Ecosystem Health

Science-Based Solutions

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

Report No. 1-6

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Scientific Supporting Document

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NOTE TO READERS

The *Ecosystem Health: Science-based Solutions* series is dedicated to the dissemination of scientific knowledge, information and tools for monitoring, assessing, and reporting on ecosystem health to support Canadians in making sound decisions. Documents published in this series include the scientific basis, methods, approaches and frameworks for environmental guidelines and their implementation; monitoring, assessing, and rehabilitating environmental quality in Canada; and, indicator development, environmental reporting and data management. Issues in this series are published *ad libitum*.

This particular issue provides a general overview of the current understanding of nitrate ion in the Canadian aquatic environment and is based largely on the scientific supporting documents to the Canadian Environment Quality Guidelines (sediment, tissue, soil) for the nitrate ion. For additional information regarding this document, please contact:

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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). The guidelines in this document are based on the best available toxicity data at the time of writing, January 2002.

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 $NO_3^-·L^{-1}$. Concentrations less than 0.4 mg $NO_3^-·L^{-1}$ are indicative of oligotrophic lakes and streams. Concentrations exceeding 4 mg $NO_3^-·L^{-1}$ 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 $NO_3^-·L^{-1}$. Nitrate levels in marine waters are usually lower than in fresh waters. In Canadian coastal waters, ambient nitrate concentrations rarely exceed 0.5 mg $NO_3^-·L^{-1}$, but in estuaries draining agricultural land, nitrate concentrations can reach 12 mg $NO_3^-·L^{-1}$. Levels of nitrate in Canadian groundwater can range from 1 to 1100 mg $NO_3^-·L^{-1}$, but in the absence of anthropogenic contamination, levels are generally less than 13 mg $NO_3^-·L^{-1}$.

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. Evidence suggests that in tests with ammonium nitrate, toxic effects observed are due to the ammonium ion, rather than the 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.

An interim water quality guideline of 13 mg $NO_3^-{\cdot}L^{-1}$ is recommended for the protection of all stages of freshwater life against the adverse effects of the nitrate ion. This guideline was derived by multiplying the lowest observable adverse effect concentration of 133 mg $NO_3^-{\cdot}L^{-1}$, reported for growth reduction in the Pacific treefrog (*Pseudacris regilla*), by a safety factor of 0.1. An interim water quality guideline of 16 mg $NO_3^-{\cdot}L^{-1}$ is recommended for the protection of marine aquatic life against the adverse effects of the nitrate ion. This guideline was derived by multiplying the median lethal concentration of 329 mg $NO_3^-{\cdot}L^{-1}$ for the temperate marine polychaete (*Nereis grubei*) by a safety factor of 0.05. A more 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 non-indigenous tropical species exposed to much lower nitrate concentrations.

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

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). Les recommandations ici présentées sont fondées sur les meilleures données sur la toxicité disponibles en janvier 2002, 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 $NO_3^- \cdot L^{-1}$. Des concentrations inférieures à 0,4 mg de $NO_3^- \cdot L^{-1}$ indiquent des lacs et des cours d'eau oligotrophes. Des concentrations supérieures à 4 mg de $NO_3^- \cdot L^{-1}$ 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 $NO_3^- \cdot L^{-1}$. 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 $NO_3^- \cdot L^{-1}$, mais dans les estuaires qui drainent des terres agricoles, les concentrations peuvent atteindre 12 mg de $NO_3^- \cdot L^{-1}$. Dans les eaux souterraines du Canada, les concentrations de nitrates peuvent varier de 1 à 1

100 mg de $NO_3^-·L^{-1}$, mais en l'absence de contamination anthropique, elles sont généralement inférieures à 13 mg de $NO_3^-·L^{-1}$.

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 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.

Une valeur provisoire de 13 mg de $NO_3^-·L^{-1}$ pour la qualité des eaux est recommandée en vue de la protection de tous les stades de vie aquatique d'eau douce contre les effets nocifs de l'ion nitrate. Cette valeur a été calculée en multipliant la plus faible concentration produisant un effet nocif observable signalée pour la réduction de la croissance de la rainette du Pacifique (*Pseudacris regilla*), soit 133 mg de $NO_3^-·L^{-1}$, par un facteur de sécurité de 0,1. Une valeur provisoire de 16 mg de $NO_3^-·L^{-1}$ est recommandée pour la protection de la vie aquatique marine contre les effets nocifs de l'ion nitrate. Cette valeur a été calculée en multipliant la concentration létale médiane de 329 mg de $NO_3^-·L^{-1}$ pour le polychète marin des régions tempérées

(*Nereis grubei*) par un facteur de sécurité de 0,05. Un facteur de sécurité plus prudent a été utilisé afin de calculer la valeur recommandée pour la vie marine parce que le polychète utilisé dans l'étude critique n'a pas été testé à son stade de vie le plus sensible, que le paramètre critique, bien que chronique, était fondé sur un effet létal médian plutôt que sur un effet sublétal faible, et que des effets nocifs ont été observés chez des espèces tropicales non indigènes exposées à des concentrations de nitrates beaucoup plus faibles.

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.

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
MDL	method detection limit
MWWTPs	municipal wastewater treatment plants
N_2	molecular nitrogen
NADH	nicotinamide adenine dinucleotide, reduced form
$NaNO_3$	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 selected nitrate salts to aquatic life is also presented to evaluate environmental hazards posed by these chemicals. Together, this information is used, in accordance with "A Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life", (CCME 1991) to derive numerical water quality guidelines (WQGs) for aquatic organisms.

It should be noted that nitrate concentrations are generally reported in this document in terms of the nitrate ion rather than as nitrate-nitrogen (i.e., mg $NO_3^-{\cdot}L^{-1}$, not mg NO_3^--$N{\cdot}L^{-1}$). Where source publications have used other units, these have been converted for consistency to mg $NO_3^-{\cdot}L^{-1}$ 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).

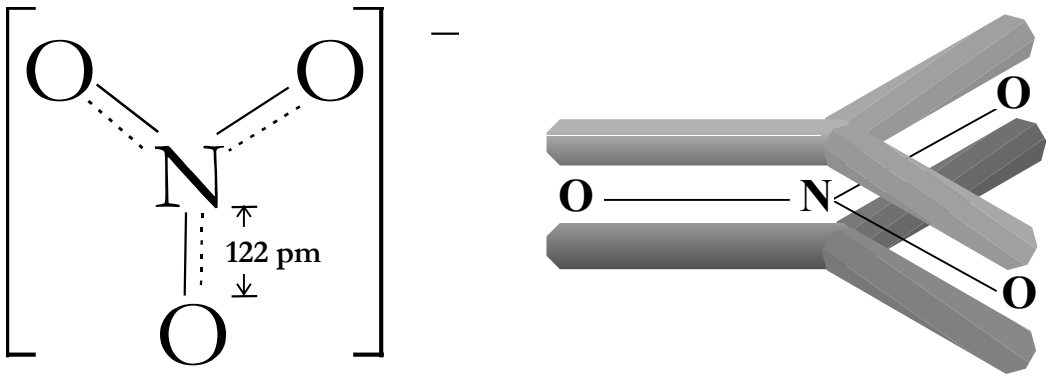


Figure 2.1. Chemical structure of the nitrate ion. The Lewis diagram on the left is adapted from McQuarrie and Rock (1991). The diagram on the right, adapted from Petrucci (1989), depicts the delocalized pi molecular orbital.

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 cold water ($g \cdot mL^{-1}$)	—	0.921	0.133	1.183	CRC (1986)
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.$\cdot L^{-1}$ *	62.0×10^3
ppm NO_3^-	1
ppb NO_3^-	10^{-3}

*note: for these units the conversion factor is the same whether they're 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]

	Technique	Analytical detector	Detection range ($mg\ NO_3^-{\cdot}L^{-1}$)	Sample precision (mean ± CV%) ($mg\ NO_3^-{\cdot}L^{-1}$)	Potential sources of interference	Protocol reference
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).

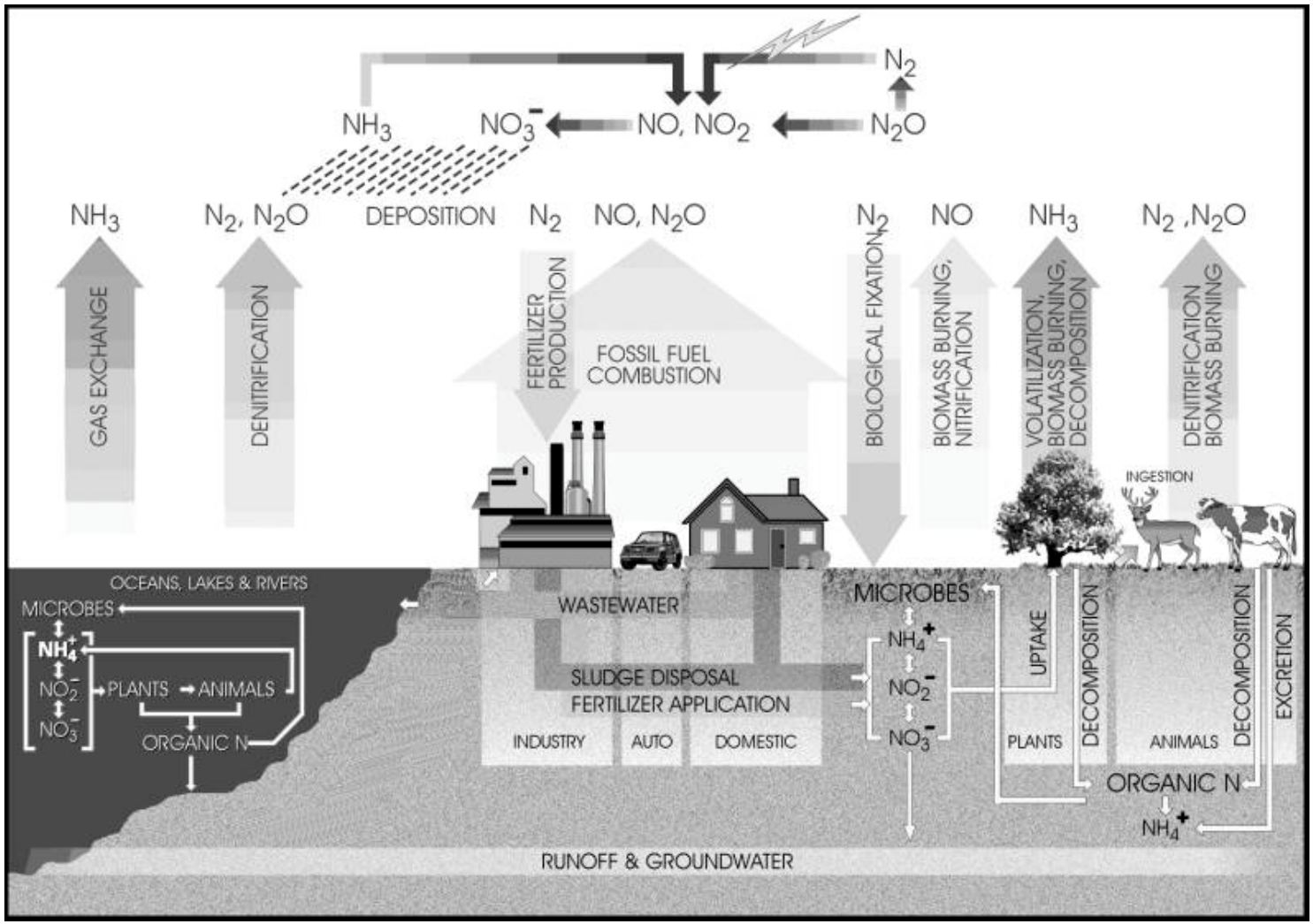
3. NITRATE PRODUCTION AND RELEASE TO THE ENVIRONMENT

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 out gassed 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 may be a conservative estimate 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 that 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

All forms of inorganic nitrogen 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). 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)

3.3.1 Municipal Wastewaters

Humans excrete virtually all nitrogen obtained in protein from food sources. This translates 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·a^{-1} from municipal water treatment plants, 11.8 kt·a^{-1} from storm sewers and combined stormwater overflows, and 15.4 kt·a^{-1} from septic systems (Table 3.1, Chambers et al. 2001).

Among the facilities in the Canadian NPRI database which reported 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). 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.

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 was 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 crop land 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 $NO_3^- \cdot t^{-1}$, while composted manure allowed to undergo nitrification can contain 5.33 kg $NO_3^- \cdot t^{-1}$ (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 that larger surpluses of nitrogen were 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 $N \cdot ha^{-1}$ (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 that showed an average nitrogen surplus of 486 kg $N \cdot ha^{-1}$. In a study of the effect of fertilizer type on nitrate levels in agricultural runoff, Eghball and Gilley (1999) found that 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 $N \cdot a^{-1}$ (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 $N \cdot ha^{-1}$ 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 $N \cdot L^{-1}$. 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 $N \cdot L^{-1}$ 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 modelling, 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 1997, but by the 1990s, nitrate and sulphate ions were present in precipitation at almost equal amounts (Dillon and Evan 20021).

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).

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.

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 that 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., clayey) 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 that 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) speculate that 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 hours 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 that 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 that 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 that 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 that include a fallow season (Campbell et al. 1984). Goss et al. (1998b) also found that, 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 that 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 that were razed by fire showed that 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 that affects 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 that followed 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 that 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 that 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 that 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 that exhibit 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 that 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 that 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 (i.e., nitrate, ammonium, dinitrogen) and organic nitrogen (i.e, 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 Nielsen 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 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 to 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).

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 that 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 that occurs 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

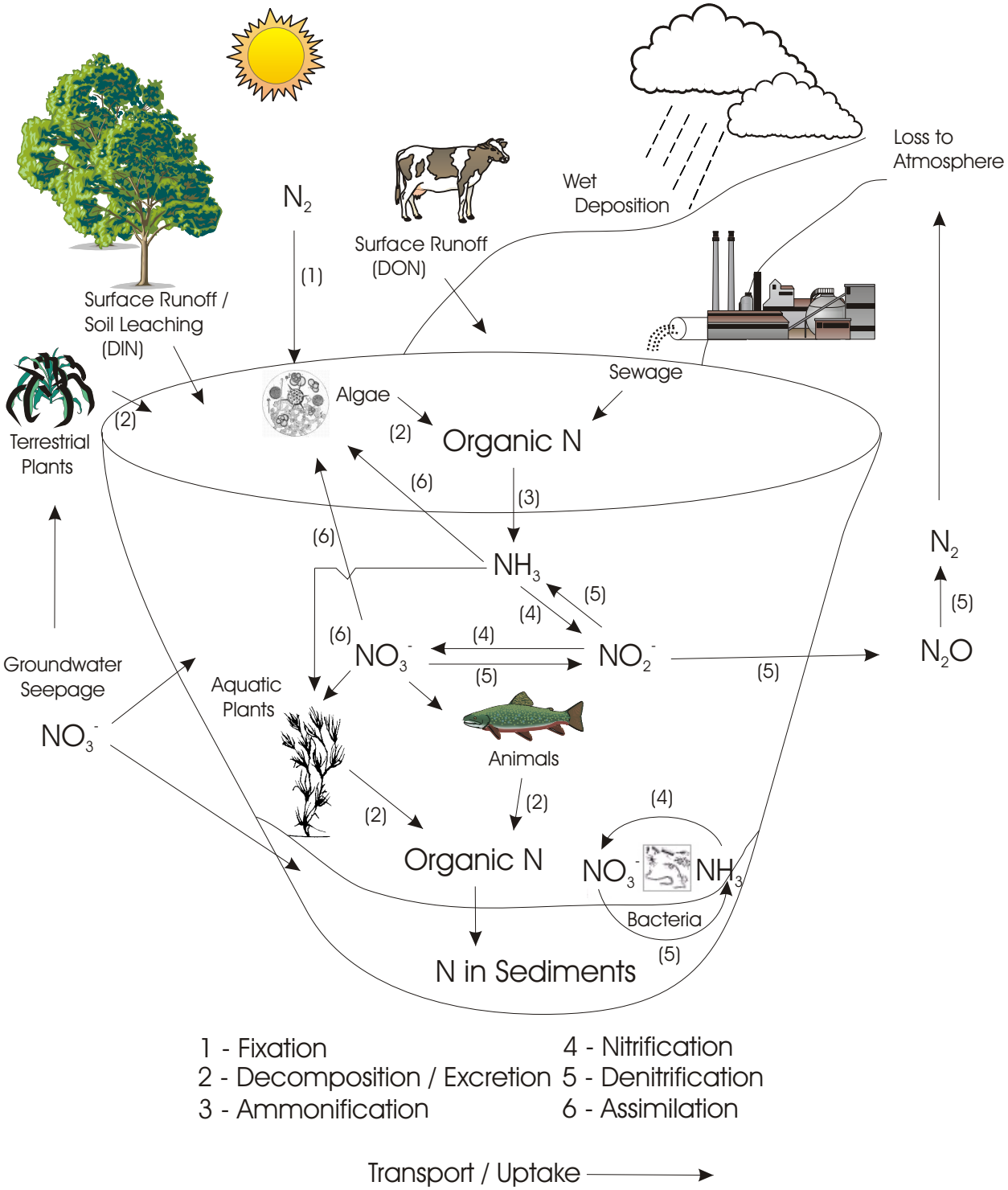
4.3.1 Physico-chemical Factors and Nitrogen Speciation

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 that 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 that are present in surface waters. In fresh and marine waters, seasonal variations in nitrate concentrations occur. Numerous researchers in northern temperate climates have found that 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).



(Adapted from NRC 1978)

Figure 4.1 Schematic representation of the nitrogen cycle emphasizing aquatic transformations.

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 that 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 that 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; Bock 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):

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

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):

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

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 that occur 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 that 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 that denitrification rates increase with increasing temperature (Cavari and Phelps 1977; Messer and Brezonik 1984). Other factors that affect 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 $N \cdot m^{-2} \cdot h^{-1}$ (Seitzinger 1988). The reported range of denitrification rates for coastal marine sediments is greater, ranging from 0 to 14.9 mg $N \cdot m^{-2} \cdot h^{-1}$, but are most commonly between 0.7 and 3.5 mg $N \cdot m^{-2} \cdot h^{-1}$ (Seitzinger 1988).

Christensen et al. (2000) found that 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 that 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 $NO_3^- \cdot L^{-1}$, 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 that 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 $NO_3^-{\cdot}L^{-1}$, prior to establishment of the wetland, to 1 mg $NO_3^-{\cdot}L^{-1}$ 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 $NO_3^-{\cdot}m^{-2}{\cdot}a^{-1}$ (= 1.92 kg $NO_3^-{\cdot}ha^{-1}{\cdot}a^{-1}$) (McGreer and Belzer 1999). Meteorological sampling between 1995 and 1998 suggested that 9.2 (± 1.6) kg $N{\cdot}ha^{-1}{\cdot}a^{-1}$ 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 $N{\cdot}ha^{-1}{\cdot}a^{-1}$ occurring east of the Manitoba-Ontario border, compared to 0.80 kg $N{\cdot}ha^{-1}{\cdot}a^{-1}$ west of the border (Chambers et al. 2001).

Heidorn (1979) showed a link between days with high nitrate deposition in suspended particulate matter (> 9.9 $\mu g{\cdot}m^{-3}$) 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 $NO_3^-{\cdot}L^{-1}$, resulting in loading estimates in excess of 20 kg $NO_3^-{\cdot}ha^{-1}{\cdot}a^{-1}$ for this region, compared to less than 1 kg $NO_3^-{\cdot}ha^{-1}{\cdot}a^{-1}$ 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 $NO_3^-{\cdot}L^{-1}$ (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 $NO_3^-{\cdot}L^{-1}$ in Snare Rapids, Northwest Territories to 2.23 mg $NO_3^-{\cdot}L^{-1}$ 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 $NO_3^-·L^{-1}$ (Table 5.1). In oligotrophic lakes and streams, where primary productivity is low, nitrate concentrations are generally < 0.4 mg $NO_3^-·L^{-1}$ (NRC 1978; Nordin and Pommen 1986). High nitrate concentrations (i.e., exceeding 4 mg $NO_3^-·L^{-1}$) tend to be associated with eutrophic conditions and waters experiencing algal blooms (NRC 1978). In the U.S., stream nitrate concentrations above the national background level of 2.7 mg $NO_3^-·L^{-1}$ are considered to have been affected by human activities (USGS 1999). In a study of streams in agricultural regions of Alberta in 1996, flow-weighted mean nitrate concentrations were 5.3 mg $NO_3^-·L^{-1}$ in regions of high agricultural intensity compared to 0.10 mg $NO_3^-·L^{-1}$ 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 $NO_3^-·L^{-1}$ (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 $NO_3^-·L^{-1}$ (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 that has been 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 $NO_3^-·L^{-1}$, and levels above 45 mg $NO_3^-·L^{-1}$ 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 $NO_3^-·L^{-1}$ (Eghball and Gilley 1999). Sewage treatment plants may also contribute to elevated nitrate levels; concentrations ranging from 19 to 42 mg $NO_3^-·L^{-1}$ 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).

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),

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

Province/Territory	Water Body	$[NO_3^-]$ (mg $NO_3^- \cdot L^{-1}$)‡	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)*
Alberta	Athabasca River	0.27	NLET (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)*
	Grand River	13.4 (1.22 - 29.0)	OMOE (2001)*
	Lake Ontario	1.46 - 2.04	NLET (2000)*
	Lake Superior	1.38	Bennett (1982)
	Mississagi River	0.56 (0.02 [trace] - 1.57)	OMOE (2001)*
	Turkey Lakes	0.252 - 61.0	NLET (2000)*
Quebec	Richelieu River	13.7	NLET (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)*

Province/Territory	Water Body	[NO_3^-] (mg $NO_3^-{\cdot}L^{-1}$)‡	Reference
Table 5.1 continued			
	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; 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)

declined in the summer and autumn months to 0.84 and 1.06 mg $NO_3^-{\cdot}L^{-1}$, 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 $NO_3^-{\cdot}L^{-1}$ (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 $NO_3^-{\cdot}L^{-1}$ in 1966 to 0.04 - 18.0 mg $NO_3^-{\cdot}L^{-1}$ 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. 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). 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). Nitrogen-to-phosphorus ratios (N:P) are increasing in Lake Ontario (ratios, expressed on a weight basis, currently range from 36 to 40) due to decreasing phosphorus and increasing nitrogen concentrations. This 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 2001b).

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 $NO_3^-{\cdot}L^{-1}$, whereas in the lowlands concentrations ranged from 0.4 to 22 mg $NO_3^-{\cdot}L^{-1}$ (MENV 2001b).

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 that 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 $NO_3^-{\cdot}L^{-1}$) were greater relative to surface waters (~0.10 mg $NO_3^-{\cdot}L^{-1}$). 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 that more biologically available nitrogen is lost through denitrification than 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 $NO_3^-{\cdot}L^{-1}$ (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 $NO_3^-{\cdot}L^{-1}$ (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 $NO_3^-{\cdot}L^{-1}$) being found in the winter months (Petrie et al. 1999). By mid-spring, nitrate is largely depleted in the surface waters (~0.038 mg $NO_3^-{\cdot}L^{-1}$) due to biological assimilation, with increasing concentrations (up to ~1.24 mg $NO_3^-{\cdot}L^{-1}$), 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 that 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 that 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 and absence of major physiological effects (Russo 1985; Jensen 1996).

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, amphibians and invertebrates 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 73 to 7752 mg $NO_3^-{\cdot}L^{-1}$ for amphibians, from 24 to 3070 mg $NO_3^-{\cdot}L^{-1}$ for invertebrates 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). Nitrate concentration ranges at which chronic effects occur are comparable for these three taxonomic groups.

Early life stages are 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 that concentrations as low as 10 and 20 mg $NO_3^-{\cdot}L^{-1}$ could significantly increase egg and fry mortality in these species. In addition, early instars of two net-spinning caddisfly species had consistently lower LC_{50}s when exposed to $NaNO_3$ relative to late instar stages (Camargo and Ward 1992).

There is very little information on the influence of water quality parameters such as water hardness, pH, temperature and DO on nitrate toxicity to aquatic organisms (Scott and Crunkilton 2000), and no studies found to date have tested these potential interactions specifically. There is, however, anecdotal evidence in the literature for influences from some of these variables.

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 30 ºC. It should be noted, however, that this is a small range of temperatures, and catfish are fairly robust.

Anecdotal evidence suggests that nitrate uptake may be pH-limited. While Jensen (1996) reported that the freshwater crayfish *Astacus astacus* exhibited limited nitrate uptake at pH $\approx$ 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.

6.1 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 between 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 suggests that the toxicity of ammonium nitrate compounds are due to the influence of the ammonium ion rather than nitrate (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 that 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 6.1). 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 6.1. Relative toxicity of sodium and potassium nitrate salts to freshwater organisms.

			$[NO_3^-]$ (mg $NO_3^- \cdot L^{-1}$)		
Organism	**Duration (h)**	**Endpoint**	**K^+ Salt**	**Na^+ Salt**	**Reference**
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 6.2). 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 that 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 6.2. 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 that the concentrations at which toxic effects are observed in freshwater organisms exposed to KNO_3 are primarily a function of the potassium ion. This is also supported by Demaël et al. (1980) who stated that the metabolic and hormonal effects, indicative of osmoregulatory stress, that they observed when the freshwater fish *Tinca tinca* (tench) was exposed to potassium nitrate at 8.5 mg $K^+ \cdot L^{-1}$, were due to the K^+, not the 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 $NO_3^- \cdot L^{-1}$ (Schuytema and Nebeker 1999b), the corresponding concentration of Na^+ was 48 mg $Na^+ \cdot L^{-1}$, 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 $NO_3^- \cdot L^{-1}$; Marco et al. 1999), the corresponding potassium concentration of 35 mg $K^+ \cdot L^{-1}$ is approximately 25 times higher than ambient levels.

In contrast, potassium ion concentrations found in marine toxicity studies which elicit a response are within natural ranges of K^+ in seawater (Stumm and Morgan 1981). For example, the nitrate LC_{50} of 496 mg $NO_3^- \cdot L^{-1}$ observed in polychaetous annelids exposed to KNO_3 (Reish 1970), corresponds to a K^+ concentration of 312 mg$\cdot L^{-1}$, which is within ambient potassium levels for seawater. The levels of potassium administered to freshwater test organisms at nitrate concentrations required to elicit a response may be an order of magnitude higher than those normally encountered, while marine organisms respond to nitrate toxicity at potassium levels normally encountered. Therefore, it appears that potassium concentrations encountered in toxicity tests are unlikely to cause adverse effects in marine organisms, and any adverse effects observed are likely due to the nitrate ion.

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

6.2 Modes of Action

6.2.1 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 $NO_3^- \cdot L^{-1}$ (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 $NO_3^-{\cdot}L^{-1}$ in control fish to 12.4 mg $NO_3^-{\cdot}L^{-1}$ 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 $NO_3^-{\cdot}L^{-1}$) and tissue concentrations in the tropical marine prawn *Penaeus monodon*. At lower exposure levels (i.e., 48 and 226 mg $NO_3^-{\cdot}L^{-1}$), 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 $NO_3^-{\cdot}L^{-1}$) (Cheng et al. 2002). At the lowest exposure level of 48 mg $NO_3^-{\cdot}L^{-1}$, 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 shown 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.

6.2.2 Direct Toxicity

6.2.2.1 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 that is 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 that 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 could 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 that induced

hemolytic anaemia (abnormally low haemoglobin levels) resulted in a 34 to 40% reduction in maximum sustained swimming speeds for rainbow trout.

Long-term sub-lethal 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 that 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 that 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) suggest that 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 $NO_3^-{\cdot}L^{-1}$ resulted in an increase of succinic dehydrogenase activity, and a decrease in lactate dehydrogenase activity. These changes indicate that 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).

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 that 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 $NO_3^-{\cdot}L^{-1}$ 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 sub-lethal histopathological changes may affect the survival of larval forms in the environment (Muir et al. 1991).

6.2.2.2 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 $NO_3^-{\cdot}L^{-1}$ (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 that acutely toxic levels of $NaNO_3$ for marine fish (24-h LC_{50}s > 15 283 mg $NO_3^-{\cdot}L^{-1}$) 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 m*M* in fresh- and marine waters, while ambient concentrations range from approximately 0.3 m*M* in fresh waters to 520 m*M* 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.

6.2.3 Indirect Toxicity

6.2.3.1 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 accepted that phosphorus is the nutrient that most 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 discussion paper (CCME 2002).

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) 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 that 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 that are toxic to consumers, including humans (Smith et al. 1999). Of the algae that produce toxins, 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 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 which are 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 that occur 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 positively 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 that decreasing N:P ratios in Tolo Harbour, Hong Kong were associated with an increase in red tide events, and that 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 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 that are 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 6.3). 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 the 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 6.3. 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]; fromSmith 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 that *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 that 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 that increasing nitrate levels in the Great Lakes would therefore affect algal species composition in these waters (Pappas and Stoermer 1995).

In coastal regions, phytoplankton has 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 $NO_3^- \cdot L^{-1}$) 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 $P \cdot L^{-1}$) (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 $NO_3^- \cdot L^{-1}$ in highly saline waters (23 - 30‰) increased Chl *a* levels from ~5 µg $\cdot L^{-1}$ in controls to ~18 µg $\cdot L^{-1}$ (Tomasky et al. 1999). However, in fresh (0‰), and brackish waters (10 - 19‰), phytoplankton growth responded to phosphorus additions only (Tomasky et al. 1999).

6.2.3.2 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 $NO_3^-{\cdot}L^{-1}{\cdot}a^{-1}$ 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 is a neutral ion. This suggests that HNO_3 precipitated in snow contributed the H^+ which was 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 suggests that 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).

6.3 Toxicity to Freshwater Life

6.3.1 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).

Results from the tissue analysis of half a dozen macrophyte species suggest that 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).

No studies were located that directly tested nitrate toxicity to aquatic primary producers. Incubation studies using the alga *Scenedesmus subspicatus* showed that 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.

6.3.2 Invertebrates

Freshwater invertebrates are relatively sensitive to nitrate exposure, with primary studies showing LOEC values being comparable to those of amphibians (see Appendix A). Toxic responses include mortality, reduction in fecundity and immobilisation.

Available studies on benthic invertebrates include caddisflies, hydra, and planaria. Short-term (up to 120-h) static bioassays for caddisflies in soft water (hardness = 42.7 mg $CaCO_3 \cdot L^{-1}$) were used to determine the toxicity of nitrate (as $NaNO_3$) to two species of common North American benthic insect larvae (Camargo and Ward 1992). Acute LC_{50} values decreased with increasing exposure time (72- to 120-h) and from last to early instar stage. For early instars of *Hydropsyche occidentalis*, and *Cheumatopsyche pettiti*, the 120-h LC_{50}s were 290 and 472 mg $NO_3^- \cdot L^{-1}$, 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 $Na^+ \cdot L^{-1}$). As no mortality was observed, it is likely that 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 $NO_3^- \cdot L^{-1}$, respectively (Camargo and Ward 1995). These values are lower than estimated safe concentrations for salmonid fish at 25 to 35 mg $NO_3^- \cdot L^{-1}$ (see Westin 1974, Section 6.4.3).

Jones (1940, 1941) 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 $NO_3^-{\cdot}L^{-1}$ (Jones 1941) and 2697 mg $NO_3^-{\cdot}L^{-1}$ (Jones 1940).

Sodium and potassium salts were used to determine the toxicity of nitrate to the growth of hydra (*Hydra attenuata*) populations (Tesh et al. 1990) and the survival of *Lymnea* snails (Dowden and Bennett 1965). The no-effect level of the nitrate ion on hydra population growth when exposed to KNO_3 was > 150 mg $NO_3^-{\cdot}L^{-1}$, based on NOECs of between 150 and 250 mg $NO_3^-{\cdot}L^{-1}$; with $NaNO_3$, the NOEC for population growth was less than 50 mg $NO_3^-{\cdot}L^{-1}$ (Tesh et al. 1990). Snails also 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 $NO_3^-{\cdot}L^{-1}$, 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.

Effects data for cladocerans exposed to nitrate ranged from 189 to 6205 mg $NO_3^-{\cdot}L^{-1}$ (Appendix A). Scott and Crunkilton (2000) exposed two common North American cladocerans, *Ceriodaphnia dubia* and *Daphnia magna* to sodium nitrate in daily renewal tests at concentrations up to 501 mg $NO_3^-{\cdot}L^{-1}$ in moderately hard waters (150 mg $CaCO_3{\cdot}L^{-1}$, pH 7.5). *Ceriodaphnia dubia* was more susceptible to nitrate toxicity than *D. magna*, with 7-d LOECs for neonate production of 189 and 3176 mg $NO_3^-{\cdot}L^{-1}$, respectively. The LOEC for *C. dubia* is within the range reported for surface waters draining agricultural lands (44 to 266 mg $NO_3^-{\cdot}L^{-1}$; McCoy 1972). Scott and Crunkilton (2000) also determined the short-term toxicity (48-h LC_{50}) of *C. dubia* and *D. magna* neonates to be 1657 and 2047 mg $NO_3^-{\cdot}L^{-1}$, respectively. 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 $NO_3^-{\cdot}L^{-1}$, 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 $NO_3^-{\cdot}L^{-1}$ (Anderson 1944, 1946, respectively).

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 $NO_3^-{\cdot}L^{-1}$) 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, the EC_{50} was 775 mg $NO_3^-{\cdot}L^{-1}$ and the LC_{50} was 709 mg $NO_3^-{\cdot}L^{-1}$ (Wickins 1976). The author noted that the EC_{50} value may have been slightly elevated due to the extremely slow growth of the prawns; perhaps with a longer exposure period, growth effects would have been seen at a lower concentration.

6.3.3 Fish

Eggs were found to be the most sensitive stage of freshwater fish to nitrate exposure. 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 anadromous 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 clearly 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. 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 resistant to nitrate exposure than egg stages. 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 6.4.3.]

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 that 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). 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 $NO_3^-·L^{-1}$ (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). 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*.

Concentrations of nitrate which affect larval and juvenile stages of common bluegill (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) 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 $CaCO_3·L^{-1}$; pH 7.4 to 8.8) to be 8753 mg $NO_3^-·L^{-1}$. Similarly, the 96-h LC_{50} for larval fathead minnows exposed to sodium nitrate was 5941 mg $NO_3^-·L^{-1}$ (Scott and Crunkilton 2000). 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 following studies also suggest that juvenile stages of 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 $NO_3^-·L^{-1}$, 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 $NO_3^-·L^{-1}$ in hard water (310 mg $NO_3^-·L^{-1}$) (Tomasso and Carmichael 1986). 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). 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 $NO_3^-·L^{-1}$, respectively (Rubin and Elmaraghy 1977).

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 that uptake of iodide (a trace element required for normal physiological functioning in fish) (Heath 1995), is not affected at environmental levels of nitrate.

6.3.4 Amphibians

Amphibians are susceptible to water pollution as they have permeable skin and rely on aquatic habitats for reproduction, larval development and hibernation (Hecnar 1995). 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 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).

Primary studies on amphibian embryos and larvae (including tadpoles) exposed to $NaNO_3$ showed acute toxic responses (4 to 16-d LC_{50}s) ranging from 1179 to 7752 mg $NO_3^-{\cdot}L^{-1}$, and chronic responses (10 to 56-d LOECs) from 129 to 2190 mg $NO_3^-{\cdot}L^{-1}$ (Appendix A). Of the primary studies available, red-legged frog embryos (*Rana aurora*) collected from the Cascade mountains of western Oregon, USA, were the most susceptible with a LOEC of < 29.1 mg NO_3^--N$\cdot L^{-1}$ (129 mg $NO_3^-{\cdot}L^{-1}$) significantly reducing overall length after 16 days exposure in soft well water (Schuytema and Nebeker 1999b). Growth of the common northern leopard frog (*Rana pipiens*) was also significantly reduced (p = 0.019) by ~2 mm over a 9 week period from exposure to 133 mg $NO_3^-{\cdot}L^{-1}$ in hard water (324 mg $CaCO_3{\cdot}L^{-1}$) (Allran and Karasov 2000).

Schuytema and Nebeker (1999a,c) demonstrated that younger (embryonic) amphibian life stages can be more sensitive to nitrates than more developed larval forms. Ninety-six hour LC_{50} estimates for the Pacific tree frog (*Pseudacris regilla*) were 2849 and 7752 mg $NO_3^-{\cdot}L^{-1}$ for embryos and larvae, respectively (Schuytema and Nebeker 1999a, 1999c; Appendix A). Length of developing embryos of *P. regilla* was also restricted at lower nitrate levels (10-d LOEC = 492 mg $NO_3^-{\cdot}L^{-1}$) than tadpoles (10-d LOEC = 1148 mg $NO_3^-{\cdot}L^{-1}$) (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 $NO_3^-{\cdot}L^{-1}$ for changes in weight, length and deformities, respectively (Schuytema and Nebeker 1999a). 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 chronic mortality estimate for *P. regilla* larvae (10-d LC_{50} = 1179 mg $NO_3^-{\cdot}L^{-1}$) was ~15% of the acute value (96-h LC_{50} = 7752 mg $NO_3^-{\cdot}L^{-1}$) (Schuytema and Nebeker 1999c).

Larvae of tree frogs (*Litoria caerulea*), and the common toad (*Bufo bufo*) were highly sensitive to $NaNO_3$ exposure in distilled water. Following 13 days of exposure to 40 mg $NO_3^-{\cdot}L^{-1}$, 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 $NO_3^-{\cdot}L^{-1}$ 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 $NO_3^-{\cdot}L^{-1}$ for a maximum of 44 days in irrigated pond water (Laposata and Dunson 1998). A deformity that involved 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 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 $NO_3^-{\cdot}L^{-1}$), ultraviolet radiation (UV-B; 280 - 315 nm) and low pH (pH 5), while not being significantly affected by high nitrate levels alone (Hatch and Blaustein 2000).

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 $NO_3^-{\cdot}L^{-1}$ for the Oregon spotted frog (*Rana pretiosa*) and 104 mg $NO_3^-{\cdot}L^{-1}$ for the northwestern salamander (*Ambystoma gracile*) (Marco et al. 1999). At higher nitrate exposures (up to 111 mg $NO_3^-{\cdot}L^{-1}$), 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).

6.4 Toxicity to Marine Life

6.4.1 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 $NO_3^-{\cdot}L^{-1}$ (Admiraal 1977). No inhibition was seen in marine diatom cultures (*Nitzschia pungens*) grown at 13.6 to 54.6 mg $NO_3^-{\cdot}L^{-1}$ (Bates et al. 1993). Naidoo (1990) found that 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 $NO_3^-{\cdot}L^{-1}$.

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.

6.4.2 Invertebrates

The tropical prawn *Penaeus monodon* was the marine species most sensitive to nitrate exposure (Appendix B). Penaeid larvae were exposed to potassium and sodium nitrate salts at 1, 10 and 100 mg $NO_3^-{\cdot}L^{-1}$ in 40-h static tests (Muir et al. 1991). 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).

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 $NO_3^-{\cdot}L^{-1}$, and the 48-h LC_{50} for five species of penaeids (pooled) was 15 062 mg $NO_3^-{\cdot}L^{-1}$. Adult penaeid shrimp (*Penaeus paulensis*) were similarly tolerant to high nitrate exposure, with a 96-h LC_{50} of 9621 mg $NO_3^-{\cdot}L^{-1}$ (Cavalli et al. 1996).

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 $NO_3^-{\cdot}L^{-1}$, respectively, and 329 mg $NO_3^-{\cdot}L^{-1}$ 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 ($g \cdot kg^{-1} \cdot d^{-1}$) in sea urchin (*Paracentrotus lividus*) and abalone (*Haliotis tuberculata*) in response to increasing nitrate concentrations (0 to 1108 mg $NO_3^- \cdot L^{-1}$). Safe levels resulting in 1% mortality, were determined to be around 443 mg $NO_3^- \cdot L^{-1}$ for *P. lividus*, and between 443 and 1108 mg $NO_3^- \cdot L^{-1}$ for *H. tuberculata*. At 1108 mg $NO_3^- \cdot L^{-1}$, 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 $NO_3^- \cdot L^{-1}$ 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 $NO_3^- \cdot L^{-1}$, suggesting that this taxa may benefit from typical environmental levels of nitrate in the sea water (Basuyaux and Mathieu 1999).

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 $NO_3^- \cdot L^{-1}$ as $NaNO_3$, suggesting that these species are insensitive to acute exposures of environmentally relevant levels (Epifanio and Srna 1975). Likewise, juvenile Australian crayfish (*Cherax quadricarinatus*) exposed to $NaNO_3$ concentrations up to 4430 mg $NO_3^- \cdot L^{-1}$ 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).

6.4.3 Fish

There are few studies available on nitrate toxicity to marine fish. Frakes and Hoff Jr. (1982) found that survival of larval anemonefish (*Amphiprion ocellaris*), 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).

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 $NO_3^- \cdot L^{-1}$ for the planehead filefish (*Monacanthus hispidus*) to > 13 290 mg $NO_3^- \cdot L^{-1}$ for beaugregory (*Pomacentrus leucostictus*) (Pierce et al. 1993).

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

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 $NO_3^-{\cdot}L^{-1}$, resulting in a 7-d TL_m of 4000 mg $NO_3^-{\cdot}L^{-1}$, for both species. All trout exhibited acute signs of toxic stress after 2 days of exposure; however, chinook exposed at $\leq$ 4400 mg $NO_3^-{\cdot}L^{-1}$ 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 $NO_3^-{\cdot}L^{-1}$ for hatchery-reared salmonids based on $1/100^{th}$ of the 7-d LC_{10} at 15‰ salinity (not reported in Appendix B).

6.5 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 that the photolysis of aromatic compounds in the presence of an aqueous nitrate solution (73 mg $NO_3^-{\cdot}L^{-1}$) 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 that 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).

6.6 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 Protection of Aquatic Life

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 ancillary (CCME 1991). Because the nitrate ion is non-volatile and tends to remain in solution (NRC 1978), and studies that monitored 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 still 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). Only studies using species resident to Canadian waters were included in the freshwater guideline derivation. Marine species included non-native temperate-dwelling organisms as per the CCME (1991) protocol. Only toxicity data for sodium nitrate were used in deriving the freshwater guidelines, while 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 Freshwater Guideline Derivation

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. Amphibian larvae and invertebrates tend to be more susceptible to nitrate exposure than larval fish (Appendix A).

Key primary studies used in guideline derivation with environmentally relevant endpoints included mortality, growth, physical deformities and reproduction. A series of primary studies by Schuytema and Nebeker (1999a-c) examined the impact of sodium nitrate addition on embryo and tadpole mortality and growth parameters for several species of frogs endemic to North America. 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 $NO_3^-{\cdot}L^{-1}$, compared to a 4-d LC_{50} value of 7752 mg $NO_3^-{\cdot}L^{-1}$ (Schuytema and Nebeker 1999c). NOECs and LOECs based on frog weight were observed at < 133 mg $NO_3^-{\cdot}L^{-1}$ (Schuytema and Nebeker 1999c). Another primary study on northern leopard frog larvae (*Rana pipiens*) found that 133 mg $NO_3^-{\cdot}L^{-1}$ significantly slowed the growth of larvae ($F_{2,213}$ = 4.04, p = 0.019), which 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). Invertebrates were found to be similarly sensitive to nitrate exposure. The common freshwater cladoceran, *Ceriodaphnia dubia*, experienced significantly reduced neonate production (7-d LOEC) at 189 mg $NO_3^-{\cdot}L^{-1}$ (Scott and Crunkilton 2000), and the 120-h LC_{50} value for the caddisfly, *Hydropsyche*

occidentalis, was 290 mg $NO_3^-·L^{-1}$ (Camargo and Ward 1992). Although there are demonstrable nitrate effects on fish well below 100 mg $NO_3^-·L^{-1}$ (see Appendix A), the lowest effect acceptable for guideline development was a 7-d LOEC of 3176 mg $NO_3^-·L^{-1}$ for fathead minnows (*Pimephales promelas*) (Scott and Crunkilton 2000).

The critical study used to determine the 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·L^{-1}, total hardness = 58.4 ± 9.5 mg·L^{-1} as $CaCO_3$, total alkalinity = 52.0 ± 7.0 mg·L^{-1} as $CaCO_3$, conductivity = 156.0 ± 15.1 µS·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 $NO_3^-·L^{-1}$ and 1148 mg $NO_3^-·L^{-1}$, respectively (Schuytema and Nebeker 1999c). The former LOEC was used in developing the guideline. The test organisms exposed to 133 mg $NO_3^-·L^{-1}$ 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 that 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).

Several studies exposing freshwater organisms to $NaNO_3$ had effect concentrations below the critical study, but were not considered for guideline derivation. Reasons for excluding each of these studies are described below.

Significant mortality was found for chinook salmon fry at 20 mg $NO_3^-·L^{-1}$ and Lahontan cutthroat trout 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 clearly demonstrated egg sensitivity to early salmonid life stages, additional egg mortalities caused by *Saprolegnia* fungal infestations could not be segregated from the data by the authors and were therefore not useable for guideline development.

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 $NO_3^-·L^{-1}$ (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.

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).

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%.

Population levels of the freshwater hydra (*Hydra attenuata*) declined with increasing nitrate concentrations (up to 150 mg $NO_3^- \cdot L^{-1}$) 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.

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). Although physiological endpoints may be considered for secondary data sources (CCME 1991), 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.

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

In a 16-day chronic study, Schuytema and Nebeker (1999b) examined the toxicity of sodium nitrate to embryos and larvae of the red-legged frog (*Rana aurora*). Statistically significant decreases in length ($p \leq 0.05$) were seen at concentrations as low as 129 mg $NO_3^-·L^{-1}$ (Schuytema and Nebeker 1999b). The difference in length between the control group and the test organisms exposed at 129 mg $NO_3^-·L^{-1}$ was only 3%. This study was not selected for guideline development because although the LOEC was statistically significant, the effects were small enough that their ecological significance was questionable.

A similar rationale was employed in the decision not to derive the guideline based on the study by Allran and Karasov (2000). In this chronic study, larvae of the northern leopard frog (*Rana pipiens*) were exposed to sodium nitrate. Statistically significant reductions in length ($p = 0.02$) were observed at 133 mg $NO_3^-·L^{-1}$, but the amount of reduction, compared to control organisms, was only 6%. The authors noted that these effects may not be ecologically significant as many other natural environmental variables can affect amphibian larval growth to a greater degree (Allran and Karasov 2000).

7.2.1 Recommended Freshwater Guideline

The recommended interim freshwater guideline for the nitrate ion is 13 mg $NO_3^-·L^{-1}$. This value is based on the lowest observable adverse effect concentration of 133 mg $NO_3^-·L^{-1}$ reported for the Pacific treefrog (*Pseudacris regilla*) (Schuytema and Nebeker 1999c). A safety factor of 0.1 was applied to the LOEC in accordance with the CCME (1991) protocol, and the final result was rounded up.

Support for this guideline can be drawn from the fact that three other studies reported 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 $NO_3^-·L^{-1}$ (Schuytema and Nebeker 1999a) and 133 mg $NO_3^-·L^{-1}$ (Allran and Karasov 2000), respectively. The water flea *Ceriodaphnia dubia* was similarly susceptible, with a 7-d LOEC for reduced reproduction at 189 mg $NO_3^-·L^{-1}$ (Scott and Crunkilton 2000). The guideline is also comparable with estimates that have been 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 $NO_3^-{\cdot}L^{-1}$.

Temperate streams and rivers draining regions that support intensive agricultural production and human populations can often exceed 13 mg $NO_3^-{\cdot}L^{-1}$ in Canada, the U.S., and Europe (Rouse et al. 1999; Van Herpe and Troch 2000; Goolsby et al. 2001; OMOE 2001). If surface water nitrate concentrations approach guideline levels, site-specific monitoring of ecological effects is recommended.

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 that can be taken up by primary producers, and therefore other forms of nitrogen may also contribute to eutrophication effects. 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).

7.2.2 Data Gaps / Research Recommendations

The current freshwater data set of acceptable primary and secondary acute nitrate toxicity studies contains five species of fish, three species of amphibians and four species of invertebrates (Appendix A). Chronic studies include three species of fish, four species of amphibians and two species of invertebrates (all planktonic) (Appendix A).

To fulfill the requirements for full freshwater guideline status, one chronic invertebrate study on a non-planktonic organism is required. As nitrate is a required nutrient to stimulate plant growth, the plant toxicity requirements of the CCME protocol (CCME 1991) have been waived. This rationale has also been adopted by Australia and New Zealand (Environment Australia 2000b).

To ensure that the nitrate freshwater guideline is sufficiently protective of all aquatic organisms in Canada, it is recommended that additional toxicity tests be conducted for fish and invertebrate species that are 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. 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.

Potential influences of other parameters on the toxicity of nitrate are currently not well understood. Further research is needed on the interactions of nitrate with potassium, ammonia, UV and low pH.

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.

7.2.3 Summary of Existing Guidelines

The previous Canadian water quality guidelines for nitrate, developed in 1987, consisted of a narrative statement that nitrate concentrations which will stimulate weed growth should be avoided (CCREM 1987). British Columbia is 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). 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 greatly exceeds the *moderate reliability* 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). Australian/New Zealand guidelines also propose that a nitrate concentration of 17 mg NO_3^-·L^{-1}, similar to the proposed Canadian guidelines, would be protective of 80% of the freshwater species (Environment Australia 2000a). This level however, exceeds a 9-d NOEC of 14 mg NO_3^-·L^{-1} for the Australian freshwater fish *Mogurnda adspersa*, and therefore

was deemed not to be protective for some key Australian species (Environment Australia 2000b).

The US EPA does not currently have a formal guideline for nitrate for the protection of aquatic life. Based on observations by Knepp and Arkin (1973), however, the US EPA has 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).

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).

7.3 Marine Guideline Development

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 (see Appendix B).

Mortality responses in marine fish tend to be similar to that of freshwater fish, with acute and chronic LC_{50} 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.

The following three primary studies were acceptable for guideline derivation. Growth (as % daily weight gain) of the purple sea urchin (*Paracentrotus lividus*) and the abalone (*Haliotis tuberculata*) was statistically significantly reduced at an exposure to 1108 mg NO_3^-·L^{-1} ($p < 0.001$) for 15 days, but was also substantially reduced for *P. lividus* at 443 mg NO_3^-·L^{-1} (Basuyaux and Mathieu 1999). The larval tiger shrimp (*Penaeus monodon*) was the most sensitive marine test organism found, with statistically significant increases in mortality occurring at 1 mg NO_3^-·L^{-1} when exposed to either sodium or potassium salts (Muir et al. 1991). At this level of exposure, Muir et al. (1991) also found various sublethal histopathological changes that could decrease the fitness of larvae, thereby decreasing their chances of survival. Although

P. monodon was the most sensitive marine receptor species, the Muir et al. (1991) study was not used to derive the guideline value as this is a non-resident, tropical prawn species. The only primary vertebrate study examined the ability of tropical and subtropical fish to withstand high nitrate concentrations in captive, recirculating water (Pierce et al. 1993). Acute mortalities (96-h LC_{50}s) for fish exposed to $NaNO_3$ ranged from 2538 mg $NO_3^-·L^{-1}$ to > 13 290 mg $NO_3^-·L^{-1}$ (see Appendix B; Pierce et al. 1993).

The following secondary studies were considered for guideline development. Four species of subtropical fish larvae reared from ocean-collected pelagic eggs and exposed to nitrate showed delayed development to first feeding after yolk sac absorption, but only at very high concentrations, with 24-h EC_{50}s ranging from 2658 to 4582 mg $NO_3^-·L^{-1}$ (Brownell 1980). In addition, Brownell (1980) demonstrated that at nitrate concentrations required to elicit mortality in 24h LC_{50}s (15 280 to 22 370 mg $NO_3^-·L^{-1}$), the toxic response could just as easily be attributed to elevated salinity, rather than the nitrate ion.

Chinook salmon (*O. tshawytscha*) and rainbow trout (*O. mykiss*) were the only temperate fish species endemic to Canadian marine or brackish waters, for which toxicity data were located. Fingerlings were exposed to $NaNO_3$ in static (96-h) or static-renewal (7-d) tests in brackish waters (15‰ salinity) (Westin 1974). Both salmonid species had very similar median tolerance limits (96-h and 7-d TL_m), ranging from 4000 to 4650 mg $NO_3^-·L^{-1}$.

Interim guidelines for the protection of marine life were derived, 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, 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$·L^{-1}$ (329 mg $NO_3^-·L^{-1}$) 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).

7.3.1 Recommended Marine Guideline

The recommended interim marine guideline for the nitrate ion is 16 mg $NO_3^-·L^{-1}$. This value is based on a 28-day median lethal concentration of 329 mg $NO_3^-·L^{-1}$ for the temperate marine polychaete *Nereis grubei* (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 $NO_3^-·L^{-1}$, 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 $NO_3^-{\cdot}L^{-1}$. 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.

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

7.3.2 Data Gaps / Research Recommendations

The current marine dataset of acceptable primary and secondary acute toxicity studies contains eleven species of fish from three studies, and three species of invertebrates from one study (Appendix B). Chronic studies include two temperate species of fish from one study, and four species of invertebrates from two studies (Appendix B).

To fulfill the requirements for full marine guideline status, at least one primary chronic fish study on one other temperate species is required. The requirements for invertebrates have been met, however, as the distributions of both the purple sea urchin (*P. lividus*) and the abalone (*H. tuberculata*) lie within European waters, and the three polychaete species were obtained from the Californian coast, it would be preferable to include at least one study on a marine invertebrate endemic to Canadian waters. As nitrate is a required nutrient for plant growth, no marine plant toxicity studies were required for guideline development.

7.3.3 Summary of Existing Guidelines

Australia and New Zealand have adopted their *moderate reliability* freshwater guideline of 0.70 mg $NO_3^-{\cdot}L^{-1}$ as the marine *low reliability* trigger value (Environment Australia 2000b). Although a *low reliability* trigger level of 13 mg $NO_3^-{\cdot}L^{-1}$ 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 $NO_3^-{\cdot}L^{-1}$ (Speijers et al. 1989). This value is based on a recommended limit of 0.1 mg $N{\cdot}L^{-1}$ 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).

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.

CWQG values are calculated conservatively, 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 live 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 interim 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 be 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 (Figures 8.1 a,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 Objectives

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 the most sensitive species for which toxicity data is available, with an extra margin of safety to account for the unknown, such as the possibility of an unstudied species that is more sensitive. Nonetheless, in spite of the safety factor, in locations where highly sensitive or endangered species occur, 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. For example, the critical study used to derive the freshwater guideline is based on the toxicity of nitrate to amphibians. In water bodies where amphibians are known not to occur naturally, and which are not likely to become amphibian habitat, managers might consider developing a site-specific objective that is based on the most sensitive fish or invertebrate species instead.

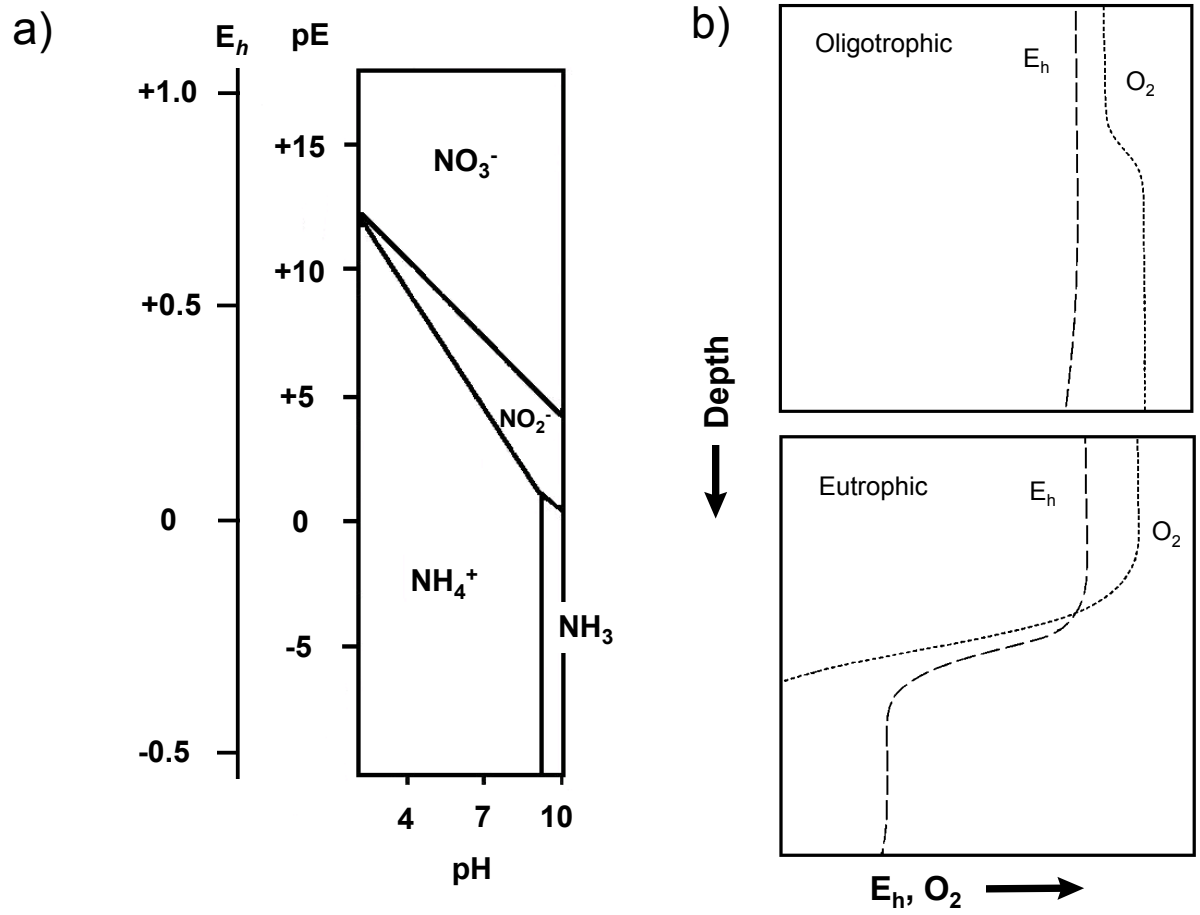


Figure 8.1 a) Redox potential (E_h) and electron potential (pE) for various species of inorganic nitrogen, as a function of pH (note: N_2 is treated as a redox inert compound). b) Generalized vertical distribution of redox potential and dissolved oxygen in stratified lakes of very low and very high productivity. [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 brook trout spawning areas, managers may want to consider setting more conservative site-specific nitrate objectives.

General guidance on the site-specific application of Canadian Water Quality Guidelines is currently being drafted for the CCME (MacDonald et al. 2002).

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). 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).

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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													
Ceriodaphnia dubia (water flea)	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	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	
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	
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	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^--L^{-1})	Test Type*	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking**	Notes
	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	$[Ca^{2+}]$ = 31 $mg \cdot L^{-1}$	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
Hydra attenuata (hydra)	Adult	Na^+	12-d LOEC (mortality)	50	S	ND	ND	ND	ND	ND	Tesh et al. 1990	A	c,d
	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	
Lymnea spp. (snail)	Eggs	K^+	96-h TL_m	671	S	ND	ND	ND	ND	ND	Dowden and Bennett 1965	A	a,b,c
	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

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^--L^{-1})	Test Type*	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking**	Notes
Macrobrachium rosenbergii (prawn)	Juvenile	Na^+	21-d LC_{50}	709	S	28.0	Sat	ND	ND	ND	Wickins 1976	2	e
	Juvenile	Na^+	21-d EC_{50} (growth)	775	S	28.0	Sat	ND	ND	ND	Wickins 1976	2	e
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
FISH													
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
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
	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	

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
	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
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)	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	
	fingerlings	Na^+	96-h LC_{50}	6000	S	13 - 16.8	Sat	ND	ND	ND	Westin 1974	2	
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^+	7-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	
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^+	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	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^--L^{-1})	Test Type*	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking**	Notes
	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^+	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^+	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	
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
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

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
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
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 (formerly *Hyla regilla*) (Pacific treefrog)	Larvae	K^+	15-d LOEC (mortality)	>111	R	15	ND	ND	ND	7?	Marco et al. 1999	1	h
	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	

Organism	Life Stage	Cation	Endpoint	Effect concentration (mg NO_3^--L^{-1})	Test Type*	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking**	Notes
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
	Larvae	Na^+	72-h LOEC (mortality)	> 4425	R	ND	ND	ND	ND	7.5	Johansson et al. 2001	A	e,l
Xenopus laevis (African tree frog)	Embryo	Na^+	120-h 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^+	120-h 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^+	120-h 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^+	120-h 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^+	120-h 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^+	120-h 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^+	120-h 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^+	120-h 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	

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 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

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													
Cherax quadricarinatus (Australian crayfish)	Juvenile	Na^+	120-h LOEC (mortality)	> 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	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}	>19840	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}	>19840	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

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
	Larvae	K^+	40-h LOEC (cellular changes)	1	S	28.0	Sat	NR	ND	8.2	Muir et al. 1991	1	d
	Early Instar	Na^+	21-d NOEC (growth)	886	F	28	ND	30 - 34	ND	ND	Wickins 1976	A	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}	15062	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
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
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	

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
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