeding rate for sea urchins of 46% ($p < 0.001$). For abalone, a slight (but not statistically significant) increase in growth was seen up to 222 mg $NO_3^-·L^{-1}$, suggesting this taxa may benefit from typical environmental levels of nitrate in the sea water (Basuyaux and Mathieu 1999).

Juvenile Australian crayfish (*Cherax quadricarinatus*) exposed to $NaNO_3$ concentrations up to 4430 mg $NO_3^-·L^{-1}$ in 120-h renewal tests did not exhibit any significant differences in oxygen consumption rates or mortality during the exposure period (Meade and Watts 1995).

The following studies were not included in the long-term dataset for CWQG derivation. The giant freshwater prawn (*Macrobrachium rosenbergii*), a native of the Indo-Pacific region is grown extensively in aquaculture operations (Eldredge 2001). The tolerance of *M. rosenbergii* to high sodium nitrate levels (up to 4483 mg $NO_3^-·L^{-1}$) in recirculating aquaculture tanks was determined under freshwater - brackish conditions (salinity ranging from 0.5 - 4‰) (Wickins 1976). For a three week exposure period using growth as the endpoint, 2 separate experiments were conducted. With the first experiment, a clear-dose response was apparent, and the following effect concentrations were calculated: an EC_{50} of 534 mg $NO_3^-·L^{-1}$ and an LC_{50} of 857 mg $NO_3^-·L^{-1}$ (Wickins 1976, Appendix A). In a second experiment, a clear dose-response was not evident and so only the EC_{50} of 872 mg $NO_3^-·L^{-1}$ was calculated (Wickins 1976, Appendix A). The author provided a combined (from both experiments) EC_{50} of 775 mg $NO_3^-·L^{-1}$ and LC_{50} of 709 mg $NO_3^-·L^{-1}$ but these were considered to be unreliable (Wickins 1976, Appendix A).

The prawn larvae in the Muir et al. (1991) study moulted from Protozoea I to Protozoea II stage during the trials. As crustaceans are reportedly more susceptible to toxins during the sensitive ecdysis stage (moulting), the increased susceptibility to nitrate found by Muir et al. (1991) is likely due to developmental sensitivity. This level of sensitivity to nitrate exposure is not seen in older penaeid shrimp. Wickins (1976), found that the growth of juvenile P. monodon (0.5 - 1.5 g live wt.) was not affected after 3 to 5 weeks exposure to concentrations over 886 mg $NO_3^-·L^{-1}$.

6.5.2.2 Fish

To address the data gaps identified during the derivation of the 2003 interim NO_3^- CWQG for the protection of marine life, a toxicity test was commissioned for a long-term fish study on a temperate species. Full results are reported elsewhere in Stantec (2006). Briefly, nitrate toxicity to topsmelt (*Atherinops affinis*) was assessed according to US EPA's 7-d growth and survival

test (US EPA 1995; Stantec, 2006). Nitrate toxicity tests were conducted following standard toxicological laboratory methods involving either static or static renewal exposure conditions, and were run using sodium nitrate ($NaNO_3$). As well, all tests satisfied the minimum requirements for test validity as outlined in the specific test methods.

Results of the definitive test with topsmelt are provided in Table 6.13. The result values were typical of other studies on similar species (Environment Canada, 2003; Appendix B).

Table 6.13. The results of the nitrate (as $NaNO_3$) toxicity tests to topsmelt (*Atherinops affinis*). Table values are expressed in mg $NO_3^-{\cdot}L^{-1}$ and include 95% confidence limits in parentheses.

	Life Stage	Endpoint	Effect Concentration (mg $NO_3^-{\cdot}L^{-1}$)	Reference
Atherinops affinis (Topsmelt)	Juvenile	7-d IC_{25} (Mortality)	2554 (486-5886)	Stantec, 2006
	Juvenile	7-d IC_{25} (Biomass)	2609 (186-6563)	Stantec, 2006

A search of the primary literature for nitrate toxicity studies published after 2001 was also conducted. This date allowed for overlap with the end of the literature search conducted for the 2003 interim guideline derivation. Web of Science was searched using keywords including: nitrate, toxicity, marine, saltwater, salinity and aquatic. No additional long-term toxicity data was found for marine fish.

Overall, there are few studies available on nitrate toxicity to marine fish. A total of 4 effect concentrations for 4 fish species were included in the short-term dataset. The most sensitive of the fish species was the anemonefish (*Amphiprion ocellaris*). Frakes and Hoff Jr. (1982) found survival of larval anemonefish reared in high-nitrate conditions (~443 mg $NO_3^-{\cdot}L^{-1}$) for 72 days, was 25% lower than larvae reared in low-nitrate treatments (~71 mg $NO_3^-{\cdot}L^{-1}$). The mean total length of juvenile anemonefish was 8% lower under high nitrate levels and these fish had noticeably faded coloration, decreasing their commercial marketability (Frakes and Hoff Jr. 1982).

The three other fish species in the long-term dataset all had similar sensitivities to nitrate. The topsmelt (*Atherinops affinis)*, the rainbow trout (Oncorhynchus mykiss), and the Chinook salmon (*Oncorhynchus tshawytscha*) had respective effect concentrations of a 7-d IC_{25} of 2554 mg $NO_3^-{\cdot}L^{-1}$ (Stantec 2006), a 7-d IC_{10} of 2954 mg $NO_3^-{\cdot}L^{-1}$ (Westin 1974) and a 7-d IC_{10} of 3510 mg $NO_3^-{\cdot}L^{-1}$ (Westin 1974).

6.5.2.3 Algae and Plants

In a review of inhibitory concentrations of nitrogen compounds for marine and freshwater algae, none were reported for nitrate (Admiraal 1977). The growth of ten species of marine benthic diatoms (expressed as a percent increase in chlorophyll *a*) under varying nitrate concentrations (as KNO_3) was either not inhibited, or only slightly inhibited, even at the highest concentration

tested, at 1048 mg $NO_3^-·L^{-1}$ (Admiraal 1977). No inhibition was seen in marine diatom cultures (*Nitzschia pungens*) grown at 13.6 to 54.6 mg $NO_3^-·L^{-1}$ (Bates et al. 1993). Naidoo (1990) found, not only did sodium nitrate have no adverse effects on the growth of the tropical marine mangrove (*Bruguiera gymnorrhiza*), it actually increased total propagule biomass, with maximum growth occurring at 44 mg $NO_3^-·L^{-1}$.

High nitrate levels might indirectly lead to metal toxicity in marine plants and algae. Wang and Dei (2000, 2001) found that nitrate additions to marine phytoplankton cultures increased concentration factors for selected metals (Cd, Se, Zn) in phytoplankton cells. Addition of ammonium nitrate fertilizer has also been observed to cause increased cadmium accumulation in terrestrial plants such as flax (Grant et al. 2000) Nutrient enrichment may therefore influence trace metal uptake at the base of the food chain.

Plant toxicity data were not included in the development of the nitrate guideline values as nitrate is a plant nutrient.

6.6 Genotoxicity of Nitrate

The carcinogenicity of the nitrate ion, nitric acid, ammonium nitrate, sodium nitrate or potassium nitrate is not classified under the International Agency for Research on Cancer (IARC) system (WHO 2001), or by the U.S. National Toxicology Program (NRC 1978; NTP 2001).

Although nitrate and its associated salts are unlikely to be carcinogenic themselves, they may be indirectly involved in mutagenesis. Suzuki et al. (1982) found the photolysis of aromatic compounds in the presence of an aqueous nitrate solution (73 mg $NO_3^-·L^{-1}$) resulted in products that were mutagenic to *Salmonella typhimurium* in Ames assays, whereas no mutagenicity was found when a non-nitrate aqueous solution was used. By carrying out these experiments in wavelengths from 250 to 577 nm and in > 300 nm, Suzuki et al. (1982) found that the majority of the mutagenicity was induced in exposure to ultraviolet light (i.e., < 300 nm wavelength).

It is also suspected elevated gastric pH levels (i.e., pH > 4) in mammals (including humans) may lead to the proliferation of denitrifying bacteria that would break down nitrate to nitrite which may ultimately form N-nitroso compounds (Packer 1995) through the following pathway:

A) nitrite is converted to nitrous acid:

$$NO_2^- + H^+ \leftrightarrow HNO_2$$

B) 2 molecules of nitrous acid reversibly form one molecule of nitrous acid anhydride:

$$2\ HNO_2 \leftrightarrow N_2O_3 + H_2O$$

C) which then reacts with non-ionized secondary amines to form N-nitrosamines:

$$R,R'NH + N_2O_3 \rightarrow R,R'N_2O + HNO_2$$

(from NRC 1978)

Most N-nitroso compounds are carcinogens and nitrosamines have induced cancer in every species of animal tested, including zebra fish (*Brachydanio rerio*), rainbow trout (*O. mykiss*), and guppy (*P. reticulata*); however, as little or no information exists on environmental exposure levels or uptake and metabolic fate, any assessment of ecological hazards will remain highly uncertain (NRC 1978; Russo 1985). In a study of nitrosating agents present in water, levels of sodium nitrate up to 8000 $mg \cdot L^{-1}$ (= 5840 mg $NO_3^- \cdot L^{-1}$) were found not to induce clastogenic responses (i.e., the induction of micronuclei in red blood cells) in newt larvae (*Pleurodeles waltl*), under varying environmental factors such as pH and lighting conditions (L'Haridon et al. 1993).

6.7 Toxicity to Semi-Aquatic Animals

No studies were located on the effects of ambient nitrate concentrations on marine or freshwater mammals or birds.

7 CANADIAN WATER QUALITY GUIDELINES

7.1 Canadian Water Quality Guidelines and Benchmark Concentrations for the Protection of Freshwater and Marine Aquatic Life

The Protocol for the Deriviation of Canadian Water Quality Guidelines includes a guideline value for long-term exposure (CWQG) and a benchmark concentration for short-term exposure (CCME 2007). The long-term CWQG is designed to protect all species at all life stages over an indefinite exposure to a substance in water. Continuous releases may occur from point or non-point sources, gradual release from soils/sediments and gradual entry through groundwater/runoff, and long-range transport. Canadian Water Quality Guidelines for the Protection of Aquatic Life are nationally accepted threshold values for substances and other attributes (such as pH and temperature) in water. These values are determined such that no adverse toxic effects are expected in aquatic plants and animals. A CWQG for the protection of aquatic life can either be numerical or narrative and is developed using the most current scientific information available at the time of derivation. Data available from algae, macrophytes, invertebrates, and vertebrates are all considered. The development of a CWQG is based on the toxicity data. Implementation issues (e.g. technological and economic feasibility) are not taken into consideration. A CWQG is not a regulatory instrument, but can be used to derive Water-Quality-Based effluent limits, which are legally enforceable (e.g. Certificates of Approval for waste dischargers). A CWQG can be the basis for the derivation of site-specific guidelines (e.g. derived using site-specific aquatic receptors). The guidelines are management tools constructed to ensure that anthropogenic stresses, such as the introduction of toxic substances, do not result in the degradation of Canadian waters. The development of a CWQG for nitrate will assist environmental risk assessors and risk managers to better assess the potential impacts of nitrate to aquatic ecosystems.

The short-term benchmark concentration is an estimator of severe effects to the aquatic ecosystem and is 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/nonpersistent 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, but rather indicate the level where severe effects are likely to be observed.

While separate data sets are used to calculate long-term guidelines and short-term benchmark concentrations, both are derived using one of three approaches. The three methods are:

1) Statistical approach (Type A or SSD approach),
2) Lowest endpoint approach using only primary data with a safety factor (Type B1),
3) Lowest endpoint approach using primary and/or secondary data with a safety factor (Type B2).

The minimum data requirements for each of these three methods are presented in Tables 7.1 for freshwater environments.

Table 7.1. Minimum data set requirements for the generation of a short-term freshwater benchmark concentration and a long-term freshwater CWQG following the 2007 CCME guideline protocol (CCME 2007).

Derivation Method	Minimum Toxicity Dataset
Type A Guideline	Toxicity tests required for the generation of an SSD, broken out as follows: Fish: 3 studies on 3 different species including 1 salmonid, 1 non-salmonid. Invertebrates: 3 studies on 3 different species including 1 planktonic crustacean, 2 others. For semi-aquatic invertebrates, the life stages tested must be aquatic. It is desirable, but not necessary, that one of the aquatic invertebrate species be either a mayfly, caddisfly, or stonefly. Plant/Algae: For short-term guidance: none (for non-phytotoxic substances), 2 studies (for phytotoxic substances). For long-term guidance: At least one study on a freshwater vascular plant or freshwater algal species (for non-phytotoxic substances), 3 studies (for phytotoxic substances) Toxicity data for amphibians are highly desirable, but not necessary. Data must represent fully aquatic stages. Acceptable endpoints for short-term guidance: LC/EC_{50} (severe effects) Acceptable endpoints for long-term guidance: Most appropriate ECx/ICx representing a no-effects threshold > EC_{10}/IC_{10} > $EC_{11\text{-}25}/IC_{11\text{-}25}$ > MATC > NOEC > LOEC > $EC_{26\text{-}49}/IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50}. Note: Primary or secondary no- and low-effects data are acceptable to meet the minimum data requirements.

Derivation Method	Minimum Toxicity Dataset
Type B1 Guideline	Toxicity tests required for the generation of a Type B1 guideline, broken out as follows: Fish: 3 studies on 3 different species including 1 salmonid, 1 non-salmonid. Invertebrates: 3 studies on 3 different species including 1 planktonic crustacean, 2 others. For semi-aquatic invertebrates, the life stages tested must be aquatic. It is desirable, but not necessary, that one of the aquatic invertebrate species be a mayfly, caddisfly, or stonefly. Plant/Algae: For short-term guidance: none (for non-phytotoxic substances), 2 (for phytotoxic substances). For long-term guidance: At least one study on a freshwater vascular plant or freshwater algal species (for non-phytotoxic substances), 3 studies (for phytotoxic substances) Toxicity data for amphibians are highly desirable, but not necessary. Data must represent fully aquatic stages. Acceptable endpoints for short-term guidance: LC/EC_{50} (severe effects) Acceptable endpoints for long-term guidance: Most appropriate ECx/ICx representing a low-effects threshold > $EC_{15\text{-}25}/IC_{15\text{-}25}$ > LOEC > MATC > $EC_{26\text{-}49}/IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50} > LC_{50}. Note: only primary data are acceptable. Only short-term studies for short-term guidance, and long-term for long-term.
Type B2 Guideline	Toxicity tests required for the generation of a Type B2 guideline, broken out as follows: Fish: 2 short-term or long-term studies on two or more fish species, including 1 salmonid, 1 non-salmonid. Invertebrates: 2 short-term or long-term studies on 2 or more invertebrate species from different classes, including 1 planktonic sp. Plants: For short-term guidance: none (for non-phytotoxic substances), 2 (for phytotoxic substances) For long-term guidance: none (for non-phytotoxic substances), 2 (for phytotoxic substances) Acceptable endpoints for short-term guidance: LC/EC_{50} (severe effects) Acceptable endpoints for long-term guidance: Most appropriate ECx/ICx representing a low-effects threshold > $EC_{15\text{-}25}/IC_{15\text{-}25}$ > LOEC > MATC > $EC_{26\text{-}49}/IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50} > LC_{50}. Note: primary or secondary data are acceptable. Only short-term studies for short-term guidance, and short or long-term for long-term guidance.

Table 7.2. Minimum data set requirements for the generation of a short-term marine benchmark concentration and a long-term marine CWQG following the 2007 CCME guideline protocol (CCME 2007).

Derivation Method	**Minimum Toxicity Dataset**
Type A Guideline	Toxicity tests required for the generation of an SSD, broken out as follows: Fish: 3 studies on 3 different species including 1 temperate species. Invertebrates: 2 studies on 2 different species from different classes including 1 temperate species. Plant/Algae: For short-term guidance: 1 study on a temperate marine vascular plant or algal species (for non-phytotoxic substances), 2 studies (for phytotoxic substances). For long-term guidance: 1 study on a temperate marine vascular plant or algal species (for non-phytotoxic substances), 3 studies (for phytotoxic substances) Acceptable endpoints for short-term guidance: LC/EC_{50} (severe effects) Acceptable endpoints for long-term guidance: Most appropriate ECx/ICx representing a no-effects threshold > EC_{10}/IC_{10} > $EC_{11\text{-}25}$/$IC_{11\text{-}25}$ > MATC > NOEC > LOEC > $EC_{26\text{-}49}$/$IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50}. Note: Primary or secondary no- and low-effects data are acceptable to meet the minimum data requirements.
Type B1 Guideline	Toxicity tests required for the generation of a Type B1 guideline, broken out as follows: Fish: 3 studies on 3 different species including 1 temperate species. Invertebrates: 2 studies on 2 different species from different classes including 1 temperate species. Plant/Algae: 1 study on a temperate marine vascular plant or algal species (for non-phytotoxic substances), 2 studies (for phytotoxic substances). Acceptable endpoints for short-term guidance: LC/EC_{50} (severe effects) Acceptable endpoints for long-term guidance: Most appropriate ECx/ICx representing a low-effects threshold > $EC_{15\text{-}25}$/$IC_{15\text{-}25}$ > LOEC > MATC > $EC_{26\text{-}49}$/$IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50} > LC_{50}. Note: only primary data are acceptable to meet the minimum data requirements. The value used to set the guideline must be primary. Only short-term studies for short-term guidance, and long-term for long-term.

Derivation Method	Minimum Toxicity Dataset
Type B2 Guideline	Toxicity tests required for the generation of a Type B2 guideline, broken out as follows: Fish: 2 studies on 2 different species including 1 temperate species. Invertebrates: 2 studies on 2 different species. Plants: For short-term guidance: data for marine plants desirable but not necessary (for non-phytotoxic substances), 2 studies (for phytotoxic substances) For long-term guidance: none (for non-phytotoxic substances), 2 studies (for phytotoxic substances) Acceptable endpoints for short-term guidance: LC/EC_{50} (severe effects) Acceptable endpoints for long-term guidance: Most appropriate ECx/ICx representing a low-effects threshold > EC_{15-25}/IC_{15-25} > LOEC > MATC > EC_{26-49}/IC_{26-49} > nonlethal EC_{50}/IC_{50} > LC_{50}. Note: primary or secondary data are acceptable. The value used to set the guideline must be secondary. Only short-term studies for short-term guidance, and short or long-term for long-term guidance.

The statistical approach (which is the preferable method if the minimum data requirements are attained) involves the use of species sensitivity distributions (SSDs) which represent the variation in sensitivity of species to a substance by a statistical or empirical distribution function of responses for a sample of species. The basic assumption of the SSD concept is that the sensitivities of a set of species can be described by some distribution, usually a parametric sigmoidal cumulative distribution function. The data points used in the SSD are most commonly those derived from laboratory-based studies. Emphasis is placed on plotting organism-level effects, such as survival, growth, and reproduction, which can be more confidently used to predict ecologically-significant consequences at the population level (Meador 2000; Forbes and Calow 1999; Suter et al. 2005). Therefore, another assumption of the SSD is that the distribution of sensitivities of laboratory species to a substance reflects the sensitivity of species in natural aquatic environments to that same substance. The SSD method involves modelling the cumulative SSD and estimating the 95% confidence interval. The guideline is defined as the intercept of the 5th percentile of the species sensitivity distribution (CCME, 2007) and so would be interpreted as protecting 95% of species. However, CCME (2007) states that no effect (e.g. EC/IC10, NOEC) data are to be used primarily, with low effect (e.g. EC/IC25, LOEC) data being less preferable, but still acceptable if no-effect data is unavailable, for guideline derivation. By using mostly no- and some low-effect data, and setting the guideline value—as the 5th percentile, this guideline is expected to maintain aquatic community structure and function. SSD derived guidelines are referred to as Type A guidelines. The use of SSDs has become common in ecological risk assessment. SSDs are also used in the development of environmental quality guidelines within the European Union, Australia and New Zealand as well as the USA. Each jurisdiction has developed its own protocol (policies) with respect to WQC development using an SSD (e.g. some use only no effect data, some apply safety factors to the 5th percentile value, some may plot multiple endpoints for one species, some only plot NOEC survival data, etc), and

therefore the approaches used are not completely identical between jurisdictions. In the case of nitrate, suitable short-term and long-term datasets were provided for the development of a Type A guideline. Freshwater and marine SSDs for both freshwater and marine biota were derived for both exposure durations following the CCME Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life (CCME, 2007).

When multiple data points for effects (*e.g.*, growth, mortality, reproduction) were available for the same species professional judgment was utilized to select a representative species effect concentration (*e.g.*, lowest value or geomean). Only one endpoint per species was plotted on the SSD. Using a customized Microsoft Excel-based software package, SSD Master Version 2.0 (Rodney *et al*., 2008), a total of five cumulative distribution functions (Normal, Logistic, Gompertz, Weibull, Fisher-Tippett) were fit to the data using regression techniques. Model fit was assessed using statistical and graphical techniques. The best model was selected based on goodness-of-fit and model feasibility. Model assumptions were verified graphically. The concentration of nitrate in freshwater at which 5% of species are predicted to be affected was determined for both short-term and long-term scenarios with 95% confidence intervals on the mean (expected) value.

Each species for which appropriate toxicity data were available was ranked according to sensitivity (from lowest to highest value), and its centralized position on the SSD (Hazen plotting position) was determined using the following standard equation (Aldenberg *et al.*, 2002; Newman *et al.*, 2002):

$$\text{Hazen Plotting Position} = \frac{i - 0.5}{N}$$

where:

i = the species rank based on ascending toxicity values

N = the total number of species included in the SSD derivation

7.1.1 Summary of Existing Water Quality Guidelines for the Protection of Freshwater Aquatic Life

A Canadian water quality guideline for nitrate was developed in 1987 and consisted of a narrative stating nitrate concentrations that will stimulate weed growth should be avoided (CCREM 1987). This 1987 guideline was updated in 2003 when an interim CWQG for the protection of freshwater life to prevent direct nitrate toxicity to aquatic organisms of 13 mg NO_3^- $\cdot L^{-1}$ was published. The 2003 interim guideline was derived following the 1991 CCME guideline derivation protocol by applying an arbitrary safety factor to the most sensitive endpoint (CCME 1991).

The critical study used to determine the 2003 interim freshwater guideline for the protection of aquatic life from nitrate was Schuytema and Nebeker (1999c). This 10-day chronic study examined the toxicity of sodium nitrate to the Pacific treefrog (*Pseudacris regilla*). Tests

followed standard procedures from ASTM (1997 a,b) and solutions were renewed daily. The following water quality parameters were monitored throughout the tests: temperature = 22 ± 1°C, dissolved oxygen = 7.2 ± 0.1 $mg \cdot L^{-1}$, total hardness = 58.4 ± 9.5 $mg \cdot L^{-1}$ as $CaCO_3$, total alkalinity = 52.0 ± 7.0 $mg \cdot L^{-1}$ as $CaCO_3$, conductivity = 156.0 ± 15.1 $\mu S \cdot cm^{-1}$, and pH = 7.0-7.6. Statistically significant decreases in weight and length ($p \leq 0.05$) were seen at concentrations as low as 133 mg $NO_3^- \cdot L^{-1}$ and 1148 mg $NO_3^- \cdot L^{-1}$, respectively (Schuytema and Nebeker 1999c). The former LOEC was used in developing the guideline. The test organisms exposed to 133 mg $NO_3^- \cdot L^{-1}$ experienced a mean decrease in weight of 15% when compared with the control group. This effect is likely to have ecological significance as predation on amphibian larvae is size-dependent (Licht 1974; Caldwell et al. 1980; Travis 1983; Wilbur 1984; Carey and Bryant 1995; Werner 1986). Other authors have reported amphibian larval size decreases of 11 and 17% can affect fitness, with observed effects including decreased juvenile survival, decreased size at maturity, and longer time to first reproduction (Smith 1987; Berven 1990). A safety factor of 0.1 was applied to the LOEC in accordance with the CCME (1991) protocol and the final result was rounded to 13 mg $NO_3^- \cdot L^{-1}$.

Support for the freshwater guideline was drawn from three other studies reporting LOECs within a similar range. Decreased length was observed in larvae of the red-legged frog and the northern leopard frog at LOECs of 129 mg $NO_3^- \cdot L^{-1}$ (Schuytema and Nebeker 1999a) and 133 mg $NO_3^- \cdot L^{-1}$ (Allran and Karasov 2000), respectively. The water flea *Ceriodaphnia dubia* was similarly susceptible, with a 7-d LOEC for reduced reproduction at 189 mg $NO_3^- \cdot L^{-1}$ (Scott and Crunkilton 2000). The guideline is also comparable with estimates made for safe nitrate concentrations for invertebrates. By converting 72-, 96- and 120-h mortality data to probit values and then to $LC_{0.01}$s, (Camargo and Ward 1995) calculated lifetime safe concentrations for hydropsychid larvae (= 8760-h $LC_{0.01}$) of 6.2 to 15.5 mg $NO_3^- \cdot L^{-1}$.

The 2003 CWQG for the protection of freshwater life from the toxic effects of the nitrate ion were considered ‘interim’ due to data gaps identified during their derivation (Environment Canada, 2003). It was recommended additional toxicity tests be conducted for fish and invertebrate species known to be highly sensitive. For example, although toxicity data was available for caddisflies, generally mayflies and stonesflies are considered more sensitive to contaminants; therefore, nitrate toxicity tests with these other invertebrates would be useful. Effects of nitrate on brook trout, particularly the egg and juvenile stages, should be studied as the spawning habits of this species could make it particularly susceptible. Brook trout (as well as other fish species) seek out groundwater upwelling areas for spawning, and may be at risk of exposure to high levels of nitrate in these upwellings. At present there are no existing nitrate toxicity data available for brook trout, so comments cannot be made about the sensitivity of this species. It is possible that brook trout eggs are more susceptible to nitrate toxicity than other fish eggs discussed in this document (e.g., fathead minnow, rainbow trout, salmon), because they have a longer incubation period (Morris 2001). Also, hatching of brook trout eggs occurs in March and April when groundwater levels of nitrate peak. Further investigation of nitrate toxicity to fish eggs, in general, is also needed as this may be a particularly sensitive life stage. For example, two ancillary studies (Kincheloe et al. 1979; Bieniarz et al. 1996) reported adverse effects on fish eggs at concentrations lower than the critical study on which the guideline was based.

Prior to the publication of the 2003 guidelines, British Columbia was the only Canadian jurisdiction to have developed guidelines for the protection of aquatic life from nitrate toxicity, with a maximum exposure of 200 mg NO_3^--N·L^{-1} (= 886 mg NO_3^-·L^{-1}) and a 30-day average exposure of 40 mg NO_3^--N·L^{-1} (= 177 mg NO_3^-·L^{-1}) (Nordin and Pommen 1986). These values were based on 50% and 10%, respectively, of the lowest 96-h LC_{50} reported in the literature (Nordin and Pommen 1986). Québec has also adopted these values for provincial guideline use (MEF 1998). The BC nitrate guideline has recently been updated and has resulted in a more conservative guideline value. The maximum exposure concentration is now 31.3 mg NO_3^--N·L^{-1} (= 139 mg NO_3^-·L^{-1}), which was derived by applying a safety factor of 0.5 to the most sensitive invertebrate endpoint (96-h LC50 of 62.5 mg NO_3^--N·L^{-1} for the amphipod *E. echinosetosus* (Nordin and Pommen 1986, 2009). The 30-day average exposure concentration is 3.0 mg NO_3^--N·L^{-1} (= 13 mg NO_3^-·L^{-1}), derived by multiplying the 10-d LOEC of 30 mg NO_3^--N·L^{-1} for the Pacific treefrog (*Pseudacris regilla*) by a safety factor of 0.1 (Nordin and Pommen 1986, 2009). BC MOE also used the McGurk et al. (2006) study to support the development of this new long-term guideline. Based on the 146-d MATC (wet weight) for the lake trout *Salvelinus namaycush* of 14 mg NO_3^-·L^{-1}, the new guideline value is protective of this sensitive coldwater salmonid species. Alberta's surface water quality guidelines have a maximum allowable concentration for total nitrogen (total inorganic plus total organic) of 1.0 mg N·L^{-1}; however, this nitrate concentration is not considered directly toxic, rather the guideline is to protect against deleterious influences of nitrate on conditions that affect aquatic life (AEP 1999).

The freshwater CWQG for the protection of aquatic life (13 mg NO_3^-·L^{-1}) greatly exceeds the *moderate reliability* (95% protection) trigger value developed by Australia and New Zealand (0.70 mg NO_3^-·L^{-1}) (Environment Australia 2000b). The Australian/New Zealand guideline was derived by applying a default acute-to-chronic ratio (ACR) to the 95% distribution of toxicity data for potassium and sodium nitrate salts which included native Australian fish and invertebrates (Environment Australia 2000b). The certainty of this low trigger value was evaluated in 2002 by the New Zealand National Institute of Water and Atmospheric Research based on the following concerns. The 95% and 99% species protection trigger values for nitrate were 1.3 and 19 times lower, respectively, than the 95% and 99% ammonia trigger values. This indicated that nitrate was more toxic when compared to ammonia, which resulted in the re-evaluation of the nitrate guideline value (Hickey 2002). The 2002 re-calculated guideline value is 31.9 mg NO_3^-·L^{-1} (95% level of protection), which is closer in value to the CWQG value of 13 mg NO_3^-·L^{-1}. Both the 2000 published and 2002 revised guideline values were re-evaluated in 2009 by the New Zealand National Institute of Water and Atmospheric Research in order to determine if they were applicable to the surface and groundwaters of the region of Canterbury (Hickey and Martin 2009). A decision was made to re-calcuate the guideline using studies that only employed the use of sodium nitrate salts. Studies that used potassium nitrate salts were excluded since K has been shown to be much more toxic when compared to Na for a range of invertebrate and fish species.

The US EPA does not currently have a numeric criterion for nitrate for the protection of aquatic life. However, the Minnesota Pollution Control Agency released a draft Aquatic Life Water Quality Standards Technical Support Document for Nitrate in November 2010 (Monson 2010). The draft acute value (maximum standard for 1-day duration) is 41 mg NO_3^--N·L^{-1}

(= 182 mg NO_3^-·L^{-1}), and the draft chronic value is 4.9 mg NO_3^--N·L^{-1} (= 21.7 mg NO_3^-·L^{-1}). For surface waters designated as Class 2A[1] (surface waters which are cold-water fisheries, trout waters, and also protected as a source of drinking water), the proposed chronic value in this case is 3.1 mg NO_3^--N·L^{-1} (= 13.7 mg NO_3^-·L^{-1}), which is comparable to the CWQG of 13 mg NO_3^-·L^{-1}. Based on observations by Knepp and Arkin (1973), however, the US EPA had suggested that nitrate levels below 90 mg NO_3^--N·L^{-1} (= 399 mg NO_3^-·L^{-1}) will be protective of warmwater fish (US EPA 1986). The US EPA does have Nutrient Criteria technical guidance manuals published, to provide guidance on the setting of numeric criteria for lakes and reservoirs (US EPA 2000b), rivers and streams (US EPA 2000a), wetlands (US EPA 2008) as well as estuarine and coastal marine waters (US EPA 2001). The approaches provided in the guidance manuals for developing numeric nutrient criteria include, but are not limited to: (1) stressor-response analysis, (2) the reference condition approach, and (3) mechanistic modeling. The US EPA is currently proposing numeric nutrient criteria (nitrogen and phosphorus) for the following four water body types in the state of Florida - lakes, streams, springs and clear streams, and canals – in order to protect people's health, aquatic life and the long term recreational uses. The proposed nutrient critieria can be accessed in the Federal Register (US EPA 2010a).

Currently the European Union has no nitrate guideline for the protection of aquatic life. The Netherlands, however, has proposed a maximum allowable concentration for nitrate of 2.0 mg NO_3^-·L^{-1} in eutrophic waters to protect against direct toxicity (Speijers et al. 1989). In addition, the Netherlands recommends a maximum allowable nitrate concentration of 0.04 mg NO_3^-·L^{-1} in oligotrophic waters to protect against eutrophication impacts (Speijers et al. 1989).

7.1.2 Evaluation of Toxicological Data

In accordance with the CCME protocol for the derivation of water quality guidelines for the protection of aquatic life, toxicity studies were classified as primary, secondary or unacceptable (CCME 2007). Because the nitrate ion is non-volatile and tends to remain in solution (NRC 1978) and studies monitoring nitrate levels over time did not report any significant losses from their experimental systems (Muir et al. 1991; Scott and Crunkilton 2000; Elphick 2011; Nautilus Environmental 2011), some studies using static test conditions were classified as primary. Primary and secondary studies were considered for guideline development. The relationship between nitrate toxicity and water hardness was investigated, and it was concluded that the relationship of decreasing toxicity with increasing water hardness has not been definitively demonstrated. The short-term and long-term exposures conducted by Elphick (2011) indicate a trend of decreasing toxicity with increasing hardness, however the study by Nautilus Environmental (2011) – 40 day embryo-alevin-fry rainbow trout exposure – indicate that toxicity can be greater at higher hardness when compared to lower hardness exposure conditions (see

[1] As per the US Clean Water Act, all states must designate beneficial uses for all waters within their jurisdiction and develop water quality standards to protect each use. The vast majority of surface waters in the state of Minnesota are designated as Class 2 – protected for aquatic life and recreation. Class 2 surface waters are further sub-divided into sub-classes: Class 2A (Cold-water fisheries, trout waters, also protected as a source of drinking water), Class 2Bd (Cool- and warm-water fisheries, also protected as a source of drinking water), Class 2B (Cool- and warm-water fisheries (not protected for drinking water)), Class 2C (Indigenous fish and associated aquatic community (not protected for drinking water)), and Class 2D (Wetlands (not protected for drinking water)) (MPCA 2011).

Section 6.1 – Effects of water quality parameters on toxicity – for a more thorough discussion). The relationship between nitrate toxicity and temperature was also investigated by Moore and Poirier (2010) (see Section 6.1.3 - Evaluating the Temperature-Toxicity Relationship for Nitrate – Short-Term Exposures Only – for more detailed information). 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. Yet other studies have indicated that temperature does not appear to affect the toxicity of nitrate to freshwater fish. As there have been no conclusive relationships drawn between nitrate toxicity and ambient levels of various water quality variables,studies that did not report some variables, but had adequate survivorship in controls, were included. Studies using distilled and/or deionized water to hold test organisms were not included due to potential ionic influences on survival (Anderson 1944). Studies using species resident to Canadian waters or temperate non-native species were preferentially included in the freshwater guideline derivation as per the CCME (2007) protocol. Only toxicity data for sodium nitrate were used in deriving the freshwater guidelines.

7.1.3 Freshwater Aquatic Life Guideline Derivation

The Protocol for the Deriviation of Canadian Water Quality Guidelines includes a guideline value for long-term exposure and a benchmark concentration for short-term exposure (CCME 2007). The long-term exposure guideline is designed to protect all species at all life stages over an indefinite exposure to a substance in water. Continuous releases may occur from point or non-point sources, gradual release from soils/sediments and gradual entry through groundwater/runoff, and long-range transport. The short-term benchmark concentration value does *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, but rather indicate the level where severe effects are likely to be observed.

While separate data sets are used to calculate short-term benchmark concentrations and long-term guidelines, both are derived using either a statistical approach without the application of a safey factor (Type A or Species Sensitivity Distribution), or one of two assessment factor approaches. The first assessment factor approach (Type B1) applies a safety factor to the lowest endpoint from a primary study, and the second approach (Type B2) applies a safety factor to the lowest endpoint from a primary and/or secondary study. The three approaches are detailed in CCME (2007).

All toxicity data for freshwater organisms can be found in appendix A. For the derivation of the short-term benchmark concentration and the long-term CWQG for the nitrate ion, this list was pared down to include data only from studies classified as primary or secondary following CCME (2007). Acceptable toxicity data were found to be available for the following aquatic species: water fleas *Ceriodaphnia dubia* and *Daphnia magna*; caddisflies *Cheumatopsyche pettiti* and *Hydropsyche occidentalis*; the stoneflies *Amphinemura delosa* and *Allocapnia vivpara*; the amphipod *Hyalella azteca*; New Zealand mudsnail (*Potamopyrgus antipodarum)*; ; midge (*Chironomus dilutus*); fatmucket mussel (*Lampsilis siliquoidea*); fingernail clam (*Sphaerium simile*); washboard mussel (*Megalonaias nervosa*); lake whitefish *(Coregonus clupeaformis)*; channel catfish (*Ictalurus punctatus)*; bluegill (*Lepomis macrochirus)*; rainbow trout *(Oncorhynchus mykiss)*; chinook salmon (*Oncorhynchus tshawytscha)*; fathead minnow (*Pimephales promelas*); lake trout (*Salvelinus namaycush*); topeka shiner (*Notropis topeka*);

arctic char (*Salvelinus alpinus*); Pacific tree frog (*Pseudacris regilla*); red-legged frog (*Rana aurora*); and African clawed frog (*Xenopus laevis*) (Tables 7.3 and 7.8).

7.1.3.1 Derivation of the Freshwater Short-term Benchmark Concentration

For many studies, results from several study durations were reported. In these situations, 96-h studies were selected as the preferred study duration following CCME (2007). The exception is crustacean zooplankton species with a shorter life cycle. In these cases, CCME (2007) recommends using 48-h studies. Where studies reported data for multiple life stages, the most sensitive lifestage was chosen for inclusion in the SSD. Following these selection criteria, 23 data points were used to derive a short-term benchmark concentration using an SSD (Table 7.3). Short-term nitrate toxicity values range from a 96-h LC_{50} of 431 mg $NO_3^-{\cdot}L^{-1}$ for the caddisfly *Hydropsyche occidentalis* to a 96-h LC_{50} of 8753 mg $NO_3^-{\cdot}L^{-1}$ for juvenile Bluegill (*Lepomis macrochirus*) (Camargo and Ward, 1992; Trama, 1954).

Table 7.3. Final freshwater nitrate toxicity data selected for short-term SSD development.

Sensitivity Ranking	Organism	Life Stage	Endpoint	Hardness of Exposure Water ($mg \cdot L^{-1}$ as $CaCO_3$)	Effect Concentration (mg $NO_3^- \cdot L^{-1}$)	Reference
1	*Hydropsyche occidentalis* Caddisfly	Early instar	96-h LC_{50}	42.7	431	Camargo and Ward 1992
2	*Cheumatopsyche pettiti* Caddisfly	Early instar	96-h LC_{50}	42.7	503	Camargo and Ward 1992
3	*Hyalella azteca* Amphipod	Juvenile	96-h LC_{50}	80-84; 110-124; 100	774*	US EPA 2010; Soucek and Dickinson 2011; Elphick 2011
4	*Chironomus dilutus* Midge	10d old	48-h LC_{50}	84-136	1582	US EPA 2010b
5	*Lampsilis siliquoidea* Fatmucket mussel	<5 day old juvenile	96-h LC_{50}	90-92	1582	Soucek and Dickinson 2011
6	*Sphaerium simile* Fingernail clam	Juvenile	96-h LC_{50}	90-92	1644	Soucek and Dickinson 2011
7	*Ceriodaphnia dubia* Water flea	Neonates	48h LC_{50}	156-172	1657	Scott and Crunkilton 2000
8	*Amphinemura delosa* Stonefly	Field-collected nymphs	96h LC_{50}	88-92	2020	Soucek and Dickinson 2011
9	*Daphnia magna* Water flea	Neonates	48h LC_{50}	156-172	2047	Scott and Crunkilton 2000
10	*Pseudacris regilla* Pacific tree frog	Embryo	96h LC_{50}	70-80	2849	Schuytema and Nebeker 1999a
11	*Pimephales promelas* Fathead minnow	Larvae	96-h LC_{50}	156-172; 136-140	3304*	Scott and Crunkilton 2000; US EPA 2010

12	*Oncorhynchus mykiss* Rainbow trout	Fingerlings	96-h LC_{50}	106-127; 90	3638*	Moore and Poirier 2010; Elphick 2011
13	*Allocapnia vivipara* Stonefly	Field-collected nymphs	96h LC_{50}	98-100	3703	Soucek and Dickinson 2011
14	*Megalonaias nervosa* Washboard mussel	<5 day old juvenile	96h LC_{50}	90-92	4151	Soucek and Dickinson 2011
15	*Potamopyrgus antipodarum* New Zealand mudsnail	Adult	96h LC_{50}	90.8	4616	Alonso and Camargo 2003
16	*Coregonus clupeaformis* Lake whitefish	Fry	96h LC_{50}	106-127	4730	Moore and Poirier 2010
17	*Salvelinus namaycush* Lake trout	Fry	96h LC_{50}	10-16	4968	McGurk et al. 2006
18	*Oncorhynchus tshawytscha* Chinook salmon	Fingerlings	96h LC_{50}	na	5800	Westin 1974
19	*Notropis Topeka* Topeka shiner	Juvenile	96h LC_{50}	210-230	5994	Adelman et al 2009
20	*Ictalurus punctatus* Channel catfish	Fingerlings	96h LC_{50}	102	6200	Colt and Tchobanoglous 1976
21	*Salvelinus alpinus* Arctic char	Fingerlings	96h LC_{50}	106-127	6650	Moore and Poirier 2010
22	Xenopus laevis African clawed frog	Tadpole	96h LC_{50}	21	7335	Schuytema and Nebeker 1999c
23	*Lepomis macrochirus* Bluegill	Juvenile	96h LC_{50}	45-50	8753	Trama 1954

* Value shown is the geometric mean of comparable values (see Table 7.4)

Table 7.4. Studies used to derive geometric means for short-term data in Table 7.3.

Organism	Life Stage	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Geomean	Reference
Hyalella azteca	Juvenile	96-h LC_{50}	73		US EPA 2010b[1]
Amphipod	Juvenile	96-h LC_{50}	2955	774	Soucek and Dickinson 2011[1]
	Juvenile	96-h LC_{50}	2149		Elphick 2011[1]
Pimephales promelas	Larvae	96-h LC_{50}	5941	3304	Scott and Crunkilton 2000[2]
Fathead minnow	Larvae	96-h LC_{50}	1838		US EPA 2010b[2]
Oncorhynchus mykiss	Fingerlings	96-h LC_{50}	1690	3638	Moore and Poirier 2010[3]
Rainbow trout	Fingerlings	96-h LC_{50}	7832		Elphick 2011[3]

[1]Tested using water that falls within the CCME designation of moderately hard water (61-120 mg L^{-1} as $CaCO_3$).
[2]Tested using water that falls within the CCME designation of hard water (121-180 mg L^{-1} as $CaCO_3$).
[3]Tested using water that falls within the CCME designation of moderately hard water (61-120 mg L^{-1} as $CaCO_3$).

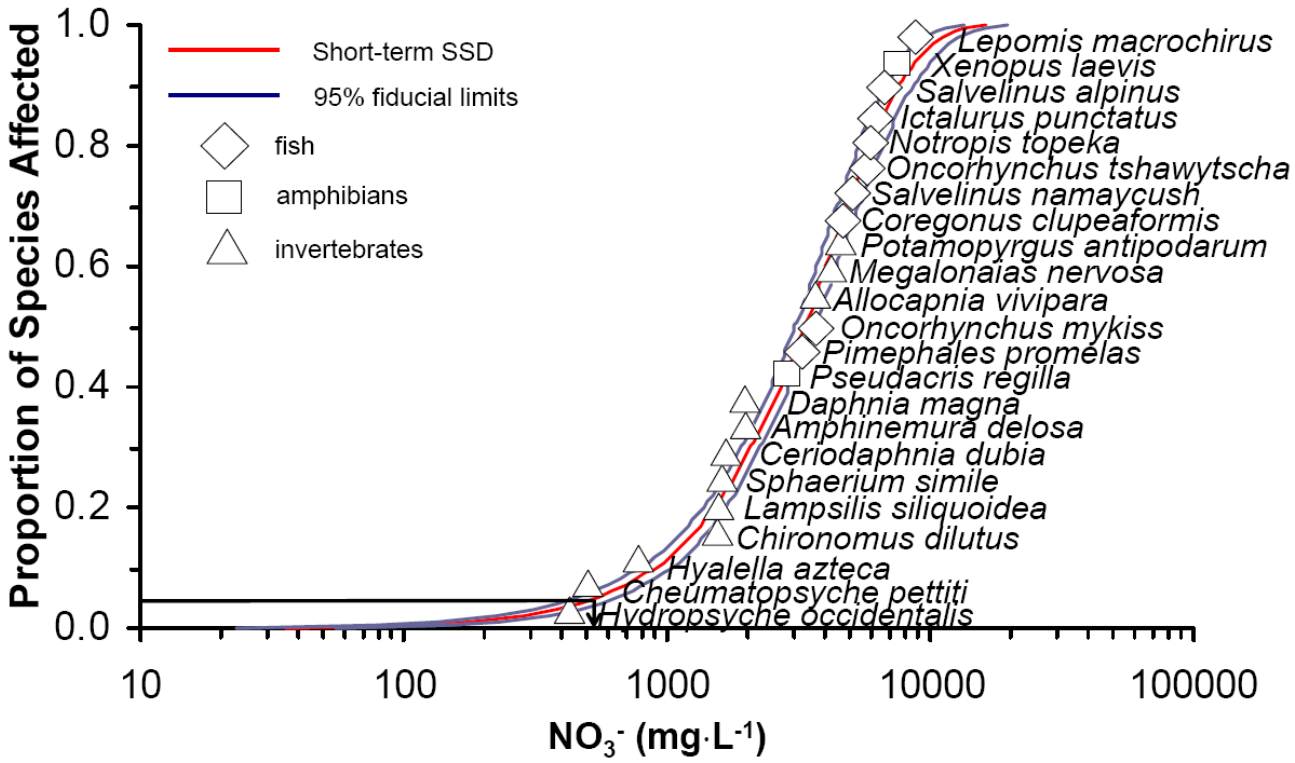


Figure 7.1. SSD of short-term LC_{50} toxicity data for the nitrate ion in freshwater derived by fitting the Gompertz model to the logarithm of acceptable toxicity data for 23 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding short-term benchmark concentration value.

The short-term SSD was fitted using the data in

Table 7.3. To create the SSD, each species was ranked by sensitivity and its centralized position on the SSD was determined using the Hazen plotting position (Aldenberg et al. 2002; Newman et al. 2002). Five cumulative distribution functions (normal, logistic, Gompertz, Weibull, Fisher-Tippett) were then fit to the data in both arithmetic and logarithmic space following standard regression techniques. Modelling assumptions were verified graphically and through assessment of statistical goodness-of-fit tests. If model residuals were found to be normally distributed, the model with the lowest Anderson-Darling goodness-of-fit (A2) score was considered to have the best fit. Following these criteria, the log-Gompertz model (A2 = 0. 261) was determined to best fit the short-term freshwater dataset (Figure 7.1).

The equation for the Gompertz model is given below:

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

Where, for the fitted model: x = log (concentration) of nitrate (mg/L), $f(x)$ is the proportion of species affected, μ = 3.6322 and s = 0.3013. Summary statistics for the SSD curve are presented in Table 7.5.

The 5th percentile on the short-term SSD is 546 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 7.5). The lower fiducial limit (5%) on the 5th percentile is 457 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 546 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. **Therefore, the short-term exposure benchmark concentration indicating the potential for severe effects (e.g. lethality or immobilization) to sensitive freshwater life during transient events is the 5th percentile of 546 mg $NO_3^-{\cdot}L^{-1}$. This value rounded to two significant digits is the short-term benchmark concentration of 550 mg $NO_3^-{\cdot}L^{-1}$.**

Table 7.5. Short-term benchmark concentration value for the nitrate ion in freshwater.

Short-term Benchmark Concentration	Concentration (mg $NO_3^-{\cdot}L^{-1}$)	Concentration (mg $NO_3^-N{\cdot}L^{-1}$)
SSD 5th percentile	550	124
LFL (5%)	457	103
UFL (95%)	652	147

Plotting species sensitivies to nitrate on an SSD can reveal interesting information about the nature of NO_3^- toxicity to aquatic animals. In the short-term, freshwater curve, invertebrate

species are mostly arranged at the bottom with fish species mostly arranged at the top (Figure 7.1). This suggests that, generally speaking, freshwater invertebrates are more sensitive to acute events, such as spills, when compared to fish. The two most sensitive fish species are the fathead minnow (*Pimephales promelas*) and the rainbow trout (*Oncorhynchus mykiss*). The two amphibian species are plotted midway in the SSD (Pacific tree frog, *Pseudacris regilla)* as well as near the upper tail end of the SSD *(*African clawed frog, *Xenopus laevis)*, indicating moderate to higher tolerance of short-term exposures to nitrate, compared to invertebrates.

Two data points fall below the short-term benchmark of 550 mg NO_3^-/L. These include the 96-hour LC_{50} of 431 mg NO_3^-/L for the caddisfly *Hydropsyche occidentalis (*Camargo and Ward 1992) and the 96-hour LC_{50} of 503 mg NO_3^-/L for the caddisfly *Cheumatopsyche pettiti* (Camargo and Ward 1992). From all the invertebrate studies used in deriving the short-term guideline 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 *may* pose the greatest hazard to the sensitive caddisflies. Note that meeting the long-term guideline will protect from severe effects.

7.1.3.2 Derivation of the Long-term Canadian Freshwater Guideline

When reviewing all of the acceptable (for guideline derivation) long-term nitrate toxicity studies, the list was pared down for inclusion in SSD by first selecting studies greater than 7 d in duration and then selecting endpoints appropriate following CCME (2007). The order of preference for the use of long-term endpoints is: the most appropriate EC_x/IC_x representing a no-effects threshold > EC_{10}/IC_{10} > $EC_{11\text{-}25}/IC_{11\text{-}25}$ > MATC > NOEC > LOEC > $EC_{26\text{-}49}/IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50}. In the case where both NOEC and LOEC were reported, the MATC was calculated as the geometric mean of the NOEC and LOEC (Table 7.6). When a study reported more than one lifestage or toxicity endpoint, the most sensitive lifestage was chosen for inclusion.

Many older studies only reported LC_{50} values. As LC_{50} is not an appropriate endpoint for inclusion in the long-term SSD, these values were recalculated where possible to LC_{10}, the preferred endpoint (see data order of preference in paragraph above as well as CCME 2007).

Table 7.7 includes all studies for which sufficient data were available for calculation of an LC_{10}. The full long-term, freshwater SSD dataset can be found in Table 7.8.

Following CCME (2007), the long-term SSD was fitted using data for a variety of species and endpoints. Values range from a 146-d MATC of 14 mg $NO_3^- \cdot L^{-1}$ for Lake trout (*Salvelinus namaycush*) fry wet weight to a 10-d LC_{10} of 3142 mg $NO_3^- \cdot L^{-1}$ for survival of chinook salmon (*Oncorhynchus tshawytscha*) (Table 7.7; McGurk et al., 2006; Westin 1974).

Table 7.6**.** Studies requiring the calculation of MATCs as the geometric mean of LOECs and NOECs for inclusion in the long-term dataset (see Table 7.7).

Organism	Endpoint	Effect Concentration (mg NO_3^-·L^{-1})	MATC (mg NO_3^-·L^{-1})	Reference
Daphnia magna	7-d LOEC (reproduction)	3176	2244	Scott and Crunkilton 2000
Daphnia magna	7-d NOEC (reproduction)	1586		Scott and Crunkilton 2000
Salvelinus namaycush	146-d LOEC (delay to swim-up and wet weight)	28	14	McGurk et al. 2006
Salvelinus namaycush	146-d NOEC (delay to swim-up and wet weight)	7		McGurk et al. 2006
Notropis topeka	30-d LOEC (growth)	1186	1594	Adelman et al 2009
Notropis topeka	30-d NOEC (growth)	2152		Adelman et al 2009
Xenopus laevis	10-d LOEC (weight)	560	404	Schuytema and Nebeker 1999c
Xenopus laevis	10-d NOEC (weight)	291		Schuytema and Nebeker 1999c
Rana aurora	16-d LOEC (weight)	1041	734	Schuytema and Nebeker 1999b
Rana aurora	16-d NOEC (weight)	517		Schuytema and Nebeker 1999b

Table 7.7. Studies for which LC_{10}s were calculated from published data and the statistical method used to calculate the LC_{10}.

Organism	Test Duration	Calculated LC_{10} concentration (mg NO_3^-·L^{-1})	LC_{10} Statistical Method	Reference
Pseudacris regilla	10-d	864.7 (580.1 - 1136.0)	Linear Regression	Schuytema and Nebeker 1999a
Pseudacris regilla	10-d	328.4 (196.8 - 461.7)	Probit	Schuytema and Nebeker 1999c
Xenopus laevis	10-d	4472.4	Logit	Schuytema and Nebeker 1999b
Rana aurora	16-d	1825	Nonlinear Regression	Schuytema and Nebeker 1999b
Oncorhynchus mykiss	8-d	3138.0 (1384.0 - 3782.0)	Linear Regression	Westin 1974
Oncorhynchus tshawytscha	10-d	3142.0 (2653.0 - 3437.0)	Linear Regression	Westin 1974

Table 7.8. Final freshwater nitrate toxicity data selected for long-term freshwater quality guideline development.

Sensitivity Ranking	Organism	Life Stage	Endpoint	Hardness of Exposure Water ($mg \cdot L^{-1}$ as $CaCO_3$)	Effect Concentration (mg $NO_3^- \cdot L^{-1}$)	Reference
1	*Salvelinus namaycush* Lake trout	Fry	146-d MATC (delay to swim-up wet weight)	10-16	14*	McGurk et al. 2006
2	*Ceriodaphnia dubia* Water flea	Neonates	7-d IC_{25} (reproduction)	44	50	Elphick 2011
3	*Hyalella azteca* Amphipod	Juvenile	14-d IC_{25} (growth)	46	57	Elphick 2011
4	*Oncorhynchus mykiss* Rainbow trout	Fry	41-d EC_{10} (proportion reaching swim-up)	10	58	Nautilus Environmental 2011
5	*Pimephales promelas* Fathead minnow	Larvae	32-d EC_{10} (survival)	132-180	207	US EPA 2010
6	*Chironomus dilutus* Midge	3rd instar	10-d IC_{25} (growth)	46	217	Elphick 2011
7	*Pseudacris regilla* Pacific treefrog	Tadpole	10-d LC_{10}	70-80	328	Schuytema and Nebeker 1999a
8	*Xenopus laevis* African clawed frog	Tadpole	10-d MATC (weight)	21	404*	Schuytema and Nebeker 1999c
9	*Rana aurora* Red-legged frog	Embryo	16-d MATC (weight)	26	734*	Schuytema and Nebeker 1999b

10	*Notropis topeka* Topeka shiner	Juvenile	30-d MATC (growth)	210-230	1594*	Adelman et al. 2009
11	*Daphnia magna* Water flea	Neonates	7-d MATC (reproduction)	156-172	2244*	Scott and Crunkilton 2000
12	*Oncorhynchus tshawytscha* Chinook salmon	fingerlings	10-d LC_{10}	na	3142	Westin 1974

* Value shown is the geometric mean of comparable values (see Table 7.6)

The long-term SSD was fitted using the data in Table 7.8. To create the SSD, each species was ranked according to sensitivity and its centralized position on the SSD was determined using the Hazen plotting position (Aldenberg et al. 2002; Newman et al. 2002). Five cumulative distribution functions (normal, logistic, Gompertz, Weibull, Fisher-Tippett) were then fit to the data in both arithmetic and logarithmic space following standard regression methods. Modelling assumptions were verified graphically and through assessment of statistical goodness-of-fit tests. If model residuals were found to be normally distributed, the model with the lowest Anderson-Darling goodness-of-fit (A^2) score was considered to have the best fit. Following these criteria, the log-Normal model (A^2 = 0.211) was determined to best fit the long-term freshwater dataset. The fitted SSD was therefore derived using the Normal model.

The equation for 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), $f(x)$ is the proportion of species affected, μ = 2.4307, σ = 0.7992 and *erf* is the error function (a.k.a. the Gauss error function). The long-term SSD is shown in Figure 7.2 with the summary statistics for the SSD curve presented in Table 7.9.

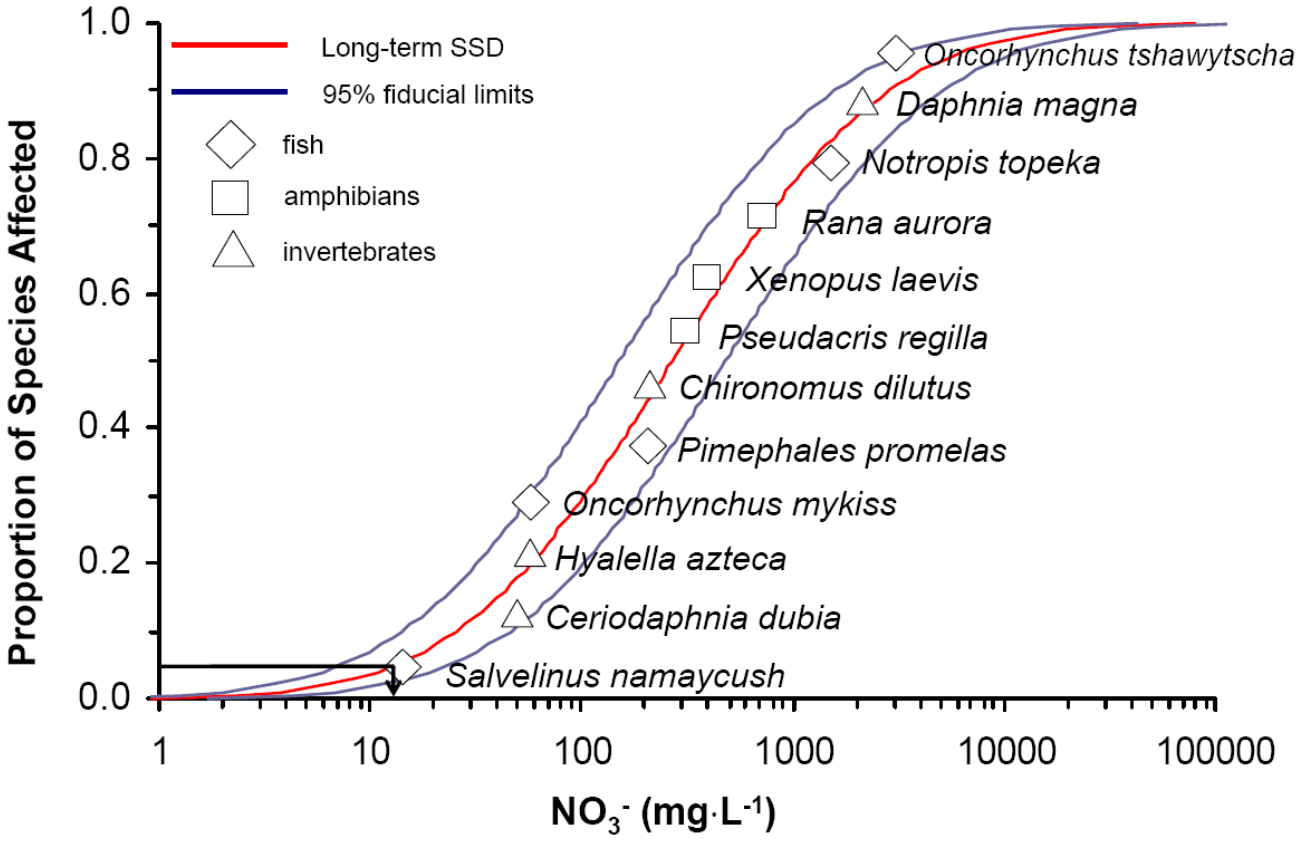


Figure 7.2. SSD of long-term no- and low-effect endpoint toxicity data for the nitrate ion in freshwater derived by fitting the Normal model to the logarithm of acceptable data for 12 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding long-term Canadian Water Quality Guideline value.

The 5th percentile on the long-term SSD is 13 mg NO_3^-·L^{-1}, and is the long-term Canadian water quality guideline (see Table 7.9). 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 life in surface waters is 13 mg NO_3^-·L^{-1}.

Table 7.9. Long-term CWQG for the nitrate ion in freshwater.

Long-term CWQG	Concentration (mg NO_3^-·L^{-1})	Concentration (mg NO_3^-N·L^{-1})
SSD 5th percentile	13	3.0
LFL (5%)	7	1.6
UFL (95%)	24	5.4

When looking at the distribution of species on the long-term freshwater SSD, the datapoints indicate a scatter or mix of invertebrate, amphibian and fish species along the curve, with effect levels ranging by more than two orders of magnitude from most to least sensitive (Figure 7.2). The early life-stage of cold-water fish species (lake trout and rainbow trout) appear to be particularly sensitive. It is of interest to note that the exposures for both lake trout and rainbow trout were conducted using very soft water (10-16 $mg \cdot L^{-1}$ as $CaCO_3$). One of the most tolerant fish (Topeka shiner) was exposed to very hard water (210-230 $mg \cdot L^{-1}$ as $CaCO_3$). The most sensitive of the 4 invertebrates are the water flea (*C. dubia*), amphipod and midge, with all tests being conducted in soft water as well (44-46 $mg \cdot L^{-1}$ as $CaCO_3$). The most tolerant was tested using hard water (156-172 $mg \cdot L^{-1}$ as $CaCO_3$). In comparison, the most sensitive amphibian (Pacific treefrog) was exposed to moderately hard water (70-80 $mg \cdot L^{-1}$ as $CaCO_3$) whereas the more tolerant amphibians (African treefrog and red-legged frog) were exposed to soft water (21-26 $mg \cdot L^{-1}$ as $CaCO_3$). Even though a definitive relationship between water hardness and nitrate toxicity was not established (see Section 6.1 – Effects of water quality parameters on toxicity), it is of interest to note the range of hardnesses of exposure water utilized in the studies selected for inclusion in the dataset for long-term guideline derivation.

It is important to note that one toxicity endpoint lies just above the guideline value (Figure 7.2), which is the 146-d MATC (developmental delay to swim-up stage and growth as wet weight) 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 both delay to swim-up stage and growth (as wet weight), of lake trout swim-up fry to be reduced at 28 $mg\ NO_3^- \cdot L^{-1}$ (low effect, or LOEC) whereas no effect (NOEC) was observed at 7 $mg\ NO_3^- \cdot L^{-1}$. Because the 2007 CCME protocol prefers the inclusion of MATC values over LOEC and NOEC values, the geometric mean (14 $mg\ NO_3^- \cdot L^{-1}$) was included in the SSD calculations. McGurk et al. (2006) also reported a MATC for lake trout mortality at 886 $mg\ NO_3^- \cdot L^{-1}$ (Appendix A). Since the CWQG of 13 $mg\ NO_3^- \cdot L^{-1}$ is below the LOEC value of 28 $mg\ NO_3^- \cdot L^{-1}$, no direct effects on developmental delays to swim-up stage, growth or survival would be expected.

7.1.4 Data Gaps / Research Recommendations

The Canadian Water Quality Guideline for the Protection of Aquatic Life for nitrate was derived solely from data on direct toxic responses to freshwater organisms and is not intended to protect against potential indirect toxic effects. Nitrate is only one of the forms of inorganic nitrogen taken up by primary producers, and therefore other forms of nitrogen may also contribute to eutrophication. An examination of the role of nitrogen and nitrogen-to-phosphorus ratios in eutrophication processes in freshwater is presented in a separate discussion paper (CCME 2002; NAESI 2005).

Potential influences of other parameters such as hardness and temperature on the toxicity of nitrate have been recently investigated (see Section 6.1 – Effects of water quality paramters on toxicity – for more details). Other potential influences, such as pH and DO, and not well understood (Section 6.1). Further research is needed on the interactions of nitrate with potassium, ammonia, UV and low pH. Recall in Section 6.3.2.3 – Amphibians – that a study by Hatch and Blaustein (2000) investigated survival and activity levels in larval Cascades frogs (*Rana cascadae*) from Oregon. The study showed that survival and activity was significantly reduced in the presence of high levels of nitrate (20 $mg\ NO_3^- \cdot L^{-1}$), ultraviolet radiation (UV-B;

280 - 315 nm) and low pH (pH 5), while not being significantly affected by high nitrate levels alone.

In general, the toxicity of the nitrate ion is well studied in the freshwater environment; however, long-term toxicity data for invertebrates are limited, particularly for non-planktonic invertebrates such as mayflies or stoneflies. In short-term studies, invertebrates appear to be the most sensitive to nitrate toxicity and it would be interesting to know if this trend continues with longer exposure to nitrate.

As discussed in Section 6.1, toxicity tests using potassium nitrate and ammonium nitrate were excluded from derivation of the freshwater guidelines because the greater toxicity observed in these studies was likely due to the K^+ and NH_4^+ ions, rather than the NO_3^-. Where the main inputs of nitrate to a freshwater system are in the form of KNO_3 and/or NH_4NO_3, adhering to the nitrate guidelines alone may not protect against adverse effects. The Canadian Water Quality Guidelines for ammonia should be followed to protect against effects from NH_4NO_3 (CCME 2000). We would also recommend that a Canadian Water Quality Guideline be developed to protect freshwater aquatic life from the adverse effects of potassium.

An interesting area for future research would be to pursue field validation of the guideline. Such validation would need to be conducted in areas where nitrate does not co-occur with other contaminants, such as those found in sewage or animal wastes, as these could have an additional effect on the aquatic community beyond the effect attributable to nitrate.

7.2 Canadian Water Quality Guideline for the Protection of Marine Life

7.2.1 Summary of Existing Water Quality Guidelines for the Protection of Marine Life

Prior to 2003, there was no guideline for the protection of aquatic life from the adverse effects of the nitrate ion for marine environments. In 2003 an interim guideline of 16 mg $NO_3^-\cdot L^{-1}$ was proposed for marine environments. The guideline for the protection of marine life was deemed interim as both primary and secondary data were included in the minimum dataset requirements (CCME 1991). The key secondary study used for guideline development exposed temperate marine adult polychaetes (Phylum: Annelida) to potassium nitrate as part of an effort to determine their susceptibility to inorganic factors present at marine sewage outfalls (Reish 1970). This static test was conducted in seawater with 19.2‰ chlorinity (or 15.5 ‰ salinity), 5.9 ppm dissolved oxygen, and a temperature range of 22° to 25°C. Of the three species of polychaetes with acceptable control mortality, the lowest 28-d TL_m (= LC_{50}) was 5.3 mg-at$\cdot L^{-1}$ (329 mg $NO_3^-\cdot L^{-1}$) for *Nereis grubei* (Reish 1970). This species is also indicative of healthy zones surrounding sewage outfalls and is generally not found directly beneath the outfall zone (Reish 1970) A safety factor of 0.05 was applied to the LC_{50}. The CCME (1991) protocol for deriving water quality guidelines recommends a safety factor of 0.1 for guidelines derived from a chronic study, and a safety factor of 0.01 for guidelines derived from an acute study. An intermediate safety factor of 0.05 was chosen for this guideline because, although it is based on a chronic study, the endpoint was an LC_{50}; therefore, low levels of mortality would have been observed at concentrations less than 329 mg $NO_3^-\cdot L^{-1}$, and sublethal effects may have occurred at even lower concentrations. The authors of the critical study noted that the test organisms used

were of adult size and, as the early larval stage is the most sensitive phase in the life history of marine invertebrates (Thorson 1956), therefore may not have represented the most conservative estimates of toxicity (Reish 1970). Further support for a conservative safety factor comes from the fact that Muir et al. (1991) observed mortality effects for juvenile tropical prawns at 1 mg $NO_3^-·L^{-1}$. Although these sensitive tropical prawns are not found in Canadian marine waters, this study flags the possibility that there may be temperate species with similarly high sensitivity to nitrate for which toxicity tests have not yet been conducted.

In other jurisdictions, Australia and New Zealand have adopted their *moderate reliability* freshwater guideline of 0.70 mg $NO_3^-·L^{-1}$ as the marine *low reliability* trigger value (Environment Australia 2000b). Although a *low reliability* trigger level of 13 mg $NO_3^-·L^{-1}$ for marine animals was derived using an uncertainty factor of 200, the more conservative *moderate reliability* freshwater value was adopted according to protocol (Environment Australia 2000b). The Netherlands have proposed a maximum acceptable concentration of 0.4 mg $NO_3^-·L^{-1}$ (Speijers et al. 1989). This value is based on a recommended limit of 0.1 mg $N·L^{-1}$ to prevent eutrophication impacts and the assumption that all nitrogen present is in the form of nitrate. This level is also deemed protective against direct toxicity to marine organisms (Speijers et al. 1989).

7.2.2 Evaluation of Toxicological Data

In accordance with the CCME protocol for the derivation of water quality guidelines for the protection of aquatic life, toxicity studies were classified as either primary, secondary or unacceptable (CCME 2007). Because the nitrate ion is non-volatile and tends to remain in solution (NRC 1978) and studies monitoring nitrate levels over time did not report any significant losses from their experimental systems (Muir et al. 1991; Scott and Crunkilton 2000), some studies using static test conditions were classified as primary. Primary and secondary studies were considered for guideline development. As there have been no conclusive relationships drawn between nitrate toxicity and ambient levels of various water quality variables (Scott and Crunkilton 2000), studies that did not report some variables, but had adequate survivorship in controls, were included. Studies using distilled and/or deionized water to hold test organisms were not included due to potential ionic influences on survival (Anderson 1944). Marine species included non-native temperate-dwelling organisms as per the CCME (2007) protocol. Toxicity data for both sodium nitrate and potassium nitrate were used in deriving the marine guidelines. The rationale for this decision was discussed in Section 6.1.

7.2.3 Marine Aquatic Life Guideline Derivation

The Protocol for the Deriviation of Canadian Water Quality Guidelines includes a guideline value for long-term exposure and a benchmark concentration for short-term exposure (CCME 2007). The long-term exposure guideline is designed to protect all species at all life stages over an indefinite exposure to a substance in water. Continuous releases may occur from point or non-point sources, gradual release from soils/sediments and gradual entry through groundwater/runoff, and long-range transport. The short-term benchmark concentration value does *not* provide guidance on protective levels of nitrate in the aquatic environment, as short-term benchmark concentrations are levels which *do not* protect against adverse effects, but rather

indicate the level where severe effects are likely to be observed.

While separate data sets are used to calculate short-term benchmark concentrations and long-term guidelines, both are derived using either a statistical approach without the application of a safey factor (Type A or Species Sensitivity Distribution), or one of two assessment factor approaches. The first assessment factor approach (Type B1) applies a safety factor to the lowest endpoint from a primary study, and the second approach (Type B2) applies a safety factor to the lowest endpoint from a primary and/or secondary study. The three approaches are detailed in CCME (2007).

All toxicity data for marine organisms can be found in appendix B. For the derivation of the short-term benchmark concentration and the long-term CWQG for the nitrate ion, this list was pared down to include data only from studies classified as primary or secondary following CCME (2007). Acceptable toxicity data were found to be available for the following aquatic species: polychaetes (*Capitella capitella*, *Dorvillea articulata*, *Neanthes arenaceodentata*, *Nereis grubei*); Australian crayfish (*Cherax quadricarinatus*); Abalone (*Haliotis tuberculata*); Purple sea urchin (*Paracentrotus lividus*); Tiger shrimp (*Penaeus monodon*); Prawn (*Penaeus paulensis*); Pacific purple sea urchin (*Strongylocentrotus purpuratus*); Anemonefish (*Amphiprion ocellaris*); Topsmelt (*Atherinops affinis*); Gulf black sea bass (*Centropristis striata*); Planehead filefish (*Monacanthus hispidus*); Rainbow trout (*Oncorhynchus mykiss*); Chinook salmon (*Oncorhynchus tshawytscha*); Beaugregory (*Pomacentrus leucostictus*); Clearnose skate (*Raja eglanteria*); and Florida pompano (*Trachinotus carolinus*) (Tables 7.10 and 7.14).

Ancillary studies for the oyster *Crassostrea virginica* and the hard clam *Mercinaria mercinaria* were not included in short-term benchmark concentration derivation due to insufficient details related to testing and water quality, as well as a lack of statistics supporting the results (Appendix B).

7.2.3.1 Derivation of the Marine Short-term Benchmark Concentration

There were sufficient data to derive a short-term benchmark concentration using an SSD (Table 7.10). Following CCME (2007), the short-term SSD was fitted using LC50 data and the final short-term benchmark concentration for nitrate was derived from the 5th percentile of the short-term SSD. Values reported in Table 7.10 range from a 96-hEC_{50} (larval development) of 1384 mg $NO_3^-{\cdot}L^{-1}$ for the purple sea urchin (*Strongylocentrotus purpuratus*) (Stantec 2006) to a 96h-LC_{50} of 13 290 mg $NO_3^-{\cdot}L^{-1}$ for the Beaugregory (*Pomacentrus leucostictus*) (Pierce et al. 1993). A geometric mean value was calculated for the Tiger shrimp, *Penaeus monodon*, because more than one LC_{50} value was available for inclusion in the SSD (Table 7.11). Effect concentrations reported for the remaining species were taken from single studies.

Table 7.10. Final marine aquatic toxicity data selected for short-term SSD development. ND means “no data”.

Sensitivity Ranking	Organism	Life Stage	Endpoint	Effect Concentration ($mg\ NO_3^-{\cdot}L^{-1}$)	Reference
1	*Strongylocentrotus purpuratus* Purple sea urchin	Larvae	96-h EC_{50} (larval development)	1384	Stantec 2006
2	*Monacanthus hispidus* Planehead filefish	ND	96-h LC_{50}	2538	Pierce et al. 1993
3	*Raja eglanteria* Clearnose skate	ND	96-h LC_{50}	>4253[1]	Pierce et al. 1993
4	*Oncorhynchus tshawytscha* Chinook salmon	Fingerling	96-h LC_{50}	4400	Westin 1974
5	*Trachinotus carolinus* Florida pompano	ND	96-h LC_{50}	4430	Pierce et al. 1993
6	*Oncorhynchus mykiss* Rainbow trout	Fingerling	96-h LC_{50}	4650	Westin,1974
7	*Penaeus monodon* Tiger shrimp	Juveniles	96-h LC_{50}	7717*	Tsai and Chen 2002
8	*Penaeus paulensis* Prawn	Adult	96-h LC_{50}	9621	Cavalli et al. 1996
9	*Centropristis striata* Gulf black sea bass	ND	96-h LC_{50}	10632	Pierce et al. 1993
10	*Pomacentrus leucostictus* Beaugregory	ND	96-h LC_{50}	13290	Pierce et al. 1993

[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 values (see Table 7.11).

Table 7.11. Geometric means derived from the results of Tsai and Chen 2002 for inclusion in the SSD.

Organism	Life Stage	Endpoint	Effect concentration ($mg\ NO_3^-{\cdot}L^{-1}$)	Geomean	Reference
Penaeus monodon Tiger shrimp	Juvenile	96-h LC_{50}	6419	7717	Tsai and Chen 2002
	Juvenile	96-h LC_{50}	6977		Tsai and Chen 2002
	Juvenile	96-h LC_{50}	10260		Tsai and Chen 2002

The short-term SSD was fitted using the data in Table 7.10 and the final benchmark concentration value for the nitrate ion was determined as the 5th percentile of the curve. To create the SSD, five cumulative distribution functions (normal, logistic, Gompertz, Weibull, Fisher-Tippett) were fit to the data in both arithmetic and logarithmic space following standard regression techniques. Modelling assumptions were verified graphically and through assessment of statistical goodness-of-fit tests. If the model residuals were found to be normally distributed, the model with the lowest Anderson-Darling goodness-of-fit (A^2) score was considered to have the best fit. Following these criteria, the Logistic model (A^2 = 0.303) was determined to best fit the short-term marine dataset. The fitted SSD was therefore derived using the log-logistic model.

The equation for the Logistic model is:

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

Where for the fitted model: x = log (concentration) of nitrate (mg/L), y is the proportion of species affected, μ = 3.7290 and σ = 0.1881. Data for marine aquatic organisms are presented in Figure 7.3, with the summary statistics for the SSD curve is presented in Table 7.12.

The 5th percentile of the short-term SSD is 1497 mg $NO_3^-{\cdot}L^{-1}$. 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 within the range of the data (to which the model was fit). Therefore, the 5th percentile and its confidence limits are interpolations. The short-term benchmark concentration value for nitrate is the 5th percentile of the SSD and is therefore 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 7.12).

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 $NO_3^-{\cdot}L^{-1}$.

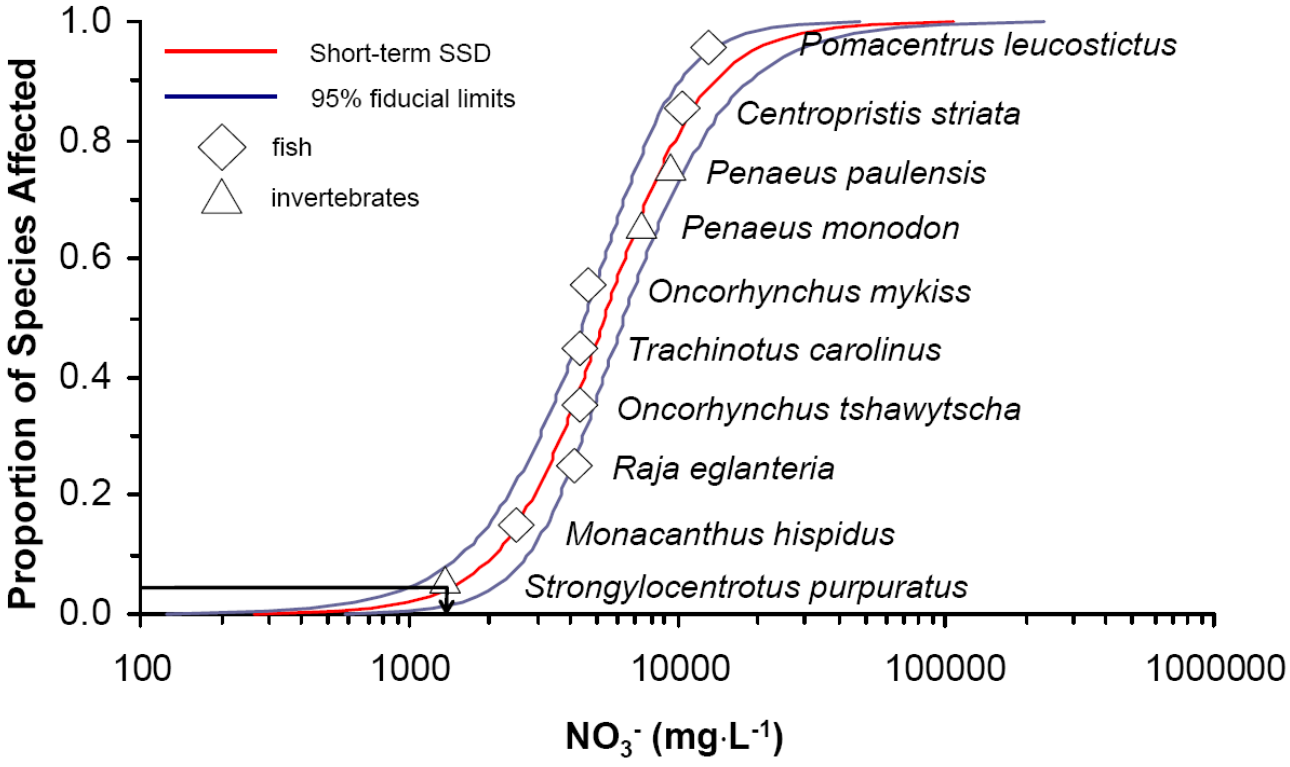


Figure 7.3. SSD of short-term L/EC50 toxicity data for the nitrate ion in saltwater derived by fitting the Logistic model to the logarithm of acceptable toxicity data for 10 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding short-term benchmark concentration value.

Table 7.12. Short-term benchmark concentration for the nitrate ion in marine ecosystems.

Short-term Benchmark Concentration	**Concentration (mg NO_3^-·L^{-1})**	**Concentration (mg NO_3^--N·L^{-1})**
SSD 5th percentile	1500	339
LFL (5%)	1046	236
UFL (95%)	2141	483

In the short-term marine SSD curve, 5 of the 7 fish species are arranged at the bottom with 2 invertebrate species plotted midway, and 2 other fish species plotted at the top. The most sensitive organism is an invertebrate, the purple sea urchin *S. purpuratus*, plotted at the lower tail end of the SSD (Figure 7.3). This data point for the purple sea urchin (96-hour EC_{50} of 1384 mg NO_3^-/L [Stantec 2006]) falls below the short-term SSD 5th percentile value of 1497 mg NO_3^-/L. Based on the short-term SSD, short-term exposures to levels of nitrate exceeding the benchmark

concentration of 1500 mg NO_3^-/L *may* pose the greatest hazard to the sensitive purple sea urchin. Note that meeting the long-term guideline will protect from severe effects.

7.2.3.2 Derivation of the Long-term Canadian Marine Guideline

There were several long-term nitrate toxicity studies for marine species from which to select data to derive a long-term CWQG using an SSD (Appendix B). The list was pared down by first selecting the endpoints appropriate for inclusion in the SSD following CCME (2007). The order of preference for the use of long-term endpoints is: most appropriate EC_x/IC_x representing a no-effects threshold > EC_{10}/IC_{10} > $EC_{11\text{-}25}/IC_{11\text{-}25}$ > MATC > NOEC > LOEC > $EC_{26\text{-}49}/IC_{26\text{-}49}$ > nonlethal EC_{50}/IC_{50}.

Many older studies only report LC_{50} values. As LC_{50} is not an appropriate endpoint for inclusion in the long-term SSD, these values were recalculated where possible to LC_{10}, the preferred endpoint. Table 7.13 includes all the studies for which sufficient data were available for calculation of an LC_{10}. The final aquatic toxicity data selected for long-term CWQG development for marine environments can be found in Table 7.14.

Table 7.13. Marine studies for which LC_{10}s were calculated from published data and the statistical method used to calculate the LC_{10}.

Organism	**Test Duration**	**Calculated LC_{10} concentration (mg $NO_3^-{\cdot}L^{-1}$)**	**LC_{10} Statistical Method**	**Reference**
Capitella capitella	28-d	660.4	Probit	Reish, 1970
Dorvillea articulata	28-d	699.6 (553.6 - 764.4)	Probit	Reish, 1970
Neanthes arenaceodentata	28-d	439.5 (259.3 - 490.7)	Probit	Reish, 1970
Nereis grubei	28-d	214.0 (26.3 – 315.8)	Probit	Reish, 1970
Oncorhynchus mykiss	7-d	2954 (1491 - 3503)	Linear Regression	Westin, 1974
Oncorhynchus tshawytscha	7-d	3510 (2894 - 3781)	Linear Regression	Westin, 1974

Table 7.14. Final aquatic toxicity data selected for long-term SSD development for marine environments. 'ND' indicate no data available.

Sensitivity Ranking	Organism	Life Stage	Endpoint	Effect Concentration (mg $NO_3^-·L^{-1}$)	Reference
1	*Nereis grubei* Polychaete	ND	28-d LC_{10}	214	Reish, 1970
2	*Neanthes arenaceodentata* Polychaete	ND	28-d LC_{10}	440	Reish, 1970
3	*Amphiprion ocellaris* Anemonefish	Larvae	72-d LOEC (growth, mortality)	443	Frakes and Hoff Jr., 1982
4	*Capitella capitella* Polychaete	ND	28-d LC_{10}	660	Reish, 1970
5	*Dorvillea articulate* Polychaete	ND	28-d LC_{10}	700	Reish, 1970
6	*Haliotis tuberculata* Abalone	ND	15-d LOEC (growth)	1108	Basuyaux and Mathieu, 1999
7	*Paracentrotus lividus* Purple Sea Urchin	ND	15-d LOEC (growth / feeding)	1108	Basuyaux and Mathieu, 1999
8	*Strongylocentrotus purpuratus* Pacific purple sea urchin	Larvae	4-d IC_{25} (larval development)	1178	Stantec, 2006
9	*Atherinops affinis* Topsmelt	Adult	7-d LC_{25}	2554	Stantec, 2006
10	*Oncorhynchus mykiss* Rainbow trout	Fingerling	7-d LC_{10}	2954	Westin, 1974
11	*Oncorhynchus tshawytscha* Chinook salmon	Fingerling	7-d LC_{10}	3510	Westin, 1974
12	*Cherax quadricarinatus* Australian crayfish	Juvenile	5-d NOEC (respiration)	4430	Meade and Watts, 1995

Values reported in Table 7.14 range from a 28-d EC_{10} of 214 mg $NO_3^-·L^{-1}$ for the polychaete worm, *Nereis grubei*, to a 5-d LOEC of 4430 mg $NO_3^-·L^{-1}$ for the Australian crayfish, *Cherax quadricarinatus* (Reish, 1970; Meade and Watts, 1995). The long-term SSD was fitted using the data in Table 7.14.

To create the SSD, each species was ranked according to sensitivity and its centralized position on the SSD was determined using the Hazen plotting position (Aldenberg et al. 2002; Newman et

al. 2002). Five cumulative distribution functions (normal, logistic, Gompertz, Weibull, Fisher-Tippett) were fit to the data in both arithmetic and logarithmic space following standard regression techniques. Modelling assumptions were verified graphically and through assessment of statistical goodness-of-fit tests. If the model residuals were found to be normally distributed, the model with the lowest Anderson-Darling goodness-of-fit (A^2) score was considered to have the best fit. Following these criteria, the log-Normal model ($A^2 = 0.252$) was determined to best fit the long-term marine dataset.

The equation for the log-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), $f(x)$ is the proportion of species affected, $\mu = 3.0385$, $\sigma = 0.4539$ and *erf* is the error function (a.k.a. the Gauss error function). Data for marine aquatic organisms are presented in Figure 7.4, with the summary statistics for the SSD curve is presented in Table 7.15.

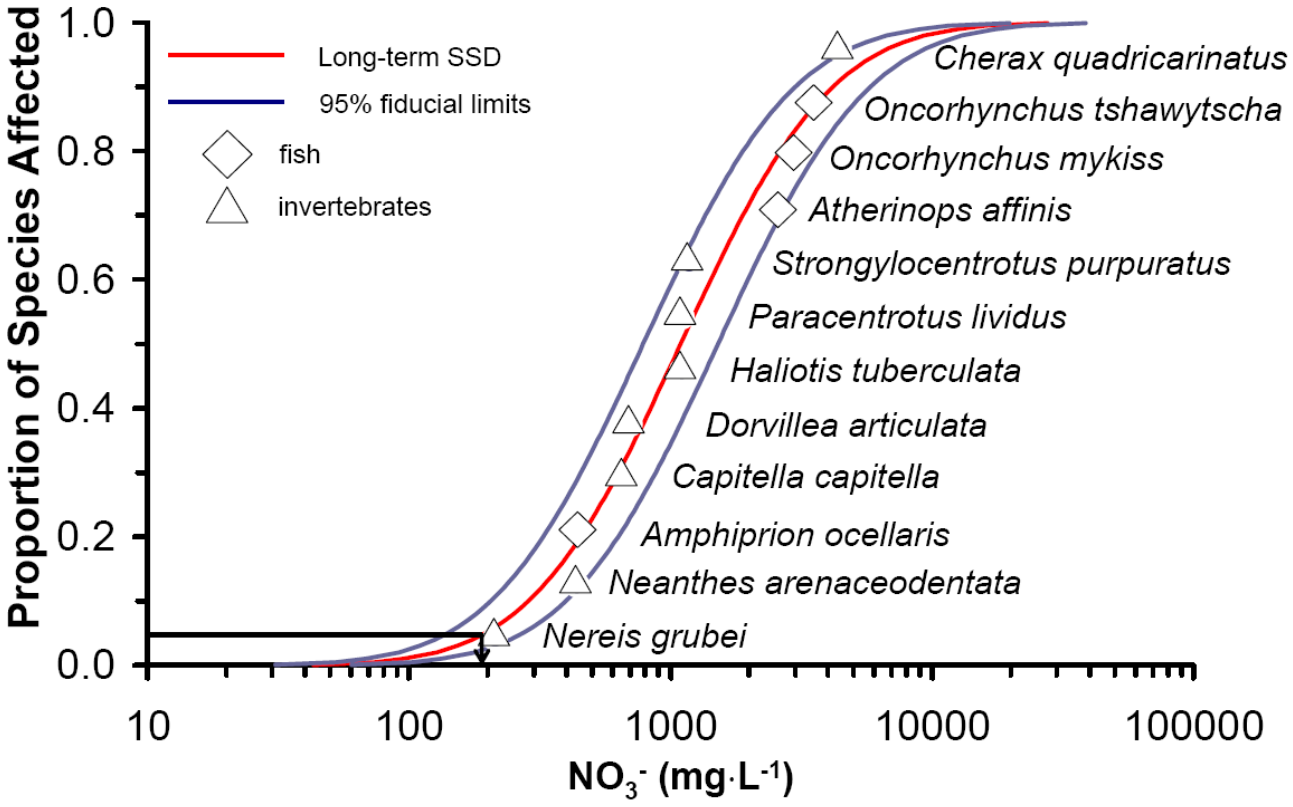


Figure 7. 4. SSD of long-term no- and low-effect endpoint toxicity data for nitrate in saltwater derived by fitting the Normal model to the logarithm of acceptable data for 12 aquatic species versus Hazen plotting position (proportion of species affected). The arrow at the bottom of the graph denotes the 5th percentile and the corresponding long-term Canadian Water Quality Guideline value.

Table 7.15. Long-term CWQG for the nitrate ion in marine ecosystems.

Long-term CWQG	Concentration (mg $NO_3^-·L^{-1}$)	Concentration (mg NO_3^--N·L^{-1})
SSD 5th percentile	200	45
LFL (5%)	141	32
UFL (95%)	273	62

The 5th percentile of the long-term SSD is 196 mg $NO_3^-·L^{-1}$. The lower fiducial limit (5%) on the 5th percentile is 141 mg $NO_3^-·L^{-1}$, and the upper fiducial limit (95%) on the 5th percentile is 273 mg $NO_3^-·L^{-1}$. The long-term CWQG for the nitrate ion is the 5th percentile of the SSD and is therefore 196 mg $NO_3^-•L^{-1}$. This value is rounded up to 2 significant digits to generate the final guideline value of 200 mg $NO_3^-•L^{-1}$. **Therefore, the proposed long-term CWQG for the protection of marine life in surface waters is 200 mg $NO_3^-·L^{-1}$.**

In the long-term marine SSD curve, there is a better mix of invertebrate and fish species along the curve (Figure 7.4). This result suggests that all marine animals are similarly affected by NO_3^- through time.

No data points fall below the 5th percentile value, or long-term CWQG, indicating that protection from adverse long-term impacts should be afforded to marine aquatic life when long-term concentrations do not exceed 200 mg $NO_3^-·L^{-1}$.

The CCME guideline derivation protocol (2007) indicates that for the derivation of long-term guidelines, acceptable data include non-lethal endpoints from test durations of ≥ 96-h for shorter-lived invertebrates (e.g., *Ceriodaphnia dubia)* non-lethal endpoints of ≥ 7 days duration for longer-lived invertebrates (e.g., crayfish), and lethal endpoints from tests of ≥ 21 days duration for longer-lived invertebrates (CCME 2007). Due to the paucity of high quality long-term studies with marine organisms, it was decided to include the 4-d IC_{25} effect concentration for the larval life stage of the purple sea urchin, as well as the 5-d LOEC effect concentration for the juvenile Australian crayfish (Table 7.14). These data points were not the most sensitive in the dataset. In the development of a marine CWQG, regulators want to have as much certainty as possible that protection will be afforded to all marine species at all life stages for indefinite exposure periods, and this translates to including as many data points as possible into the SSD. As a verification, the SSD was in fact calculated with n=10 datapoints (where the larval purple sea urchin 4-d IC_{25} and Australian crayfish 5-d LOEC effect concentrations were removed). The log-Normal model fit best, with a resulting 5th percentile value of 177 mg $NO_3^-·L^{-1}$, with respective LFL (5%) and UFL (95%) of 118 and 264 mg $NO_3^-·L^{-1}$. The two datasets essentially derive the same guideline value, since the 5th percentile value of 177 mg $NO_3^-·L^{-1}$ (derived with n=10 datapoints) falls within the fiducial limits of the 5th percentile value derived using n=12 datapoints, as provided in Table 7.14 (which includes the larval purple sea urchin 4-d IC_{25} and Australian crayfish 5-d LOEC effect concentrations).

7.2.4 Data Gaps / Research Recommendations

The current marine dataset of acceptable primary and secondary acute toxicity studies contains seven species of fish from two studies, and three species of invertebrates from three studies

(Appendix B). Chronic studies include four temperate species of fish from three studies, and nine species of invertebrates from five studies (Appendix B).

Both the short-term and long-term marine data sets meet the minimum data requirements for the development of respective short-term benchmark concentration and long-term CWQG values. However, these guideline values are based on relatively small datasets of 9 species for the short-term studies of which 7 are fish, and 3 invertebrates. In the case of the long-term dataset, 12 marine species are respresented, of which 4 are fish species, and 8 are invertebrates. More studies of both marine fish and invertebrates (especially those endemic to Canadian waters) may serve to increase confidence in the guideline values as derived by the SSDs. As well, more studies of the relationship between nitrate toxicity and salinity would be useful for the development of guideline values for nearshore marine environments. As nitrate is a required nutrient for plant growth, no marine plant toxicity studies were required for guideline development.

7.3 General Discussion

Nitrate is the oxidation product by micro-organisms in plants, soil or water and, to a lesser extent, by electrical discharges such as lightning of many nitrogenous materials. The nitrate ion is also very mobile as its salts are water soluble. In agricultural landscapes, it is ubiquitous. All nitrogen sources including organic nitrogen, ammonia and fertilizers are potential sources of nitrates.

In water, nitrate is transformed through nitrification and denitrification of total ammonia (NH_{3+4}) and nitrite (NO_2^-) and may be removed through assimilation by primary producers. High levels of un-ionized ammonia (NH_3) and nitrite (NO_2^-) are very toxic to aquatic animals including nitrifying bacteria, which can hamper nitrification (Camargo and Alonso, 2006). Of the three inorganic nitrogen forms, nitrate is the most benign. Before it can become toxic, it must be converted to nitrite under internal body conditions (Camargo and Alonso, 2006). Overall, nitrate uptake in aquatic animals is more limited than nitrite uptake, which contributes to the relatively low toxicity of nitrate (Camargo and Alonso, 2006).

An abundance of toxicity data allowed for the use of species-sensitivity distributions for the derivation of short-term benchmark concentrations and long-term CWQGs for both freshwater and marine environments. This application of the SSD has resulted in no change in the freshwater NO_3^- standards from the 2003 interim values, whereas a substantial increase was observed in the marine NO_3^- standard from the 2003 interim value (Environment Canada, 2003). There are several likely underlying reasons for the substantial change in the marine long-term guideline value. The 2003 interim guideline was derived using the 1991 CCME guideline derivation protocol (CCME, 1991). When the 1991 protocol was created, toxicity tests were frequently only available for a limited number of aquatic species. Recognizing the vast range of plants and animals not tested, guidelines were derived by applying a safety factor to the most sensitive endpoint – referred to as the "lowest endpoint derivation approach" (CCME 2007). The safety factor was applied to account for differnces in sensitivity to a chemical variable due to differences in species (intra- and inter-species variability), exposure conditions (laboratory versus field, varying environmental conditions), and test endpoints, as well as a paucity of toxicological data, cumulative exposures and policy requirements (in particular, extrapolating

from a low-effect toxicological threshold to a protective environmental management benchmark) (CCME 2007). The result was the development of guidelines that were frequently considered to be either too conservative (e.g. were below method detection limits), as there was no evidence more sensitive species requiring the extra protection existed in the environment, or at times not conservative enough (e.g. CWQG set with a very limited dataset). As well, the old CCME 1991 protocol provided methodology to choose the one study with the most sensitive endpoint (referred to as the critical study endpoint), placing a great deal of emphasis on one study, one species and one toxicity endpoint.

The 2003 interim guideline for the protection of marine life was derived from a study in which temperate marine adult polychaete worms were exposed to potassium nitrate in an effort to determine their susceptibility to inorganic factors present at marine sewage outfalls (Reish 1970). Of the three polychaete species, the lowest 28-d LC_{50} was 329 mg $NO_3^-{\cdot}L^{-1}$ for *Nereis grubei* (Reish 1970). An intermediate safety factor of 0.05 was applied to this value to produce a guideline of 16 mg $NO_3^-{\cdot}L^{-1}$. An intermediate safety factor was chosen for this guideline because, although it was based on a chronic study, the endpoint was an LC_{50}; therefore, low levels of mortality would have been observed at concentrations less than 329 mg $NO_3^-{\cdot}L^{-1}$, and sublethal effects may have occurred at even lower concentrations. The current CWQG calculated using a species sensitivity distribution has resulted in an increased value of 200 mg $NO_3^-{\cdot}L^{-1}$, with all minimum dataset requirements for the development of a CWQG fulfilled. It must be noted that the 2003 CWQG was interim, meaning that the required dataset was not fulfilled (one chronic study on a marine invertebrate endemic to Canadian waters was missing). 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 CWQG of 200 mg $NO_3^-{\cdot}L^{-1}$,to the data for temperate marine species in Appendix B 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.

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 the CCME (1991) protocol and the final result was rounded to 13 mg $NO_3^-{\cdot}L^{-1}$. The current CWQG calculated using a species sensitivity distribution has resulted in an unchanged guideline value of 13 mg $NO_3^-{\cdot}L^{-1}$, with all minimum dataset requirements for the development of a CWQG 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). In addition to this, testing was conducted on the early life stage of two species of sensitive salmonids, including the lake trout and rainbow trout (McGurk et al. 2006). Test results indicated that the CWQG of 13 mg $NO_3^-{\cdot}L^{-1}$ would be protective of these sensitive fish species. Therefore, the freshwater CWQG value is still considered to abide

by the guiding principle of protecting all aquatic organisms at all life stages during indefinite exposure periods.

Another cause of the slight increase in the full CWQG from the interim CWQGs is the splitting of the toxicological data into short- and long-term standards. The short-term benchmark concentration is an estimator of severe effects to the aquatic ecosystem and is 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/nonpersistent 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, but rather indicate the level where severe effects are likely to be observed. The long-term CWQG is designed to protect against any adverse effect. For nitrate, long-term exposure generally tends to be more deleterious to aquatic animals as it is the accumulation of nitrite in the blood that impairs oxygen transport, hence weakening animals (Camargo and Alonso, 2006).

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 (although the guiding principle of protecting all species at all life stages is met due to the inclusion of mostly no-effect data into the SSD). The long-term freshwater CWQG is a case where the lowest data point exists just above the 5th percentile. McGurk et al. (2006) observed both delay to swim-up stage and growth (as wet weight), of lake trout swim-up fry to be reduced at 28 mg $NO_3^-{\cdot}L^{-1}$ whereas no effect (NOEC) was observed at 7 mg $NO_3^-{\cdot}L^{-1}$. Because the 2007 CCME protocols prefers the inclusion of MATC values over LOEC and NOEC values, the geometric mean (14 mg $NO_3^-{\cdot}L^{-1}$) was included in the SSD calculations. The equivalent endpoint (MATC) for delay to swim-up stage for rainbow trout swim-up fry is 58 mg $NO_3^-{\cdot}L^{-1}$ (Nautilus Environmental, 2011). Since the CWQG of 13 mg $NO_3^-{\cdot}L^{-1}$ is below the LOEC of 28 mg $NO_3^-{\cdot}L^{-1}$ for the lake trout (McGurk et al 2006), and since the MATC for lake trout mortality is 886 mg $NO_3^-{\cdot}L^{-1}$ (Appendix A), no effects on delay to swim-up, growth or survival are expected at the level of the derived CWQG.

One study noted NO_3^- toxicity endpoints just below and above the value of the long-term freshwater CWQG of 13 mg $NO_3^-{\cdot}L^{-1}$. Kincheloe et al. (1979) found concentrations as low as 10 and 20 mg $NO_3^-{\cdot}L^{-1}$ could significantly increase egg and fry mortality in Coho salmon (*Oncorhynchus kisutch*) and Rainbow trout (*Oncorhynchus mykiss*). Although this study demonstrated sensitivity of eggs and early salmonid life stages to nitrate, additional egg mortalities caused by *Saprolegnia* fungal infestations could not be separated from the data by the authors and the results of this study were not considered useable for CWQG development.

The SSDs reveal interesting information about the nature of NO_3^- toxicity to aquatic animals. In the short-term, freshwater curve, invertebrate species are arranged at the bottom with fish species at the top (Figure 7.1), whereas the long-term freshwater (Figure 7.2) and both marine curves (Figures 7.3 and 7.4) show a better mix of invertebrate, amphibians and fish species along the curve – although cold-water salmonids appear to be most sensitive with respect to long-term exposures.

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). The guideline values are so high they represent NO_3^- concentrations 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.

8 GUIDANCE ON APPLICATION OF THE GUIDELINES

8.1 General Guidance on the Use of Guidelines

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.

The short-term benchmark concentration and long-term CWQG for nitrate are set to provide protection for short- and long-term exposure periods, respectively. The short-term guideline is 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) and is an estimator of severe effects to the aquatic ecosystem. 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 long-term CWQG is intended to protect all forms of aquatic life for indefinite exposure periods. Both the short-term and long-term guideline values are based on generic environmental fate and behaviour and toxicity data. The 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 it is mostly based on toxicity tests using naïve (i.e., non-tolerant) laboratory organisms, the guideline may not be relevant for areas with a naturally elevated concentration of nitrate and associated adapted ecological community (CCME 2007). Thus, if an exceedence of the guideline is observed (due to anthropogenically enriched water or because of elevated natural background concentrations), 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).

Fiducial limits are reported along with the 5^{th} percentile or guideline value. Fiducial limits (or inverse confidence limits) represent the range in concentration at which a certain proportion of taxa are expected to be affected by a substance (the confidence around the independent variable,

in this case, being the concentration of nitrate). Note that only the 5th percentile is used as the guideline value.

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 the CCME do not necessarily endorse these methods.

8.2 Monitoring and Analysis of Nitrate Levels

In comparing field measurements of nitrate to the Canadian water quality guidelines, it is important to be aware of potential seasonal and meteorological impacts at the time of sampling. Nitrate concentrations in surface waters can peak for short periods of time during storm events and spring melt. As these pulses often occur in the spring when the most sensitive life stages (e.g., larvae) for many organisms are present, their relationship to the guideline should be considered. A stream may normally have a low baseline concentration of nitrate, but during and immediately following (1-2 days) one of these events, the nitrate concentrations could exceed the guideline value. The exceedance could result from one of two scenarios. First, the increase in nitrate could occur as a result of a natural increase in background levels, for example due to snow melt in a pristine area. Second, the source of the nitrate in storm- or meltwater may not be natural; for example, it could be due to runoff from agricultural fields where nitrate fertilizer has been applied, or due to greater inputs from combined sewer overflows. In the former case the guidelines do not strictly apply (because a guideline cannot be set lower than natural background levels for a naturally occurring substance). Nonetheless, we recommend that if nitrate levels are found to exceed the recommended guideline values, that data on the frequency and severity of the exceedances should be evaluated on a site-specific basis to determine whether they warrant any preventative or remedial actions.

For monitoring long-term temporal trends in nitrate levels, an undue weighting should not be given to samples that were collected during, or immediately following a storm event, or during the spring thaw. Due to seasonal variability in nitrate levels, comparison of long-term trend data should occur between standardized collection intervals over similar time periods (i.e, spring, summer, fall, winter).

Depending on the analytical methods used, water samples are sometimes analysed for the total concentration of nitrate plus nitrite. In most cases, these measured nitrate + nitrite concentrations consist almost entirely of nitrate, and therefore may be directly compared to the guidelines recommended in this document, which are given for concentrations of nitrate. Most natural ambient waters are sufficiently aerobic that nitrite concentrations are negligible, with the nitrite

being readily oxidised to nitrate by nitrifying bacteria (NRC 1978; Halling-Sorensen and Jorgensen 1993). Where direct comparison might not be appropriate, due to the possibility of elevated levels of nitrite, is with water samples obtained from highly reducing environments. Low redox potentials (E_h) which would promote nitrite formation are associated with elevated pH and waters nearing anoxia (Figure 8.1a,b). These conditions are often found at the sediment-water interface, at the bottom of permanently stratified meromictic lakes, or in bogs and bog lakes with very high levels of reducing humic acids (Wetzel 2001).

8.3 Developing Site-Specific Guidelines and Objectives

National guidelines, such as the one for nitrate, can be the basis for the derivation of site-specific guidelines (e.g. derived with site-specific scientific data) as well as objectives (e.g. derived with site-specific scientific data as well as consideration of technological, site-specific socioeconomic, or management factors) (CCME 2007). There are some cases in which the development of site-specific objectives for nitrate should be considered. The guidelines were derived to be protective of all forms of aquatic life and all aspects of the aquatic life cycles, including the most sensitive life stage of the most sensitive species over the long term. However, in locations where highly sensitive or endangered species occur, or in areas where species of commercial / recreational importance occur, water managers may wish to consider the use of a more conservative site-specific objective. Conversely, where certain sensitive species are historically absent, the use of less conservative site-specific objectives for those particular areas could be justified.

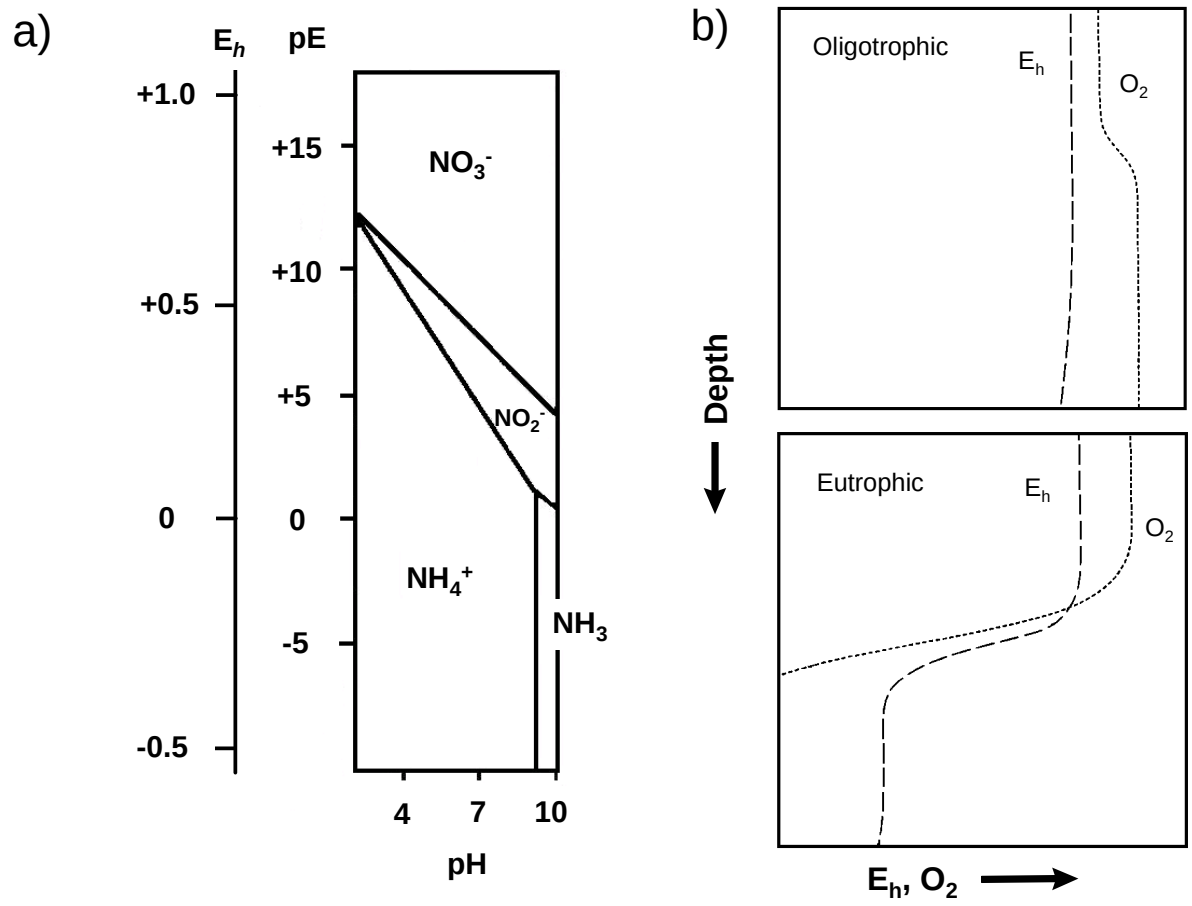


Figure 8.1. a) Redox potential (E_h) and electron potential (pE) for various species of inorganic nitrogen, as a function of pH (note: N_2 is treated as a redox inert compound). b) Generalized vertical distribution of redox potential and dissolved oxygen in stratified lakes of very low and very high productivity. [from a) Stumm and Morgan 1981; b) Wetzel 2001]

Managers of surface water bodies where there are groundwater upwellings should note that elevated levels of nitrate (i.e., above the recommended guideline values) in the immediate vicinity of the upwelling could pose a potential risk to some aquatic life. In particular, brook trout, and other fish species that seek out groundwater upwelling areas for spawning may be at risk. At present there are no existing nitrate toxicity data available for brook trout, so comments cannot be made about the sensitivity of this species. It is possible that brook trout eggs are more susceptible to nitrate toxicity than other fish eggs discussed in this document (e.g., fathead minnow, rainbow trout, salmon), because they have a longer incubation period (Morris 2001). Also, hatching of brook trout eggs occurs in March and April when groundwater levels of nitrate peak. In fish spawning areas, managers may want to consider setting more conservative site-specific nitrate objectives.

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).

8.4 Trophic Status Management

The nitrate WQGs developed in this document are intended to protect aquatic life from direct toxic effects. Nitrate concentrations that are below these levels, however, may still contribute to increased primary production within a waterbody, and could therefore result in indirect toxic effects that are associated with eutrophication. Due to the wide range in responses seen in algal biomass and species composition as a result of increased nitrate supply, and the simultaneous influence of other factors in regulating primary production (e.g., phosphorus levels, light availability, water retention times), it may not be feasible to propose threshold levels for inorganic nitrogen in fresh waters which will protect against nuisance algal growth (CCME 2002; NAESI 2005). To assess the role of nitrate in regulating production in a specific waterbody, nitrogen-to-phosphorus ratios could be used to first determine potential nutrient limitation, followed by nutrient bioassays with resident water sources to determine the impact from increased nitrate levels (see CCME 2002; NAESI 2005).

9.0 GUIDELINE SUMMARY

The short-term freshwater data met the toxicological and statistical requirements for the Type A guideline derivation method (Table 7.1). The Gompertz model was used for short-term benchmark concentration derivation. As seen in Table 7.3, the data requirements for the SSD were surpassed, and a total of 23 data points from 23 species (fish, amphibians and invertebrates) were used in the derivation of the benchmark concentration. Only LC_{50} values were used in the derivation.

The long-term freshwater data met the toxicological and statistical requirements for the Type A guideline derivation method (Table 7.1). The Normal model was used for long-term guideline derivation. As seen in Table 7.8, the data requirements for the SSD were surpassed, and a total of 12 data points from 12 species (fish, amphibians and invertebrates) were used in the derivation of the guideline.

The short-term marine data met the toxicological and statistical requirements for the Type A guideline derivation method (Table 7.2). The Logistic model was used for short-term benchmark concentration derivation. As seen in Table 7.10, the data requirements for the SSD were surpassed, and a total of 10 data points from 10 species (fish and invertebrates) were used in the derivation of the benchmark concentration. Mostly LC_{50} and one EC_{50} value was used in the derivation.

The long-term marine data met the toxicological and statistical requirements for the Type A guideline derivation method (Table 7.2). The Normal model was used for long-term guideline derivation. As seen in Table 7.14, the data requirements for the SSD were surpassed, and a total of 13 data points from 12 species (fish and invertebrates) were used in the derivation of the guideline.

The following Canadian water quality guidelines (CWQGs) are recommended to protect aquatic biota from harmful exposure to nitrate in water.

Canadian Water Quality Guideline and Benchmark Concentration for Nitrate for the Protection of Aquatic Life[‡]

	Long-Term[c] Water Quality Guideline (95% fiducial limits)	Short-Term[d] Benchmark Concentration (95% fiducial limits)
2011 update		
Freshwater[a]	13 mg NO_3^-·L^{-1} (7, 24)	550 mg NO_3^-·L^{-1} (457, 652)
	3.0 mg NO_3^--N·L^{-1} (1.6, 5.4)	124 mg NO_3^--N·L^{-1} (103, 147)
Marine[b]	200 mg NO_3^-·L^{-1} (141, 273)	1500 mg NO_3^-·L^{-1} (1046, 2141)
	45 mg NO_3^--N·L^{-1} (32, 62)	339 mg NO_3^--N·L^{-1} (236, 483)

[‡] = 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).

REFERENCES

AAFC (Agriculture and Agri-Food Canada). 2000. The health of our water: Toward sustainable agriculture in Canada. Coote, D. R. and L. J. Gregorich (eds.). Report no. Publication 2020/E. Research Branch, Agriculture and Agri-Food Canada. 173 pp.

Adelman, I. R., L. I. Kusilek, J. Koehle and J. Hess. 2009. Acute and chronic toxicity of ammonia, nitrite and nitrate to the endangered topeka shiner (*Notropis topeka*) and fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. 28(10):2216-2223.

Admiraal, W. 1977. Tolerance of estuarine benthic diatoms to high concentrations of ammonia, nitrite ion, nitrate ion and orthophosphate. Mar. Biol. 43: 307-315.

AEP (Alberta Environmental Protection). 1999. Surface water quality guidelines for use in Alberta. Report no. T/483. Environmental Sciences Division, Alberta Environment. Edmonton, AB. iii + 20 pp.

Ahn, O., R. J. Petrell, and P. J. Harrison. 1998. Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. J. Appl. Phycol. 10: 333-340.

Alkema, H. 2000. Personal Communication. Quality Assurance Specialist, National Laboratory for Environmental Testing, Environment Canada, National Water Research Institute, Burlington, ON.

Alleman, J. E., V. Keramida, and L. Pantea-Kiser. 1987. Light induced *Nitrosomonas* inhibition. Wat. Res. 21(4): 499-501.

Allran, J. W. and W. H. Karasov. 2000. Effects of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae exposed in the laboratory from posthatch through metamorphosis. Environ. Toxicol. Chem. 19(11): 2850-2855.

Alonso, A. and J.A. Camargo. 2003. Short-term toxicity of ammonia, nitrite, and nitrate to the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca). Bulletin of Environmental Contamination and Toxicology. 70: 1006-1012.

Anderson, A. M., D. O. Trew, R. D. Neilson, N. D. MacAlpine, and R. Borg. 1998. Impacts of agriculture on surface water quality in Alberta, Part II: Provincial stream survey. Canada-Alberta Environmentally Sustainable Agriculture Agreement. Government of Canada, Government of Alberta. 91 pp.

Anderson, B. G. 1944. The toxicity thresholds of various substances found in industrial wastes as determined by the use of *Daphnia magna*. Sew. Works J. 16(6): 1156-1165.

-----. 1946. The toxicity thresholds of various sodium salts determined by the use of *Daphnia magna*. Sew. Works J. 18(1): 82-87.

ANL (Argonne National Laboratory). 2005. Potassium-40. Human Health Fact Sheet. August 2005. Accessed at: http://www.ead.anl.gov/pub/doc/potassium.pdf

Antonakos, A. and N. Lambrakis. 2000. Hydrodynamic characteristics and nitrate propagation in Sparta Aquifer. Wat. Res. 34(16): 3977-3986.

Anuradha, S. and V. Subburam. 1995. Role of sewage bacteria in methemoglobin formation in *Cyprinus carpio* exposed to nitrate. J. Environ. Biol. 16(2): 175-179.

APHA (American Public Health Association). 1998. Inorganic nonmetallic constituents. *In*: Standard methods for the examination of water and wastewater. (20th Ed.). Franson, M. A. H. (ed.). American Public Health Association. Washington D.C. pp. 4-1-4-181.

ASTM (American Society for Testing Materials). 1997a. Standard guide for conducting the frog embryo teratogenesis assay - *Xenopus* (Fetax). Philedelphia, PA. Vol. ASTM E. 1439-1491.

-----. 1997b. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Philadelphia, PA. Vol. ASTM. 729-796.

-----. 2000a. Standard test methods for nitrite-nitrate in water. *In*: Annual Book of ASTM Standards, Section 11: Water and Environmental Technology, Vol. 11.01: Water (1). American Society for Testing and Materials. West Conshohocken, PA. pp. 365-372.

-----. 2000b. Standard test method for anions in water by chemically suppressed ion chromatography. *In*: Annual Book of ASTM Standards, Section 11: Water and Environmental Technology; Volume 11.01, Water (1). American Society for Testing and Materials. West Conshohocken, PA. pp. 422-428.

-----. 2000c. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. *In*: Annual Book of ASTM Standards 2000, Section 11: Water and Environmental Technology, Vol. 11.05: Biological Effects and Environmental Fate; Biotechnology; Pesticides. American Society for Testing Materials. West Conshohocken, PA. pp. 213-233.

Baker, J. and V. Waights. 1993. The effect of sodium nitrate on the growth and survival of toad tadpoles (*Bufo bufo*) in the laboratory. Herpetol. J. 3(4): 147-148.

-----. 1994. The effects of nitrate on tadpoles of the tree frog *(Litoria caerulea)*. Herpetol. J. 4(3): 106-108.

Basuyaux, O. and M. Mathieu. 1999. Inorganic nitrogen and its effect on growth of the abalone *Haliotis tuberculata* Linnaeus and the sea urchin *Paracentrotus lividus* Lamarck. Aquaculture. 174: 95-107.

Bates, S. S., C. J. Bird, A. S. W. de Freitas, R. Foxall, M. Gilgan, L. A. Hanic, G. R. Johnson, A. W. McCulloch, P. Odense, R. Pocklington, M. A. Quilliam, P. G. Sim, J. C. Smith, D. V. Subba Rao, E. C. D. Todd, J. A. Walter, and J. L. C. Wright. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can. J. Fish. Aquat. Sci. 46: 1203-1215.

Bates, S. S., J. Worms, and J. C. Smith. 1993. Effects of ammonium and nitrate on growth and domoic acid production by *Nitzschia pungens* in batch culture. Can. J. Fish. Aquat. Sci. 50: 1248-1254.

BCMELP (British Columbia Ministry of Environment, L. a. P. 1986. Principles for preparing water quality objectives in British Columbia. British Columbia Ministry of Environment, Lands, and Parks, Water Management Branch, Resource Quality Section.

Bennett, E. B. 1982. The nitrifying of Lake Superior, 1880-2000. Bayfield Laboratory, Department of Fisheries and Oceans. Burlington, ON. ii + 26 pp.

Bergstrom, L. and R. Johansson. 1991. Leaching of nitrate from monolith lysimeters of different types of agricultural soils. J. Environ. Qual. 20: 801-807.

Berven, K. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology. 71(4): 1599-1608.

Bieniarz, K., P. Epler, M. Sokolowska-Mikolajczyk, J. Chyb, and W. Popek. 1996. Carp reproduction in highly eutrophic pond conditions. Bull. Environ. Contam. Toxicol. 57: 842-848.

Biksey, T. M. and E. Brown. 2001. The hyporheic zone: Linking groundwater and surface water -- Understanding the paradigm. Remediation. 12(1): 55-62.

Bock, E. 1978. Lithoautotrophic and chemoorganotrophic growth of nitrifying bacteria. *In*: Microbiology-1978. Schlessinger, D. (ed.). American Society for Microbiology. Washington, D.C. pp. 310-314.

Bondoux, G., P. Jandik, and W. Jones. 2000. New approach to the analysis of low levels of anions in water. J. Chromatography. 602: 79-88.

Bone, Q. and N. B. Marshall. 1986. Biology of Fishes. Blackie and Son Ltd. London, U.K. 247 pp.

Böhlke, J. K. and J. M. Denver. 1995. Combined use of groundwater dating, chemical, and isotopic analyses to resolve the history and fate of nitrate contamination in two agricultural watersheds, Atlantic coastal plain, Maryland. Wat. Resources Res. 31(9): 2319-2339.

Brandi-Dohrn, F. M., R. P. Dick, M. Hess, S. M. Kauffman, D. D. Hemphill Jr., and J. S. Selker. 1997. Nitrate leaching under a cereal rye cover crop. J. Environ. Qual. 26: 181-188.

Brauner, C. J., A. L. Val, and D. J. Randall. 1993. The effect of graded methemoglobin levels on the swimming performance of chinook salmon (*Oncorhynchus tshawytscha*). J. Exp. Biol. 185: 121-135.

Bredemeier, M., K. Blanck, Y.-J. Xu, A. Tietema, A. W. Boxman, B. Emmett, R. Moldan, P. Gundersen, P. Schleppi, and R. F. Wright. 1998. Input-output budgets at the NITREX sites. Forest Ecology and Management. 101: 57-64.

Brinkhoff, H. C. 1978. Analysis of nitrate content of surface water and effluent water. Application of a plastic-membrane nitrate-selective electrode in water quality monitors. Environ. Sci. Technol. 12(13): 1392-1394.

Brownell, C. L. 1980. Water quality requirements for first-feeding in marine fish larvae. I. Ammonia,

nitrite and nitrate. J. Exp. Mar. Biol. Ecol. 44: 269-283.
Bulger, A. J., L. Lien, B. J. Cosby, and A. Henrikson. 1993. Brown trout (*Salmo trutta*) status and chemistry from the Norwegian Thousand Lake Survey: Statistical analysis. Can. J. Fish. Aquat. Sci. 50: 575-585.
Burns, D. A. 1998. Retention of NO_3^- in an upland stream environment: A mass balance approach. Biogeochem. 40: 73-96.
Burton, D. L., Younie, M. F., Beauchamp, E. G., Kachanoski, R. G., and Loro, P. 1994. Impact of manure application on nitrate leaching to groundwater. *In*: Papers presented at the 37th Annual Manitoba Society of Soil Science Meeting. 190-201. Winnipeg.
Caldwell, J. P., J. H. Thorp, and T. O. Jervey. 1980. Predator-prey relationships among larval dragonflies, salamanders, and frogs. Oecologia. 46: 285-289.
Calleja, M. C., G. Persoone, and P. Geladi. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of *in vitro* cytotoxicity chemicals to aquatic non-vertebrates. Arch. Environ. Contam. Toxicol. 26: 69-78.
Camargo, J. A. and J. V. Ward. 1992. Short-term toxicity of sodium nitrate ($NaNO_3$) to non-target freshwater invertebrates. Chemosphere. 24(1): 23-28.
-----. 1995. Nitrate (NO_3-N) toxicity to aquatic life: A proposal of safe concentrations for two species of nearctic freshwater invertebrates. Chemosphere. 31(5): 3211-3216.
Camargo, J.A., A. Alonso and A. Salamanca. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere. 58(9):1255-1267.
Camargo, J.A. and A. Alonso. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environment International. 32:831-849.
Campbell, C. A., R. de Jong, and R. P. Zentner. 1984. Effect of cropping, summerfallow and fertilizer nitrogen on nitrate-nitrogen lost by leaching on a brown chernozemic loam. Can. J. Soil Sci. 64: 61-74.
Canada Gazette. 2010. Wastewater systems effluent regulations - Regulatory impact analysis statement. Vol. 144, No. 12 – March 20, 2010. http://www.gazette.gc.ca/rp-pr/p1/2010/2010-03-20/html/reg1-eng.html
Carey, C. and C. J. Bryant. 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environ. Health Perspect. 103(Suppl. 4): 13-17.
Carrol JJ, SJ Ellis, WS Oliver. 1979. Influence of hardness constituents on the acute toxicity of cadmium to brook trout (*Salvelinus fontinalis*). Bulletin of Environmental Contamination and Toxicology. 22:575-581.
Castillo, M. M., J. D. Allan, and S. Brunzell. 2000. Nutrient concentrations and discharges in a midwestern agricultural catchment. J. Environ. Qual. 29: 1142-1151.
Cavalli, R. O., W. Wasielesky Jr., C. S. Franco, and K. M. Filho. 1996. Evaluation of the short-term toxicity of ammonia, nitrite and nitrate to *Penaeus paulensis* (Crustacea, Decopoda) broodstock. Arq. Biol. Tecnol. 39(3): 567-575.
Cavari, B. Z. and G. Phelps. 1977. Denitrification in Lake Kinneret in the presence of oxygen. Freshwat. Biol. 7: 385-391.
CCME (Canadian Council of Ministers of the Environment). 1991. Appendix IX - A protocol for the derivation of water quality guidelines for the protection of aquatic life (April 1991). *In*: Canadian water quality guidelines. Canadian Council of Resource and Environment Ministers, 1987. Prepared by the Task Force on Water Quality Guidelines. [Updated and reprinted with minor revisions and editorial changes in Canadian environmental quality guidelines, Chapter 4, Canadian Council of Ministers of the Environment, 1999, Winnipeg.].
-----. 2000. Canadian water quality guidelines for the protection of aquatic life: Ammonia. *In*: Canadian Environmental Quality Guidelines, 1999. Canadian Council of Ministers of Environment. Winnipeg.
-----. 2002. Indirect toxic effects of nitrogen and the role of nitrogen-to-phosphorus ratios (N:P) in regulating primary production in freshwater ecosystems. Canadian Council of Ministers of the Environment. Winnipeg, MB.

-----. 2003. Canadian water quality guidelines for the protection of aquatic life: Guidance on the Site-Specific Application of Water Quality Guidelines in Canada: Procedures for Deriving Numerical Water Quality Objectives. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

-----. 2007. A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian water quality guidelines. Task Force on Water Quality Guidelines of the Canadian Council of Ministers of the Environment. Winnipeg, MB.

Chambers, P. A., M. Allard, S. L. Walker, J. Marsalek, J. Lawrence, M. Servos, J. Busnarda, K. S. Munger, K. Adare, C. Jefferson, R. A. Kent, and M. P. Wong. 1997. Impacts of municipal wastewater effluents on Canadian waters: A review. Water Qual. Res. J. Canada. 32(4): 659-713.

Chambers, P. A., M. Guy, E. S. Roberts, M. N. Charlton, R. A. Kent, C. Gagnon, G. Grove, and N. Foster. 2001. Nutrients and their impact on the Canadian environment. Agriculture and Agri-Food Canada, Environment Canada, Fisheries and Oceans Canada, Health Canada and Natural Resources Canada. 241 pp.

Charles, A. M., Markich, S. J., Stauber, J. L., and De Filippis, L. F. 2002. The effect of water hardness on the toxicity of uranium to a tropical freshwater alga (*Chlorella* sp.). Aquatic Toxicology 60: 61-73.

Cheminfo. 2011. Cheminfo: Chemical profiles created by the Canadian Centre for Occupational Health and Safety. http://ccinfoweb.ccohs.ca/chempendium/search.html

Cheng, S.-Y., S.-J. Tsai, and J.-C. Chen. 2002. Accumulation of nitrate in the tissues of *Penaeus monodon* following elevated ambient nitrate exposure after different time periods. Aquat. Tox. 56: 133-146.

Christensen, P. B., S. Rysgaard, N. P. Sloth, T. Dalsgaard, and S. Schwaerter. 2000. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. Aquat. Microb. Ecol. 21: 73-84.

Clifford, D. and X. Liu. 1993. Ion exchange for nitrate removal. J. Am. Wat. Works Assoc. 85(4): 135-143.

CNACD (Canadian National Atmospheric Chemistry Database). 2001. Precipitation Data. Environment Canada, Meteorological Service of Canada, Air Quality Research Branch. Toronto, ON.

Coleman, V. L. and J. M. Burkholder. 1994. Community structure and productivity of epiphytic microalgae on eelgrass *(Zostera marina L.)* under water-column nitrate enrichment. J. Exp. Mar. Biol. Ecol. 179: 29-48.

Collins, M. T., D. L. Dawe, and J. B. Gratzek. 1976. Immune response of channel catfish under different environmental conditions. J. Am. Vet. Med. Assoc. 169(9): 991-994.

Colt, J. and D. A. Armstrong. 1981. Nitrogen toxicity to crustaceans, fish, and molluscs. Bio-Engineering Symposium for Fish Culture. 1: 34-37.

Colt, J. and G. Tchobanoglous. 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture. 8: 209-224.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2002. Assessment and Update Status Report on the Pugnose Shiner (*Notropis anogenus*) in Canada. Endangered 2002.
http://dsp-psd.pwgsc.gc.ca/Collection/CW69-14-309-2003E.pdf

Cook, H. F. 1999. Sensitive areas, vulnerable zones and buffer strips: A critical review of policy in agricultural nitrate control. *In*: Managing Risks of Nitrates to Humans and the Environment. Wilson, W. S., A. S. Ball, and R. H. Hinton (eds.). The Royal Society of Chemistry. Cambridge, UK. pp. 189-205.

Cooke, S. E. and E. E. Prepas. 1998. Stream phosphorus and nitrogen export from agricultural and forested watersheds on the Boreal Plain. Can. J. Fish. Aquat. Sci. 55: 2292-2299.

Cooper, A. B. 1990. Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia. 202: 13-26.

Correll, D. L., T. E. Jordan, and D. E. Weller. 1995. Livestock and pasture land effects on the water quality of Chesapeake Bay watershed streams. *In*: Animal Waste and the Land-Water Interface. Steele, K. (ed.). CRC Press, Inc. Boca Raton, FL. pp. 107-117.

Crawford, N. M. and A. D. M. Glass. 1998. Molecular and physiological aspects of nitrate uptake in plants. Trends in Plant Science. 3(10): 389-395.

CRC (Chemical Rubber Company). 1986. CRC Handbook of Chemistry and Physics: A Ready-Reference Book of Chemical and Physical Data. (66th Ed.). Weast, R. C., M. J. Astle, and W. H. Beyer (eds.). CRC Press, Inc. Boca Raton, FL.

Creed, I. F., L. E. Band, N. W. Foster, I. K. Morrison, J. A. Nicolson, R. S. Semkin, and D. S. Jeffries. 1996. Regulation of nitrate-N release from temperate forests: A test of the N flushing hypothesis. Wat. Resources Res. 32(11): 3337-3354.

Cresswell, R. C. and P. J. Syrett. 1981. Uptake of nitrate by the diatom *Phaeodactylum tricornutum*. J. Exp. Bot. 32(126): 19-25.

Crouzet, P., J. Leonard, S. Nixon, Y. Rees, W. Parr, L. Laffon, J. Bogestrand, P. Kristensen, C. Lallana, G. Izzo, T. Bokn, J. Bak, and T. J. Lack. 1999. Nutrients in European ecosystems. Thyssen, N. (ed.). Report no. Environmental Assessment Report no. 4. European Environment Agency. Copenhagen, Denmark.

Crumpton, W. G., T. M. Isenhart, and S. W. Fisher. 1993. Fate of non-point source nitrate loads in freshwater wetlands: Results from experimental wetland mesocosms. *In*: Constructed Wetlands for Water Quality Improvement. Moshiri, G. A. (ed.). Lewis Publishers. Boca Raton. pp. 283-291.

Dalziel, J. A., P. A. Yeats, and B. P. Amirault. 1998. Inorganic chemical analysis of major rivers flowing into the Bay of Fundy, Scotian Shelf and Bras D'Or lakes. Report no. 2226. Can. Tech. Rep. of Fish. Aquat. Sci. Department of Fisheries and Oceans. vii + 140 pp.

Demaël, A., D. Garin, and G. Pérès. 1980. Response of the tench (*Tinca tinca* L.) to potassium nitrate enriched water. J. Fish Biol. 16: 15-22.

deSaint-Blanquat, G. 1980. Aspects toxicologiques et nutritionnels des nitrates et des nitrites. Ann. Nutr. Alim. 34: 827-864.

Devito, K. J. and P. J. Dillon. 1993. Importance of runoff and winter anoxia to the P and N dynamics of a beaver pond. Can. J. Fish. Aquat. Sci. 50: 2222-2234.

Devito, K. J., D. Fitzgerald, A. R. Hill, and R. Aravena. 2000. Nitrate dynamics in relation to lithology and hydrologic flow path in a river riparian zone. J. Environ. Qual. 29: 1075-1084.

DFO (Department of Fisheries and Oceans). 1998. Preliminary 1996 Canadian aquaculture production statistics. www.dfo-mpo.gc.ca/communic/statistics/aquacult/AQUA96.htm

Dillon, P. J. and Evans, H. E. 2001. Long-term changes in the chemistry of a soft-water lake under changing acid deposition rates and climate fluctuations. Verh. Internat. Verein Limnol. 27: 2615-2619.

Dillon, P. J., M. Lusis, R. Reid, and D. Yap. 1988. Ten-year trends in sulphate, nitrate and hydrogen deposition in Central Ontario. Atmosph. Environ. 22(5): 901-905.

Dillon, P. J. and L. A. Molot. 1989. The role of ammonium and nitrate retention in the acidification of lakes and forested catchments. *In*: The Role of Nitrogen in the Acidification of Soils and Surface Waters. Malanchuck, J. L. and J. Nilsson (eds.). Nordic Council of Ministers. Denmark. pp. 1-25.

Dise, N. B. and R. F. Wright. 1995. Nitrogen leaching from European forests in relation to nitrogen deposition. Forest Ecology and Management. 71: 153-161.

DND (Department of National Defence).1998. An evaluation and interpretation of stormwater quality data from federal airport facilities. Department of National Defence, Transport Canada, Environment Canada. Ottawa, ON. 50 pp.

Doblander, C. and R. Lackner. 1997. Oxidation of nitrite to nitrate in isolated erythrocytes: a possible mechanism for adaptation to environmental nitrite. Can. J. Fish. Aquat. Sci. 54: 157-161.

Dodds, W. K., J. R. Jones, and E. B. Welch. 1998. Suggested classification of stream trophic state: Distributions of temperate stream types by chlorophyll, total nitrogen and phosphorus. Wat. Res.

32(5): 1455-1462.
Dowden, B. F. and H. J. Bennett. 1965. Toxicity of selected chemicals to certain animals. J. Waste Poll. Control Fed. 37(9): 1308-1316.
Driscoll, C. T. and R. Van Dreason. 1993. Seasonal and long-term temporal patterns in the chemistry of Adirondack lakes. Water Air Soil Poll. 67: 319-344.
Druliner, A. D. 1989. Overview of the relations of nonpoint-source agricultural chemical contamination to local hydrogeological, soil, land-use, and hydrochemical characteristics of the High Plains aquifer of Nebraska. *In*: U.S. Geological Survey Toxic Substances Hydrology Program - Proceedings of the Technical Meeting. Mallard, G. E. and S. E. Ragone (eds.). Phoenix, AZ, 26 - 30 Sept. pp. 411-435.
Dvir, O., J. vanRijn, and A. Neori. 1999. Nitrogen transformations and factors leading to nitrite accumulation in a hypertrophic marine culture system. Mar. Ecol. Prog. Ser. 181: 97-106.
Earl, J.E. and H. Whiteman. 2009. Effects of pulsed nitrate exposure on amphibian development. Environ Toxicol Chem. 28(6):1331-1337.
Edwards, T.M., K.A. McCoy, T.Barbeau, M.W. McCoy, J.M. Thro, and L.J. Guillette Jr. 2006. Environmental context determines nitrate toxicity in Southern toad (*Bufo terrestris*) tadpoles. Auqatic Toxicology. 78:50-58.
Eghball, B. and J. E. Gilley. 1999. Phosphorus and nitrogen in runoff following beef cattle manure or compost application. J. Environ. Qual. 28: 1201-1210.
Elder, F. C. 1984. Effects of nitrate on the acidification of the aquatic system. Report no. EPS 2/TS/1. Canada Centre for Inland Waters, Environment Canada. pp. 1-14.
Eldredge, L. G. 2001. Introductions of commercially significant aquatic organisms to the Pacific Islands. *In*: Perspectives in Aquatic Exotic Species Management in the Pacific Islands 1994: Volume 1. SPC (Secretariat of the Pacific Community). 91-101. www.spc.org.nc/coastfish/Reports/IFRP/Introd/introd.htm
Eliassen, R., B. M. Wyckoff, and C. D. Tonkin. 1965. Ion exchange for reclamation of reusable supplies. J. Am. Wat. Works Assoc. 57(9): 1113-1122.
Elphick, J. 2011. Evaluation of the role of hardness in modifying the toxicity of nitrate to freshwater organisms. Nautilus Environmental. 4 April 2011.
Environment Australia. 2000a. Australian and New Zealand guidelines for fresh and marine water quality. Volume 1: The guidelines. National Water Quality Management Strategy. Australian and New Zealand Environmental and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand.
-----. 2000b. Australian and New Zealand guidelines for fresh and marine water quality. Volume 2: Aquatic ecosystems - Rationale and background information. National Water Quality Management Strategy. Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand.
Environment Canada. 1998. Biological test method: Toxicity tests using early life stages of salmonid fish (rainbow trout). Environmental Protection Series EPS 1/RM/28, 2nd ed. Conservation and Protection, Ottawa, ON.
-----. 1999. Municipal Water Use Database (MUD). Environmental Economics, Policy and Communications, Environment Canada. Hull, Quebec.
-----. 2001a. National Pollutant Release Inventory. www.ec.gc.ca/pdb/npri/npri_home_e.cfm
-----. 2010a. National Pollutant Release Inventory. www.ec.gc.ca/inrp-npri/
-----. 2010b. Freshwater Quality Monitoring – Pacific Yukon Water Quality Data. http://waterquality.ec.gc.ca/waterqualityweb/searchtext.aspx
Epifanio, C. E. and R. F. Srna. 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Mar. Biol. 33: 241-246.
Eriksson, P. G. and S. E. B. Weisner. 1999. An experimental study on effects of submersed macrophytes on nitrification and denitrification in ammonium-rich aquatic systems. Limnol. Oceanogr. 44(8): 1993-1999.
Evans, M. S. 1997. Limnological investigations in the west basin of Great Slave Lake, March 1994.

Report no. 131. Northern River Basins Study. Edmonton, AB.
Everall NC, NAA Macfarlane, RW Sedgwick. 1989. The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout *Salmo trutta*. Journal of Fish Biology. 35:27-36.
Ezeonu, F. C. and A. N. Okaka. 1996. Seasonal profile of nitrate and nitrite in potable water sources in Onitsha, Nigeria. Environ. Toxicol. Wat. Qual. 11: 99-103.
Findlay, D. L. and S. E. M. Kasian. 1987. Phytoplankton community responses to nutrient addition in lake 226, experimental lakes area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 35-46.
Forbes, V.E. and P. Calow. 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environmental Toxicology and Chemistry. 18(7): 1544-1556.
Forsberg, C. 1975. Nitrogen as a growth factor in freshwater. *In*: Conference on nitrogen as a water pollutant. IAWPR. Copenhagen, Denmark. Vol. 2.
Fowler, D., R. I. Smith, U. M. Skiba, M. Coyle, C. Flechard, C. E. R. Pitcairn, and M. A. Sutton. 1999. The atmospheric nitrogen cycle and the role of anthropogenic activity. *In*: Managing Risks of Nitrates to Humans and the Environment. Wilson, W. S., A. S. Ball, and R. H. Hinton (eds.). The Royal Society of Chemistry. Cambridge, U.K. pp. 121-138.
Frakes, T. and F. H. Hoff Jr. 1982. Effect of high nitrate-N on the growth and survival of juvenile and larval anemonefish, *Amphiprion ocellaris*. Aquaculture. 29(155): 158.
Galloway, J. N. and P. J. Dillon. 1983. Effects of acid deposition: The importance of nitrogen. Report no. SNV PM 1636. *In*: Ecological Effects of Acid Deposition: Report on Background Papers - 1982 Stockholm Conference on the Acidification of the Environment; Expert Meeting 1. National Swedish Environment Protection Board. pp. 145-160.
Gangbazo, G., A. R. Pesant, G. M. Barnett, J. P. Charuest, and D. Cluis. 1995. Water contamination by ammonium nitrogen following the spreading of hog manure and mineral fertilizers. J. Environ. Qual. 24: 420-425.
Gold, A. J., K. Addy, D. Q. Kellogg, and A. E. Rosenblatt. 1999. Nitrate removal in shallow groundwater. Contribution No. 3757 of the Rhode Island Agricultural Experiment Station. pp. 185-196.
Goolsby, D. A., W. A. Battaglin, B. T. Aulenbach, and R. P. Hooper. 2001. Nitrogen input to the Gulf of Mexico. J. Environ. Qual. 30: 329-336.
Goss, M. J., D. A. J. Barry, and D. L. Rudolph. 1998a. Contamination in Ontario farmstead domestic wells and its association with agriculture: 1. Results from drinking water wells. J. Contam. Hydrol. 32: 267-293.
Goss, M. J. and D. Goorahoo. 1995. Nitrate contamination of groundwater: Measurement and prediction. Fert. Res. 42: 331-338.
Goss, M. J., K. R. Howse, D. G. Christian, J. A. Catt, and T. J. Pepper. 1998b. Nitrate leaching: Modifying the loss from mineralized organic matter. Europ. J. Soil Sci. 49: 649-659.
Goss, M. J., K. R. Howse, P. W. Lane, D. G. Christian, and G. L. Harris. 1993. Losses of nitrate-nitrogen in water draining from under autumn-sown crops established by direct drilling or mouldboard phoughing. J. Soil Sci. 44: 35-48.
Government of Canada. 1996. The State of Canada's Environment: 1996. Minister of Public Works and Government Services (ed.). Gilmour Printing. Gloucester, ON.
Government of Saskatchewan. 2008. Livestock Health and Nitrate Toxicity. September 2008. http://www.agriculture.gov.sk.ca/Nitrate_Toxicity
Grabda, E., T. Einszporn-Orecka, C. Felinska, and R. Zbanyszek. 1974. Experimental methemoglobinemia in rainbow trout. Acta Ichthyologica et Piscatoria. 4(2): 43-70.
Grant, C. A., J. C. P. Dribnenki, and L. D. Bailey. 2000. Cadmium and zinc concentrations and ratios in seed and tissue of solin (cv Linola™ 947) and flax (cvs McGregor and Vimy) as affected by nitrogen and phosphorus fertiliser and Provide (*Penicillium bilaji*). J. Sci. Food Agric. 80: 1735-1743.
Grimaldi, C. and V. Chaplot. 2000. Nitrate depletion during within-stream transport: Effects of exchange processes between streamwater, the hyporheic and riparian zones. Water Air Soil Poll. 124: 95-112.

Hall, G. H. 1986. Nitrification in lakes. *In*: Nitrification. Prosser, J. I. (ed.). IRL Press. Oxford. pp. 127-156.

Hall, K. J., P. Kiffney, R. Macdonald, D. McCallum, G. Larkin, J. Richardson, H. Schrier, J. Smith, P. Zandbergen, P. Keen, W. Belzer, R. Brewer, M. Sekela, and B. Thompson. 1999. Non-point source contamination in the urban environment of Greater Vancouver: A case study of the Brunette River Watershed. *In*: Health of the Fraser River aquatic ecosystem, Vol. 2: A synthesis of research conducted under the Fraser River Action Plan. Gray, C. and T. Tuominen (eds.). Department of the Environment, Fraser River Action Plan, Publication No.DOE FRAP 1998-11.

Halling-Sorensen, B. and S. E. Jorgensen. 1993. The Removal of Nitrogen Compounds From Wastewater. Elsevier Science Publishers. Amsterdam, NL. 443 pp.

Harvey, H. H., R. C. Pierce, P. J. Dillon, J. R. Kramer, and D. M. Whelpdale. 1981. Acidification in the Canadian environment: Scientific criteria for assessing the effects of acidic deposition on aquatic ecosystems. Report no. NRCC 18475. National Research Council Canada. Ottawa, ON. 369 pp.

Hatch, A. C. and A. R. Blaustein. 2000. Combined effects of UV-B, nitrate, and low pH reduce the survival and activity level of larval Cascades frogs (*Rana cascadae*). Arch. Environ. Contam. Toxicol. 39: 494-499.

Hattori, A. 1983. Denitrification and dissimilatory nitrate reduction. *In*: Nitrogen in the Marine Environment. Carpenter, E. J. and D. G. Capone (eds.). Academic Press. New York, USA. pp. 191-232.

Haycock, N. E. and T. P. Burt. 1993. The sensitivity of rivers to nitrate leaching: The effectiveness of near-stream land as a nutrient retention zone. *In*: Landscape Sensitivity. Thomas, D. S. G. and R. J. Allison (eds.). John Wiley and Sons Ltd. Chichester, England. pp. 261-272.

Håkanson, L. 1994. A review of effect-dose-sensitivity models for aquatic ecosystems. Int. Rev. Gesamten Hydrobiol. 79: 621-667.

Health Canada. 1998. Blue-green algae (cyanobacteria) and their toxins. It's Your Health. Ottawa. www.hc-sc.gc.ca/ehp/ehd/catalogue/general/iyh/algae.htm

Heath, A. G. 1995. Water Pollution and Fish Physiology. (2nd Ed.). CRC Press Inc., Lewis Publishers. Boca Raton.

Heathwaite, A. L., P. J. Johnes, and N. E. Peters. 1996. Trends in nutrients. Hydrol. Process. 10: 263-293.

Hecnar, S. J. 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. Environ. Toxicol. Chem. 14(12): 2131-2137.

Heidorn, K. C. 1979. Incidents of elevated nitrate in Southern Ontario. Water Air Soil Poll. 12: 417-429.

Heijerick DG, KAC De Schamphelaere, CR Janssen. 2002. Predicting acute zinc toxicity for *Daphnia magna* as a function of key water chemistry characteristics: development and validation of a biotic ligand model. Environmental Toxicology and Chemistry. 21:1309-1315.

Hemond, H. F. and E. J. Fechner. 1994. Chemical Fate and Transport in the Environment. Academic Press. San Diego, CA. 338 pp.

Hickey, C. W. and M. L. Martin. 2009. A review of nitrate toxicity to freshwater aquatic species. Prepared for Environment Canterbury. New Zealand National Institute of Water and Atmospheric Research Ltd. June 2009. Report No. R09/57. ISBN 978-1-86937-997-1.

Hickey, C. W. 2002. Nitrate guideline values in ANZECC 2000. Memorandum to James Court, MFE. 30 September 2002. Retrieved at: http://www.mfe.govt.nz/publications/water/anzecc-water-quality-guide-02/anzecc-nitrate-correction-sep02.html

Hilborn, D. and C. Brown. 1996. 10 Steps to complete a nutrient management plan for livestock and poultry manure. Ontario Ministry of Agriculture, Food and Rural Affairs. www.gov.on.ca/OMAFRA/english/livestock/swine/facts/96-053.htm

Hill, A. R. 1978. Factors affecting the export of nitrate-nitrogen from drainage basins in Southern Ontario. Wat. Res. 12: 1045-1057.

Hill, M. J. 1999. Nitrate toxicity: myth or reality? Br. J. Nutr. 81: 343-344.

Hodgkiss, I. J. and K. C. Ho. 1997. Are changes in N:P ratios in coastal waters the key to increased red

tide blooms? Hydrobiologia. 352: 141-147.
Hooda, P. S., A. C. Edwards, H. A. Anderson, and A. Miller. 2000. A review of water quality concerns in livestock farming areas. Sci. Total Env. 250: 143-167.
Huckabee, J. W. and N. A. Griffith. 1974. Toxicity of mercury and selenium to the eggs of carp (*Cyprinus carpio*). Trans. Amer. Fish. Soc.(4): 822-825.
Hudon, C. and A. Sylvestre. 1998. Qualité de l'eau en aval de l'archipel de Montréal 1994-1996. Report no. ST-170. Environnement Canada - Région du Québec, Conservation de l'environnement, Centre Saint-Laurent.
Huey, D. W. and T. L. Beitinger. 1980a. Toxicity of nitrite to larvae of the salamander *Ambystoma texanum*. Bull. Environ. Contam. Toxicol. 25(6): 909-912.
-----. 1980b. Hematalological responses of larval *Rana catesbiana* to sublethal nitrite exposures. Bull. Environ. Contam. Toxicol. 25: 574-577.
Huey, D. W. and T. L. Beitinger. 1982. A methemoglobin reductase system in channel catfish *Ictalurus punctatus*. Can. J. Zool. 60: 1511-1513.
Hund, K. 1997. Algal growth inhibition test - Feasibility and limitations for soil assessment. Chemosphere. 35(5): 1069-1082.
Hunt, P. G., K. C. Stone, F. J. Humenik, and J. M. Rice. 1995. Impact of animal waste on water quality in an eastern coastal plain watershed. *In*: Animal Waste and the Land-Water Interface. Steele, K. (ed.). CRC Press, Inc. Boca Raton. pp. 257-264.
Iizumi, H., A. Hattori, and C. P. McRoy. 1980. Nitrate and nitrite in interstitial waters of eelgrass beds in relation to the rhizosphere. J. Exp. Mar. Biol. Ecol. 47: 191-201.
Intrinsik Science. 2010. Synthesis of research and methods for developing water quality guidelines for the Canadian environmental sustainability indicators (CESI) freshwater quality indicator. Prepared for Environment Canada, National Guidelines and Standards Office. February 2010.
Izaurralde, R. C., Y. Feng, J. A. Robertson, W. B. McGill, N. G. Juma, and B. M. Olson. 1995. Long-term influence of cropping systems, tillage methods, and N sources on nitrate leaching. Can. J. Soil Sci. 75: 497-505.
Jackson BP, PJ Lasier, WP Miller, PW Winger. 2000. Effects of calcium, magnesium and sodium on alleviating cadmium toxicity to *Hyalella azteca*. Bulletin of Environmental Contamination and Toxicology. 64:279-286.
Jeffries, D. S. and R. G. Semkin. 1983. Changes in snowpack, stream and lake chemistry during snowmelt in the Turkey Lakes Watershed. Report no. TLW-83-10. Turkey Lakes Watershed Study: Unpublished Report Series. Environmental Contaminants Division, National Water Research Institute, Environment Canada. Burlington, ON. 23 pp.
Jensen, F. B. 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). Aquat. Tox. 34: 95-104.
Johansson, M., K. Räsänen, and J. Merilä. 2001. Comparison of nitrate tolerance between different populations of the common frog, *Rana temporaria*. Aquat. Tox. 54: 1-14.
Johnes, P. J. and T. P. Burt. 1993. Nitrate in surface waters. *In*: Nitrate: Processes, Patterns and Management. Burt, T. P., A. L. Heathwaite, and S. T. Trudgill (eds.). John Wiley & Sons Ltd. Chichester, England. pp. 269-317.
Jones, D. R. 1971. The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 55: 541-551.
Jones, J. R. E. 1939. The relation between the electrolytic solution pressures of the metals and their toxicity to the stickleback (*Gasterosteus aculeatus* L.). J. Exp. Biol. 16(4): 425-437.
Jones, J. R. E. 1940. A further study of the relation between toxicity and solution pressure, with *Polycelis nigra* as test animal. J. Exp. Biol. 17: 408-415.
Jones, J. R. E. 1941. A study of the relative toxicity of anions, with *Polycelis nigra* as test animal. J. Exp. Biol. 18: 170-181.
Jury, W. A. and D. R. Nielsen. 1989. Nitrate transport and leaching mechanisms. *In*: Nitrogen

Management and Ground Water Protection. Follett, R. F. (ed.). Elsevier Science Publishing Co. Inc. New York. pp. 139-157.
Kamstra, A., J. A. Span, and J. H. van Weerd. 1996. The acute toxicity and sublethal effects of nitrite on growth and feed utilization of European eel, *Anguilla anguilla* (L.). Aquacult. Res. 27: 903-911.
Kaplan, W. A. 1983. Nitrification. *In*: Nitrogen in the Marine Environment. Carpenter, E. J. and D. G. Capone (eds.). Academic Press. New York, USA. pp. 139-190.
Kapoor, A. and R. Viraraghavan. 1997. Nitrate removal from drinking water - Review. J. Environ. Engineering. 123(4): 371-380.
Kaur, K., A. Dhawan, and H. S. Toor. 1993. Toxic effects of industrial effluents on hatchability and viability of *Cyprinus carpio* eggs. Bull. Environ. Contam. Toxicol. 50: 640-645.
Keeney, D. R., R. L. Chen, and D. A. Graetz. 1971. Importance of denitrification and nitrate reduction in sediments to the nitrogen budgets of lakes. Nature. 233: 66-67.
Keith, L. H. 1992. Water quality parameters. *In*: Compilation of E.P.A.'s Sampling and Analysis Methods. Keith, L. H. (ed.). Lewis Publishers, Inc. Chelsea, Michigan. pp. 739-792.
Keizer, P. D., D. V. Bugden, S. Rao, and P. M. Strain. 1996. Long-term monitoring program: Indian Point and Sambro, Nova Scotia, for the period July 1992 to December 1994. Report no. Can. Data Rep. Fish. Aquat. Sci. 980. Department of Fisheries and Oceans, Canada. Dartmouth, N.S. v + 20 pp.
Kelly, W. R. and S. D. Wilson. 2000. Movement of bromide, nitrogen-15, and atrazine through flooded soils. J. Environ. Qual. 29: 1085-1094.
Khangarot, B. S. and P. K. Ray. 1989. Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea *Daphnia magna* Straus. Ecotoxicol. Environ. Saf. 18: 109-120.
Killham, K. 1986. Heterotrophic nitrification. *In*: Nitrification. Prosser, J. I. (ed.). IRL Press. Oxford. pp. 117-126.
Kincheloe, J. W., G. A. Wedemeyer, and D. L. Koch. 1979. Tolerance of developing salmonid eggs and fry to nitrate exposure. Bull. Environ. Contam. Toxicol. 23: 575-578.
Knepp, G. L. and G. F. Arkin. 1973. Ammonia toxicity levels and nitrate tolerance of channel catfish. Prog. Fish Cult. 35(4): 221-224.
Kolpin, D., M. R. Burkart, and E. M. Thurman. 1994. Herbicides and Nitrate in Near-Surface Aquifers in the Midcontinental United States, 1991. Report no. USGS Water-Supply Paper 2413. United States Geological Survey. 34 pp.
Korol, M. and G. Rattray. 2000. Canadian Fertilizer Consumption, Shipments and Trade 1998/1999. Agriculture and Agri-Food Canada. pp. 1-32.
L'Haridon, J., M. Fernandez, V. Ferrier, and J. Bellan. 1993. Evaluation of the genotoxicity of *N*-nitrosoatrazine, *N*-nitrosodiethanolamine and their precursors *in vivo* using the newt micronucleus test. Wat. Res. 27(5): 855-862.
Lahti, E., M. Harri, and O. V. Lindqvist. 1985. Uptake and distribution of radioiodine, and the effect of ambient nitrate, in some fish species. Comp. Biochem. Physiolog. 80A(3): 337-342.
Lamontagne, S., S. L. Schiff, and R. J. Elgood. 2000. Recovery of ^{15}N-labelled nitrate applied to a small upland boreal forest catchment. Can. J. For. Res. 30: 1165-1177.
Lanyon, L. E. 1996. Does nitrogen cycle? Agric. Consult.(February): 17.
Laposata, M. M. and W. A. Dunson. 1998. Effects of boron and nitrate on hatching success of amphibian eggs. Arch. Environ. Contam. Toxicol. 35: 615-619.
Laudon, H., O. Westling, and K. Bishop. 2000. Cause of pH decline in stream water during spring melt runoff in northern Sweden. Can. J. Fish. Aquat. Sci. 57: 1888-1900.
Levine, S. N. and D. W. Schindler. 1989. Phosphorus, nitrogen, and carbon dynamics of Experimental Lake 303 during recovery from eutrophication. Can. J. Fish. Aquat. Sci. 46: 2-10.
Lewis, Jr. W. M. and D. P. Morris. 1986. Toxicity of nitrite to fish: A review. Transactions of the American Fisheries Society. 115(2): 183-195.
Licht, L. E. 1974. Survival of embryos, tadpoles, and adults of the frogs *Rana aurora aurora* and *Rana pretiosa pretiosa* sympatric in southwestern British Columbia. Can. J. Zool. 52: 613-627.

Likens, G. E., F. H. Bormann, N. M. Johnson, D. W. Fisher, and R. S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecological Monographs. 40: 23-47.

Lilius, H., B. Isomaa, and T. Holmstrom. 1994. A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. Aquat. Tox. 30: 47-60.

Louanchi, F. and R. G. Najjar. 2000. A global monthly climatology of phosphate, nitrate, and silicate in the upper ocean: Spring-summer export production and shallow remineralization. Global Biogeochem. Cycles. 14(3): 957-977.

Lovett, G. M., K. C. Weathers, and W. V. Sobczak. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. Ecol. App. 10(1): 73-84.

Lowrance, R. 1992. Nitrogen outputs from a field-size agricultural watershed. J. Environ. Qual. 21: 602-607.

Lucey, K. J. and D. A. Goolsby. 1993. Effects of climatic variations over 11 years on nitrate-nitrogen concentrations in the Raccoon River, Iowa. J. Environ. Qual. 22: 38-46.

MacDonald, D. D., J. B. Kemper, D. E. Smorong, and D. A. Levy. 2002. Guidance on the site-specific application of water quality guidelines in Canada: Procedures for deriving numerical water quality objectives. Report prepared for the Canadian Council of Ministers of the Environment and Environment Canada.

MacDonald, K. B. 2000a. Residual nitrogen. MacRea, T., C. A. S. Smith, and L. J. Gregorich (eds.). *In*: Environmental Sustainability of Canadian Agriculture: Report of the Agri-environmental Indicator Project. Research Branch, Policy Branch, Praire Farm Rehabilitation Administration, Agriculture and Agri-Food Canada.

-----. 2000b. Risk of water contamination by nitrogen. MacRea, T., C. A. S. Smith, and L. J. Gregorich (eds.). *In*: Environmental Sustainability of Canadian Agriculture: Report of the Agri-environmental Indicator Project. Research Branch, Policy Branch, Praire Farm Rehabilitation Administration, Agriculture and Agri-Food Canada.

Madramatoo, C. A., W. R. Johnston, and L. S. Willardson. 1997. Management of agricultural drainage water quality. Water Reports (no. 13). International Commission on Irrigation and Drainage, Food and Agriculture Organization of the United Nations. Rome.

Magette, W. L., P. A. Pacheco, and F. W. Wheaton. 1990. Ion exchange treatment of subsurface drainage water. Agric. Water Manage. 18: 121-133.

Magilligan, F. J. and P. F. McDowell. 1997. Stream channel adjustments following elimination of cattle grazing. J. Am. Water Res. Assoc. 33: 867-878.

Makarewicz, J. C., P. Bertram, and T. W. Lewis. 2000. Chemistry of the offshore surface waters of Lake Erie: Pre- and post - *Dreissena* introduction (1983 - 1993). J. Great Lakes Res. 26(1): 82-93.

Makepeace, D. K., D. W. Smith, and S. J. Stanley. 1995. Urban stormwater quality: Summary of contaminant data. Crit. Rev. Environ. Sci. Technol. 25(2): 93-139.

Manitoba Conservation. 2000. Water quality: Station status summary report. Compiled by: Williamson, D. Water Quality Management Section, Manitoba Conservation. 123 Main St., Suite 160, Winnipeg, MB. R3C 1A5.

Manzano, C., P. Candau, C. Gomez-Moreno, A. M. Relimpio, and M. Losada. 1976. Ferrodoxin-dependent photsynthetic reduction of nitrate and nitrite by particles of *Anacystis nidulans*. Mol. Cell Biochem. 10: 161-168.

Marco, A., C. Quilchano, and A. R. Blaustein. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. Environ. Toxicol. Chem. 18(12): 2836-2839.

Martin, T. L., N. K. Kaushik, H. R. Whiteley, S. Cook, and J. W. Nduhiu. 1999. Groundwater nitrate concentrations in the riparian zones of two southern Ontario streams. Can. Wat. Resour. J. 24(2): 125-138.

Marubini, F. and M. J. Atkinson. 1999. Effects of lowered pH and elevated nitrate on coral calcification. Mar. Ecol. Prog. Ser. 188: 117-121.

Mattice, J. S., S. C. Tsai, M. B. Burch, and J. J. Beauchamp. 1981. Toxicity of trihalomethanes to

common carp embryos. Trans. Amer. Fish. Soc. 110: 261-269.
Mazumder, A. and K. E. Havens. 1998. Nutrient-chlorophyll-Secchi relationships under contrasting grazer communities of temperate versus subtropical lakes. Can. J. Fish. Aquat. Sci. 55: 1652-1662.
McBeath, I. 1987. Review of the Candian Fertilizer Industry and Evaluation of Control Technology. Report no. EPS 2/AG/1. Environment Canada. pp. 1-231.
McCoy, E. F. 1972. Role of bacteria in the nitrogen cycle in lakes. Report no. 16010 EHR 03/72. U.S. Environmental Protection Agency. vii + 23 pp.
McDonald, D. D., L. E. Fidler, and D. Valiela. 1987. Site specific water quality criteria for fish and aquatic life in the Canadian portion of the Flathead River basin: Nitrate, nitrite and ammonia. Inland Waters and Lands, Pacific and Yukon Region, Environment Canada. Vancouver, BC. 127 pp.
McEachern, P., E. E. Prepas, J. J. Gibson, and W. P. Dinsmore. 2000. Forest fire induced impacts on phosphorus, nitrogen, and chlorophyll a concentrations in boreal subarctic lakes of northern Alberta. Can. J. Fish. Aquat. Sci. 57(Suppl. 2): 73-81.
McGreer, E. R. and W. Belzer. 1999. Contaminant sources. *In*: Health of the Fraser River Aquatic Ecosystem, Vol. 1: A Synthesis of Research Conducted Under the Fraser River Action Plan. Gray, C. and T. Tuominen (eds.). Department of the Environment, Fraser River Action Plan; Publication No. DOE FRAP 1998-11.
McGurk, M. D., F. Landry, A. Tang, and C. H. Hanks. 2006. Acute and chronic toxicity of nitrate to early life stages of lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*). Environ. Toxicol. Chem. 25(8):2187-2196.
McMahon, B. R. and S. A. Stuart. 1989. The physiological problems of crayfish in acid waters. *In*: Acid toxicity and Aquatic Animals. Morris, R., E. W. Taylor, D. J. A. Brown, and J. A. Brown (eds.). Cambridge University Press. Cambridge. pp. 171-199.
McQuarrie, D. A. and P. A. Rock. 1991. General Chemistry (3rd Ed.). W. H. Freeman and Company. New York, NY. 1083 pp.
Meade, M. E. and S. A. Watts. 1995. Toxicity of ammonia, nitrite, and nitrate to juvenile Australian crayfish, *Cherax quadricarinatus*. J. Shellfish Res. 14(2): 341-346.
Meador, J.P. 2000. An analysis in support of tissue and sediment based threshold concentrations of polychlorinated bipenyls (PCBs) to protect juvenile salmonids listed by the endangered species act. NOAA White Paper, Northwest Fisheries Science Center, Environmental Conservation Division, Seattle, Washington.
Meeuwig, J. J., P. Kauppila, and H. Pitkanen. 2000. Predicting coastal eutrophication in the Baltic: a limnological approach. Can. J. Fish. Aquat. Sci. 57: 844-855.
MEF (Ministère de l'Environnement et de la Faune du Québec). 1998. Critères de qualité de l'eau de surface au Québec. Direction des ecosystèmes aquatiques, Ministère de l'Environnement et de la Faune, Québec. 387 pp.
Melillo, J. M., J. D. Aber, P. A. Steudler, and J. P. Schimel. 1983. Denitrification potentials in a successional sequence of northern hardwood forest stands. Ecological Bulletin (Stockholm). 35: 217-228.
Mengis, M., S. L. Schiff, M. Harris, M. C. English, R. Aravena, R. J. Elgood, and A. MacLean. 1999. Multiple geochemical and isotopic approaches for assessing ground water NO3- elimination in a riparian zone. Ground Wat. 37(3): 448-457.
MENV (Ministère de l'Environnement du Québec). 2001. Portrait global de la qualité des eaux au Québec. Ministère de l'Environnement du Québec. www.mddep.gouv.qc.ca/eau/sys-image/global/index.htm
MEQ (Ministère de l'Environnement du Québec). 1991. Méthode de calculation des objectifs environnementaux de rejet pour les contaminants du milieu aquatique (Révisé 1996 et 2001). *In*: Direction du suivi de l'état de l'environnement. Ministerè de l'Environnement du Québec. Québec. 26 pp.
Merck. 1996. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals (12th Ed.). Budavari, S., M. J. O'Neil, A. Smith, P. E. Heckelman, and J. F. Kinneary (eds.). Merck and Co., Inc.

Whitehous Station, NJ. 1741 p. + tables.
Messer, J. J. and P. L. Brezonik. 1984. Laboratory evaluation of kinetic parameters for lake sediment denitrification models. Ecol. Modelling. 21: 277-286.
Millman, J. D. 1999. Development and evaluation of a nitrogen transfer-recycle-input-output model framework for dairy farms. University of Guelph. 313 pp.
Mitchell, M. J., C. T. Driscoll, J. S. Kahl, G. E. Likens, P. S. Murdoch, and L. H. Pardo. 1996. Climatic control of nitrate loss from forested watersheds in the northeast United States. Environ. Sci. Technol. 30: 2609-2612.
Moerman, D. and D. Briggins. 1994. Nova Scotia farm well water quality assurance study - Final report. Nova Scotia Department of Agriculture and Fisheries. www.gov.ns.ca/nsaf/rs/envman/wellrep.shtml
Monson, P. 2010. Aquatic life water quality standards technical support document for nitrate. Minnesota Pollution Control Agency. Draft for External Review, November 12, 2010. http://www.pca.state.mn.us/index.php/view-document.html?gid=14949
Moore, D and D. Poirier. 2010. The influence of temperature on the toxicity of nitrate and nitrite to 4 species of fish: lake whitefish (*Coregonus clupeaformis*), rainbow trout (*Oncorhynchus mykiss*), arctic char (*Salvelinus alpinus*), and lake trout (*Salvelinus namaycush*). Laboratory Services Branch, Ontario Ministry of the Environment. Unpublished data.
Morris, R. 2001. Personal Communication. Fisheries Biologist, Credit Valley Conservation Authority, 1255 Old Derry Road West, Meadowvale, ON L5N 6R4.
Mount, D. R., D. D. Gulley, J. R. Hockett, T. D. Garrison, and J. M. Evans. 997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnow). Environ. Toxicol. Chem. 16(10): 2009-2019.
MPCA (Minnesota Pollution Control Agency). 2011. Water Quality standards. Accessed at: http://www.pca.state.mn.us/index.php/water/water-monitoring-and-reporting/water-quality-and-pollutants/water-quality-standards.html (Last modified on Monday, December 12, 2011).
Mueller, D. K., B. C. Ruddy, and W. A. Battaglin. 1997. Logistic model of nitrate in streams of the upper-midwestern United States. J. Environ. Qual. 26: 1223-1230.
Muir, P. R., D. C. Sutton, and L. Owens. 1991. Nitrate toxicity to *Penaeus monodon* protozoea. Mar. Biol. 108: 67-71.
Muscutt, A. D., G. L. Harris, S. W. Bailey, and D. B. Davies. 1993. Buffer zones to improve water quality: A review of their potential use in UK agriculture. Agric. Ecosyst. Env. 45: 59-77.
Nadelhoffer, K. J., B. A. Emmett, P. Gundersen, O. J. Kjonaas, C. J. Koopmans, P. Schleppi, A. Tietema, and R. F. Wright. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature. 398 (March 11): 145-148.
NAESI (National Agri-Environmental Standards Initiative). 2005. N and P interactions and N:P ratios as a tool for N management of freshwater ecosystems. Technical Series 2005. Report No. 1-25. December 2005.
Nagaraju, T. and J. V. Ramana Rao. 1983. Levels of dehydrogenases of mosquito fish, *Gambusia affinis* (Baird and Girard) subjected to nitrate and nitrite stress. Indian J. Environ. Health. 25(3): 175-178.
-----. 1985. Effect of nitrate and nitrite stress on the transaminases of mosquito fish, *Gambusia affinis* (Baird and Girard). Indian J. Com. Anim. Physiol. 3(1): 29-32.
Naidoo, G. 1990. Effects of nitrate, ammonium and salinity on growth of the mangrove *Bruguiera gymnorrhiza* (L.) Lam. Aquat. Bot. 38: 209-219.
Nautilus Environmental. 2010. The role of water hardness in toxicity of nitrate: implications for water quality guidelines. J. Elphick, personal communication. Unpublished data presented at the 37th Annual Aquatic Toxicity Workshop in Toronto, October 2010..
Nautilus Environmental. 2011. Evaluation of the role of hardness in modifying the toxicity of nitrate to rainbow trout. Final Report Submitted to Mining Association of BC. 12 September 2011.
Neill, M. 1989. Nitrate concentrations in river waters in the south-east of Ireland and their relationship with agricultural practice. Wat. Res. 23(11): 1339-1355.
NLET (National Laboratory for Environmental Testing). 1994. Nutrients. *In*: Manual of Analytical

Methods, Volume 1: Major Ions and Nutrients. National Laboratory for Environmental Testing, Canada Centre for Inland Waters, Environment Canada. Burlington, ON.
NLET (National Laboratory for Environmental Testing). 2000. Nitrate + nitrite data in Canadian waters. Compiled by: Alkema, H. Environment Canada. National Water Research Institute, Burlington ON.
Nordin, R. N. 1982. The effect on water quality of explosives use in surface mining. Volume 2: The effect on algal growth. Planning and Resource Management Division, B.C. Ministry of Environment. Victoria, B.C. http://wlapwww.gov.bc.ca/wat/wq/studies/explode2.pdf
Nordin, R. N. and L. W. Pommen. 1986. Water quality criteria for nitrogen (nitrate, nitrite, and ammonia): Technical appendix. Water Quality Criteria. Water Management Branch, British Columbia Ministry of Environment and Parks. Victoria. 83 pp.
Nordin, R. N., L. W. Pommen and C. L. Meays. 2009. Water quality guidelines for nitrogen (nitrate, nitrite, and ammonia): Addendum to Technical Appendix. Water Quality Criteria. Water Management Branch, British Columbia Ministry of Environment and Parks. Victoria. 4 pp. http://a100.gov.bc.ca/pub/eirs/finishDownloadDocument.do?subdocumentId=7221
NRC (National Research Council). 1972. Accumulation of Nitrate. Committee on Nitrate Accumulation, Agriculture Board, National Academy of Sciences. Washington, DC.
-----. 1978. Nitrates: An Environmental Assessment. Scientific and Technical Assessments of Environmental Pollutants. National Academy of Sciences. Washington, D.C. 723 pp.
NRCAN (Natural Resources Canada). 1978. Hydrological Atlas of Canada. Water Quality of Surface Waters – hardness. Accessible at: http://atlas.nrcan.gc.ca/auth/english/maps/archives/hydrological_atlas_1978/water_quality/28_Water_Quality_Surface_Waters_1978/#download
NSDEL (Nova Scotia Department of the Environment and Labour). 2001. Water quality data for selected Nova Scotia lakes, 1982 to 1985. Compiled by: Taylor, D. Nova Scotia Department of the Environment and Labour. Halifax, NS.
NTP (National Toxicity Program). 2001. 9th Report on carcinogens; Revised January 2001. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. http://ehis.niehs.nih.gov.roc.html
Nürnberg, G. K. 1996. Trophic state of clear and colored, soft- and hardwater lakes with special consideration of nutrients, anoxia, phytoplankton and fish. J. Lake Reserv. Manage. 12(4): 432-447.
Oldham, R. S., D. M. Latham, D. Hilton-Brown, M. Towns, A. S. Cooke, and A. Burn. 1997. The effect of ammonium nitrate fertiliser on frog (*Rana temporaria*) survival. Agric. Ecosyst. Env. 61: 69-74.
OMAFRA (Ontario Ministry for Agriculture, F. a. R. A. 1996. Ten steps to complete a nutrient management plan for livestock and poultry manure. Report no. Order No. 96-053, Agdex No. 743/530. Ontario Ministry for Agriculture, Food and Rural Affairs. http://www.gov.on.ca/OMAFRA/english/livestock/swine/facts/96-053.htm
OMOE (Ontario Ministry of the Environment). 1983. Rondeau Bay Watershed data collection summary. Ontario Ministry of the Environment, Southwest Region, London ON.
-----. 1994. Water Management: Policies, Guidelines, and Provincial Water Quality Objectives of the Ministry of Environment and Energy. July 1994.
-----. 2001. Provincial Water Quality Monitoring Network Database. Compiled by: Todd, A. Water Monitoring Section, Environmental Monitoring and Reporting Branch, Ontario Ministry of the Environment. Etobicoke, ON.
-----. 2010a. Municipal Wastewater and Landfill Leachate Characterization Study. April 2010. Unpublished.
-----. 2010b. Provincial Water Quality Monitoring Network Data. Compiled by: Todd, A., Water Monitoring Section, Environmental Monitoring and Reporting Banch, Ontario Ministry of the Environment, Etobicoke, ON.
-----. 2010c. Great Lakes Nearshore Data, Compiled by: Boyd, D., Water Monitoring Section, Environmental and Reporting Branch, Ontario Ministry of the Environment, Etobicoke, ON.
Oyen, F. G. F., L. E. C. M. M. Camps, and S. E. Wendelaar Bonga. 1991. Effect of acid stress on the

embryonic development of the common carp (Cyprinus carpio). Aquat. Tox. 19: 1-12.
Packer, P. J. 1995. Nitrate pharmacology and toxicology. *In*: Role of Gut Bacteria in Human Toxicology and Pharmacology. Hill, M. J. (ed.). Taylor and Francis, Inc. Bristol, PA. pp. 59-67.
Padarauskas, A., V. Olsauskaite, V. Paliulionyte, and B. Pranaityte. 2000. Simultaneous separation of nitrate, nitrite and ammonium by capillary electrophoresis. Chromatographia. 52(3/4): 133-136.
Paerl, H. W. 1993. Emerging role of atmospheric nitrogen deposition in coastal eutrophication: Biogeochemical and trophic perspectives. Can. J. Fish. Aquat. Sci. 50: 2254-2269.
Pappas, J. L. and E. F. Stoermer. 1995. Effects of inorganic nitrogen enrichment on Lake Huron phytoplankton: An experimental study. J. Great Lakes Res. 21(2): 178-191.
Paquin PR, JW Gorsuch, S Apte, GE Batley, KC Bowles, PGC Campbell, CG Delos, DM DiToro, RL Dwyer, F Galvez, RW Gensemer, GG Goss, C Hogstrand, CR Janssen, JC McGeer, RB Naddy, RC Playle, RC Santore, U Schneider, WA Stubblefield, CM Wood, and KB Wu. 2002. The biotic ligand model: a historical overview. Comparative Biochemistry and Physiology C. 133:3-35.
Part P, O Svanberg, A Kiessling. 1985. The availability of cadmium to perfused rainbow trout gills in different water qualities. Water Research. 19:427-434.
Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science. 292(April 6): 86-90.
Petrie, B., P. A. Yeats, and P. M. Strain. 1999. Nitrate, silicate and phosphate atlas for the Scotian Shelf and Gulf of Maine. Report no. Can. Tech. Report of Hydrog. Ocean Sci. 203. Bedford Institute of Oceanography. Dartmouth, N.S. vii + 96 pp.
Petrucci, R. H. 1989. General Chemistry: Principles and Modern Applications. (5th Ed.). Macmillan Publishing Company. New York, NY. 1042 pp.
Pierce, R. H., J. M. Weeks, and J. M. Prappas. 1993. Nitrate toxicity to five species of marine fish. J. World Aquacult. Soc. 24(1): 105-107.
Pinar, G., E. Duque, A. Haidour, J.-M. Oliva, L. Sanchez-Barbero, V. Calvo, and J. L. Ramos. 1997. Removal of high concentrations of nitrate from industrial wastewaters by bacteria. Appl. Environ. Microbiol. 63(5): 2071-2073.
Pommen, L. W. 1983. The effect on water quality of explosives use in surface mining. Volume 1: Nitrogen sources, water quality, and prediction and managment of impacts. Report no. MOE Technical Report 4. British Columbia Ministry of Environment. Victoria, B.C. 149 + pp. pp.
Post, W. M., J. Pastor, P. J. Zinke, and A. G. Strangenberger. 1985. Global patterns of soil nitrogen storage. Nature. 317(17): 613-616.
Prepas, E., B. Pinel-Alloul, D. Planas, G. Methot, S. Paquet, and S. Reedyk. 2001. Forest harvest impacts on water quality and aquatic biota on the Boreal Plain: Introduction to the TROLS lake program. Can. J. Fish. Aquat. Sci. 58: 421-436.
Présing, M., K. V.-Balogh, L. Vörös, and H. M. Shafik. 1997. Relative nitrogen deficiency without occurrence of nitrogen fixing blue-green algae in a hypertrophic reservoir. Hydrobiologia. 342/343: 55-61.
Randall, G. W., D. R. Huggins, M. P. Russelle, D. J. Fuchs, W. W. Nelson, and J. L. Anderson. 1997. Nitrate losses through subsurface tile drainage in conservation reserve program, alfalfa, and row crop systems. J. Environ. Qual. 26: 1240-1247.
Reish, D. J. 1970. The effects of varying concentrations of nutrients, chlorinity, and dissolved oxygen on polychaetous annelids. Wat. Res. 4: 721-735.
Rhodes, A. L., R. M. Newton, and A. Pufall. 2001. Influences of land use on water quality of a diverse New England watershed. Environ. Sci. Technol. 35: 3640-3645.
Ritter, L., K. Solomon, P. Sibley, and K. Hall. 2001. Sources, pathways and relative risks of contaminants in water. Walkerton Inquiry. 180 Dundas St. W., 22nd Fl., Toronto, ON M5G 1Z8. ix + 167 pp.
RIVM (National Institute of Public Health and the Environment). 1991. Mapping critical loads for Europe. Hettelingh, J.-P., R. J. Downing, and P. A. M. de Smet (eds.). Report no. CEC Technical

Report No. 1; RIVM Report No. 259101001. Coordination Center for Effects, National Institute of Public Health and Environmental Protection. Bilthoven, The Netherlands.

-----. 2001. Modelling and mapping of critical thresholds in Europe: Status Report 2001. Posch, M., P. A. M. de Smet, J.-P. Hettelingh, and R. J. Downing (eds.). Report no. RIVM 259101010. Coordination Center for Effects, National Institute for Public Health and the Environment. Bilthoven, Netherlands. iv + 188 pp.

Ro, C., R. Vet, D. Ord, and A. Holloway. 1995. National Atmospheric Chemistry Database, 1993 Annual Report: Acid Precipitation in Eastern North America. Atmospheric Environmental Service, Environment Canada. Downsview, ON.

Robertson, W. D., D. W. Blowes, C. J. Ptacek, and J. A. Cherry. 2000. Long-term performance of in situ reactive barriers for nitrate remediation. Ground Wat. 38(5): 689-695.

Robertson, W. D., B. M. Russell, and J. A. Cherry. 1996. Attenuation of nitrate in aquitard sediments of sourthern Ontario. J. Hydrol. 180: 267-281.

Rodvang, S. J., R. Schmidt-Bellach, and L. I. Wassenaar. 1998. Nitrate in groundwater below irrigated fields in southern Alberta. Alberta Agriculture, Food and Rural Development. Lethbridge, AB.

Romansic, J.M., K.A. Diez, E.M. Higashi, and A.R. Blaustein. 2006. Effects of nitrate and pathogenic water mold *Saprolegnia* on survival of amphibian larvae. Diseases of Aquatic Organisms. 68:235-243.

Rott, E., H. C. Duthie, and E. Pipp. 1998. Monitoring organic pollution and eutrophication in the Grand River, Ontario, by means of diatoms. Can. J. Fish. Aquat. Sci. 55: 1443-1453.

Rouse, J. D., C. A. Bishop, and J. Struger. 1999. Nitrogen pollution: An assessment of its threat to amphibian survival. Environ. Health Perspect. 107: 799-803.

Roy, J. W., G. W. Parkin, and C. Wagner-Riddle. 2000. Timing of nitrate leaching from turfgrass after multiple fertilizer applications. Water Qual. Res. J. Canada. 35(4): 735-752.

Rubin, A. J. and G. A. Elmaraghy. 1977. Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. Wat. Res. 11: 927-935.

Rudolph, D. L., D. A. J. Barry, and M. J. Goss. 1998. Contamination in Ontario farmstead domestic wells and its association with agriculture: 2. Results from multilevel monitoring well installations. J. Contam. Hydrol. 32: 295-311.

Russo, R. C. 1985. Ammonia, nitrite, and nitrate. *In*: Fundamentals of Aquatic Toxicology. Rand, G. and S. R. Petrocelli (eds.). Hemisphere Publishing Corp. Washington,D.C. pp. 455-471.

Russo, R. C., R. V. Thurston, and K. Emerson. 1981. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): Effects of pH, nitrite species and anion species. Can. J. Fish. Aquat. Sci. 38: 387-393.

Ryden, J. C., P. R. Ball, and E. A. Garwood. 1984. Nitrate leaching from grassland. Nature. 311: 50-53.

Ryther, J. H. and W. M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science. 171: 1008-1013.

Schindler, D. W., F. A. J. Armstrong, S. K. Holmgren, and G. J. Brunskill. 1971. Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate. J. Fish. Res. Board Can. 28(11): 1763-1782.

Schindler, D. W., P. J. Dillon, and H. Schreier. 2006. A review of anthropogenic sources of nitrogen and their effects on Canadian aquatic ecosystems. Biogeochemistry. 79: 25-44.

Schuh, W. M., D. L. Klinkebiel, J. C. Gardner, and R. F. Meyer. 1997. Tracer and nitrate movement to groundwater in the Northern Great Plains. J. Environ. Qual. 26: 1335-1347.

Schuytema, G. S. and A. V. Nebeker. 1999a. Comparative effects of ammonium and nitrate compounds on Pacific treefrog and African clawed frog embryos. Arch. Environ. Contam. Toxicol. 36: 200-206.

-----. 1999b. Effects of ammonium nitrate, sodium nitrate, and urea on red-legged frogs, Pacific tree frogs and African clawed frogs. Bull. Environ. Contam. Toxicol. 63: 357-364.

-----. 1999c. Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles. Environ. Toxicol. Chem. 18(10): 2251-2257.

Scott, G. and R. L. Crunkilton. 2000. Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia*, and *Daphnia magna*. Environ. Toxicol. Chem. 19(12): 2918-2922.

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnol. Oceanogr. 33(4, part 2): 702-724.
SENES Consultants Ltd. 2001. Review of the Canadian Fertilizer Industry and Evaluation of Control Technology. SENES Consultants Ltd. Richmond Hill, ON. vii + 114 pp.
Senkbeil, E. G. and J. C. Wriston. 1981a. Haemocyanin synthesis in the American lobster *Homarus americanus*. Comp. Biochem. Physiolog. 68: 163-171.
-----. 1981b. Catabolism of haemocyanin in the American lobster *Homarus americanus*. Comp. Biochem. Physiolog. 69: 781-790.
Sharp, J. 1983. The distribution of inorganic nitrogen and dissolved and particulate organic nitrogen in the sea. *In*: Nitrogen in the Marine Environment. Carpenter, E. J. and D. G. Capone (eds.). Academic Press. New York, NY.
Shimura, R., K. Ijiri, R. Mizuno and S. Nagaoka. 2002. Aquatic animal research in space station and its issues: focus on support technology on nitrate toxicity. Advances in Space Research. 30(4): 803-808.
Siddiqi, M. Y., A. D. M. Glass, and T. J. Ruth. 1991. Studies of the uptake of nitrate in barley: III. Compartmentation of NO_3^-. J. Exp. Bot. 42: 1455-1463.
Siu, G. K. Y., M. L. C. Young, and D. K. O. Chan. 1997. Environmental and nutritional factors which regulate population dynamics and toxin production in the dinoflagellate *Alexandrium catenella*. Hydrobiologia. 352: 117-140.
Slater, J. M. and D. G. Capone. 1987. Denitrification in aquifer soil and nearshore marine sediments influenced by groundwater nitrate. Appl. Environ. Microbiol. 53(6): 1292-1297.
Smith, D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology. 68(2): 344-350.
Smith, J. C., R. Cormier, J. Worms, M. A. Bird, R. Quilliam, R. Pocklington, R. Angus, and L. Hanic. 1990. Toxic blooms of the domoic acid containing diatom *Nitzschia pungens* in the Cardigan River, Prince Edward Island. *In*: Toxic Marine Phytoplankton. Granéli, E., B. Sundström, L. Edler, and D. M. Anderson (eds.). Elsevier Science Publishing Co., Inc. New York, NY. pp. 227-232.
Smith, R. A., R. B. Alexander, and M. G. Wolman. 1987. Water-quality trends in the nation's rivers. Science. 235: 1607-1615.
Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science. 221: 669-671.
Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine and terrestrial ecosystems. Env. Poll. 100: 179-196.
Soucek, D.J. and A. Dickinson. 2011. Acute toxicity of nitrate and nitrite to sensitive freshwater insects, molluscs, and a crustacean. Arch. Environ. Contam. Toxicol. DOI 10.1007/s00244-011-9705-8.
Spalding, R. F. and Exner, M. E. 1991. Nitrate contamination in the contiguous United States. *In*: Nitrate Contamination: Exposure, Consequence, and Control. NATO ASI Series. Bogardi, I., R. D. Kuzelka, and W. G. Ennenga (eds). Vol. G 30: 13-48. Springer-Verlag. Berlin, Germany.
Speijers, G. J. A., G. F. van Went, M. E. van Appeldoorn, G. K. Montizaan, J. A. Janus, J. H. Canton, C. A. M. van Gestel, C. A. van der Heijden, E. Heijna-Merkus, A. G. A. C. Knaap, R. Luttik, and D. deZwart. 1989. Integrated criteria document nitrate (Appendix: Effects). Report no. 758473012 (Appendix). Research for Man and Environment. National Institute of Public Health and Environmental Protection. Bilthoven, Netherlands. 198 pp.
Spencer, C. P. 1975. The micronutrient elements. *In*: Chemical Oceanography (2nd Ed.). Riley, J. P. and G. Skirrow (eds.). Academic Press. London. pp. 245-300.
Spruill, T. B. 2000. Statistical evaluation of effects of riparian buffers on nitrate and ground water quality. J. Environ. Qual. 29: 1523-1538.
Stammers, W. N., J. B. Robinson, H. R. Whiteley, N. Kaushik, and P. Sain. 1978. Modeling nitrate transport in a small upland stream. Water Poll. Res. Canada. 13: 161-174.
Stantec Consulting Ltd. 2006. Report on the toxicity of nitrate to Canadian fish and aquatic invertebrate species. Prepared for: Environment Canada, National Guidelines Standards Office. Stantec Project No. 162704276.

Statutes of Alberta. 2001. Agricultural Operation Practices Amendment Act. SA 2001 c16. Government of Alberta. Came into force January 1, 2002.

Stoddard, J. L. 1994. Long-term changes in watershed retention of nitrogen: Its causes and aquatic concerns. *In*: Environmental Chemistry of Lakes and Reservoirs. Baker, L. A. (ed.). American Chemical Society. Washington,D.C. pp. 223-284.

Stoddard, J. L. and J. H. Kellog. 1993. Trends and patterns in lake acidification in the state of Vermont: Evidence from the long-term monitoring project. Water Air Soil Poll. 67: 301-317.

Stormer, J., F. B. Jensen, and J. C. Rankin. 1996. Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: effects on ionic balance. Can. J. Fish. Aquat. Sci. 53: 1943-1950.

Strain, P. M. and P. A. Yeats. 1999. The relationships between chemical measures and potential predictors of the eutrophication status of inlets. Mar. Poll. Bull. 38(12): 1163-1170.

Stumm, W. and J. J. Morgan. 1981. Aquatic Chemistry (2nd Ed.). John Wiley and Sons. New York, NY. 780 pp.

Suter, G.W., S.B. Norton and A. Fairbrother. 2005. Individuals versus organisms versus populations in the definition of ecological assessment endpoints. Integrated Environmental Assessment and Management. 1: 397-400.

Sutton, A. L., D. W. Nelson, J. D. Hoff, and V. B. Mayrose. 1982. Effects of injection and surface applications of liquid swine manure on corn yield and soil composition. J. Environ. Qual. 11(3): 468-472.

Sutton, A. L., D. W. Nelson, V. B. Mayrose, and J. C. Nye. 1978. Effects of liquid swine waste applications on corn yield and soil chemical composition. J. Environ. Qual. 7(3): 325-333.

Suzuki, J., H. Okazaki, Y. Nishi, and S. Suzuki. 1982. Formation of mutagens by photolysis of aromatic compounds in aqueous nitrate solution. Bull. Environ. Contam. Toxicol. 29: 511-516.

Swank, W. T., J. B. Waide, D. A. Crossley, Jr., and R. L. Todd. 1981. Insect defoliation enhances nitrate export from forest ecosystems. Oecologia. 51: 297-299.

Swedish EPA. 2000. Environmental quality criteria: coasts and seas. Report, vol. 5052. Stockholm, Sweden: Swedish Environmental Protection Agency (as cited in: Camargo and Alonso 2006).

Taylor, D., S. Nixon, S. Granger, and B. Buckley. 1995. Nutrient limitation and the eutrophication of coastal lagoons. Mar. Ecol. Prog. Ser. 127: 235-244.

Tesh, A. E. C., O. K. Wilby, and J. M. Tesh. 1990. Effects of inorganic nitrates on growth and development of *Hydra*. Toxic. in Vitro. 4(4/5): 614-615.

Thorson, G. 1956. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the sound (ø resund). Report no. 4. Komm. Danmarks Fisk. - Havundersgelser Ser. Plankton, Meddel. pp. 1-523.

Tilak, K.S., S.J. Lakshmi and T.A. Susan. 2002. The toxicity of ammonia, nitrite and nitrate to the fish, *Catla catla* (Hamilton). Journal of Environmental Biology. 23(2): 147-149.

Times Books. 1999. The Times Atlas of the World. (10th Comprehensive Ed.). Times Books Ltd. (ed.). Random House Inc. New York.

Tobias, C. R., S. A. Macko, I. C. Anderson, E. A. Canuel, and J. W. Harvey. 2001. Tracking the fate of a high concentration groundwater nitrate plume through a fringing marsh: A combined groundwater tracer and in situ isotope enrichment study. Limnol. Oceanogr. 46(8): 1977-1989.

Tomasky, G., J. Barak, I. Valiela, P. Behr, L. Soucy, and K. Foreman. 1999. Nutrient limitation of phytoplankton growth in Waquoit Bay, Massachusetts, USA; A nutrient enrichment study. Aquat. Ecol. 33: 147-155.

Tomasso, J. R. and G. J. Carmichael. 1986. Acute toxicity of ammonia, nitrite, and nitrate to the Guadalupe bass, *Micropterus treculi*. Bull. Environ. Contam. Toxicol. 36: 866-870.

Trama, F. 1954. The acute toxicity of some common salts of sodium, potassium and calcium to the common bluegill (*Lepomis macrochirus* Rafinesque). Proc. Acad. Nat. Sci. Philadelphia. 106: 185-205.

Travis, J. 1983. Variation in growth and survival of *Hyla gratiosa* larvae in experimental enclosures. Copeia. 1983(1): 232-237.

Tsai, S-J. and J-C Chen. 2002. Acute toxicity of nitrate on *Penaeus monodon* juveniles at different salinity levels. Aquaculture. 213:163–170.

Tucker, C. S. and T. A. Debusk. 1983. Seasonal variation in the nitrate content of water hyacinth (*Eichhornia crassipes* [Mart.] Solms). Aquat. Bot. 15: 419-422.

USEPA (U.S.Environmental Protection Agency). 1986. Quality Criteria for Water, 1986. Report no. EPA 440/5-86-001. US Environmental Protection Agency, Office of Water Regulations and Standards. Washington, DC.

-----. 2000. Nutrient Criteria Technical Guidance Manual: Estuarine and Coastal Marine Waters. Report no. EPA-822-B-01-003. Office of Water, United States Environmental Protection Agency.

-----. 2000a. Nutrient Criteria Technical Guidance Manual: Rivers and Streams. Report no. EPA-822-B-00-002. Office of Water, Office of Science and Technology, United States Environmental Protection Agency. Washington, DC. xiv + 152 pp.

-----. 2000b. Nutrient criteria technical guidance manual: Lakes and reservoirs, 1st Ed. Report no. EPA-822-B00-001. United States Environmental Protection Agency, Office of Water, Office of Science and Technology. Washington, D.C.

-----. 2001. 2001 Update of ambient water quality criteria for cadmium. Washington, D.C., Office of Water.

-----. 2002. Chemical/name index to EPA Test Methods (December 2001) N-Se. World Wide Web. http://www.epa.gov/epahome/index/nameindx3.htm

-----. 2002b. http://www.epa.gov/waterscience/criteria/nutrient/guidance/index.html (and as cited in: Camargo and Alonso 2006).

-----. 2008. Nutrient Criteria Technical Guidance Manual: Wetlands. Report no. EPA-822-B-08-003. Office of Water, United States Environmental Protection Agency.

-----. 2010a. Water quality standards for the state of Florida's lakes and flowing waters. Federal Register. Vol. 75, No. 16. Tuesday January 26, 2010. Proposed Rules.
http://edocket.access.gpo.gov/2010/pdf/2010-1220.pdf
http://www.epa.gov/waterscience/standards/rules/florida/

-----. 2010b. Final report on acute and chronic toxicity of nitrate, nitrite, boron, manganese, fluoride, chloride and sulfate to several aquatic animal species. Report No. EPA 905-R-10-002. November 2010. US EPA Office of Science and Technology, Health and Ecological Criteria Division and US EPA Region 5 Water Division.

USGS (U.S.Geological Survey). 1999. The quality of our nation's waters: Nutrients and pesticides. Report no. 1225. U.S. Geological Survey. Reston,VA. pp. 1-82.

Vadeboncoeur, Y., D. M. Lodge, and S. R. Carpenter. 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. Ecology. 82(4): 1065-1077.

van der Hoek, J. P., P. J. M. van der Ven, and A. Klapwijk. 1988. Combined ion exchange/biological denitrification for nitrate removal from ground water under different process conditions. Wat. Res. 22(6): 679-684.

van der Leeden, F., F. L. Troise, and D. K. Todd. 1990. The Water Encyclopedia, 2nd Ed. Lewis Publishers. Chelsea, Michigan. 808 pp.

van Duijvenbooden, W. and A. J. C. M. Matthijsen. 1989. Integrated criteria document nitrate. Report no. 758473012. Research for Man and Environment. National Institute of Public Health and Environmental Protection. Bilthoven, Netherlands.

Van Herpe, Y. and P. A. Troch. 2000. Spatial and temporal variations in surface water nitrate concentrations in a mixed land use catchment under humid termperate climatic conditions. Hydrol. Process. 14: 2439-2455.

Verstraete, W. and M. Alexander. 1973. Heterotrophic nitrification in samples of natural ecosystems. Environ. Sci. Technol. 7(1): 39-42.

Viets, F. G., Jr. 1965. The plant's need for and use of nitrogen. *In*: Soil Nitrogen. Bartholomew, W. V. and F. E. Clark (eds.). American Society of Agronomy, Inc. Madison, WI. pp. 503-548.

Wang, W.-X. and R. C. H. Dei. 2000. Metal uptake in a coastal diatom influenced by major nutrients (N,

P, and Si). Wat. Res. 35(1): 315-321.
Wang, W.-X. and R. C. H. Dei. 2001. Effects of major nutrient additions on metal uptake in phytoplankton. Env. Poll.(111): 233-240.
Wassenaar, L. I. 1994. Evaluation of the origin and fate of nitrate in the Abbotsford aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. Report no. NHRI Contribution No. CS - 94009. National Hydrology Research Centre. Saskatoon, SK.
Watson, S. W., F. W. Valois, and J. B. Waterbury. 1981. The family nitrobacteraceae. *In*: The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Starr, M. P., H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (eds.). Springer-Verlag. Berlin. pp. 1005-1022.
Webb, J. R., B. J. Cosby, F. A. Deviney, Jr., K. N. Eshleman, and J. N. Galloway. 1995. Change in the acid-base status of an Appalachian mountain catchment following forest defoliation by the gypsy moth. Water Air Soil Poll. 85: 535-540.
Wedemeyer, G. A. and W. T. Yasutake. 1978. Prevention and treatment of nitrite toxicity in juvenile steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 35: 822-827.
Welsh PG. 1996. Influence of dissolved organic carbon on the speciation, bioavailability and toxicity of metals to aquatic biota in soft water lakes. PhD Thesis. University of Waterloo. Waterloo, Ontario.
Werner, E. E. 1986. Amphibian metamorphosis: Growth rate, predation risk, and optimal size at transformation. Am. Nat. 128(3): 319-341.
Westin, D. T. 1974. Nitrate and nitrite toxicity to salmonid fishes. Prog. Fish Cult. 36(2): 86-89.
Wetzel, R. G. 1983. Limnology (2nd Ed.). Saunders College Publishing. Toronto, Canada. 767 pp.
Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems (3rd Ed.). Academic Press. San Diego, CA. 1006 pp.
Whitney,F. 2001. Personal Communication. Institute of Ocean Sciences, Department of Fisheries and Oceans Canada, Sidney, BC.
WHO (World Health Organization). 1986. Health hazards from nitrates in drinking water. Environmental Health. World Health Organization, Regional Office for Europe. Copenhagen. 102 pp.
WHO (World Health Organization). 1996. Inorganic Constituents and Physical Parameters. *In*: Guidelines for Drinking-Water Quality: Volume 2 Health Criteria and Other Supporting Information. (2nd Ed.). World Health Organization. Geneva. pp. 313-322.
-----. 2001. International Agency for Research on Cancer. http://www.iarc.fr/
Wickins, J. F. 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture 9: 19-37.
Wilbur, H. M. 1984. Complex life cycles and community organization in amphibians. *In*: A new ecology: Novel approaches to interactive systems. Price, P. W., C. N. Slobodchikoff, and W. S. Gaud (eds.). John Wiley & Sons. New York. pp. 195-22?
Williams, D. J. and K. W. Kuntz. 1999. Lake Superior Surveillance Program, 1996-1997 Results: Spatial and Temporal Trends. Report no. EHD/ECB-OR/99-01/I. Environmental Conservation Branch/Ontario Region, Ecosystem Health Division, Environment Canada. iv + 31 pp.
Williams, D. J., K. W. Kuntz, S. L'Italien, and V. Richardson.1998a. Lake Ontario Surveillance Program, 1992-1993 Results: Spatial and Temporal Trends. Report no. EHD/ECB-OR/98-04/I. Environmental Conservation Branch/Ontario Region, Ecosystem Health Division, Environment Canada. iv + 24 pp.
-----. 1998b. Lake Erie Surveillance Program, 1994-1995 Results: Spatial and Temporal Trends. Report no. EHD/ECB-OR/98-05/I. Environmental Conservation Branch/Ontario Region, Ecosystem Health Division, Environment Canada. iv + 29 pp.
Winter, J. G., Dillon, P. J., Futter, M. N., Nicholls, K. H., Scheider, W. A., and Scott, L. D. 2002. Total phosphorus budgets and nitrogen loads: Lake Simcoe, Ontario (1990 - 1998). J. Great Lakes Res. 28(3): 301-314.
Winter, T. C., J. W. Harvey, O. L. Franke, and W. M. Alley. 1998. Ground water and surface water: A single resource. Report no. U.S. Geological Survey Circular 1139. U.S. Geological Survey. Denver, Colorado. 79 pp. water.usgs.gov/pubs/circ/circ1139/pdf/circ1139.pdf

Wolfe, R. L., N. I. Lieu, G. Izaguirre, and E. G. Means, III. 1990. Ammonia-oxidizing bacteria in a chloraminated distribution system: Seasonal occurrence, distribution, and disinfection resistance. Appl. Environ. Microbiol. 56(2): 451-462.

Wolfe, R. L., E. G. Means, III, K. D. Marshall, and S. E. Barrett. 1988. Biological nitrification in covered reservoirs containing chloraminated water. J. Am. Waste Wat. Assoc. Sept.: 109-114.

World Atlas. 2002. World Wide Web.www.worldatlas.com/aatlas/world.htm

Wright DA. 1980. Cadmium and calcium interactions in the freshwater amphipod *Gammarus pulex*. Freshwater Biology. 10:123-133.

Yamaguchi, M. and S. Itakura. 1999. Nutrition and growth kinetics in nitrogen- or phosphorus-limited cultures of the noxious red tide Dinoflagellate *Gymnodinium mikimotoi*. Fish. Sci. 65(3): 367-373.

Zayed, G. and J. Winter. 1998. Removal of organic pollutants and of nitrate from wastewater from the dairy industry by denitrification. Appl. Microbiol. Biotechnol. 49: 469-474.

APPENDIX A. SUMMARY OF FRESHWATER TOXICITY STUDIES.

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
INVERTEBRATES													
Amphinemura delosa (Stonefly)	Field-collected nymphs	Na^+	96-h LC_{50}	2020	S	11.9-12.8	8.80-9.97	88-92	60-62	7.8-8.0	US EPA 2010b (study completed by Soucek and Dickinson 2011)	1	
Allocapnia vivipara (Stonefly)	Field-collected nymphs	Na^+	96-h LC_{50}	3703	S	11 ± 1.0	10.3 ± 0.4	99 ± 1.0		7.9 ± 1.0	Soucek and Dickinson 2011	1	
Ceriodaphnia dubia (water flea)	Neonates	Na^+	48-h LC_{50}	1657	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Neonates	Na^+	7-d NOEC (reproduction)	94	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Neonates	Na^+	7-d LOEC (reproduction)	189	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Neonates (<24h old)	Na^+	7-d LC50	196	R	25 ± 1.0	6.8-8.2	44	30	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LC50	523	R	25 ± 1.0	6.8-8.2	98	62	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LC50	536	R	25 ± 1.0	6.8-8.2	166	108	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d IC25 (reproduction)	50	R	25 ± 1.0	6.8-8.2	44	30	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d IC25 (reproduction)	106	R	25 ± 1.0	6.8-8.2	98	62	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d IC25 (reproduction)	192	R	25 ± 1.0	6.8-8.2	166	108	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d NOEC (survival)	177	R	25 ± 1.0	6.8-8.2	44	30	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d NOEC (survival)	354	R	25 ± 1.0	6.8-8.2	98	62	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d NOEC (survival)	354	R	25 ± 1.0	6.8-8.2	166	108	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LOEC (survival)	354	R	25 ± 1.0	6.8-8.2	44	30	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LOEC (survival)	709	R	25 ± 1.0	6.8-8.2	98	62	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LOEC (survival)	709	R	25 ± 1.0	6.8-8.2	166	108	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d NOEC (reproduction)	44	R	25 ± 1.0	6.8-8.2	44	30	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d NOEC (reproduction)	89	R	25 ± 1.0	6.8-8.2	98	62	7.3-8.3	Elphick 2011	1	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Neonates (<24h old)	Na^+	7-d NOEC (reproduction)	177	R	25 ± 1.0	6.8-8.2	166	108	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LOEC (reproduction)	89	R	25 ± 1.0	6.8-8.2	44	30	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LOEC (reproduction)	177	R	25 ± 1.0	6.8-8.2	98	62	7.3-8.3	Elphick 2011	1	
	Neonates (<24h old)	Na^+	7-d LOEC (reproduction)	354	R	25 ± 1.0	6.8-8.2	166	108	7.3-8.3	Elphick 2011	1	
Cheumatopsyche pettiti (caddisfly)	Early Instar	Na^+	8760-h $LC_{0.01}$	11	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Last Instar	Na^+	8760-h $LC_{0.01}$	16	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Early Instar	Na^+	120-h $LC_{0.01}$	30	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Last Instar	Na^+	120-h $LC_{0.01}$	43	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Early instar	Na^+	120-h LC_{50}	472	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Early instar	Na^+	96-h LC_{50}	503	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Last instar	Na^+	120-h LC_{50}	527	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Last instar	Na^+	96-h LC_{50}	733	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Early instar	Na^+	72-h LC_{50}	846	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Last instar	Na^+	72-h LC_{50}	930	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
Chironomus dilutus (midge)	10d old	Na^+	48-h LC_{50}	1582	S	21.3-22.7	7.4-8.0	84-136	60-90	7.8-7.99	US EPA 2010b	1	
	3rd instar	Na^+	10-d LC50	505	R	23 ± 1.0	7.5-8.3	46	22	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LC50	975	R	23 ± 1.0	7.5-8.3	86	48	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LC50	1493	R	23 ± 1.0	7.5-8.3	172	100	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d IC25 (growth)	217	R	23 ± 1.0	7.5-8.3	46	22	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d IC25 (growth)	447	R	23 ± 1.0	7.5-8.3	86	48	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d IC25 (growth)	771	R	23 ± 1.0	7.5-8.3	172	100	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d NOEC (survival)	177	R	23 ± 1.0	7.5-8.3	46	22	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d NOEC (survival)	709	R	23 ± 1.0	7.5-8.3	86	48	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d NOEC (survival)	709	R	23 ± 1.0	7.5-8.3	172	100	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LOEC (survival)	354	R	23 ± 1.0	7.5-8.3	46	22	7.1-8.0	Elphick 2011	1	o

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	3rd instar	Na^+	10-d LOEC (survival)	1418	R	23 ± 1.0	7.5-8.3	86	48	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LOEC (survival)	1418	R	23 ± 1.0	7.5-8.3	172	100	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d NOEC (growth)	177	R	23 ± 1.0	7.5-8.3	46	22	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d NOEC (growth)	354	R	23 ± 1.0	7.5-8.3	86	48	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d NOEC (growth)	709	R	23 ± 1.0	7.5-8.3	172	100	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LOEC (growth)	354	R	23 ± 1.0	7.5-8.3	46	22	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LOEC (growth)	709	R	23 ± 1.0	7.5-8.3	86	48	7.1-8.0	Elphick 2011	1	o
	3rd instar	Na^+	10-d LOEC (growth)	1418	R	23 ± 1.0	7.5-8.3	172	100	7.1-8.0	Elphick 2011	1	o
Daphnia magna (water flea)	ND	K^+	96-h TL_m	24	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
	ND	K^+	48-h TL_m	299	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
	ND	Na^+	96-h TL_m	485	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
	ND	K^+	96-h TL_m	549	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	b,c
	Neonates	Na^+	7-d NOEC (reproduction)	1586	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Neonates	Na^+	48-h LC_{50}	2047	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	ND	Na^+	48-h TL_m	2614	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,c
	ND	Na^+	96-h TL_m	3070	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	b,c
	Neonates	Na^+	7-d LOEC (reproduction)	3176	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Early Instar	Na^+	48-h EC (immobilization)	3650	S	25	ND	[Ca2+] = 31 mg·L^{-1}	97 - 100	ND	Anderson 1946	A	c
	Early Instar	Na^+	16-h EC (immobilization)	6205	S	25	ND	ND	97 - 100	ND	Anderson 1944	A	c
Eulimnogammarus toletanus (amphipod)	Adult	Na^+	48-h LC_{10}	209	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	48-h LC_{50}	798	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	72-h LC_{10}	126	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	72-h LC_{50}	483	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Adult	Na^+	96-h LC_{10}	98	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	96-h LC_{50}	377	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	120-h $LC_{0.01}$	19	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	120-h LC_{10}	85	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	120-h LC_{50}	324	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
Echinogammarus echinosetosus (amphipod)	Adult	Na^+	48-h LC_{10}	72	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	48-h LC_{50}	473	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	72-h LC_{10}	51	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	72-h LC_{50}	331	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	96-h LC_{10}	42	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	96-h LC_{50}	277	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	120-h $LC_{0.01}$	12	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	120-h LC_{10}	38	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
	Adult	Na^+	120-h LC_{50}	249	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c, e
Hyalella azteca (freshwater scud)	Juvenile	Na^+	96-h LC_{50}	744	S	23	>80% saturation	44 (CCME soft)	30	7.2-7.7	Elphick 2011	1	
	Juvenile	Na^+	96-h LC_{50}	2149	S	23	>80% saturation	100 (CCME mod hard)	58	7.4-7.9	Elphick 2011	1	
	Juvenile	Na^+	96-h LC_{50}	4080	S	23	>80% saturation	164 (CCME hard)	90	7.8-8.3	Elphick 2011	1	
	10 days old	Na^+	96-h LC_{50}	73	S	21.9-22.9	8.0-9.0	80-84 (CCME mod hard)	60	7.80-8.26	US EPA 2010b	1	
	7-14 days old	Na^+	96-h LC_{50}	2955	S	22.5 ± 0.2	8.1 ± 0.1	117 ± 7 (CCME mod hard)		8.0	Soucek and Dickinson 2011	1	

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	Juvenile	Na^+	10-d LOEC (Survival)	2083	S	23	6.9-8.3	310	230	8.2-8.6	Stantec, Ltd. 2006	1	
	Juvenile	Na^+	10-d NOEC (Survival)	1018	S	23	6.9-8.3	310	230	8.2-8.6	Stantec, Ltd. 2006	1	
	Juvenile	Na^+	10-d LC_{50} (Survival)	2725	S	23	6.9-8.3	310	230	8.2-8.6	Stantec Ltd., 2006	1	
	Juvenile	Na^+	10-d LOEC (Growth)	4274	S	23	6.9-8.3	310	230	8.2-8.6	Stantec Ltd., 2006	1	
	Juvenile	Na^+	10-d NOEC (Growth)	2083	S	23	6.9-8.3	310	230	8.2-8.6	Stantec Ltd., 2006	1	
	Juvenile	Na^+	10-d IC_{25} (Growth)	830	S	23	6.9-8.3	310	230	8.2-8.6	Stantec Ltd., 2006	1	
	6-8 day old	Na^+	14-d LC50	558	R	23 ± 1	7.1-8.5	46	22	7.1-8.0	Elphickl 2011	1	o
	6-8 day old	Na^+	14-d LC50	1271	R	23 ± 1	7.1-8.5	86	48	7.1-8.0	Elphickl 2011	1	o
	6-8 day old	Na^+	14-d LC50	>2835	R	23 ± 1	7.1-8.5	172	100	7.1-8.0	Elphickl 2011	1	o
	6-8 day old	Na^+	14-d IC25 (growth)	57	R	23 ± 1	7.1-8.5	46	22	7.1-8.0	Elphickl 2011	1	o
	6-8 day old	Na^+	14-d IC25 (growth)	518	R	23 ± 1	7.1-8.5	86	48	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d IC25 (growth)	806	R	23 ± 1	7.1-8.5	172	100	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d NOEC (survival)	354	R	23 ± 1	7.1-8.5	46	22	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d NOEC (survival)	709	R	23 ± 1	7.1-8.5	86	48	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d NOEC (survival)	2835	R	23 ± 1	7.1-8.5	172	100	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d LOEC (survival)	709	R	23 ± 1	7.1-8.5	46	22	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d LOEC (survival)	1418	R	23 ± 1	7.1-8.5	86	48	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d LOEC (survival)	>2835	R	23 ± 1	7.1-8.5	172	100	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d NOEC (growth)	44	R	23 ± 1	7.1-8.5	46	22	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d NOEC (growth)	354	R	23 ± 1	7.1-8.5	86	48	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d NOEC (growth)	709	R	23 ± 1	7.1-8.5	172	100	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d LOEC (growth)	89	R	23 ± 1	7.1-8.5	46	22	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d LOEC (growth)	709	R	23 ± 1	7.1-8.5	86	48	7.1-8.0	Elphick 2011	1	o
	6-8 day old	Na^+	14-d LOEC (growth)	1418	R	23 ± 1	7.1-8.5	172	100	7.1-8.0	Elphick 2011	1	o
Hydra attenuata (hydra)	Adult	Na^+	12-d LOEC (mortality)	50	S	ND	ND	ND	ND	ND	Tesh et al. 1990	A	c,d

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Adult	K^+	13-d NOEC (mortality)	150 - 250	S	ND	ND	ND	ND	ND	Tesh et al. 1990	A	c,d
Hydropsyche occidentalis (caddisfly)	Early Instar	Na^+	8760-h $LC_{0.01}$	6	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Last Instar	Na^+	8760-h $LC_{0.01}$	10	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Early Instar	Na^+	120-h $LC_{0.01}$	20	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Last Instar	Na^+	120-h $LC_{0.01}$	29	S	18	9.6	42.7	35	7.9	Camargo and Ward 1995	1	a
	Early instar	Na^+	120-h LC_{50}	290	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Last instar	Na^+	120-h LC_{50}	342	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Early instar	Na^+	96-h LC_{50}	431	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Last instar	Na^+	96-h LC_{50}	483	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Early instar	Na^+	72-h LC_{50}	658	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
	Last instar	Na^+	72-h LC_{50}	813	S	18	9.6	42.7	35	7.9	Camargo and Ward 1992	1	
Hydropsyche exocellata (caddisfly)	Adult	Na^+	48-h LC_{10}	278	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	48-h LC_{50}	2622	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	72-h LC_{10}	177	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	72-h LC_{50}	1551	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	96-h LC_{10}	141	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	96-h LC_{50}	1194	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	120-h $LC_{0.01}$	53	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	120-h LC_{10}	123	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
	Adult	Na^+	120-h LC_{50}	1019	S	17.9	7.7	293	ND	7.8	Camargo et al. 2005	2	c,e
Lampsilis siliquoidea (fatmucket mussel)	<5 day old juveniles	Na^+	96-h LC_{50}	1582	S	19.8-20.1	7.72-8.12	90-92	60-62	7.9-8.0	US EPA 2010b (study completed by Soucek and Dickinson 2011)	1	
Lymnea spp. (snail)	Eggs	K^+	96-h TL_m	671	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Eggs	K^+	48-h TL_m	910	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
	Eggs	Na^+	96-h TL_m	2373	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
	Eggs	Na^+	48-h TL_m	4716	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
Macrobrachium rosenbergii (prawn)	Juvenile	Na^+	21-d LC_{50} (combined from 2 experiments)	709	F	28.0	Sat	ND	ND	ND	Wickins 1976	2 (unreliable effect concentration)	e
	Juvenile	Na^+	21-d EC_{50} (growth) (combined from 2 experiments)	775	F	28.0	Sat	ND	ND	ND	Wickins 1976	2 (unreliable effect concentration)	e
	Juvenile	Na^+	21-d LC_{50} Experiment 1	857	F	28.0	Sat	ND	ND	ND	Wickins 1976	2	e
	Juvenile	Na^+	21-d EC_{50} (growth) Experiment 1	534	F	28.0	Sat	ND	ND	ND	Wickins 1976	2	e
	Juvenile	Na^+	21-d LC_{50} Experiment 2	na (no clear dose response)	F	28.0	Sat	ND	ND	ND	Wickins 1976	2	e
	Juvenile	Na^+	21-d EC_{50} (growth) Experiment 2	872	F	28.0	Sat	ND	ND	ND	Wickins 1976	2	e
Megalonaias nervosa (washboard mussel)	<5 day old juveniles	Na^+	96-h LC_{50}	4151	S	20.8-20.9	7.90-8.43	90-92	60-62	7.8-8.2	US EPA 2010b (study completed by Soucek and Dickinson 2011)	1	
Potamopyrgus antipodarum (New Zealand mudsnail)	Adult	Na^+	96-h LC_{50}	4616	R	20.4	6.7	90.8	ND	8.3	Alonso and Camargo 2003	1	
Polycelis nigra (planaria)	ND	Na^+	48-h LC_{50}	2666	S	15 - 18	ND	ND	ND	6.4	Jones 1941	A	c,f
	ND	Na^+	48-h LC_{50}	2697	S (R?)	15 -18	ND	ND	ND	6.4	Jones 1940	A	c,f
Sphaerium simile (fingernail clam)	Juvenile	Na^+	96-h LC_{50}	1644	S	22.5-23.0	4.52-8.31	90-92	60-62	7.8-8.1	US EPA 2010b (study completed by Soucek and Dickinson 2011)	1	
FISH													
Catla catla (Indian major carp)	Juvenile	Na^+	24-h-LC_{50}	6935	S	28	8 – 10	232	472	8.4	Tilak et al. 2002	A	c,e
	Juvenile	Na^+	24-h-LC_{50}	2144	R	28	8 – 10	232	472	8.4	Tilak et al. 2002	A	c,e

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
Carassius carassius (crucian carp)	Juvenile	Na^+	64-d LOEC (iodine uptake inhibition)	0.9	ND	5 - 6	ND	ND	ND	ND	Lahti et al. 1985	A	c,g
	ND	Na^+	24-h TL_m	8870	S	ND	Sat	ND	ND	7.9	Dowden and Bennett 1965	A	b,c
Coregonus clupeaformis (lake whitefish)	Alevin	Na^+	96-h LC_{50}	9683	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	2	j
	Fry	Na^+	96-h LC_{50}	8429	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	2	
	Fry (1 g)	Na^+	24h LC50	4730	S	10	10-11.2	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	24h LC50	9840	S	15	10.0-10.96	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	48h LC50	4730	S	10	10-11.2	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	48h LC50	8440	S	15	10.0-10.96	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	72h LC50	4730	S	10	10-11.2	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	72h LC50	5110	S	15	10.0-10.96	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	96h LC50	4730	S	10	10-11.2	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	96h LC50	5110	S	15	10.0-10.96	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Egg to Embryo	Na^+	90-d LOEC (survival)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Egg to Embryo	Na^+	90-d NOEC (survival)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Embryo to Alevin	Na^+	90-d LOEC (% survival)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Embryo to Alevin	Na^+	90-d NOEC (% survival)	111	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Eyed-Embryo to Alevin	Na^+	90-d LOEC (% survival)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Eyed-Embryo to Alevin	Na^+	90-d NOEC (% survival)	111	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Embryo to Fry	Na^+	120-d LOEC (% survival)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Embryo to Fry	Na^+	120-d NOEC (% survival)	111	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j
	Eyed-Embryo to Fry	Na^+	120-d LOEC (% survival)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i.j

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Eyed-Embryo to Fry	Na^+	120-d NOEC (% survival)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Embryo to Alevin	Na^+	90-d LOEC (hatching)	111	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Embryo to Alevin	Na^+	90-d NOEC (hatching)	28	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Embryo to Fry	Na^+	120-d LOEC (development)	111	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Embryo to Fry	Na^+	120-d NOEC (development)	28	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Alevin	Na^+	120-d LOEC (behaviour)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Alevin	Na^+	120-d NOEC (behaviour)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Alevin	Na^+	120-d LOEC (deformation)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Alevin	Na^+	120-d NOEC (deformation)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Fry	Na^+	120-d LOEC (behaviour)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Fry	Na^+	120-d NOEC (behaviour)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Fry	Na^+	120-d LOEC (deformation)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
	Fry	Na^+	120-d NOEC (deformation)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	A	i,j
Cyprinus carpio (common carp)	Egg	Na^+	5-d LOEC (hatching success)	15	R	ND	8 - 9	300 - 310	ND	7.5	Bieniarz et al. 1996	A	,j
	Sperm	Na^+	2-h LOEC (reduced motility)	8860	S	4	ND	ND	ND	ND	Epler et al. 2000	2	n
Gambusia affinis (mosquito fish)	Juvenile	Na^+	96-h LOEC (enzyme induction)	29	S	ND	Sat	ND	ND	ND	Nagaraju and Ramana Rao 1985	A	c,e
	Juvenile	Na^+	96-h LOEC (enzyme induction)	29	S	ND	Sat	ND	ND	ND	Nagaraju and Ramana Rao 1983	A	c,e
Gasterosteus aculeatus (stickleback)	ND	K^+	10-d NOEC (mortality)	79	R	15 - 18	ND	ND	ND	6.0 - 6.8	Jones 1939	A	h,i
	ND	Na^+	10-d NOEC (mortality)	1348	R	15 - 18	ND	ND	ND	6.0 - 6.8	Jones 1939	A	h,i
Ictalurus punctatus (channel catfish)	Juvenile	none	164-d LOEC (growth, feeding)	> 400	R	ND	ND	ND	ND	ND	Knepp and Arkin 1973	A	i
	Juvenile	none	10-wk LOEC (physiological)	>1280	R	26	6.1 - 6.8	ND	ND	6.4- 6.7	Collins et al. 1976	A	g

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	fingerlings	Na^+	96-h LC_{50}	6200	S	22,26,30	Sat	102	220	8.6 - 8.8	Colt and Tchobanoglous 1976	2	
Lepomis macrochirus (bluegill)	Juvenile	K^+	96-h LC_{50}	1840	S	22 ± 1.0	4.8 - 8.3	46 - 49	50 - 58	7.5 - 8.4	Trama 1954	2	h
	ND	K^+	24-h TL_m	3355	ND	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	b,c
	Juvenile	Na^+	96-h LC_{50}	8753	S	22 ± 1.0	4.6 - 6.6	45 - 50	51 - 56	7.4 - 8.8	Trama 1954	2	
	ND	Na^+	24-h TL_m	9344	ND	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	b,c
Micropterus salmoides (largemouth bass)	Juvenile	none	164-d LOEC (growth, feeding)	> 400	R	ND	ND	ND	ND	ND	Knepp and Arkin 1973	A	i
Micropterus treculi (Guadalupe bass)	Juvenile	Na^+	96-h LC_{50}	5586	S	22	Sat	222 - 203	183 -163	7.9 - 8.4	Tomasso and Carmichael 1986	A	c
Notropis topeka (Topeka shiner)	Adult (32 months, lt = 64.5-67.9 mm)	Na^+	96-h LC50	6902 (6251 – 7614)	F	23.9 ± 0.04	>6.0	210 - 230	215 - 230	8.16-8.27	Adelman et al. 2009	2	
	Juvenile (19 months, lt = 44.7-47.9 mm)	Na^+	96-h LC50	5994 (5644 – 6362)	F	24.5 ± 0.04	>6.0	210 - 230	215 - 230	8.15-8.28	Adelman et al. 2009	2	
	Juvenile (10 months; wt = 0.77-0.81 g)	Na^+	30-d NOEC (growth)	1186 (26.6)	F	23.4 ± 0.04	>6.0	210 - 230	215 - 230	8.24-8.26	Adelman et al. 2009	2	
	Juvenile (10 months; wt = 0.77-0.81 g)	Na^+	30-d LOEC (growth)	2152 (106)	F	23.4 ± 0.04	>6.0	210 - 230	215 - 230	8.24-8.26	Adelman et al. 2009	2	
	Juvenile (10 months; wt = 0.77-0.81 g)	Na^+	30-d MATC (growth)	1594	F	23.4 ± 0.04	>6.0	210 - 230	215 - 230	8.24-8.26	Adelman et al. 2009	2	
Oncorhynchus kisutch (coho salmon)	Egg	Na^+	> 30-d LOEC (survivorship)	>20	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i
	Fry	Na^+	> 30-d LOEC (survivorship)	>20	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i
Oncorhynchus mykiss (steelhead trout)	Egg	Na^+	> 30-d LOEC (survivorship)	5	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i
	Fry	Na^+	> 30-d LOEC (survivorship)	>20	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i
Oncorhynchus mykiss (rainbow trout)	fingerlings	Na^+	96-h LC_{50}	6000	S	13 - 16.8	Sat	ND	ND	ND	Westin 1974	2	

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	fry	Na^+	96-h LC_{50}	3061	S	15 ± 1	>80% saturation	11 (CCME very soft)	12	6.8-7.2	Elphick 2011	1	
	fry	Na^+	96-h LC_{50}	6361	S	15 ± 1	>80% saturation	54 (CCME soft)	38	7.1-7.5	Elphick 2011	1	
	fry	Na^+	96-h LC_{50}	7832	S	15 ± 1	>80% saturation	90 (CCME mod hard)	66	7.5-7.8	Elphick 2011	1	
	fry	Na^+	96-h LC_{50}	7832	S	15 ± 1	>80% saturation	164 (CCME hard)	116	7.7-8.1	Elphick 2011	1	
	Fry (1 g)	Na^+	24h LC50	8010	S	5	10.2-13.6	106-127 (CCME mod hard)		6.8-8.3	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	24h LC50	7710	S	10	9.7-11.3	106-127		7.8-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	24h LC50	2640	S	15	9.9-10.4	106-127		7.8-8.1	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	48h LC50	5710	S	5	10.2-13.6	106-127		6.8-8.3	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	48h LC50	5720	S	10	9.7-11.3	106-127		7.8-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	48h LC50	2020	S	15	9.9-10.4	106-127		7.8-8.1	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	72h LC50	3980	S	5	10.2-13.6	106-127		6.8-8.3	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	72h LC50	5720	S	10	9.7-11.3	106-127		7.8-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	72h LC50	1690	S	15	9.9-10.4	106-127		7.8-8.1	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	96h LC50	2790	S	5	10.2-13.6	106-127		6.8-8.3	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	96h LC50	3580	S	10	9.7-11.3	106-127		7.8-8.2	Moore and Poirier, 2010	1	
	Fry (1 g)	Na^+	96h LC50	1690	S	15	9.9-10.4	106-127		7.8-8.1	Moore and Poirier, 2010	1	
	Fry (40d EAF test)	Na^+	40-d LC_{10}	651	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{10}	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	

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	Fry (40d EAF test)	Na^+	40-d LC_{10}	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{10}	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{25}	815	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{25}	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{25}	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{25}	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{50}	1041	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{50}	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{50}	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LC_{50}	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (survival)	199	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (survival)	598	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (survival)	1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (survival)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (survival)	1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	

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	Fry (40d EAF test)	Na^+	40-d LOEC (survival)	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (survival)	1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (survival)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (weight, wet wt)	421	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (weight, wet wt)	780	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (weight, wet wt)	585	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (weight, wet wt)	1484	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (weight, wet wt)	>1794	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (weight, wet wt)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (weight, wet wt)	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (weight, wet wt)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (weight, wet wt)	>1794	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (weight, wet wt)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (weight, wet wt)	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (weight, wet wt)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	

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	Fry (40d EAF test)	Na^+	40-d NOEC (weight, wet wt)	199	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (weight, wet wt)	598	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (weight, wet wt)	598	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (weight, wet wt)	1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (weight, wet wt)	199	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (weight, wet wt)	598	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (weight, wet wt)	199	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (weight, wet wt)	598	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (length)	492	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (length)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (length)	1085	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{10} (length)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (length)	>1794	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (length)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{25} (length)	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	

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	Fry (40d EAF test)	Na^+	40-d IC_{25} (length)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (length)	>1794	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (length)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (length)	>1794	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d IC_{50} (length)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (length)	66	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (length)	199	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (length)	66	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (length)	199	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (length)	199	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (length)	598	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (length)	1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (length)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{10} (proportion reaching swim-up)	58	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{10} (proportion reaching swim-up)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	

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	Fry (40d EAF test)	Na^+	40-d EC_{10} (proportion reaching swim-up)	235	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{10} (proportion reaching swim-up)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{25} (proportion reaching swim-up)	142	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{25} (proportion reaching swim-up)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{25} (proportion reaching swim-up)	306	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{25} (proportion reaching swim-up)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{50} (proportion reaching swim-up)	315	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{50} (proportion reaching swim-up)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{50} (proportion reaching swim-up)	474	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d EC_{50} (proportion reaching swim-up)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (proportion reaching swim-up)	66	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	

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	Fry (40d EAF test)	Na^+	40-d LOEC (proportion reaching swim-up)	199	R	14	>80% saturation	10	5-15	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (proportion reaching swim-up)	1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (proportion reaching swim-up)	>1794	R	14	>80% saturation	50	30-40	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (proportion reaching swim-up)	199	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (proportion reaching swim-up)	598	R	14	>80% saturation	92	60-70	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d NOEC (proportion reaching swim-up)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Fry (40d EAF test)	Na^+	40-d LOEC (proportion reaching swim-up)	>1794	R	14	>80% saturation	176	110-120	6.8-7.5	Nautilus Environmental 2011	1	
	Juvenile	Na^+	64-d LOEC (iodine uptake inhibition)	1.5	ND	5 - 6	ND	ND	ND	ND	Lahti et al. 1985	A	c,g
	Egg	Na^+	> 30-d LOEC (survivorship)	10	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i,j
	Fry	Na^+	> 30-d LOEC (survivorship)	10	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i
	2-yr olds	Ca^{2+}	77-d EC (physiological)	26	R	11 - 15	3.1 - 7.8	ND	ND	6.8 - 7.0	Grabda et al. 1974	A	d,g,i
	2-yr olds	K^+	77-d EC (physiological)	31	R	11 - 15	3.1 - 7.8	ND	ND	6.8 - 7.0	Grabda et al. 1974	A	d,g,h,i
	fingerlings	Na^+	7-d LC_{50}	4700	R	13 - 16.8	Sat	ND	ND	ND	Westin 1974	2	
	Egg	Na^+	34-d EC25	2168	S-R	13 - 15	5.6 – 9.8	310	230	8.1 – 8.5	Stantec Ltd. 2006	1	
	Alevin	Na^+	64-d LC50	2023	S-R	13 – 15	5.6 – 9.8	310	230	8.1 – 8.5	Stantec Ltd. 2006	1	
	Fry	Na^+	64-d LOEC	1062	S-R	13 - 15	5.6 – 9.8	310	230	8.1 – 8.5	Stantec Ltd. 2006	1	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Fry	Na^+	64-d NOEC	511	S-R	13 - 15	5.6 – 9.8	310	230	8.1 – 8.5	Stantec Ltd. 2006	1	
	Fry	Na^+	64-d IC25 (growth)	718 (563-899)	S-R	13 - 15	5.6 – 9.8	310	230	8.1 – 8.5	Stantec Ltd. 2006	1	
Oncorhynchus tshawytscha (chinook salmon)	Fry	Na^+	> 30-d LOEC (survivorship)	20	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i
	Egg	Na^+	> 30-d LOEC (survivorship)	>20	F	10	ND	8 - 10	25	6.2	Kincheloe et al. 1979	A	i,j
	fingerlings	Na^+	10-d LC_{50}	4800	R	13 - 16.8	Sat	ND	ND	ND	Westin 1974	2	
	fingerlings	Na^+	96-h LC_{50}	5800	S	13 - 16.8	Sat	ND	ND		Westin 1974	2	
Oryzias latipes (medaka)	Egg		124-d LOEC (hatching)	332	S	25 ± 1.0	ND	ND	ND	7.4 – 8.2	Shimura et al. 2002	2	e,c,d
	Adult		298-d NOEC (survival, growth, feeding)	111	S	25 ± 1.0	ND	ND	ND	7.4 – 8.2	Shimura et al. 2002	2	e,c,d
	Adult		298-d LOEC (survival)	443	S	25 ± 1.0	ND	ND	ND	7.4 – 8.2	Shimura et al. 2002	2	e,c,d
	Adult		298-d LOEC (growth)	332	S	25 ± 1.0	ND	ND	ND	7.4 – 8.2	Shimura et al. 2002	2	e,c,d
	Juvenile		300-d LOEC (feeding)	222	S	25 ± 1.0	ND	ND	ND	7.4 – 8.2	Shimura et al. 2002	2	e,c,d
Perca fluviatilis (perch)	Juvenile	Na^+	64-d LOEC (iodine uptake inhibition)	1.5	ND	5 - 6	ND	ND	ND	ND	Lahti et al. 1985	A	c,g
Pimephales promelas (fathead minnow)	Larvae	Na^+	96-h LC_{50}	5941	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Larvae	Na^+	96-h LC_{50}	655	R	25	>80% saturation	5-15	5-15		Nautilus Environmental 2010	1	p,q
	Larvae	Na^+	96-h LC_{50}	1505	R	25	>80% saturation	40-60	30-40		Nautilus Environmental 2010	A	p,q
	Larvae	Na^+	96-h LC_{50}	2391	R	25	>80% saturation	80-110	60-70		Nautilus Environmental 2010	A	p,q
	Larvae	Na^+	96-h LC_{50}	2594	R	25	>80% saturation	160-190	110-120		Nautilus Environmental 2010	A	p,q
	Larvae (weight 0.11g and length 16mm)	Na^+	96-h LC_{50}	1838	S	24.2-25	5.8-8.4	136-140	96-104	7.42-8.14	US EPA 2010b	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LC_{50}	501	R	25 ± 1	>80% saturation	12	16	6.8-7.4	Elphick 2011	1	

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	Larvae	Na^+	7-d LC_{50}	1014	R	25 ± 1	>80% saturation	50	36	6.9-8.1	Elphick 2011	1	
	Larvae	Na^+	7-d LC_{50}	1772	R	25 ± 1	>80% saturation	94	66	7.2-8.3	Elphick 2011	1	
	Larvae	Na^+	7-d LC_{50}	2011	R	25 ± 1	>80% saturation	168	112	7.7-8.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d IC_{25} (growth)	292	R	25 ± 1	>80% saturation	12	16	6.8-7.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d IC_{25} (growth)	908	R	25 ± 1	>80% saturation	50	36	6.9-8.1	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d IC_{25} (growth)	1506	R	25 ± 1	>80% saturation	94	66	7.2-8.3	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d IC_{25} (growth)	1741	R	25 ± 1	>80% saturation	168	112	7.7-8.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (survival)	222	R	25 ± 1	>80% saturation	12	16	6.8-7.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (survival)	443	R	25 ± 1	>80% saturation	50	36	6.9-8.1	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (survival)	886	R	25 ± 1	>80% saturation	94	66	7.2-8.3	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (survival)	886	R	25 ± 1	>80% saturation	168	112	7.7-8.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (survival)	443	R	25 ± 1	>80% saturation	12	16	6.8-7.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (survival)	886	R	25 ± 1	>80% saturation	50	36	6.9-8.1	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (survival)	1772	R	25 ± 1	>80% saturation	94	66	7.2-8.3	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (survival)	1772	R	25 ± 1	>80% saturation	168	112	7.7-8.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (growth)	222	R	25 ± 1	>80% saturation	12	16	6.8-7.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (growth)	886	R	25 ± 1	>80% saturation	50	36	6.9-8.1	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (growth)	886	R	25 ± 1	>80% saturation	94	66	7.2-8.3	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d NOEC (growth)	1772	R	25 ± 1	>80% saturation	168	112	7.7-8.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (growth)	443	R	25 ± 1	>80% saturation	12	16	6.8-7.4	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (growth)	1772	R	25 ± 1	>80% saturation	50	36	6.9-8.1	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (growth)	1772	R	25 ± 1	>80% saturation	94	66	7.2-8.3	Elphick 2011	1	
	Larvae (<24-h post-hatch)	Na^+	7-d LOEC (growth)	3544	R	25 ± 1	>80% saturation	168	112	7.7-8.4	Elphick 2011	1	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Larvae	Na^+	7-d NOEC (growth)	1586	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Larvae	Na^+	7-d LOEC (growth)	3176	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Larvae	Na^+	7-d NOEC (mortality)	3176	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Larvae	Na^+	7-d NOEC (spawning success)	3176	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Larvae	Na^+	7-d LOEC (mortality)	6353	R	25 ± 1.0	7.9 - 8.3	156 - 172	140 - 170	7.9 - 8.3	Scott and Crunkilton 2000	1	
	Juvenile (7 months, initial weight of 0.72-0.74g)	Na^+	30-d NOEC (survival)	257 (4.4)	F	23.1 ± 0.07	5.5	210 - 230	215 - 230	8.2-8.3	Adelman et al. 2009	2	
	Juvenile (7 months, initial weight of 0.72-0.74g)	Na^+	30-d LOEC (survival)	536 (13)	F	23.1 ± 0.07	5.5	210 - 230	215 - 230	8.2-8.3	Adelman et al. 2009	2	
	Juvenile (7 months, initial weight of 0.72-0.74g)	Na^+	30-d MATC (survival)	372	F	23.1 ± 0.07	5.5	210 - 230	215 - 230	8.2-8.3	Adelman et al. 2009	2	
	Embryo-larval	Na^+	30-d NOEC (growth)	695 (15)	F	23 ± 0.10	>6.0	210 - 230	215 - 230	8.2-8.3	Adelman et al. 2009	2	
	Embryo-larval	Na^+	30-d LOEC (growth)	1302 (35)	F	23 ± 0.10	>6.0	210 - 230	215 - 230	8.2-8.3	Adelman et al. 2009	2	
	Embryo-larval	Na^+	30-d MATC (growth)	952	F	23 ± 0.10	>6.0	210 - 230	215 - 230	8.2-8.3	Adelman et al. 2009	2	
	<24 hour fertilized embryos	Na^+	Embryo Percent Hatch NOEC	1954	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d LC_{50}	340	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d NOEC (survival)	217	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d LOEC (survival)	483	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d NOEC (growth)	217	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	<24 hour fertilized embryos	Na^+	32-d LOEC (growth)	483	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d LC_{25}	302	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d LC_{20}	286	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d LC_{10}	246	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d EC_{50}	404	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d EC_{25}	289	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d EC_{20}	265	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
	<24 hour fertilized embryos	Na^+	32-d EC_{10}	207	F	24.7-25.3	7.2-7.9	132-180	93-107	7.97-8.32	US EPA 2010b	1	
Poecilia reticulatus (guppy)	Fry	K^+	96-h LC_{50}	847	S	77 F	>6.0	117 - 126	25.2 - 43.8	7.4 - 7.7	Rubin and Elmaraghy 1977	1	e,h
	Fry	K^+	72-h LC_{50}	882	S	77 F	>6.0	117 - 126	25.2 - 43.8	7.4 - 7.7	Rubin and Elmaraghy 1977	1	e,h
	Fry	K^+	48-h LC_{50}	969	S	77 F	>6.0	117 - 126	25.2 - 43.8	7.4 - 7.7	Rubin and Elmaraghy 1977	1	e,h
	Fry	K^+	24-h LC_{50}	1181	S	77 F	>6.0	117 - 126	25.2 - 43.8	7.4 - 7.7	Rubin and Elmaraghy 1977	1	e,h
Salmo clarki (cutthroat trout)	Egg	Na^+	> 30-d LOEC (survivorship)	20	F	13	ND	6 - 9	39	7.6	Kincheloe et al. 1979	A	i,j
	Fry	Na^+	> 30-d LOEC (survivorship)	30	F	13	ND	6 - 9	39	7.6	Kincheloe et al. 1979	A	i
Salvelinus alpinus (arctic char)	Fry (1g)	Na^+	24h LC50	6650	S	5	9.99-12.06	106-127		6.8-8.0	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	24h LC50	14490	S	10	9.58-11.32	106-127		8.0-8.4	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	24h LC50	16120	S	15	9.68-10.43	106-127		8.0-8.3	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	48h LC50	6680	S	5	9.99-12.06	106-127		6.8-8.0	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	48h LC50	6200	S	10	9.58-11.32	106-127		8.0-8.4	Moore and Poirier, 2010	1	

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	Fry (1g)	Na^+	48h LC50	10620	S	15	9.68-10.43	106-127		8.0-8.3	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	72h LC50	5320	S	5	9.99-12.06	106-127		6.8-8.0	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	72h LC50	6650	S	10	9.58-11.32	106-127		8.0-8.4	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	72h LC50	9570	S	15	9.68-10.43	106-127		8.0-8.3	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	96h LC50	5320	S	5	9.99-12.06	106-127		6.8-8.0	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	96h LC50	6650	S	10	9.58-11.32	106-127		8.0-8.4	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	96h LC50	9570	S	15	9.68-10.43	106-127		8.0-8.3	Moore and Poirier, 2010	1	
Salvelinus namaycush (lake trout)	Alevin	Na^+	96-hr LC_{50}	10,377	S	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	96-hr LC_{50}	4968	S	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry (1g)	Na^+	24h LC50	5230	S	5	11.68-12.4	106-127		8.1-8.7	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	24h LC50	5230	S	10	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	24h LC50	4550	S	15	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	48h LC50	5230	S	5	11.68-12.4	106-127		8.1-8.7	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	48h LC50	5230	S	10	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	48h LC50	4550	S	15	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	72h LC50	5230	S	5	11.68-12.4	106-127		8.1-8.7	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	72h LC50	5230	S	10	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	72h LC50	4550	S	15	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	96h LC50	5230	S	5	11.68-12.4	106-127		8.1-8.7	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	96h LC50	5230	S	10	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Fry (1g)	Na^+	96h LC50	4550	S	15	10.41-11.32	106-127		8.0-8.2	Moore and Poirier, 2010	1	
	Egg to Embryo	Na^+	120-d LOEC (survival)	>1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Egg to Embryo	Na^+	120-d NOEC (survival)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	

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	Embryo to Alevin	Na^+	90-d LOEC (% survival)	>1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Alevin	Na^+	90-d NOEC (% survival)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Eyed-Embryo to Alevin	Na^+	90-d LOEC (% survival)	>1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Eyed-Embryo to Alevin	Na^+	90-d NOEC (% survival)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Fry	Na^+	146-d LOEC (% survival)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Fry	Na^+	146-d NOEC (% survival)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Eyed-Embryo to Fry	Na^+	146-d LOEC (% survival)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Eyed-Embryo to Fry	Na^+	146-d NOEC (% survival)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Alevin	Na^+	90-d LOEC (hatching)	>1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Alevin	Na^+	90-d NOEC (hatching)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Fry	Na^+	146-d LOEC (developmental delay)	28	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Embryo to Fry	Na^+	146-d NOEC (developmental delay)	7	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Alevin	Na^+	120-d LOEC (deformation)	>1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Alevin	Na^+	120-d NOEC (deformation)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Alevin	Na^+	120-d LOEC (behaviour)	>1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Alevin	Na^+	120-d LOEC (behaviour)	1772	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d LOEC (deformation)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d NOEC (deformation)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d LOEC (behaviour)	>443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d NOEC (behaviour)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Fry	Na^+	146-d LOEC (length)	443	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d NOEC (length)	111	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d LOEC (wet weight)	28	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
	Fry	Na^+	146-d NOEC (wet weight)	7	SR	7.5	10.4 - 12.5	10-16	10-16	6 - 7.4	McGurk et al. 2006	1	
AMPHIBIANS													
Ambystoma gracile (northwestern salamander)	Larvae	K^+	15-d LOEC (mortality)	55	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h
	Larvae	K^+	15-d LC_{50}	104	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h
Ambystoma jeffersonianum (Jefferson salamander)	Egg	Na^+	25-d LOEC (hatching success, deformities)	> 41	S	5 - 10	ND	ND	ND	6.5	Laposata and Dunson 1998	2	l
Ambystoma maculatum (spotted salamander)	Egg	Na^+	44-d LOEC (hatching success, deformities)	> 41	S	5 - 10	ND	ND	ND	6.5	Laposata and Dunson 1998	2	l
Bufo americanus (American toad)	Egg	Na^+	23-d LOEC (hatching success, deformities)	> 41	S	5 - 10	ND	ND	ND	6.5	Laposata and Dunson 1998	2	l
Bufo boreas (western toad)	Larvae	K^+	15-d LOEC (mortality)	>111	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h
Bufo bufo (common toad)	Tadpole	Na^+	16-d LOEC (mortality)	40	R	19 - 24	ND	ND	ND	5.6 - 7.5	Baker and Waights 1993	A	e,f
	Tadpole	Na^+	16-d LOEC (length)	40	R	19 - 24	ND	ND	ND	5.6 - 7.5	Baker and Waights 1993	A	e,f
Bufo terrestris (Southern toad	Tadpole (Gosner stage 25)	Na^+	Time to metamorphosis 5d earlier compared to controls	133	R	16-22	ND	ND	ND	7.6 (spring water)	Edwards et al. 2006	A	
	Tadpole (Gosner stage 25)	Na^+	Time to metamorphosis 7d later compared to controls	133	R	16-22	ND	ND	ND	6.1 (spring water)	Edwards et al. 2006	A	
Litoria caerulea (tree frog)	Tadpole	Na^+	16-d LOEC (length)	40	R	22.5 - 26	ND	ND	ND	5.6-7.6	Baker and Waights 1994	A	e,f,h
	Tadpole	Na^+	16-d LOEC (mortality)	40	R	22.5 - 26	ND	ND	ND	5.6-7.6	Baker and Waights 1994	A	e,f,h
Pseudacris regilla (Pacific treefrog)	Larvae	K^+	15-d LOEC (mortality)	>111	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Tadpole	Na^+	10-d LOEC (weight)	133	R	22 ± 0.1	7.2 ± 0.1	58.4 ± 9.5	52.0 ± 7.0	7.0 - 7.6	Schuytema and Nebeker 1999c	1	
	Embryo	Na^+	10-d NOEC (weight and length)	251	R	22 ± 0.1	7.6 ± 0.1	75.0 ± 4.6	54.0 ± 1.2	6.7	Schuytema and Nebeker 1999a	1	
	Embryo	Na^+	10-d LOEC (weight and length)	492	R	22 ± 0.1	7.6 ± 0.1	75.0 ± 4.6	54.0 ± 1.2	6.7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	10-d NOEC (length)	560	R	22 ± 0.1	7.2 ± 0.1	58.4 ± 9.5	52.0 ± 7.0	7.0 - 7.6	Schuytema and Nebeker 1999c	1	
	Tadpole	Na^+	10-d LOEC (length)	1148	R	22 ± 0.1	7.2 ± 0.1	58.4 ± 9.5	52.0 ± 7.0	7.0 - 7.6	Schuytema and Nebeker 1999c	1	
	Tadpole	Na^+	10-d LC_{50}	1179	R	22 ± 1.0	7.2 ± 0.1	58.4 ± 9.5	52.0 ± 7.0	7.0 - 7.6	Schuytema and Nebeker 1999c	1	
	Embryo	Na^+	10-d LC_{50}	2561	R	22 ± 0.1	7.6 ± 0.1	75.0 ± 4.6	54.0 ± 1.2	6.7	Schuytema and Nebeker 1999a	1	
	Embryo	Na^+	96-h LC_{50}	2849	R	22 ± 0.1	7.6 ± 0.1	75.0 ± 4.6	54.0 ± 1.2	6.7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	96-h LC_{50}	7752	R	22 ± 1.0	7.2 ± 0.1	58.4 ± 9.5	52.0 ± 7.0	7.0 - 7.6	Schuytema and Nebeker 1999c	1	
Rana aurora (red-legged frog)	Embryo	Na^+	16-d LOEC (length)	129	R	15 ± 1	8.7 ± 0.2	25.5 ± 1.7	24.2 ± 1.6	6.8	Schuytema and Nebeker 1999b	1	
	Embryo	Na^+	16-d NOEC (weight)	517	R	15 ± 1	8.7 ± 0.2	25.5 ± 1.7	24.2 ± 1.6	6.8	Schuytema and Nebeker 1999b	1	
	Embryo	Na^+	16-d LOEC (weight)	1041	R	15 ± 1	8.7 ± 0.2	25.5 ± 1.7	24.2 ± 1.6	6.8	Schuytema and Nebeker 1999b	1	
	Embryo	Na^+	16-d LC_{50}	2819	R	15 ± 1	8.7 ± 0.2	25.5 ± 1.7	24.2 ± 1.6	6.8	Schuytema and Nebeker 1999b	1	
	Embryo	Na^+	16-d EC_{100} (mortality)	4067	R	15 ± 1	8.7 ± 0.2	25.5 ± 1.7	24.2 ± 1.6	6.8	Schuytema and Nebeker 1999b	1	
Rana cascadae (Cascades frog)	Larvae	Na^+	21-d LOEC (mortality)	> 20	R	12 - 17	ND	32 - 48	15 - 20	5 or 7	Hatch and Blaustein 2000	A	l,m
	Larvae	Na^+	21-d LOEC (activity)	> 20	R	12 - 17	ND	32 - 48	15 - 20	5 or 7	Hatch and Blaustein 2000	A	l,m
Rana pipiens (northern leopard frog)	Larvae	Na^+	56-d LOEC (length)	133	R	22	11.5	324	ND	8	Allran and Karasov 2000	1	
Rana pretiosa (Oregon spotted frog)	Larvae	K^+	15-d LOEC (mortality)	55	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h
	Larvae	K^+	15-d LC_{50} (mortality)	73	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h
Rana sylvatica (wood frog)	Egg	Na^+	23-d LOEC (hatching success, deformities)	> 41	S	5 - 10	ND	ND	ND	6.5	Laposata and Dunson 1998	2	l
Rana temporaria (European common frog)	Larvae	Na^+	35 to 48-d (growth and maturation)	22	R	17.4	ND	ND	ND	7.7-7.9	Johansson et al. 2001	A	e,g

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Hardness (mg·L^{-1})	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Larvae	Na^+	72-h LOEC (mortality)	> 4425	R	ND	ND	ND	ND	7.5	Johansson et al. 2001	A	e,l
Xenopus laevis (African clawed frog)	Embryo	Na^+	5-d NOEC (weight)	110	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Embryo	Na^+	5-d LOEC (weight)	251	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Embryo	Na^+	5-d NOEC (length)	251	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	10-d NOEC (weight)	291	R	22 ± 0.1	7.2 ± 0.1	20.6 ± 0.2	26.0 ± 0.9	6.7 - 7.6	Schuytema and Nebeker 1999c	1	
	Embryo	Na^+	5-d LOEC (length)	492	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Embryo	Na^+	5-d NOEC (deformities)	492	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	10-d LOEC (weight)	560	R	22 ± 0.1	7.2 ± 0.1	20.6 ± 0.2	26.0 ± 0.9	6.7 - 7.6	Schuytema and Nebeker 1999c	1	
	Embryo	Na^+	5-d LOEC (deformities)	1021	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	10-d NOEC (length)	1148	R	22 ± 0.1	7.2 ± 0.1	20.6 ± 0.2	26.0 ± 0.9	6.7 - 7.6	Schuytema and Nebeker 1999c	1	
	Embryo	Na^+	5-d LC_{50}	1942	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	10-d LOEC (length)	2190	R	22 ± 0.1	7.2 ± 0.1	20.6 ± 0.2	26.0 ± 0.9	6.7 - 7.6	Schuytema and Nebeker 1999c	1	
	Embryo	Na^+	5-d EC_{50} (deformities)	2311	R	22 ± 0.1	7.6 ± 0.1	36.2 ± 6.5	34.5 ± 4.1	7	Schuytema and Nebeker 1999a	1	
	Tadpole	Na^+	10-d LC_{50}	5476	R	22 ± 1.0	7.2 ± 0.1	20.6 ± 0.2	26.0 ± 0.9	6.7 - 7.6	Schuytema and Nebeker 1999c	1	
	Tadpole	Na^+	96-h LC_{50}	7335	R	22 ± 1.0	7.2 ± 0.1	20.6 ± 0.2	26.0 ± 0.9	6.7 - 7.6	Schuytema and Nebeker 1999c	1	

Notes: ND = no data provided; Sat = saturation (O_2)
* Test Types: R = renewal, S = static, F = flow-through
** Ranking Scheme: 1 = primary source, 2 = secondary source, A = ancillary source
[a] $LC_{0.01}$ extrapolated from Camargo and Ward (1992) LC_{50} data, therefore not used in guideline development
[b] tests run with filtered local lake water
[c] insufficient test details / water quality information provided
[d] lack of statistical support
[e] non-resident, or tropical species
[f] distilled water used as test medium
[g] lack of clear dose-response relationship
[h] potassium salts not suitable for guideline derivation
[i] inadequate test design or conditions
[j] control mortality > 10%
[k] organisms only exposed to one test concentration
[l] lowest observable effect level beyond nitrate concentration range tested
[m] >10% change in nitrate concentration in test containers
[n] the ecological significance of this endoint is uncertain

[o]tests run with sediment
[P]survival at 96-h reported during a 7-d survival and growth toxicity test
[q]Unpublished data retrieved from a slide deck presented at the 37th Annual Aquatic Toxicity Workshop (Toronto – October 2010).

APPENDIX B. SUMMARY OF MARINE TOXICITY STUDIES.

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Salinity (‰)	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
INVERTEBRATES													
Capitella capitella (Polychaete)	NA	K^+	28-d LC_{10}	660	S	22-25	5.9	19.2	NR	NR	Reish 1970	2	
Cherax quadricarinatus (Australian crayfish)	Juvenile	Na^+	120-h LOEC (mortality)	4430 (LOEC > 4430)	R	28.0	Sat	ND	70.5 ± 5	7.5 ± 0.2	Meade and Watts 1995	2	a,f
	Juvenile	Na^+	120-h LOEC (respiration)	4430 (LOEC > 4430)	R	28.0	Sat	ND	70.5 ± 5	7.5 ± 0.2	Meade and Watts 1995	2	a,f
Crassostrea virginica (oyster)	Juvenile	Na^+	20-h LOEC (feeding)	9921	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
	Adult	Na^+	20-h LOEC (feeding)	9921	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
	Juvenile	Na^+	96-h LC_{50}	11533	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
	Adult	Na^+	96-h LC_{50}	16803	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
Dorvillea articulata (polychaete)	ND	K^+	28-d LC_{50}	880	S	22 - 25	5.9	34.7	ND	ND	Reish 1970	2	
Haliotis tuberculata (abalone)	ND	Na^+	15-d LOEC (growth)	1108	R	18.5 ± 0.5	Sat	34 ± 1	200 ± 25	8.1 ± 0.5	Basuyaux and Mathieu 1999	1	
Mercinaria mercinaria (hard clam)	Juvenile	Na^+	20-h LOEC (feeding)	2480	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
	Adult	Na^+	20-h LOEC (feeding)	9921	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
	Juvenile	Na^+	96-h LC_{50}	> 19 840	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
	Adult	Na^+	96-h LC_{50}	> 19 840	S	20 ± 2	7.0 - 8.2	35	ND	7.7 - 8.2	Epifanio and Srna 1975	A	b,c
Neanthes arenaceodentata (polychaete)	ND	K^+	28-d LC_{50}	496	S	22 - 25	5.9	34.7	ND	ND	Reish 1970	2	
Nereis grubei (polychaete)	ND	K^+	28-d LC_{50}	329	S	22 - 25	5.9	34.7	ND	ND	Reish 1970	2	
Paracentrotus lividus (purple sea urchin)	ND	Na^+	15-d LOEC (growth / feeding)	1108	R	18.5 ± 0.5	Sat	34 ± 1	200 ± 25	8.1 ± 0.5	Basuyaux and Mathieu 1999	1	
Penaeus monodon (prawn)	Larvae	Na^+	40-h LOEC (mortality)	1	S	28.0	Sat	NR	ND	8.2	Muir et al. 1991	1	d
	Larvae	Na^+	40-h LOEC (cellular changes)	1	S	28.0	Sat	NR	ND	8.2	Muir et al. 1991	1	d
	Larvae	K^+	40-h LOEC (mortality)	1	S	28.0	Sat	NR	ND	8.2	Muir et al. 1991	1	d
	Larvae	K^+	40-h LOEC (cellular changes)	1	S	28.0	Sat	NR	ND	8.2	Muir et al. 1991	1	d

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Salinity (‰)	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Early Instar	Na^+	21-d NOEC (growth)	886	F	28	ND	30 - 34	ND	ND	Wickins 1976	A	d
	Juvenile	Na^+	48-h LC_{50}	12 741	R	25	6.2	15	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	72-h LC_{50}	7633	R	25	6.2	15	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	96-h LC_{50}	6419	R	25	6.2	15	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	48-h LC_{50}	17 250	R	25	6.2	25	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	72-h LC_{50}	11 102	R	25	6.2	25	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	96-h LC_{50}	6977	R	25	6.2	25	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	48-h LC_{50}	22 017	R	25	6.2	35	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	72-h LC_{50}	15 616	R	25	6.2	35	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
	Juvenile	Na^+	96-h LC_{50}	10 260	R	25	6.2	35	ND	8.05 – 8.25	Tsai and Chen 2002	1	d
Penaeus paulensis (prawn)	Adult	Na^+	96-h LC_{50}	9621	R	27.0 ± 0.2	Sat	32 to 41	NR	7.7 ± 0.2	Cavalli et al. 1996	2	d
Penaeus spp. (prawn)	Early Instar	Na^+	48-h LC_{50}	15 062	S	26 - 28	ND	30 - 34	ND	ND	Wickins 1976	A	d
Porites compressa (coral)	Nubbin	K^+	35-d LOEC (growth)	> 0.35	F	ND	Sat	ND	1.96 meq·L^{-1}	7.1 - 8.0	Marubini and Atkinson 1999	A	d,e
Strongylocentrotus purpuratus (purple sea urchin)	Larvae	Na^+	96-h LOEC (larval development)	228	S	14	6.5-7.4	30	ND	8.3	Stantec, Ltd. 2006	1	
	Larvae	Na^+	96-h NOEC (larval development)	104	S	14	6.5-7.4	30	ND	8.3	Stantec, Ltd. 2006	1	
	Larvae	Na^+	96-h IC_{25} (larval development)	1178	S	14	6.5-7.4	30	ND	8.3	Stantec, Ltd. 2006	1	
	Larvae	Na^+	96-h IC_{50} (larval development)	1384	S	14	6.5-7.4	30	ND	8.3	Stantec, Ltd. 2006	1	
FISH													
Amphiprion ocellaris (anemonefish)	Larvae	Na^+	72-d LOEC (growth, mortality)	443	S	ND	ND	NR	ND	ND	Frakes and Hoff Jr. 1982	A	b,c,e
Atherinops affinis (topsmelt)	Adult	Na^+	7-d LOEC (mortality)	4134	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	
	Adult	Na^+	7-d NOEC (mortality)	1971	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^-·L^{-1})	Test Type*	Temp (°C)	DO (mg·L^{-1})	Salinity (‰)	Alkalinity (mg·L^{-1})	pH	Reference	Ranking**	Notes
	Adult	Na^+	7-d LC50	4430 (1971-8306)	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	
	Adult	Na^+	7-d IC25 (mortality)	2554 (486-5886)	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	
	Adult	Na^+	7-d LOEC (biomass)	8306	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	
	Adult	Na^+	7-d NOEC (biomass)	4134	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	
	Adult	Na^+	7-d IC25 (biomass)	2609 (186-6563)	S-R	20	7.5	30	ND	8.2	Stantec, Ltd. 2006	1	
Centropristis striata (Gulf black sea bass)	ND	Na^+	96-h LC_{50}	10 632	R	20 - 24	ND	32 ± 2	ND	ND	Pierce et al. 1993	1	
Diplodus sargus (white seabream)	Larvae	Na^+	24-h EC_{50} (feeding)	3455	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	2	
	Larvae	Na^+	24-h LC_{50}	15 771	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	A	f
Gaidropsarus capensis (cape rockling)	Larvae	Na^+	24-h EC_{50} (feeding)	4582	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	2	
	Larvae	Na^+	24-h LC_{50}	> 17 720	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	A	f
Heteromycteris capensis (cape sole)	Larvae	Na^+	24-h EC_{50} (feeding)	3145.3	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	2	
	Larvae	Na^+	24-h LC_{50}	22 372	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	A	f
Lithognathus mormyrus (striped seabream)	Larvae	Na^+	24-h EC_{50} (feeding)	2658	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	2	
	Larvae	Na^+	24-h LC_{50}	15 284	S	15.0	ND	34.4 - 35.7	107.5 - 122.5	7.8 - 7.9	Brownell 1980	A	f
Monacanthus hispidus (planehead filefish)	ND	Na^+	96-h LC_{50}	2538	R	20 - 24	ND	32 ± 2	ND	ND	Pierce et al. 1993	1	
Oncorhynchus mykiss (rainbow trout)	fingerling	Na^+	7-d LC_{50}	4000	S	13 - 14	> 7	15	ND	ND	Westin 1974	2	
	fingerling	Na^+	96-h LC_{50}	4650	S	13 - 14	> 7	15	ND	ND	Westin 1974	2	
Oncorhynchus tshawytscha (chinook salmon)	fingerling	Na^+	7-d LC_{50}	4000	S	13 - 14	> 7	15	ND	ND	Westin 1974	2	
	fingerling	Na^+	96-h LC_{50}	4400	S	13 - 14	> 7	15	ND	ND	Westin 1974	2	
Pomacentrus leucostictus (beaugregory)	ND	Na^+	96-h LC_{50}	> 13 290	R	20 - 24	ND	32 ± 2	ND	ND	Pierce et al. 1993	1	a
Raja eglanteria (clearnose skate)	ND	Na^+	96-h LC_{50}	> 4253	R	20 - 24	ND	32 ± 2	ND	ND	Pierce et al. 1993	1	a
Trachinotus carolinus (Florida pompano)	ND	Na^+	96-h LC_{50}	4430	R	20 - 24	ND	32 ± 2	ND	ND	Pierce et al. 1993	1	

Notes: ND = no data provided; NR = variable measured but not reported; Sat = saturation (O_2)
* Test Types: R = renewal, S = static, F = flow-through

** Ranking Scheme: 1 = primary source, 2 = secondary source, A = ancillary source
[a] lowest observable effect level beyond nitrate concentration range tested
[b] insufficient test details / water quality information provided
[c] lack of statistical support
[d] tropical species
[e] lack of clear dose-response relationship
[f] toxicity could be due to increased salinity levels