5.0 Direct and Indirect Toxic Effects of Nutrient Addition

Highlights

- Toxic forms of nitrogen include un-ionized ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and nitrogen dioxide (NO_2). Phosphorus, in the forms present in the environment, is not toxic even at high concentrations.
- Nitrates from agriculture have contributed to the recent decline in frogs and salamanders in Canada. Concentrations of nitrate greater than 60 mg/L in water kill the larvae of many amphibians.
- Elevated nitrate concentrations have been shown to stimulate the growth of a marine diatom capable of producing domoic acid, an algal toxin. In 1987, the PEI mussel fishery was closed for the remainder of the season after mussels accumulated domoic acid to levels toxic to humans.
- 26% of Canadians, approximately 8 million people, rely on ground water for domestic water supply. In most provinces, 5-20% of these wells are contaminated with unsafe levels of nitrate with up to 60% of wells contaminated in regions with high-demand crops or intensive livestock operations. Agriculture and septic beds are the largest human-derived contributors to ground water contamination in Canada.
- Methaemoglobinaemia or "Blue Baby Syndrome" occurs when nitrite (NO_2^-) reacts with the iron in haemoglobin to reduce the oxygen-carrying capacity of haemoglobin.
- Use of nitrogen fertilizers, including manure, in agriculture has resulted in incidences of nitrate poisoning in livestock. Feed crops accumulate nitrate while growing which can be passed on to livestock during feeding. The consequences of nitrate poisoning to livestock include decreased milk production, weight gain, and disease resistance.

Nitrogen and phosphorus are essential elements for all organisms. P is not directly toxic in the forms and amounts found naturally in the environment (Switzer-Howse and Coote 1984). However, elemental phosphorus (P_4) is known to be toxic to humans and other animals (Isom 1960; Idler et al. 1981). Elemental P does not occur naturally. It is produced by combining phosphate-bearing rocks, coke and silica in an electric arc furnace and is used in the production of baking powder, tooth paste, dyestuffs, pharmaceuticals, fertilizers and dozens of other products. Because elemental P quickly reacts with oxygen when dissolved in water to form non-toxic phosphate, dilution of any accidental spill of elemental P results in its rapid disappearance (Jangaard 1972); thus, cases of elemental P toxicity in Canada are extremely rare (e.g., Jangaard 1972; Idler et al. 1981). In contrast, high concentrations of certain N forms can cause adverse health effects for plants and animals including humans. This chapter describes nitrogen toxicity for aquatic and terrestrial plants and animals, including humans, and then evaluates the extent to which toxic concentrations of N and their impacts on biota occur in Canada. The role of N and P in contributing to the development of toxic algal blooms is also considered. In addition to the information in this chapter, Environment Canada is undertaking an assessment of ammonia toxicity as part of a review of compounds for inclusion in the Federal Government's *Canadian Environmental Protection Act* Priority Substances List (see *CEPA* PSL2 text box).

Canadian Environmental Protection Act Priority Substances List II: Problem formulation for ammonia in the aquatic environment

In 1995, the Ministers' Expert Advisory Panel listed ammonia in the aquatic environment as a compound that should be placed on the second *Canadian Environmental Protection Act* (*CEPA*) Priority Substances List. The rationale for its inclusion was: "Anthropogenic sources of ammonia in Canada include effluent from sewage treatment plants, steel mills, fertilizer plants, the petroleum industry, and intensive farming; and releases from the sources can result in local elevated concentrations" (Environment Canada 1997b). As a result, an assessment of ammonia in the aquatic, terrestrial and atmospheric environments was initiated by Environment Canada to evaluate the extent of the problem in Canada.

The *CEPA* assessment identifies the release and use of ammonia into the Canadian environment (Environment Canada 1999b). In addition, it assesses the fate of ammonia in the atmosphere, soil, groundwater, surface water, and sediments. Aquatic toxicity to plants, microbes, invertebrates, and vertebrates and the ecological relevance of the findings are also examined.

Preliminary findings from the *CEPA* assessment indicate that:

- the major, quantifiable, source of ammonia to aquatic ecosystems in Canada is municipal sewage effluents;
- the major industrial source of ammonia to the Canadian environment is the fertilizer industry and most of its releases are to the air;
- agricultural operations, specifically intensive livestock rearing, are a large source of ammonia to air and soil; however, contributions to water are difficult to quantify;
- further study is required to quantify accurately ammonia releases from automobile emissions.

Releases of ammonia to freshwaters have the potential to affect many aquatic organisms deleteriously. Preliminary results indicate that the acute critical toxicity value for freshwater organisms is 0.29 mg/L un-ionized ammonia, based on a lethal response of 37 freshwater species (including fish, aquatic insects, aquatic worms, crustaceans and clams). The chronic toxicity value for freshwater fish was 0.041 mg/L un-ionized ammonia, based on a 5% decline in growth/reproduction of seven fish species, an amphibian species and five aquatic invertebrate species. Due to the lack of recent marine toxicity data, it was decided to use the U.S. EPA's Genus Mean Acute Toxicity Value of 0.49 mg/L for winter flounder. A chronic toxicity value for marine species is not available (Environment Canada 1999b).

The *CEPA* assessment also noted that ammonia gas in Canadian air is generally non-toxic, except for some accidental industrial releases. Although ammonia deposition from the atmosphere onto forests, fens and moors in industrialized regions of Europe has caused terrestrial eutrophication, this phenomenon has not yet been documented as happening in Canada. There are, however, potential instances of this occurring in Canada, for example in the Lower Fraser Valley and in Ontario just north of Toronto (Environment Canada 1999b).

Environment Canada is currently completing its assessment of ammonia in the Canadian environment and Health Canada is reviewing the health-related effects from exposure to ammonia in ambient air. These technical documents will form the basis for a decision as to whether ammonia should be added to the *CEPA* List of Toxic Substances. If ammonia is added to the list, then new management policies may be implemented for the release of ammonia to aquatic environments.

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5.1. Laboratory Studies of Toxic Effects of Nitrogen

Aquatic plants

Nitrogen is essential for plant growth. It is a component of protein, chlorophyll and other organic compounds. For most plants, N can be assimilated as ammonium and nitrate; however, although ammonia is an important nutrient, it is toxic at higher concentrations.

To appreciate the toxicity of ammonia, a basic understanding of its chemical forms is necessary. In aqueous solutions, a chemical equilibrium is established between un-ionized ammonia (NH_3) and the ammonium ion (NH_4^+):

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

The total ammonia content of a solution is the sum of un-ionized ammonia (NH_3) and the ammonium ion (NH_4^+). The proportion of un-ionized ammonia in a solution is related to both the temperature and pH of the solution such that the proportion of un-ionized ammonia is greater at high temperature and high pH (i.e., alkaline solutions). Concentrations of ammonia that are toxic for aquatic animals are generally expressed as un-ionized ammonia, because un-ionized ammonia and not the ammonium ion has been found to be the toxic form (Mueller and Helsel 1996).

Data on the toxicity of ammonia for freshwater phytoplankton (e.g., Abelovich and Azov 1976; Bretthauer 1978) and vascular plant communities (Stanley 1974; Welch 1992) are limited. Results suggest that freshwater plants are more tolerant to NH_3 than invertebrates or fish (NRC 1979; Environment Canada 1997b) and that toxicity is probably due to un-ionized ammonia (NH_3) rather than ammonium (NH_4^+) (WHO 1986). The few studies available generally show that ammonia at concentrations > 2.4 mg NH_3/L inhibits photosynthesis and growth of algae (Abelovich and Azov 1976; WHO 1986). Experiments with rooted aquatic plants have also reported ammonia reduced the length and weight of roots and shoots (Stanley 1974; Litav and Lehrer 1978). Ammonia has also been investigated for its potential as an aquatic herbicide. Complete eradication of rooted aquatic plant species (water shield, *Brasenia schreberi* and American lotus, *Nelumbo* sp.) was achieved with ammonia treatment of 25.6 mg NH_3/L (Champ et al. 1973). These authors also noted a 95% reduction in phytoplankton density (dinoflagellates, diatoms, desmids, and blue-green algae) two weeks after treating a small natural pond with 3.6 mg/L ammonia (NH_3).

There have been no conclusive toxicity tests on saltwater plants. *Coccolithus huxleyi* was found to be the most sensitive Chrysophyte to ammonium sulfate (Pinter and Provasoli 1963). Ammonium sulfate was also found to be toxic to some marine dinoflagellates, but only at ammonium concentrations far exceeding those in sea water (Provasoli and McLaughlin 1963). The euryhaline alga *Pyrmnesium parvum* was effectively controlled by 100 mg N /L as ammonium sulfate as a result of the lytic effect of un-ionized ammonia (Shilo and Shilo 1953; 1955). A concentration of 0.24 mg NH_3/L retarded the growth of seven of ten species of benthic diatoms cultured for 10 days by Admiraal (1977). In a study of ammonium-induced production of the marine phycotoxin, domoic acid, in the diatom *Nitzschia pungens*, Bates et al. (1993) observed enhanced domoic acid production at concentrations of 7.92 mg NH_4^+/L, whereas concentrations of 15.84 mg NH_4^+/L prevented the growth of diatoms. Decreased cell yield and inhibition of photosynthesis were also associated with increased domoic acid output. The authors suggested that enhanced production of domoic acid is a response to ammonia toxicity.

Table 5.1. Acute effects of un-ionized ammonia on aquatic animals. All endpoints were LC_{50} (the concentration of NH_3 at which 50% of the test organisms died.)

Organism		Life Stage	Conc. (mg NH_3/L)	Exposure period	Reference
Vertebrates					
Acipenser brevirostrum	Shortnose sturgeon	fingerlings	0.58	96 h	Fontenot et al. 1998
Catastomus commersoni	White sucker		1.53	96 h	Arthur et al. 1987
Chasmites brevirostris	Shortnose sucker	larvae	1.24	96 h	Saiki et al. 1999
		juvenile	0.48	96 h	Saiki et al. 1999
Deltistes luxatus	Lost River sucker	larvae	0.51	96 h	Saiki et al. 1999
		juvenile	0.92	96 h	Saiki et al. 1999
Diplodus sargus	Porgy	larvae	0.36	24 h	Brownell 1980
Etheostoma spectabile	Orange throat darter		0.74-0.88	96 h	Hazel et al. 1979
Gaidropsarus capnesis	Cod larvae		0.46	24 h	Brownell 1980
Gasterosteus aculeatus	Threespine stickleback		0.71-0.84	96 h	Hazel et al. 1971
Ictalurus punctatus	Channel catfish	fingerlings	2.36	24 h	Robinette 1976
		juveniles	1.6-3.1	96 h	Colt and Tchobanoglous 1976
			2.4-3.8	96 h	Colt and Tchobanoglous 1976
			0.27	24 h	Spehar et al. 1981
			0.86	96 h	Arthur et al. 1987
Lepomis cyaneilus	Green sunfish	juveniles	0.5-1.73	96 h	McCormick et al. 1984
Lepomis macrochirus	Bluegill		1.17	24 h	Hazel et al. 1979
Lithognathus mormyrus	Mormora	larvae	0.38	24 h	Brownell 1980
Micropterus dolomieui	Smallmouth bass	juveniles	0.69-1.78	96 h	Broderius et al. 1985
M. treculi	Guadelupe bass	fingerlings	0.56	96 h	Tomasso and Carmichael 1986
Morone saxatillis	Striped bass		0.76-1.2	96 h	Hazel et al. 1971
			1.01	96 h	Oppenborn and Goudie 1993
Notropis lutrensis	Red shiner		2.3-2.6	96 h	Hazel et al. 1979
			0.99	96 h	Rosage et al. 1979
Oncorhynchus gorbuscha	Pink salmon	eggs & larvae	0.083	96 h	Rice and Bailey 1980
O. kisutch	Coho salmon	fingerlings	0.28-0.59	96 h	Robinson-Wilson & Seim 1975
		fingerlings	0.51	48 h	Buckley 1978
		fingerlings	0.45	96 h	Buckley 1978
O. mormyrus	Chinook salmon	parr	0.87-1.0	24 h	Harader and Allen 1983
O. mykiss	Rainbow trout	fry	0.072	24 h	Rice and Stokes 1975
			0.3-0.8	96 h	Thurston et al. 1981
			0.16-0.50	96 h	Thurston et al. 1981
			0.53	96 h	Arthur et al. 1987
O. nerka	Sockeye salmon	eggs	0.11	62 days	Spehar et al. 1981
O. tshawytscha	Chinook salmon	parr	0.36	96 h	Harader and Allen 1983
Pachymetopon blochi	Hottentot	larvae	0.42	24 h	Brownell 1980
Pimephales promelas	Fathead minnow		0.75-3.4	96 h	Thurston et al. 1983
Poecilia reticulata	Guppy	fry	0.66-2.62	24 h	Rubin and Elmaraghy 1977
		fry	1.06-1.38	48 h	Rubin and Elmaraghy 1977
		fry	1.00-1.26	96 h	Rubin and Elmaraghy 1977
Salmo clarki	Cuthroat trout		0.5-0.8	96 h	Thurston et al. 1978
		adults	0.30-0.33	96 h	Thurston et al. 1981
S. salar	Atlantic salmon		0.04-0.23	24 h	Alabaster et al. 1979
		2-year smolt	0.80-2.0	24 h	Alabaster et al. 1983

Table 5.1. Acute effects of un-ionized ammonia on aquatic animals (concluded).

Organism		Life Stage	Conc. (mg NH_3/L)	Exposure period	Reference
Sciaenops ocellatus	Red drum	post hatch larvae	0.39	96 h	Holt and Arnold 1983
Stizostedion vitreum	Walleye		0.66	96 h	Arthur et al. 1987
Tilapia aurea	Tilapia		2.4	48 h	Redner and Stickney 1979
Invertebrates					
Asellus racovitzai	Isopod		5.02	96 h	Arthur et al. 1987
Callibaetis skokianus	Mayfly		3.90	96 h	Arthur et al. 1987
Cherax quadricarinatus	Australian crayfish	juvenile	2.02	24 h	Meade and Watts 1995
			1.63	48 h	Meade and Watts 1995
			0.98	98 h	Meade and Watts 1995
Crangonyx pseudogracilis	Amphipod		3.12	96 h	Arthur et al. 1987
Crassostrea virginica	American oyster		8.36-23.46	96 h	Epifanio and Srna 1975
Helisoma trivolvis	Snail		2.37	96 h	Arthur et al. 1987
Macrobrachium rosengergii	Freshwater prawn	3-8 days old	0.66-3.58	24 h	Armstrong et al. 1978
			0.26-1.35	144 h	Armstrong et al. 1978
Mercenaria mercenaria	Quahog clam		3.06-4.49	96 h	Epifanio and Srna 1975
Musculium transversum	Fingernail clam		1.10	96 h	Arthur et al. 1987
Orconectes nais	Crayfish		3.2	24 h	Hazel et al. 1979
O. immunis	Crayfish		18.3	96 h	Arthur et al. 1987
Perna viridis	Green mussel		6.2	48 h	Reddy and Menon 1979
			11.6	96 h	Reddy and Menon 1979
Physa gyrina	Snail		1.95	96 h	Arthur et al. 1987
Philartcus quaeris	Caddisfly		10.1	96 h	Arthur et al. 1987

In conclusion, aquatic plants appear to be relatively tolerant of ammonia as compared to fish and invertebrates. Nitrates are relatively non-toxic and, therefore, their toxicity to aquatic plants has not been tested. However, with the growing interest in the use of aquatic macrophytes to treat wastewater (Pries 1994; Kadlec and Knight 1996), further studies are needed to identify ammonia (as well as nitrate and nitrite) sensitivity of fresh and salt water plant species.

Aquatic animals

The scientific literature on ammonia toxicity to aquatic animals is extensive. A complete review of that large volume of literature is beyond the scope of this report and only a small selection of the literature is presented.

In most surface waters, total ammonia concentrations greater than about 2 mg/L are toxic to aquatic animals (Mueller and Helsel 1996), although this varies among species and life stages (Table 5.1). Because seawater is typically more alkaline than freshwater and thus has a greater proportion of total ammonia in the un-ionized form, total ammonia concentrations eliciting a toxic response are likely higher in seawater than in freshwater (Environment Canada 1997b). With respect to the toxic un-ionized form, mean 96-hour LC_{50} values for fishes range from 0.083 to 3.8 mg NH_3/L. The 48-hour LC_{50} values range from 0.51 to 2.4 mg NH_3/L and 24-hour LC_{50} values range from 0.04 to 2.62 mg NH_3/L (Table 5.1). The cause of death appears to be an increase in permeability of the fish to water such that the water intake ultimately exceeds the maximum sustained urine production (NRC

Table 5.2. Acute and chronic effects of nitrate on aquatic animals. All endpoints were LC_{50} (the concentration of NO_3^- at which 50% of the test organisms died) unless otherwise noted.

Organism		Life Stage	Conc. (mg NO_3/L)	Exposure period	Reference
Fish					
Centropristis striata	Gulf Black sea bass		10629	96 h	Pierce et al. 1993
Ictalarus punctatus	Channel catfish		6200	96 h	Colt and Tchobanoglous 1976
Lepomis macrochirus	Bluegill		1860-8860	96 h	Trama 1954
Micropterus treculi	Guadelupe bass	fingerlings	1261	96 h	Tomasso and Carmichael 1986
Monacanthus hispidus	Planehead filefish		2538	96 h	Pierce et al. 1993
Oncorhynchus mykiss	Rainbow trout	fingerlings	6000	96 h	Westin 1974
		eggs	10	--	Kincheloe et al. 1979
		eggs	5	--	Kincheloe et al. 1979
O. tshawytscha	Chinook salmon	fry	5800	96 h	Westin 1974
		fry	20	--	Kincheloe et al. 1979
Poecilia reticulata	Guppy		797-886	96 h	Rubin and Elmaraghy 1977
Pomacentrus leucostritus	Beaugregory		>13286	96 h	Pierce et al. 1993
Raja eglanteria	Clearnose skate		>4252	96 h	Pierce et al. 1993
Trachinotus carolinus	Florida pompano		4429	96 h	Pierce et al. 1993
Amphibians					
Bufo americanus	American toad	tadpoles	60-175	96 h	Hecnar 1995
B. bufo	Common toad	tadpoles	1704	96 h	Xu and Oldham 1997
			1637	168 h	Xu and Oldham 1997
Litoria caerulea	Tree frog	tadpoles	100[1]	16 d	Baker and Waights 1994
		tadpoles	40[2]	16 d	Baker and Waights 1994
Pseudacris t. triseriata	Western chorus frog	tadpoles	76	96 h	Hecnar 1995
		tadpoles	11[3]	24 h	Hecnar 1995
		tadpoles	44[4]	100 d	Hecnar 1995
Rana pipiens	Northern leopard frog	tadpoles	100	96 h	Hecnar 1995
		tadpoles	44[4]	100 d	Hecnar 1995
R. clamitans	Green frog	tadpoles	144	96 h	Hecnar 1995
Triturus vulgaris	Smooth newt	larvae	77.5-387.5[5]	24 h, 48 h or 72 h	Watt and Oldham 1995

[1]Endpoint is death
[2]Endpoint is an Effective Concentration (EC) (behaviour change)
[3]Endpoint is an EC (physical and behavioural changes)
[4]Endpoint is an EC (survival during metamorphosis)
[5]Endpoint is an EC (size of metamorphs, feeding rate)

1979; Environment Canada 1984). Sublethal exposure to ammonia has been reported to cause adverse physiological and histopathological effects in fish (NRC 1979). Less information is available on the toxic effects of ammonia to invertebrates and benthic organisms. Mean 48 and 96-hour LC_{50} values for freshwater invertebrates range from < 1 to 23.46 mg NH_3/L (Hickey and Vickers 1993; Table 5.1).

Nitrate is considerably less toxic to aquatic organisms than ammonia or nitrite and, as a result, has been less studied (Russo 1985; Table 5.2). For adult fish, LC_{50} values range from 800 to 12 000 mg NO_3^-/L (Colt and Tchobanoglous 1976; Tomasso and Carmichael 1986). However, nitrate concentrations in the range of 1 to 10 mg NO_3^-/L are lethal to eggs and, to a lesser extent, fry of salmonid fish (Kincheloe et al. 1979). In addition, Kincheloe et al. (1979) showed that 31% of rainbow trout (*Oncorhynchus mykiss*) eggs and 15% of fry died when exposed to 2.3 mg NO_3^-/L. Nitrate

exposure in tadpoles from various amphibian species has led to lethality at concentrations as low as 60 mg/L (Hecnar 1995; Table 5.2). However, the behaviour and survivorship of some species were affected by concentrations as low as 11 mg NO_3^-/L (Hecnar 1995). Furthermore, tadpoles exposed to 11-44 mg NO_3^-/L for 24-h had reduced feeding activity and weight loss, swam less vigorously, showed a delayed response to prodding, and displayed disequilibrium and eventually paralysis (Hecnar 1995). Signs of developmental abnormalities were also observed and included: development of bulges in the head and thorax regions; eye deformities; complete loss of pigment; subdermal edema resulting in a large globular body shape; digestive-tract deformities; and bent tails (Hecnar 1995). In addition, the size at metamorphosis decreased with increasing nitrate in the smooth newt (*Triturus vulgaris*) (Watt and Oldham 1995), which could ultimately affect the population dynamics of these newts. It is interesting to note that amphibians appear to be more sensitive to nitrate exposure than fish (Table 5.2). Nitrate toxicity experiments conducted on invertebrates such as two species of caddisfly larvae showed relatively high LC_{50} values of 504.4 mg NO_3^-/L for *Cheumatopsyche pettiti* and 432.4 mg NO_3^-/L for *Hydropsyche occidentalis* (Camargo and Ward 1992).

Under some conditions, nitrate and ammonia can be transformed to nitrite (Marco and Blaustein 1999). Nitrite is highly toxic, more so than nitrate. It is suspected that nitrite can reach lethal levels in aquatic culture systems, ponds, waters receiving municipal wastewater effluents, and any natural system where animal biomass is high (Huey and Beitinger 1980b). However, because it rapidly oxidizes to nitrate and is therefore rarely present in high concentrations naturally, its toxicity has not been well studied. Of the few studies that exist, the 96-hour LC_{50} values for fish (0.19 to 188 mg NO_2^-/L; Table 5.3) are, on average, higher than those reported for ammonia. Moreover, of the fish species studied, rainbow trout (*O. mykiss*) appears to be the most sensitive. Huey and Beitinger (1980b) exposed larvae of salamanders (*Ambrystoma tesnaum*) to nitrite and determined an LC_{50} of 1.09 mg NO_2^-/L. Sublethal concentrations of nitrite (11.5 mg NO_2^-/L) induced behavioural and morphological changes, retarded development, and altered the age at emergence in the cascades frog (*Rana cascadae*) (Marco and Blaustein 1999). In addition, nitrites transform haemoglobin into methaemoglobin, decreasing the oxygen carrying capacity of the blood: nitrite concentrations as low as 1 mg/L have been shown to increase the amount of methaemoglobin in the blood of bullfrog (*Rana catesbiana*) tadpoles (Huey and Beitinger 1980a). Other behavioural modifications (occupying shallow waters, bobbing behaviour, and air gulping) have been noticed in amphibians in response to high nitrite concentrations (11 – 50 mg NO_2^-/L) in surface waters (Huey and Beitinger 1980a; Marco and Blaustein 1999).

Terrestrial Plants

Anthropogenic inputs of N to vegetation are associated with air pollution, particularly nitrogen oxides, un-ionized ammonia, ammonium and nitrate. Of these compounds, nitrogen dioxide, un-ionized ammonia and ammonium are all highly phytotoxic. Nitrogen-containing air pollutants can affect vegetation indirectly via chemical reactions in the atmosphere immediately surrounding the plant, or directly from uptake after deposition on vegetation, soil or water surfaces (WHO 1997a).

Nitrogen toxicity exhibits a variety of symptoms including abscission of foliage and reductions in total assimilatory capacity (Miller et al. 1979 cited in Jacobson et al. 1990); formation of larger, thin walled cells and a “succulent” type of growth (Roelofs et al. 1985; Teshow and Anderson 1989); needle yellowing and lesions (Van Dijk and Roelofs 1988); reduction in drought hardiness due to loss of control of transpiration by stomata and to an increase in shoot/root ratios (Lucas 1990; Atkinson et al.

Table 5.3. Acute and chronic effects of nitrite on aquatic animals. All endpoints are LC_{50} (the concentration of NO_2^- at which 50% of the test organisms died) unless otherwise indicated.

Organism		Life Stage	Conc. (mg NO_2/L)	Exposure period	Reference
Fish					
Acipenser brevirostrum	Shortnose sturgeon	fingerlings	11.3	96 h	Fontenot et al. 1998
Anguilla anguilla	European eel		143.7	96 h	Kamstra et al. 1996
Ictalurus punctatus	Channel catfish		7	96 h	Palachek and Tomasso 1984
Micropterus treculi	Guadelupe bass	fingerling	187.6	96 h	Tomasso and Carmichael 1986
M. salmoides	Largemouth bass		140	96 h	Palachek and Tomasso 1984
Oncorhynchus mykiss	Rainbow trout	fingerling	0.06[1]	6 mon.	Wedemeyer and Yasatake 1978
		fingerling	0.015[2]	6 mon.	Wedemeyer and Yasatake 1978
		fingerling	0.06[1]	10 d	Russo et al. 1974
		fingerling	0.19	96 h	Russo et al. 1974
O. tshawytscha	Chinook salmon	fry	2.9	96 h	Westin 1974
Amphibians					
Rana cascadae	Cascades frog	tadpoles	11.5[3]	14 d	Marco and Blaustein 1999
Rana catesbiana	Bullfrog	tadpoles	1-50[4]	24 h	Huey and Beitinger 1980a
Tilapia aurea	Tilapia		16	96 h	Palachek and Tomasso 1984
Ambrystoma tesnaum	Smallmouth salamander	larvae	6.6	96 h	Huey and Beitinger 1980b
		larvae	1.09	96 h	Huey and Beitinger 1980b
Invertebrates					
Cherax quadricainatus	Australian crayfish	juveniles	42.9	24 h	Meade and Watts 1995
		juveniles	37.1	48 h	Meade and Watts 1995
		juveniles	25.9	96 h	Meade and Watts 1995

[1]Endpoint is a NOEC (No observed effect concentration)
[2]Endpoint is an Effective Concentration (EC) (methaemoglobin changes)
[3]Endpoint is an EC (developmental and behavioural changes)
[4]Endpoint is an EC (% methaemoglobin)

1991; Fangmeijer et al. 1994); and reduction in frost hardiness due to the disruption of the production of anti-freeze proteins and the utilization of carbohydrates (Cape et al. 1991; Hall et al. 1997). The adverse effects of N-containing compounds may be due to their interference with cellular acid-base regulation as they can influence cellular pH both before and after assimilation (Raven 1988; Wollenweber and Raven 1993).

Nitrate is thought to be essentially non-toxic to plants (Teshow and Anderson 1989; Yaremcio 1991; WHO 1997a). Certain plant species can accumulate nitrate to concentrations that present health complications in their consumers (see Terrestrial Animal section). Nitrate accumulation in plants results from uptake in excess of their capacity to reduce and assimilate nitrate into amino acids (Environment Canada 1997b). Acid precipitation, which is caused by sulfate and nitrate ions, can injure vegetation through the precipitation of major ions, increased water loss and decreased photosynthesis. However, the effects of acid precipitation do not appear to be due to nitrogen (or sulfate) directly but to the decrease in foliar pH.

When in excess, nitrogen oxides may cause toxicity (Table 5.4). The greatest direct effect on vegetation lies in the interaction of NO_2 with other pollutants and in disrupting balances with other nutrients (WHO 1997a). Woody plant species that lack the necessary enzymes to metabolize the nitrate and nitrite formed in the leaves by the absorption of nitrogen oxides may be particularly

Table 5.4. Acute and chronic effects of nitrogen (ammonia, nitrogen dioxide, and nitric oxide) on terrestrial plants.

Organism		Effect	Conc. ($\mu g/m^3$)	Exposure period	Reference
Ammonia					
Amaranthus retroflexus	Pigweed	Slight injury	8.4	4 h	NRC 1979
Brassica alba	Mustard weed	Severe injury	2.1	4 h	NRC 1979
B. rapa	Turnip	Leaf injury	2000	2 h	Benedict and Breen 1955
Coleus	Coleus	Leaf lost colour	28.2	1 h	NRC 1979
Fagopyrum esculentum	Buckwheat	50% foliar injury	704	5 min	NRC 1979
Helianthus	Sunflower	Injury	28.2	1 h	NRC 1979
Lepidium sativum	Garden cress	Reduced biomass	1000	2 wk	Van Haut and Prinz 1979
Lolium perenne	Perennial rye grass	Impact acid/base regulation	213	5 d	Wollenweber and Raven 1993
Lycoperiscon esculentum	Tomato	Injury	28.2	1 h	NRC 1979
		50% foliar necrosis	176	4 min	NRC 1979
Malus sylvestris	Apples	Dark discolouration around lenticels	>282	--	NRC 1979
Nicotiana tabacum	Tobacco	50 % foliar injury	704	8 min	NRC 1979
Pinus sylvestris	Scotch pine	Increased loss of water	53	9 mnth	Dueck et al. 1990
		Increased glutamine synthetase activity	60	14 wk	Pérez-Soba et al. 1990
Prunus persica	Peach fruit	Darkening of skin	140.8	--	NRC 1979
Pseudotsuga menziesii	Douglas fir	Imbalanced nutrient status	180	13 wk	Van der Eerden et al. 1991
Raphanus sativus	Radish seeds	Mortality	704	16 h	NRC 1979
Secale cereale	Spring rye seeds	52% germination decrease	176	16 h	NRC 1979
		Mortality	704	4 h	NRC 1979
Violion caninea	Violet	Imbalanced nutrient status	50	8 mnth	Dueck and Elderson 1992
Nitrogen dioxide					
Citrus sinesis	Orange	Increased fruit drop	120-500	9.5 mnth	Thompson et al. 1970
Glycine max	Soya bean	Enhanced dark respiration and photosynthesis	375	35 h	Sabarathnam et al. 1988
Helianthus annus	Sunflower	Reduced net assimilation rate	375	2 wk	Okano et al. 1985
Hordeum vulgare	Barley	Growth stimulation	10-43	130 d	Adaros et al. 1991
Lolium perenne	Perennial rye grass	Lower glutamate dehydrogenase activity	125	140 d	Wellburn et al. 1981
Lycoperiscon esculentum	Tomato	Lower leaf nitrate concentration	940	19 d	Taylor and Eaton 1966
Medicago sativa	Alfalfa	Inhibition of photosynthesis	1100	1.5 h	Hill and Bennet 1970
Phaseolus vulgaris	Common bean	Increased dark respiration	120	22 d	Sandhu and Gupta 1989
Picea rubens	Red spruce	Increased nitrate reductase activity	140	1 d	Norby et al. 1989
Pinus strobus	Eastern white pine	Slight needle necrosis	100	5 d	Yang et al. 1983
Pinus sylvestris	Scotch pine	Increased nitrate reductase activity	160	7 d	Wingsle et al. 1987
Pisum sativum	Pea	Emission of stress ethylene	850	7 h	Mehlhorn and Wellburn 1987
Polytrichum formosum	Polytrichium moss	Injury and changes in growth	122	37 wk	Bell et al. 1992

Table 5.4. Acute effects of nitrogen on terrestrial plants (concluded).

Organism		Effect	Conc. ($\mu g/m^3$)	Exposure period	Reference
Nitric oxide					
Avena sativa	Oats	Reduced photosynthesis	750	1 h	Hill and Bennet 1970
Capsicum annum	Sweet pepper	Decreased nitrate reductase	1875	18 h	Murray and Wellburn 1980
Ctendium molluscum	Bryophyte	Increased dark respiration	44	8-24 h	Morgan et al. 1992
Lactuca sativa	Lettuce	Induction of nitrate reductase	375	8 d	Besford and Hand 1989
Lactuca sativa		Reduced photosynthesis	2500	10 min	Capron 1989
Lactuca sativa		Reduced biomass	635	16 d	Capron et al. 1991
Lycoperiscon esculentum	Tomato	Induction of nitrate reductase	500	28 d	Wellburn et al. 1980
		Reduced biomass	500	5 d	Bruggink et al. 1988
Medicago sativa	Alfalfa	Reduced photosynthesis	750	1 h	Hill and Bennet 1970
Pisum sativum	Pea	Increased ethylene release	188	7 h	Mehlhorn and Wellburn 1987

susceptible to injury (Amundson and McLean 1982). Nitrogen dioxide in concentrations of 100-140 µg NO_2/m^3 caused slight needle necrosis in eastern white pine (Yang et al. 1983), increased dark respiration in beans (Sandhu and Gupta 1989), and increased nitrate reductase activity in red spruce (Norby et al. 1989). Nitrogen dioxide at a concentration of 375 µg NO_2/m^3 increased dark respiration and photosynthesis in soya beans (Sabarathnam et al. 1988) and reduced net N assimilation rate in sunflowers (Okano et al. 1985). NO_2 concentrations as high as 900-1100 µg NO_2/m^3 decreased leaf nitrate concentrations in tomato plants (Taylor and Eaton 1966) and inhibited photosynthesis in alfalfa (Hill and Bennet 1970).

Terrestrial plants may also show a toxic response to nitric oxide (Table 5.4). Leaves are more sensitive to nitric oxide injury under low light or dark conditions (Taylor 1973), as the process that reduces nitrates operates more slowly when plants are not photosynthesizing (Ramade 1987). Nitric oxide has been shown to increase dark respiration at concentrations as low as 44 µg NO/m^3 in bryophytes (Morgan et al. 1992), reduce plant mass in tomatoes at concentrations of 500 µg NO/m^3 (Bruggink et al. 1988), and inhibit photosynthesis in lettuce at concentrations of 2 500 µg NO/m^3 (Capron 1989).

Ammonium (NH_4^+) in plant tissues is known to be toxic at quite low concentrations. Ammonium toxicity is generally characterized by an immediate reduction in growth rate, wilting, marginal necrosis, inter-veinal chlorosis of terminal leaves and, ultimately, death of the entire plant (Maynard and Barker 1969). For example, exposure of the roots of tomato seedlings to an ammonium solution as the sole N source resulted in reduced growth, a bruised root system and considerable wilting of the leaves, which developed marginal necrosis (Pierpont and Minotti 1977). A significant effect of increased atmospheric ammonium is that it may stimulate leaching; in this regard, ammonium has been shown to increase leaching of potassium from hardwoods (Roelofs et al. 1985; Leonardi and Flückiger 1989) and calcium and magnesium ions from conifers (Wilson 1992). This leaching, in turn, can lead to nutrient

imbalances as shown in Douglas fir at concentrations of 180 µg NH_4^+/m^3 (Van der Eerden et al. 1991) and violets at concentrations of 50 µg NH_4^+/m^3 (Dueck and Elderson 1992). Atmospheric ammonium ions may also contribute to a recent decline of forests by affecting frost hardiness. Changes in frost hardiness of only a few degrees Celsius could significantly increase the risk of frost damage (Friedland et al. 1984; Sheppard et al. 1989; Cape et al. 1991; Hall et al. 1997).

Foliar uptake of un-ionized ammonia (NH_3) is also possible and high ambient concentrations can be toxic. Ammonia may exert direct effects on plant structure and function and indirect effects on soil conditions (Environment Canada 1997b; Table 5.4). Ammonia is an N source for the synthesis of proteins and, although the use of ammonia in the synthesis of organic molecules can be regarded as a process for storing a valuable nutrient, it is also important for detoxifying a potentially toxic chemical, namely ammonia. Excessive uptake of ammonia can therefore stress carbohydrate metabolism. Ammonia is also an inhibitor of photosynthetic phosphorylation, which results in reduced carbohydrate production and therefore, reduced growth (Teshow and Anderson 1989). The toxic effects of ammonia are most often seen in older leaves or needles. Symptoms often resemble those induced by drought, salts, some plant diseases, or other pollutants. Symptoms specific to ammonia include dark spots or the complete blackening of leaves, rusty marginal spotting, and dark brown lesions between the veins on leaves, which turn black the next day followed by the entire leaf drying up (NRC 1979). Colour changes in fruit and vegetable skins and brownish to black lesions also occur (NRC 1979). Injury symptoms on broad-leaved woody plants exposed to high concentrations of ammonia usually begin as large, dark green, water-soaked areas that darken after several hours to brownish-grey or black necrotic lesions widely scattered over the leaf surface (NRC 1979). On trees or shrubs with crowded or overlapping leaves, injury may be confined to particular sections of the leaf. In addition, foliar lesions can occasionally turn orange, purple, or reddish-brown, mimicking fall colouration. Conifer foliage exposed to ammonia darkens to shades of grey-brown, purple, or black and the entire part of the needle exposed to the gas is usually affected (Dueck et al. 1990). Symptoms of injury are more variable on herbaceous plants than on woody species, ranging from irregular, bleached, bifacal, necrotic lesions to reddish inter-veinal streaking or dark upper-surface discolouration (Van der Eerden 1982; Environment Canada 1984; Teshow and Anderson 1989; Hall et al. 1997; WHO 1997a; Table 5.4). In addition, concentrations as high as 704 µg NH_3/m^3 applied to radish and rye seeds caused mortality (NRC 1979). As with ammonium, a major effect of ammonia exposure to plants is increased sensitivity to cold and frost (Cape et al. 1991). Thus, elevated atmospheric ammonia concentrations may also lead to forest decline as a result of lipid saturation by ammonia in cell membranes, thereby increasing their permeability and decreasing their flexibility (Van der Eerden 1982).

Although P is not directly toxic in the forms and amounts found naturally in the environment (Switzer-Howse and Coote 1984), plants supplied with a small excess of P often develop purple to rusty-brown lesions on their dark green lower leaves (Jones 1998). Growth is not impaired when excess P is supplied in small amounts. However, plants supplied with a large excess of P lack vigour and have short stout stems. The leaves are dark green in colour and the blades of flower leaves may be almost covered by reddish-purple lesions. Eventually, the tips and margins of the blades turn brown, wither, curl upwards and die. Clark (1982) and Furlani et al. (1986a, b) described reddish purple lesions on sorghum plants growing in solution cultures, potted sand or soil, or in the field and referred to them as “red-speckling”. The concentration of P that induced this response depended on the cultural method, cultivar, age of the plants, and source of phosphate. With a susceptible cultivar, “red-speckling” appeared 3 days after 2.18 µg/L potassium dihydrogen phosphate was added to solution cultures of 7-

Table 5.5. Acute and chronic effects of nitrogen (nitrate and ammonia) on terrestrial organisms.

Organism	Endpoint	Conc. (mg/m^3)	Exposure period	Reference
Nitrate				
Guinea pigs	Lung structure. No significant effects	1	4 wk	Busch et al. 1986
Rat	Lung structure. No significant effects	1	4 wk	Busch et al. 1986
Ammonia				
Bats	No adverse physiological effects	59.8-1302.4	--	NRC 1979
Birds (Starling; Sparrow; Pigeon)	LC_{50}	1600	7 min	NRC 1979
Cat	Severe dysponea, anorexia, dehydration, bronchopneumonia after 7 days	700	10 min	Dodd and Gross 1980
Cattle (dairy)	High morbidity and mortality rates	182-1400	--	NRC 1979
Mouse	30% mice dead; nasal, eye and breathing problems	3185	1 h	MacEwen and Vernot 1972
Pigs	Excessive nasal, lacrimal and mouth secretions	70.4-105.6	5 wk	NRC 1979
Poultry	Reduced food consumption and weight gain; air sacculitis; increased susceptibility to respiratory diseases; general discomfort	14.1-35.2	--	NRC 1979
Poultry	Reduced egg production, tracheitis, keratoconjunctivitis (ocular disorder)	42.2-70.4	--	NRC 1979
Rabbit	No signs of toxicity; no lung abnormalities	40	24 hr/day for 114 days	Coon et al. 1970
Swine	Reduced growth rate	>70.4	--	NRC 1979
Swine	Frothing of the mouth, excessive secretion, and irregular breathing	196	36 h	Stombaugh et al. 1969

day-old plants (Furlani et al. 1986b) or after 0.58 µg/L ammonium dihydrogen phosphate was added to solution cultures of 2-day-old seedlings of *Sorghum bicolor* (Grundon et al. 1987). The "red-speckling" of P toxicity can be confused with the reddish purple or brown lesions developed by plants suffering from a severe deficiency of potassium or magnesium. However, P toxicity lesions are irregular in outline and the brown necrosis is confined to the margins.

Terrestrial Animals

Nitrogen toxicity in terrestrial animals is associated either with air pollution (nitrogen dioxide, ammonia or nitrate) or the consumption of vegetation with high nitrate levels. However, water-borne nitrates and nitrites have caused the induction of methaemoglobinaemia in young animals (WHO 1977).

With respect to nitrogen toxicity from atmospheric sources, studies of nitrogen dioxide have largely described effects on the respiratory tract (Cavanagh and Morris 1987; WHO 1997a), but extrapulmonary effects have also been briefly studied (e.g., WHO 1997a). Nitrogen dioxide exposure causes structural alterations in lungs, including emphysema. Ammonia studies have mostly focused on inhalation and irritation effects (Environment Canada 1984; Environment Canada 1997b). Table 5.5 shows the effects of nitrate and ammonia gas inhalation on mammals. There are no reported effects of ammonium aerosol inhalation on various mammals (Rothenberg et al. 1986; Schlesinger et al. 1990). Nitrate may act by altering intracellular pH, which can cause serious health effects (WHO 1997a). Only one inhalation study has been reported for mammals (Busch et al. 1986) and there is no information on the effect of nitrate on reptiles (Rouse et al. 1999) (Table 5.5).

Table 5.6. Plants used as livestock feed that accumulate nitrate. (Data from Bradley et al. 1940; Osweiler et al. 1985; Mulligan and Munro 1990; Yaremcio 1991; Wohlgemuth and Casper 1992; Steppuhn et al. 1994)

CROPS	WEEDS
Alfalfa (*Medicago sativa*)	Canada thistle (*Cirsium arvense*)
Barley (*Hodeum vulgare*)	Dock (*Rumex venosus*)
Canola (*Brassica rapa*)	Fireweed (*Epilobium angustifolium*)
Corn (*Zea mays*)	Jimsonweed (*Datura stramonium*)
Flax (*Linum usitatissimum*)	Johnson grass (*Sorghum halepense*)
Millet (*Panicum miliaceum*)	Kochia (*Kochia scparia*)
Oats (*Avena sativa*)	Lambsquarter (*Chenopodium album*)
Rape (*Brassica napus*)	Nightshade (*Solanum* sp.)
Rye (*Secale cereale*)	Pigweed (*Amaranthus* sp.)
Soyabean (*Glycine max*)	Russian thistle (*Salsola kali*)
Sorghum (*Sorghum* sp.)	Smartweed (*Polygonum acre*)
Sudangrass (*Sorghum sudanense*)	Wild sunflower (*Helianthus* sp.)
Sugar beets (*Beta vulgaris*)	
Sweet clover (*Melilotus alba*)	
Wheat (*Triticum aestivum*)	

Nitrate poisoning as a result of plant ingestion occurs when animals consume in excess of 0.35 to 0.45% nitrate in their diet (Kvasnicka and Krysl 1996). Livestock fed on feed containing 0 to 0.5 % nitrate (dry weight) show no signs of toxicity. However, livestock given feed with nitrate levels of 0.5 to 1.0 % (dry weight) may show sublethal symptoms. Nitrate concentrations >1.0% (dry weight) have caused death of livestock (Neilson 1974; Osweiler et al. 1985; Yaremcio 1991; Wohlgemuth and Casper 1992).

Nitrates can accumulate to high levels in certain plant species, in particular common plants used for livestock feed (Table 5.6). In general, annual forage crops tend to accumulate greater amounts of nitrate than perennial forages. A variety of conditions are associated with nitrate-enrichment of vegetation (e.g., drought, insect damage, hail, cloudy weather, crowding, shading, application of nitrogen-containing fertilizers, and farm management) (Bradley et al. 1940; Yaremcio 1991; Steppuhn et al. 1994; Kvasnicka and Krysl 1996; Lester 1998). The frequent use of nitrogen-fertilizers in recent years has resulted in increased incidence of nitrate poisoning (Kvasnicka and Krysl 1996). Plants may also accumulate livestock-toxic levels of nitrate (up to 4.7 % dry weight NO_3) if rooted on soils heavily contaminated by animal excreta, for example around water tanks, ponds and salt licks and along frequently used trails (Williams 1989).

Nitrate in vegetation is not toxic to livestock until it is converted to nitrite in the animal's digestive tract. Nitrite is absorbed into the blood and converts haemoglobin to methaemoglobin, which has no oxygen carrying capacity (Yaremcio 1991). Cattle and sheep are more susceptible to poisoning because micro-organisms in their rumen favour this conversion to nitrite (Bradley et al. 1940; Osweiler et al. 1985). Horses, pigs, and poultry are less susceptible to nitrate poisoning because they convert nitrate to nitrite in the intestine, where there is less opportunity for nitrites to be absorbed by the blood (Neilson 1974; Yaremcio 1991).

Acute nitrate poisoning usually occurs within a half to four hours after consuming toxic levels (> 1.0 % dry weight) (Wohlgemuth and Casper 1992). Signs of acute poisoning in livestock are increased heart rate; quickened respiration; muscle tremors; vomiting; weakness; blue-grey mucous membranes; blindness; excess saliva and tear production; depression; laboured or violent breathing; staggered gait; frequent urination; low body temperature; disorientation; and an inability to get up (Emerick 1974; Cowley and Collings 1977; Yaremcio 1991; El-Bahri et al. 1997). Animals are often found lying down after only a short struggle. In most cases of acute poisoning, animals are found dead before any signs of toxicity are observed.

Sublethal responses to nitrate affect livestock production and are not often recognized. They include lower milk production, reduction in weight gain and lower resistance to disease, resulting in poor animal performance and a general lack of health (Emerick 1974; Hibbs et al. 1978; El-Bahri et al. 1997; Lester 1998). Sublethal doses of nitrate also increase the incidence of stillborn calves, abortions, retained placentas, and cystic ovaries (Hibbs et al. 1978; Johnson et al. 1983; Putnam 1989). In addition, nitrate blocks the conversion of beta-carotene to true vitamin A and alpha-tocopherol to vitamin E, resulting in photosensitivity and other chronic disorders (Lester 1998).

Nitrate poisoning is treatable with intravenous administration of methylene blue shortly after ingestion of nitrate-enriched plants (Osweiler et al. 1985; Yaremcio 1991). Careful use of nitrogen-fertilizers, an awareness of which plants accumulate nitrate, silage moisture levels, the effect of weather, and cattle feed management can help reduce losses from nitrate poisoning. Prevention of nitrate poisoning is best achieved by controlling the type and quantity of forage offered to livestock. Most feeds that contain nitrate can be fed to cattle if managed properly.

Water-borne nitrate or nitrite can also cause problems for terrestrial animals. Methaemoglobinaemia is the most studied problem, although nitrate has induced prenatal mortality, resorptions, and decreased birthweight in rat and guinea pig pups whose mothers received drinking water containing sodium nitrite (Alexandrov and Jänisch 1971; Shuval and Gruener 1972; WHO 1977). Nitrate induced increases in methaemoglobin levels in rabbits have been found to increase cardiac activity (Garbuz 1971). In adult rats, elevated sodium nitrite levels in drinking water increased methaemoglobin levels but not to the point of producing overt toxic effects (Druckrey et al. 1963; Behroozi et al. 1971).

Human Health

The effect of controlled and accidental acute exposure to ammonia on human health has been widely studied (see WHO 1986 for a complete review). The data show serious health effects of ammonia; however, further research is required to identify exposure time and concentration guidelines for spill situations and to identify long-term health problems associated with acute toxic exposure. Human contact with ammonia may be in the form of gas, anhydrous liquid, or aqueous solution (Environment Canada 1997b). Inhalation is considered to be the primary route for human exposure and effects can range from mild irritation to severe corrosion of sensitive membranes of the eyes, nose, throat and lungs. The most dangerous consequence of exposure to the gas is pulmonary edema followed by severe irritation to moist skin surfaces (WHO 1986; Environment Canada 1997b; WHO 1997a;).

In addition to ammonia, nitrate can pose a serious problem to human health. In Canada, a maximum acceptable concentration of 45 mg/L for nitrate [10 mg NO_3^--N /L] in drinking water is recommended (Health Canada 1996). Nitrite, which is directly toxic and is produced from nitrate, has a recommended

maximum acceptable concentration of 3.2 mg NO_2^-/L [0.97 mg NO_2^--N/L] in drinking water (Health Canada 1996). Nitrate levels in Canadian municipal water supplies are generally less than 5 mg NO_3^-/L (Health Canada 1996). Concentrations in ground water supplies vary across the country (Table 5.10).

Ingestion of high quantities of nitrate or nitrite may result in methaemoglobinaemia ("Blue Baby Syndrome"), a condition resulting from the oxidation (by nitrite) of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron in haemoglobin, the oxygen carrier of mammalian blood. The resulting methaemoglobin has no oxygen carrying capacity. Symptoms are cyanosis, asphyxia, and death (Health Canada 1992; WHO 1997a). The most sensitive subpopulation is infants less than three months of age. Reasons for the greater susceptibility of young infants include their readily oxidizable fetal haemoglobin and their depressed methaemoglobin capacity to secrete gastric acid and increased susceptibility to gastro-enteritis, both of which permit nitrate-reducing bacteria in the gastrointestinal tract to convert nitrate to nitrite (Craun et al. 1981).

For bottle-fed infants, water used in the preparation of infant formula is generally the main source of nitrates (Health Canada 1996). Consumption of 0.6 L of drinking water containing 4.5 mg/L nitrate could contribute 2.7 mg NO_3^-/d or about 0.7 mg NO_3^-/kg body weight for a 3 to 5 kg infant (Health Canada 1992). For adults, food is generally the main source unless drinking water concentrations are elevated. Vegetables such as beets, celery, lettuce, radishes, and spinach contribute significantly to an adult's dietary intake of nitrate (Health Canada 1992), with nitrate levels ranging from 1 700 to 2 400 mg NO_3^-/kg food (Corre and Breimer 1979). Spinach can easily accumulate nitrates from the soil and consumption of spinach grown in soils heavily fertilized with nitrate leads to human health risks (methaemoglobinaemia) (Ramade 1987). There have been cases in Europe of methaemoglobinaemia due to consumption of spinach contaminated with excess nitrates (Ramade 1987). Small amounts of nitrates may also be present in fish and dairy products such as cheese, baked goods, and cereals. The total daily intake of nitrates by adults is estimated to be 51 mg (44.3 mg from food and 6.8 mg from drinking water containing nitrate at a concentration of 4.5 mg/L), or 0.7 mg/kg body weight for a 70-kg adult (Health Canada 1992).

Ingested nitrate is readily absorbed in the upper small intestine and is distributed rapidly throughout the body of which roughly 25% is recirculated into the saliva. Once in the saliva, 20% of the recirculated nitrate is reduced to nitrite by the oral microflora (Health Canada 1992). Nitrite production increases with age (Turek et al. 1980) and is enhanced during bacterial infections causing diarrhoea (Hartman 1983). Nitrites are rapidly absorbed both in the stomach and in the small intestine. In the human stomach, nitrites can react with nitro-satiable compounds, such as amides and amines, to form N-nitroso compounds (Broitman et al. 1981). Some N-nitroso compounds are potent carcinogens and corrosive substances in animal species (Broitman et al. 1981; Ramade 1987), and are therefore probably carcinogenic in humans (Fraser 1985). Some evidence for an association between the ingestion of nitrate and the incidence of stomach cancer has been obtained in descriptive epidemiological studies (Hill et al. 1973; Fraser and Chilvers 1981; Clough 1983; Gilli et al. 1984). Yet, other studies indicate no association between the ingestion of nitrate and stomach cancer (Vincent et al. 1983; Beresford 1985; NRC 1995).

Nitrate contamination of drinking water was found not to be associated with peripheral lymphocyte sister chromatid exchange frequencies in humans (Kleinjans et al. 1991). Nitrate is not mutagenic in

bacteria and mammalian cells *in vitro* (Speijers et al. 1989). However, there is some evidence that nitrites or nitro-satiable reaction products are mutagenic in rats, mice and rabbits (Luca et al. 1987). Furthermore, the results of preliminary studies (Petukhov and Ivanov 1970; Shuval and Gruener 1972; Rotton et al. 1982; Health Canada 1992) indicate that there may be some behavioural effects associated with the ingestion of drinking water with elevated nitrate concentrations; this aspect warrants further study.

5.2. Nitrogen Toxicity in Canadian Environments

Aquatic Ecosystems

Ammonia is not routinely found in Canadian surface waters at high enough concentrations to pose a wide-scale toxic threat to invertebrates or fish. Total ammonia in Canadian surface waters rarely exceeds 0.2 mg NH_3 /L and is usually below 0.1 mg NH_3 /L (Environment Canada 1997b). From 1980 to 1984, total ammonia concentrations in surface waters ranged from 0.002 to 2.00 mg/L in 2 175 samples from across Canada. The highest values were obtained in the Prairies (Environment Canada 1997b). Similarly, 92% of 63 Canadian public water supplies surveyed between 1966 and 1974 contained total ammonia at concentrations of 0.2 mg/L or less (NAQUADAT 1976). Elevated ammonia concentrations are usually associated with mixing zones near municipal and sewage discharges, or with manure or fertilizer runoff from agricultural fields. These high ammonia concentrations are usually dissipated within 10s or 100s of metres from the source because of dilution in the receiving water, conversion of ammonia to nitrate (by nitrification), and volatilization of ammonia to the atmosphere.

Because of the well-documented toxic effects of high ammonia concentrations, discharges of municipal and industrial wastewater in Canada have long been regulated to avoid acute toxicity to aquatic organisms in receiving waters. With respect to agriculture, most Canadian provinces provide recommendations for minimizing nutrient losses to the environment (e.g., Manitoba Agriculture 1994; Brisbin 1995; Brenton and Mellish 1996; NB Agriculture and Rural Development 1997; SAF 1997; AAFRD 1998; MEFQ 1998b; Ontario Farm Environmental Coalition 1998). Nevertheless, there are still incidents of fish kills related to the discharge of N-containing material and, more recently, concern has arisen regarding the role of nitrate in contributing to a decline in amphibian numbers (see Amphibians in Danger text box).

In Canada, 353 fish kills were reported to the National Environmental Emergencies Centre (NEEC) of Environment Canada from 1987 to 1997, of which 22 were caused by discharge of nutrient-containing materials (Table 5.7). It is important to note these incidents are initial pollution reports (unpublished data from NEEC 1998). The incidents are not verified at a later date and only the information actually recorded at the time of the first report is recorded on the pollution report sheets so there may be missing information for some incidents (C. Lau, DOE, personal communication). The nutrient-related fish kills were mainly caused by discharge of N-containing substances; only one incident was related to a phosphorus-containing material and it was likely not the P but another chemical in the product that resulted in toxicity. The majority of the reported fish kills were associated with the agricultural sector, particularly the release of manure. All but one of the fish kills associated with manufacturing and food processing industries were due to the routine discharge of ammonium or ammonia. Fish kills caused by municipal sewage discharge were largely due to equipment failure, discharges from combined sewers, or sewage treatment plant bypasses. A more thorough examination of fish kills caused by agricultural activity in Ontario documented 53 fish kills for a similar time period (1988 to 1998) and

Table 5.7. Fish kills caused by the discharge of nutrient-related material from 1987 to 1997 as reported to the National Environmental Emergencies Centre, Environment Canada, Ottawa. Note that where there has been no finding of fact by a court, the incidents set out in this table are alleged only.

Sector	Material	Number of Incidents	Province
Agriculture	manure	9	ON, MB
	nitrogen fertilizer	1	ON
Chemical Manufacture	anhydrous ammonia/ ammonium chloride	2	ON
Food Processing	ammonia	2	BC, NS
	manure	1	ON
General Manufacturing	concentrated shampoo	1	ON
Municipal Waste	sewage	6	NB, BC, ON

showed that most agriculture-related fish kills are caused by spray irrigation of liquid manure from swine operations (see the Fish Kills in Southwestern Ontario case study).

Nitrate is a less serious environmental problem. Although it can be found in relatively high concentrations in surface waters, it is relatively non-toxic to aquatic plants, benthic invertebrates and fish (Russo 1985; Table 5.2). Lethal concentrations of nitrate for a number of amphibian species range from 13 to 40 mg NO_3^--N/L with chronic effects occurring at concentrations as low as 2.5 mg NO_3^--N/L (Baker and Waights 1993; Baker and Waights 1994; Hecnar 1995; Watt and Oldham 1995).

Nitrite is not considered a severe environmental problem because, although extremely toxic to aquatic life, it does not usually occur in natural surface water systems at concentrations considered deleterious to aquatic organisms (Russo 1985; CCREM 1987).

Forest Ecosystems

Concerns about threats to forest health from air pollution led the Canadian Forest Service to establish the Acid Rain National Early Warning System (ARNEWS) in 1984. This national biomonitoring network was designed to detect early signs of the effects of acid rain on forests to enable action to forestall anticipated damage. The term 'acid rain' encompasses all forms of air pollution: wet and dry deposition of sulfates (SOx), nitrogen oxides (NOx), gaseous pollutants (ozone), and airborne particles.

The latest (1994) analysis of data from ARNEWS concluded there was no evidence of a large-scale decline in the health of Canadian forests (NRCan 1999). Tree mortality was mostly in the normal range of 1 to 2% and largely due to natural factors. Nevertheless, certain instances of forest damage were observed where pollutants might be involved. For example, in the Bay of Fundy area of New Brunswick, dieback on birch is coincident with the presence of acid fog and high levels of tropospheric ozone (Cox et al. 1996). Needle flecking has been observed on conifers in Nova Scotia and New Brunswick; its cause has yet to be verified. However, there is no evidence to suggest that elevated concentrations of nitrogen oxides or ammonia or direct deposition of N on vegetation have caused the decline of Canada's forests.

Amphibians in Danger: Are nitrate levels hampering their development?

Recently, it has become evident that amphibian populations around the world have suffered serious decline. In Canada alone, the population numbers for 17 of the country's 24 frog and toad species and 21 salamander species have declined considerably (Jacobs 1999). Because of the worldwide nature of this phenomenon, causes such as acid precipitation, increased ultraviolet radiation, global climate change, or the introduction of exotic species have been put forward as possible explanations (Wyman 1990; Blaustein et al. 1994). Changes in local conditions may, however, offer a better explanation. One possible reason for the declining numbers of amphibians in agricultural landscapes may be the death of their larvae caused by chemical pollutants such as nitrate salts (e.g., Baker and Waights 1993; Hecnar 1995; Marco and Blaustein 1999; Rouse et al. 1999). As well, some of their defining characteristics (permeable skin, dependence on aquatic habitat for reproduction, larval development, forage, or hibernation) contribute to their susceptibility to water pollution (Vitt et al. 1990).

Surface waters naturally contain < 4.4 mg NO_3^-/L; concentrations above 22 mg NO_3^-/L reflect anthropogenic contamination (OME 1981). In North America, nitrate concentrations in agricultural streams typically range between 9 and 178 mg NO_3^-/L (Goolsby et al. 1991; Keeney and Deluca 1993), but levels > 444 mg NO_3^-/L have been found (Rouse et al. 1999). In areas of intensive agriculture in Southern Ontario, nitrate concentrations commonly exceed the Canadian drinking water guideline of 45 mg/L nitrate (Hendry et al. 1983; OME 1983; CCREM 1987; Agriculture Canada 1992). In the laboratory, lethal and sublethal toxic effects are detected for many amphibian species at nitrate concentrations as low as 11-44 mg/L (see Section 5.1). If nitrate in the field acts on amphibians in a similar way to that determined in laboratory studies, then nitrate concentrations in surface waters in North America are very likely adversely affecting amphibian growth and survival (Rouse et al. 1999). Recent studies in the Great Lakes Basin have shown that nitrate concentrations in about 19% of surface water samples were high enough to cause developmental anomalies and 3% were high enough to kill amphibians in laboratory experiments (Rouse et al. 1999).

The primary anthropogenic sources of nitrogenous water contamination in agricultural areas are N-based fertilizers and animal wastes (Goolsby et al. 1991). Nitrogen-based fertilizers have been implicated in the deaths of amphibians in Denmark (Wederkinch 1988), Poland (Berger 1989), Britain (Oldham et al. 1997) and Canada (Hecnar 1995). Amphibian exposure to agriculturally derived nitrate occurs in many ways. In temperate North America, nitrate concentrations in water are usually highest from late fall to spring because of reduced nitrate assimilation by plants (Goolsby et al. 1991; Mason 1991; Rouse et al. 1999). This peak coincides with the period of adult frog hibernation, which leads to prolonged nitrate exposure for adult frogs, especially if they hibernate in water (Hecnar 1995). The type of fertilizer applied is also important. For example, ammonium nitrate fertilizer granules have toxic effects on frogs (*Rana temporaria*) at application rates of 4 to 7 g/m^2 (Oldham et al. 1997), which overlaps with the average application rate (6-9 g/m^2) in Canada (Statistics Canada 1997a; Agriculture and Agri-Food Canada 1998). Under normal application conditions, there is a low probability of an acute impact of ammonium nitrate on adult frogs as granules dissolve rapidly, thereby decreasing the exposure period. Furthermore, the frog's exposure is limited as fertilizer application generally occurs during daylight and most amphibian migration occurs during darkness (Oldham et al. 1997). However, under drier conditions, the toxic effects appear to be longer lasting (Oldham et al. 1997).

Advances in farming methods may also influence exposure to fertilizer. Recently, there has been a shift toward the spring as the peak period for fertilizer application (Chalmers et al. 1990). During this period, chemicals may runoff into ponds and ditches in snowmelt water. In the Prairies, spring application of fertilizer occurs after snowmelt has taken place, so fall applications are more susceptible to enter watercourses via snowmelt (B. Todd, Manitoba Agriculture and Food, personal communication). As frogs commonly migrate over agricultural land to breeding sites during spring, the risk of exposure increases if this movement coincides with a recent fertilizer application (Oldham et al. 1997). This time period also corresponds with an amphibian's vulnerable egg and tadpole stages (Hecnar 1995).

Although nitrate pollution is widespread, the toxicity of this compound is not the only cause of amphibian decline in North America. Other suggested causes include ultraviolet irradiation; genetic mutation; microbes; parasites and other diseases; and other environmental contaminants (Schmidt 1997; Kaiser 1999; Sessions et al. 1999). Nevertheless, water quality data from Canadian agricultural and urban areas suggest that concentrations of nitrate in surface waters exceed toxic levels for amphibians for extended periods of time and during sensitive periods of egg and tadpole development (Rouse et al. 1999).

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Ground Water

Approximately 26% of Canadians rely on ground water for their domestic water supply (Table 5.9). In 1981, this percentage varied from less than 1% in the Northwest Territories to 100% for Prince Edward Island. Ninety percent or more of rural domestic water supplies came from ground water in all provinces except British Columbia and the Yukon and Northwest Territories. Assuming 26% of today's population of more than 30 million people rely on ground water, about 8 million Canadians use it for their drinking water supply. Ground water is also expected to provide a significant percentage of livestock drinking-water supplies in Canada.

Under natural conditions, nitrate-N concentrations in ground water are usually less than 3 mg/L; values greater than this are usually associated with human activity (Henry and Meneley 1993; Carmichael et al. 1995). However, nitrate-N values up to 250 mg/L have been documented in semi-arid regions of Alberta, Saskatchewan, Manitoba and the western United States (Rodvang et al. 1998).

Nitrate occurs in ground water that does not receive a large amount of recharge from precipitation, as indicated by a lack of tritium and high concentrations of dissolved solids. Naturally high nitrate concentrations require the presence of a thick, oxidized, fine-textured deposit where ground water flow has not been sufficient to flush the nitrate (Rodvang et al. 1998). The naturally high concentrations may also be found in areas where native prairie grassland has been tilled. On the Canadian Prairies, the native grassland that formed the basis of the Chernozemic soils was a nitrogen-conserving system and thus, under the natural grass condition, nitrate was seldom present in ground waters of this region. However, when the land was tilled, this nitrate was released from the fibrous root system of the native prairie plants and leached to the ground water (Henry and Meneley 1993). Although nitrate was released as a result of tillage, this nitrate source is still considered to be natural because the nitrate was produced by natural mineralization in the soil.

Nitrate is the most detected ground water pollutant for which a federal drinking water guideline exists (Keeney 1987). Regional surveys of nitrate contamination in ground water wells in Canada indicate that from 1.5 to more than 60% have nitrate-N concentrations greater than the acceptable drinking-water concentration of 10 mg/L nitrate-N (Table 5.10). Aquifers particularly vulnerable to nitrate contamination are shallow (< 30 m below the Earth's surface) and/or situated in very sandy, porous soils (Figure 5.1). Many of these are located in the prime agricultural regions of Canada.

Aquifers in south-coastal British Columbia underlie areas of high rainfall and intensive agriculture and often contain nitrate levels above the safe limit (Table 5.10). Application of manure from intensive livestock operations and extensive fertilization of fruit and vegetable crops are the main contributors of nitrate, although septic systems are also suspected of contributing to the loading in shallow highly permeable aquifers in the region. Sampling of ground water quality for 192 community wells and 75 selected private wells in the Fraser Valley showed that 23 wells (9.6%) had nitrate-N values in excess of 10 mg/L during winter (November 1992 to February 1993) compared to 25 wells (10.1%) during summer1993 (Carmichael et al. 1995). Nearly all of the exceedances occurred in three heavily developed, highly vulnerable, unconfined sand and gravel aquifers: the Abbotsford/Sumas, Hopington and Langley/Brookswood aquifers (see the Abbotsford Aquifer Case Study). Highly vulnerable aquifers are a source of water supply to 50% of the community wells in the study area. During summer 1993, 21 of the 25 wells with nitrate-N over 10 mg/L were private wells.

Agricultural Water Pollution: Fish Kills in Southwestern Ontario

Natural watercourses and drainage ditches are abundant in the fertile ecoregion of the Mixedwood Plains ecozone of southern Ontario. Each year, manure spills occur in this region threatening the health of streams, rivers and lakes by killing fish and other aquatic life, degrading water quality and altering fish habitat. In addition, excessive levels of bacteria have led to concerns about the quality of recreational areas (beach closings) and the use of water by downstream farmers for livestock watering. To assess the impact of agriculture on watercourses in Ontario, data were compiled through the federally-initiated Livestock Manure Pollution Prevention Project on date of manure spill; township and county of spill; fish kill (yes or no); type of operation; route to water; and equipment used.

Data collected between 1988 and 1998 showed that 274 manure spills were reported in Ontario, ranging from 17 to 35 spills per year. Fifty-three of these spills resulted in fish kills, caused either by ammonia toxicity, high inputs of oxygen-demanding material, or both. Of the 274 manure spills, 214 occurred in Southwestern Ontario, particularly in counties with the largest number of intensive livestock operations, and resulted in 42 fish kills (Table 5.8). The majority of spills resulted from spray irrigation of liquid manure from swine operations (41% of all manure spills in Southwestern Ontario), followed by inadequately sized storage facilities (16%) and equipment failure (15%). Most (59%) of the manure spills entered the stream through a tile drain.

Table 5.8. Manure spill data for Southwestern Ontario, 1988 to 1998.

Year	Number of Spills	Fish Kills	Route to Stream		How Spill Occurred				
			Field Tile	Overland Runoff	Storage Related	Irrigation Applied	Tanker Applied	Equipment Failure	Transport Related
1988	23	6	21	2	1	21	2	2	2
1989	19	2	8	4	3	6	1	6	1
1990	29	2	22	5	3	15	5	3	2
1991	21	1	12	4	2	10	3	5	2
1992	22	4	11	6	1	11	2	3	2
1993	14	3	9	3	4	4	-	-	-
1994	15	2	9	3	3	2	3	4	2
1995	15	6	5	3	3	2		3	2
1996	20	4	7	2	-	3	2	4	3
1997	23	7	14	12	10	7	4	-	-
1998	13	5	9	4	4	6	2	1	1
Total	**214**	**42**	**127**	**50**	**34**	**87**	**24**	**31**	**17**

Overall the data indicate very few spills were truly accidental. Although causes were related to manure storage, spreading or transportation, the fundamental reason spills occurred was mismanagement. Most of the spills might have been prevented had existing best management practices been implemented, for example:

- where excessive manure was applied, split applications and weather and soil conditions should have been considered;
- if adequate storage capacity had been available in instances where storage facilities overflowed, there may have been enough flexibility to consider weather and soil conditions during application; and
- where equipment failure was a factor, proper maintenance and monitoring of equipment may have prevented spills.

Additional effort is needed to ensure that environmental farm planning, nutrient management planning, manure management planning and contingency planning are in place to minimize manure spills and their environmental impacts.

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Table 5.9. Population dependent on ground water in Canada. 1981 data from Hess (1986); 1997 data from Federal-Provincial Subcommittee on Drinking Water (M. Giddings, HC, personal communication).

	1981		1997	
	No. of people dependent on ground water	**Percentage of population**	**No. of people dependent on ground water**	**Percentage of population**
Rural population	4 010 000	17	3 million	11
Municipal population	2 210 000	9	3 to 4.8 million	11 to 17.6
Total	6 220 000	26	6 to 7.8 million	22 to 29

The higher number of exceedances for private wells is likely due to the fact that private wells are biased toward high-risk areas and many are located in rural areas where agriculture is more intensive and residents rely on septic systems. Significant nitrate leaching into ground water may also occur in an aquifer near the town of Osoyoos in the lower Okanagan Valley. About 17% of the wells sampled in 1986 and 33% of those sampled in 1987 contained nitrate-N levels higher than 10 mg/L. Fertilizer used in intensive, irrigated tree-fruit production is likely the main source of the nitrate (Reynolds et al. 1995).

The Prairies are considered to have a low risk of contamination due to a dry climate, the generally clayey texture of the soils, and a low cropping intensity. However, there are exceptions, particularly on irrigated lands and those areas near intensive livestock operations that receive large amounts of animal manure. Nitrates due to natural causes have also been reported at concentrations up to several hundred mg/L but these occurrences are highly localized and near the water table (Hendry et al. 1984; Rodvang et al. 1998). In Alberta, about 4 to 6% of domestic wells have nitrate-N > 10 mg/L (Henry and Meneley 1993; Fitzgerald et al. 1997). The low percentage of contaminated Alberta wells may be due to the fact that there are fewer shallow, saturated, surface aquifers in Alberta and that, in many parts of the province, bedrock aquifers are the preferred water source because of their softer water. In Saskatchewan, nitrates exceeded the 10 mg/L nitrate-N guideline in 66 of 184 samples (36%) from private domestic wells in shallow unconfined aquifers (Vogelsang and Kent 1997; Table 5.10). Most of the wells with nitrate exceedances had cattle and/or poultry operations near the well.

Although the results may represent a worst cast scenario for ground water quality in the province, the unconfined aquifers are important because they supply approximately 60% of all farm water supplies in Saskatchewan (Vogelsang and Kent 1997). Data from the Saskatchewan Research Council database and the University of Saskatchewan suggest that from 7 to 17% (Table 5.10) of wells may contain nitrate-N > 10 mg/L (Henry and Meneley 1993). In Manitoba, monitoring conducted by the Department of Natural Resources shows that non-point source contamination of ground water is not significant although a small but measurable increase in nitrate concentration (< 3 mg/L nitrate-N) was detected in portions of the Assiniboine Delta Aquifer where irrigated agriculture is practiced (Henry and Meneley 1993).

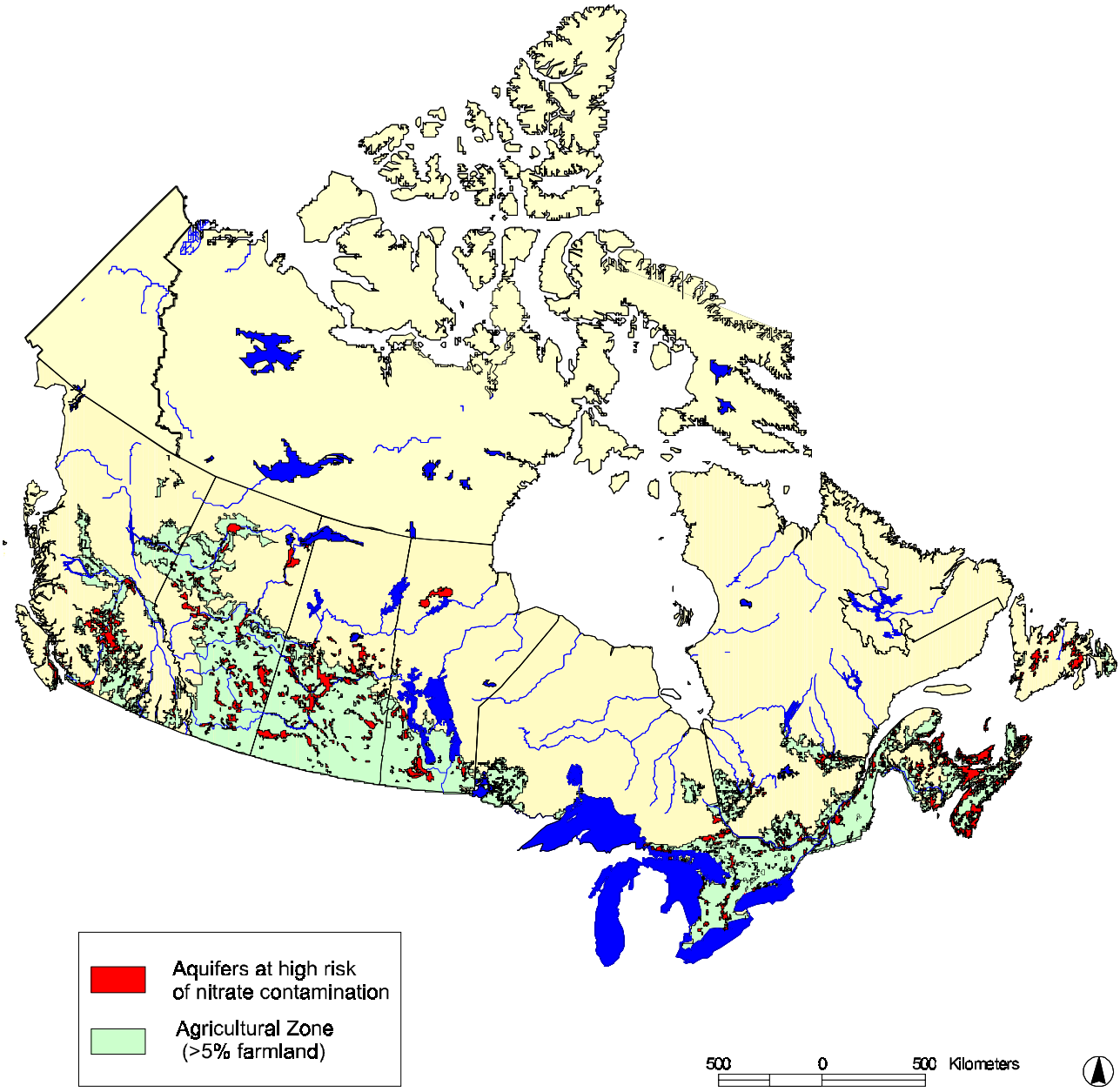

Figure 5.1. Canadian aquifers vulnerable to nitrate contamination as identified by Agriculture Canada, 1989 (AC 1989). Vulnerable aquifers are shallow (<30 m below the surface) and/or situated in very sandy, porous soils. Agricultural zone after Smith and McRae (2000).

Table 5.10. Summary of nitrates in rural wells in Canada.

Source of data	No of wells sampled	NO_3-N >10 mg/L (%)	Reference
BRITISH COLUMBIA			
Lower Fraser Valley, Winter 1992-93	240	9.6	Carmichael et al. 1995
Lower Fraser Valley, Summer 1993	238	10.1	Carmichael et al. 1995
ALBERTA			
Alberta Agriculture Database	1425	4.8	Henry and Meneley 1993
Environmental Centre Database	12342	4.3	Henry and Meneley 1993
Alberta Environment Database	1692	3.3	Henry and Meneley 1993
Farmstead Water Quality Survey	813	5.7	Fitzgerald et al. 1997
SASKATCHEWAN			
Saskatchewan Research Council Database	1968	7.2	Henry and Meneley 1993
Soil Salinity Studies	1484	17.0	Henry and Meneley 1993
Shallow Ground Water Quality Survey	184	35.9	Vogelsang and Kent 1997
MANITOBA			
Interlake Carbonate Aquifer	119	1.7	Betcher 1997
Odanah Shale Aquifer	98	19.4	Betcher 1997
Assiniboine Delta Aquifer	29	3.5	Buth et al. 1992
ONTARIO			
Ontario Farm Well Survey, Winter 1991-92	1212	12.8	Goss et al. 1998
Ontario Farm Well Survey, Summer 1992	1212	14.3	Goss et al. 1998
QUÉBEC			
Portneuf	70	41.4	Paradis et al. 1991
Potato Growing Regions	33	63.6	Giroux 1995
Portneuf	26	34.6	Paradis 1997
Montérégie	150	2.0	Gaudreau and Mercier 1997
Orléans Island	87	4.6	Chartrand et al. 1999
NEW BRUNSWICK			
Carleton County	300	11 – 18.2	Ecobichon et al. 1996
Victoria and Madawaska	300	14.5 – 26.1	Ecobichon et al. 1996
NOVA SCOTIA			
Kings County	237	13.0	Briggins and Moerman 1995
PRINCE EDWARD ISLAND			
Water Well Database	2216	1.5	Somers 1998
Dairy Farm Well Survey	146	44.0	Van Leeuwen 1998

In the Odanah Shale Aquifer, however, 19 of 98 samples had nitrate-N above 10 mg/L even though tritium was undetectable in some of the samples indicating naturally-occurring nitrate may be contributing in part to high nitrate concentrations (Betcher 1997).

Nitrate from fertilizer and manure application is a significant contaminant of ground water in Ontario and Québec. A survey of 1 212 domestic farm wells found that 12.8% contained nitrate-N concentrations > 10 mg/L during winter 1991/92 and 14.3% during summer 1992 (Agriculture Canada

1993; Goss et al. 1998; Rudolph et al. 1998) (Table 5.10). Although nitrate contamination in Ontario surveys was not related to any particular land use practice, correlation between contamination of domestic water wells and beneath nearby fields indicates widespread contamination in the aquifer. Application of fertilizer and manure to fields rather than point source contamination from barnyards and septic systems appears to be the source of the nitrate.

Nitrate contamination of ground water in Québec is associated with areas of intensive potato production. Of 70 wells sampled in potato growing areas in Portneuf between 1990 and 1991, average nitrate-N concentrations exceeded the guideline of 10 mg/L for 29 wells (41.4%) (Paradis et al. 1991). A similar study found 21 of 33 domestic wells (63.6%) in potato growing regions of Québec had nitrate-N concentrations > 10 mg/L (Giroux 1995). Investigations in other agricultural and non-agricultural areas found that only three of 150 wells in the Montérégie and four of 87 wells on Ile d'Orléans east of Québec City exceeded the nitrate guideline for drinking water (Gaudreau and Mercier 1997; Chartrand et al. 1999).

In the Atlantic Provinces, ground water contamination occurs mainly in areas where potatoes and corn are produced intensively. Leaching of nitrate can be a serious problem, although levels seem to be steady. Studies of well water quality in agricultural regions of the Saint John River valley, NB (Carleton County, Victoria and Madawaska) in the mid-1980s showed that, depending upon sampling month, up to 22% of wells were contaminated (Table 5.10). There was no correlation of nitrate contamination with distance from fields where fertilizers were used, suggesting that the contamination of ground water was widespread. The presence of nitrate appeared to be closely associated with farming activity as reflected by increasing ground water nitrate concentrations throughout the growing season, particularly following periods of heavy rainfall. Poor well construction also contributed to contamination problems (Ecobichon et al. 1996). In Nova Scotia, nitrate-N concentrations exceeded 10 mg/L for 13% of 237 wells sampled in Kings County, a highly productive agricultural area located at the eastern end of the Annapolis Valley. Point-source contamination from poor well construction and/or maintenance was the primary factor causing contamination (Briggins and Moerman 1995). In Prince Edward Island, 1.5% of 2 216 drinking-water wells had nitrate-N concentrations greater than the 10 mg/L (Somers 1998). A 1997 survey of water samples from 146 dairy farms showed that 44% of the water samples were above 10 mg/L nitrate-N (VanLeeuwen 1998). Monthly monitoring in a suite of 54 wells between 1988 and 1991 indicated that, on average, background concentrations of nitrate from "pristine sites" were three times lower than the average value for the total sample population and four times lower than for samples collected from sites in agricultural areas. Elevated nitrate concentrations were also found in subdivisions with on-site sewage disposal (Somers 1998).

Data on long term changes for nitrate concentrations in ground water in Canada are sparse. A study of ground water age and nitrate distribution within the glacial aquifer used for water supply for the cities of Kitchener-Waterloo showed elevated nitrate concentrations post-1960s, which corresponded to the period when fertilizer application rates increased and corn became a major crop in southern Ontario (Johnston et al. 1998). Of 179 sites with ground water quality monitoring for at least four and up to 25 years in PEI, 33 or 18% of the sites showed increasing nitrate levels, 26.2 or 12%, had decreasing nitrate levels and 124 showed no trend. These results suggest that nitrate levels could reach a steady state for particular types and duration of land use (Somers 1998). Similar experience in Québec indicates that low cost improvements to management practices may lower these levels but levels may

Abbotsford Aquifer

The Abbotsford-Sumas aquifer is an unconfined, sand and gravel aquifer located southwest of Abbotsford in southwestern British Columbia and northwestern Washington state. It is the largest unconfined aquifer in the Lower Fraser Valley and has an areal extent of approximately 100 km^2 in British Columbia and about 100 km^2 in Washington. The sand and gravel is covered at the surface by a thin soil horizon developed in medium textured eolian deposits.

Elevated nitrate concentrations are observed over a wide area of the aquifer (Liebscher et al. 1992; Carmichael et al. 1995; Zebarth et al. 1998). Using an interpolative technique and ground water nitrate concentrations measured in piezometers and domestic wells in February and August 1993, Wassenaar (1995) estimated that nitrate-N concentrations exceeded 10 mg/L in as much as 80% of the aquifer.

Land use above the aquifer is primarily agricultural with intensive raspberry farming and poultry production accounting for much of the agricultural activity. Urban development is spreading over parts of the aquifer; about 20% of the aquifer surface is covered by urban development (Liebscher et al. 1992). Homes outside municipal limits use septic systems for waste disposal. In many places the septic system tile fields have been installed below the soils in the permeable sand and gravel.

Agricultural production, particularly intensive poultry production, has been suggested as a major contributor to the elevated nitrate concentrations. Poultry manure is frequently applied to raspberry fields as a fertilizer and to enhance the organic content of the soils. Using ^{15}N and ^{18}O isotopes, Wassenaar (1995) concluded that nitrate in the aquifer originated primarily from poultry manure. Fertilizer did not appear to be a significant contributor. Wassenaar (1995) also noted that although nitrate derived from human wastes in domestic septic systems would be indistinguishable isotopically from animal wastes, less than 10% of the nitrate in the aquifer could be derived from septic systems based on the estimated density of septic systems above the aquifer.

Zebarth et al. (1998) used a N balance to estimate N loading from agricultural production to the Abbotsford aquifer. The nutrient balance calculation estimated a surplus of N additions over N removals from the root zone of 245 kg N/ha in 1991. If the same animal and crop management system is used over a long time period and soil N processes reach equilibrium, actual leaching losses should approach the calculated N surplus. Average annual precipitation in the Abbotsford area is about 1500 mm/yr. Approximately 70% of the total precipitation occurs during the October to March period when N removal by crops is low. Because recharge to the aquifer is primarily by infiltration of precipitation, the primary source of elevated nitrate concentrations in the aquifer is likely due to leaching of surplus N from agricultural land over the aquifer.

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still remain above the drinking water guideline for nitrate unless the land use practice is abolished or more costly management practices are adopted.

Excessive nitrate in ground water was first identified as a health problem when methaemoglobinaemia in an infant was associated with high nitrate well water in Iowa (Comly 1945). Cases from Saskatchewan (Goluboff 1948; Robertson and Riddell 1949), Manitoba and Ontario (Medovy et al. 1947) were subsequently reported in Canada. Worldwide, some 200 cases of infant-methaemoglobinaemia were reported between 1945 and 1970 (Shuval and Gruener 1972). Despite pockets of elevated nitrate concentrations in ground water drinking supplies across Canada, fatalities caused by methaemoglobinaemia are rare (A. Huegenholtz, HC, personal communication). Toxic effects caused by elevated nitrate concentrations are rare because drinking water quality guidelines incorporate a margin of safety, only a small segment of the human population (i.e., infants less than

three months old) is most sensitive to nitrate toxicity, and readily available testing methods can detect and prevent problems before they occur. In Canada, households using a ground water drinking source are advised to use bottled water for infants and young children.

Although not common, nitrate poisoning of cattle from well water has been reported in Saskatchewan (Campbell et al. 1954). Sublethal responses resulting in poor animal performance and a general lack of health may not always be recognized, however. The more serious cases of ground water contamination may result in a loss of livestock water supply in rural areas when nitrate-N exceeds the drinking water guideline of 100 mg/L for livestock. The economic costs to replace the contaminated ground water supply may be significant.

5.3. Indirect Toxic Effects

Toxic Algal Blooms

In addition to the direct effects of N on human health, nutrients, especially N, can stimulate growth of toxic species of phytoplankton in both fresh and coastal waters. All phytoplankton species, toxic or not, increase in abundance as a result of nutrient addition however blooms of toxic species are usually most noticed and reported. Consumption of the algal species that produce toxins or the organisms that accumulate algal toxins (e.g., shellfish) can affect the health of terrestrial animals, including humans. Nutrient inputs to inland and coastal waters have increased markedly in recent decades, resulting in changes in phytoplankton growth rates and species dominance (Carmichael et al. 1985; GESAMP 1990; Schramm and Nienhuis 1996; Heinze 1999). Although terrestrial inputs of N (e.g., from runoff and rivers) are the most obvious source of N to oceans, atmospheric deposition is a growing source of N to the North Atlantic Ocean and has been linked to apparent increases in the frequency of harmful algal blooms (Paerl and Whitall 1999).

In marine waters, the most common toxic algae are species of the dinoflagellate family. When certain species of dinoflagellates are present in great abundance a red colouration to the water may be seen, a phenomenon known as “red tide”. Certain dinoflagellate species produce toxins that accumulate in shellfish. Although the shellfish are only marginally affected by the toxins, a single clam can accumulate enough toxin to kill a human (Anderson 1994). The first reported “red tide” causing shellfish toxicity in Canada occurred in April 1933 in Nanaimo, British Columbia (Clemens 1935). However, there was no reported correlation between nutrient inputs and shellfish toxicity in this case. Similarly, Prakash and Taylor (1966) reported a fatal outbreak of shellfish toxicity in the Strait of Georgia in British Columbia in June 1965 from cockles. The shellfish toxins originated from a bloom of *Gonyaulax acatenalla* triggered by high vitamin B_{12} levels in an inlet. Despite the lack of relationship between nutrients and blooms in these early cases, there is now evidence of a link between increased nutrients and the density and scale of a bloom, if not the occurrence of a particular algal species (GESAMP 1990; Schramm and Nienhuis 1996) [see Contaminated Mussel case study].

As a result of concern about shellfish contamination from algae and other sources, the federal government of Canada developed the Canadian Shellfish Sanitation Program and the Canadian Shellfish Water Quality Protection Program. Their main aims are to ensure that growing areas for all bivalve molluscan shellfish (e.g., clams, mussels, oysters, whole and roe-on scallops, and other bivalve molluscs) meet approved federal water quality criteria, that pollution sources to these areas are identified, and that all shellfish sold commercially are harvested, transported and processed in an

approved manner. Shellfish are routinely tested for phytoplankton toxins that could be a serious threat to human health (Todd 1990; A. Menon, DOE, personal communication).

In fresh water, the algae causing toxic blooms are usually the blue-green algae. In temperate climates like Canada, such blooms usually occur in late summer and early autumn. Although the causes of bloom formation are not fully understood, conditions that appear to favour blue-green algae blooms include: high concentrations of nitrate, ammonia, and/or phosphate; low irradiance; water temperatures between 15 and 30 °C; and pH between 6 and 9 or higher (Skulberg et al. 1984; Reesom et al. 1994). Bloom development is initiated when warm, stratified surface water low in N undergoes P enrichment (Granéli et al. 1990; Nascimento and Azevedo 1999). Many blue-green algae species are capable of fixing atmospheric nitrogen gas in their cells; hence, in waters with low N concentrations, blue-green algae often out compete other algae and become the dominant taxa.

Not all species of blue-green algae form blooms or produce toxins (Carmichael 1994; Health Canada 1998a). Moreover, blue-green algae that can generate toxins do not always do so. The factors controlling toxin production are poorly understood and many of the factors identified as important in the formation of blue-green algal blooms (e.g., nutrient concentrations, water temperature, light, wind etc.) also appear to be important in toxin production (Carmichael 1986; Wicks and Theil 1990; Kotak et al. 1993a; Chorus and Bartram 1999). Once a bloom occurs, its toxicity can vary over time despite stable environmental conditions. In some cases, blue-green algal scum can remain toxic even after drying out on shorelines.

Blue-green algal toxins are either neurotoxic (i.e., affect the nervous system) or hepatotoxic (i.e., affect the liver) (Kotak et al. 1993b). They are released from the algae when the cells age and lyse (Carmichael 1994). Clinical signs of blue-green algal poisoning include nervous derangement, staggering, tremors and severe abdominal pain (Chorus and Bartram 1999). Ingestion of water containing blue-green algal neurotoxins can lead to death of animals in as little as five minutes. Death results from respiratory arrest due to either neuromuscular blockade (Carmichael et al. 1975) or inhibition of cholinesterase (Cook et al. 1989) (Table 5.11). Two genera of blue-green algae are responsible for the majority of neurotoxin-related poisonings: *Anabaena* and *Aphanizomenon*. In contrast to neurotoxins, blue-green algal hepatotoxins are very stable; death results from destruction of the liver and, ultimately, haemorrhagic shock (Kotak et al. 1993a, b) (Table 5.11). Hepatotoxins result in more cases of animal poisoning, both worldwide and in Canada (Kotak et al. 1995). The most common hepatotoxins are microcystins, produced by many species of blue-green algae including *Microcystis*, the species from which the toxin was first identified.

Blue-green algal toxins can cause illness or death in almost any mammal, bird, or fish which ingests enough of the toxic cells or extracellular toxins (Grant and Hughes 1953; Ingram and Prescott 1954; Gorham 1964; Schwimmer and Schwimmer 1968; Carmichael et al. 1985; Codd et al. 1992; Carmichael 1994). The amount of blue-green algal-tainted water needed to kill an animal depends on such factors as the type and amount of toxin produced by the cells, the concentration of the cells, as well as the species, size, sex, and age of the animal (Carmichael 1994; Chorus and Bartram 1999). Globally, only one confirmed case of human death has been attributed to cyanobacteria (Jochimsen et al. 1998). In 1996, 101 of 124 patients in Brazil who underwent dialysis with water containing blue-green algae toxins (microcystins) had acute liver injury and fifty died. The low incidence of human

Contaminated Mussels- A Prince Edward Island tragedy

In November and December 1987, 105 acute cases of human poisoning and three deaths resulted from the consumption of contaminated blue mussels (*Mytilus edulis* L.) from Prince Edward Island (Bates et al. 1989). Although Canadian authorities had dealt with shellfish poisoning for decades (e.g., Clemens 1935), symptoms were unfamiliar and disturbing. In addition to abdominal cramps, vomiting, diarrhoea and disorientation, some patients exhibited permanent short-term memory loss (Anderson 1994). Fittingly, this new outbreak of shellfish poisoning was termed amnesic shellfish poisoning.

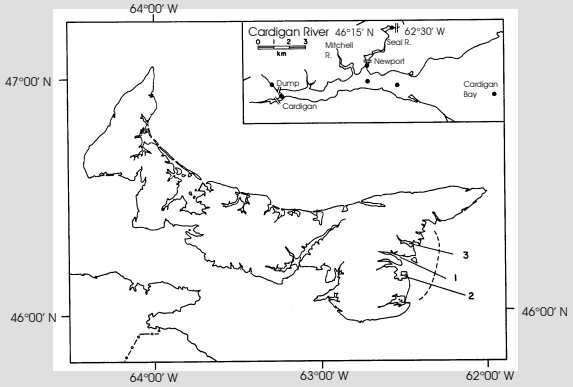

Figure 5.2. Sites where high concentrations of both *Nitszchia pungens* and domoic acid were found in Prince Edward Island in 1987. (1) Cardigan River; (2) St. Marys River; (3) Boughten River. Adapted from Bates et al. (1988).

Commercial mussel cultivation has operated since 1975 on Prince Edward Island in sheltered small river estuaries where salinity is 23-29 parts per thousand and water temperatures vary from -2°C in January to 23°C in late summer (Todd 1990). The origin of the 1987 shellfish poisoning outbreak was traced to the Cardigan River in eastern PEI (Environment Canada and PEI DOE 1990; Figure 5.2). Agriculture forms a major part of PEI's economy and the Cardigan River watershed is no exception. The main farming activities centre on production of livestock (dairy and beef cattle, hogs and foxes); tobacco; grains; potatoes; and food for livestock. As well, an aquaculture site exists on the Seal River, a tributary of the Cardigan River, and there are two land-based finfish aquaculture operations in the watershed (Environment Canada and PEI DOE 1990).

In any outbreak of food poisoning, establishing the poisonous food component and its source is often a difficult task. In mid December 1987, the toxin was identified as domoic acid (Wright et al. 1989). Mussels containing measurable levels of domoic acid were found to be restricted to the Cardigan Bay region (Bates et al. 1991). Domoic acid was not detected in mussels collected in summer and autumn of 1987; however, appreciable amounts were present in mussels collected from the affected area in mid to late October 1987 and reached maximum values in November to December 1987 (Bates et al. 1988; 1991 Figure 5.3). The total amount of domoic acid in all cultivated mussels in the affected area during the 1987 incident was estimated to be 6 kg (Wright et al. 1989). The only obvious potential source of this much domoic acid was the intense bloom of phytoplankton, dominated by the pennate diatom *Nitszchia pungens* (Bates et al. 1988; 1991; Figure 5.3). In mid December 1987, when first sampled, the bloom extended about 10 km seaward from the mouth of the Cardigan River.

The factors influencing the production of domoic acid by *N. pungens* are still not well understood. *N. pungens*, grown in culture, has been shown to produce and release domoic acid with N in the growth medium being essential for domoic acid production (Bates et al. 1991). Subsequently, the abundance of *N. pungens* was correlated with nitrate concentrations in the Cardigan River (Smith et al. 1990). When nitrate was not detected in the water column there was no *N. pungens* bloom. However, when nitrate concentrations in the water column exceeded 1.1 µg/L, a bloom of *N. pungens* developed. The mechanisms producing pulses of nitrate in the Cardigan River estuary were less clear. Rain or wind velocity or both appeared to be involved (Smith et al. 1990), with the most likely nitrate source being freshwater runoff from the agricultural land in the Cardigan

Contaminated Mussels concluded

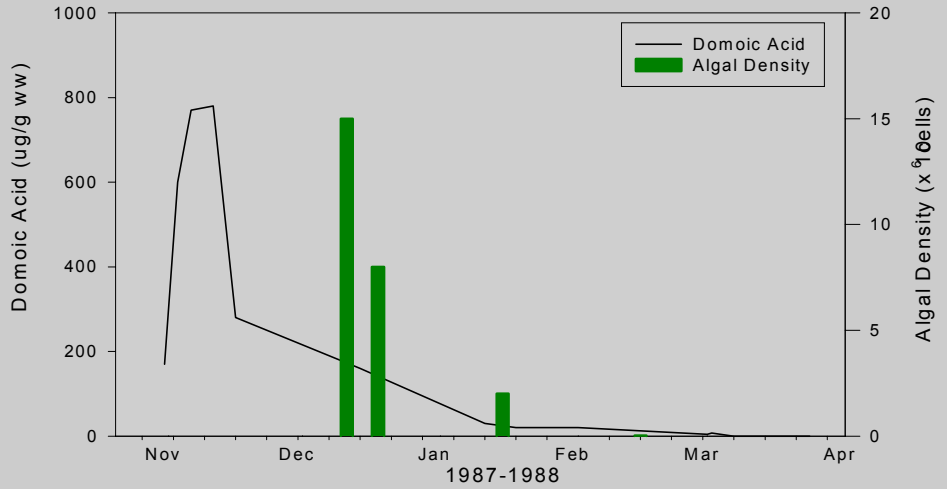

Figure 5.3. The concentration of domoic acid in the tissue of blue mussels (*Mytilus edulis* L.) from Cardigan Bay, PEI, and the density of the pennate diatom *Nitszchia pungens* during the period of mussel toxicity in 1987-1988. Adapted from Bates et al. 1989.

River basin following a rainstorm. Other hypotheses put forward were the suspension of bottom sediments by strong winds and associated release of nitrate, or upwelling of deep nutrient-rich waters. However, bottom sediments are an unlikely source of nitrates and, in the case of upwelling, there was no relationship between the direction of the wind and the timing of the nitrate pulses, nor was there any indication of intrusion of cold deep water. It is therefore thought that the massive bloom of *N. pungens* was the result of a long dry summer followed by nitrate runoff during an intensely wet (but sunny) fall. (J. Wright, DOE, personal communication).

This shellfish incident was unique in Canada in that it was the first known occurrence of human intoxication due to ingestion of domoic acid. As a result shellfish containing more than 20 μg domoic acid/ g shellfish meat is considered unsafe for human consumption (Hallegraeff 1993). The 1987 shellfish incident was a serious threat to the molluscan aquaculture industry in PEI. The establishment of a monitoring program by Department of Fisheries and Oceans and Department of Environment, however, has provided an early warning of toxic events that have protected the industry and consumers of shellfish since 1988.

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death from cyanobacterial toxins is due not to greater resistance but to an aversion by humans to drink water with algal scum (Health Canada 1998b). Serious human poisonings have, however, occurred after accidental ingestion of toxic cells and water during recreational activities (Kotak et al. 1993b; Lambert et al. 1994; Health Canada 1998b). Blue-green algal toxins can also cause gastroenteritis and contact irritation in users (Billings 1981). Lifetime consumption of water containing low concentrations of blue-green algal toxins may also contribute to the development of cancer (Falconer et al. 1983; Carmichael 1989; Falconer and Buckley 1989; Falconer 1991; Carmichael 1994). Research is currently underway to investigate this link.

Table 5.11. Acute and chronic effects of cyanobacterial toxins on terrestrial organisms (IP = administered by intraperitoneal injection; Oral = administered orally; SD = single dose; LD_{50} = the dose of toxins at which 50% of the test organisms died); EC = the effective concentration; NOEC = No observed effect concentration, and MDL_{100} = the minimum lethal dose.

Organism	Endpoint	Conc. (mg/kg)	Exposure period	Comments	Reference
***Microcystis aeruginosa* - toxic isolate-UV-006**					
Mouse	LD_{50}	32570	SD	pH= 6.5; IP	Eloff & Van Der Westhuizen 1981
Mouse	LD_{50}	51470	SD	pH= 8.0; IP	Eloff & Van Der Westhuizen 1981
Mouse	LD_{50}	36200	SD	pH= 10.5; IP	Eloff & Van Der Westhuizen 1981
***Microcystis aeruginosa* - extract of unidentified toxins**					
Mouse	MLD_{100}	40000	60 min.	IP	Østensvik and Skulberg 1981
Mouse	LD_{50}	9100	--	IP	Falconer 1991
Mouse	LD_{50}	28500	24 h	IP	Falconer et al. 1994
Mouse	LD_{50}	1700	--	IP	Falconer et al. 1988
Mouse	LD_{50}	9000	23 day	IP	Falconer et al. 1988
Mouse	LD_{50}	560000	23 day	Oral	Falconer et al. 1988
Mouse	LD_{50}	656000	--	Oral	Falconer 1991
Mouse	LD_{50}	2.6g/kg/d	21 day	Oral	Falconer et al. 1994
Pig	Liver function tests	796.0 & 1312.0 µg toxin/kg/d	71 day	Oral	Falconer et al. 1994
Rat	MLD_{100}	40000.0	3-4 h	IP	Østensvik and Skulberg 1981
***Microcystis aeruginosa* - hepatotoxin- toxic peptide**					
Mouse	LD_{50}	56.0	SD	IP	Runnegar and Falconer 1981
***Microcystis aeruginosa* - microcystin-LR**					
Amphibians (Axolotl; Smooth newt; Marsh Frog)	NOEC (survival); feeding; weight; limb development;	5 and 50 µg/L	SD	Oral to larvae	Oberemm et al. 1999
Fish (Zebra fish; Rainbow trout; Chub)	survival rates; timing of hatching; growth rate; and body length	50 µg/L	SD	Oral to embryos. No acute toxic effects	Oberemm et al. 1999
Mouse	mortality; body weight; alterations in food/water intake	35 µg/mouse	20 µg/L daily for 18 mon.	Oral. No chronic toxicity or accumulation of toxin	Ueno et al. 1999
Mouse	LD_{50}	50.0	SD: 25, 50, or 100 µg/kg toxin	IP	Kotak et al. 1993a
Mouse	LD_{50}	50.0	SD	IP	Carmichael 1988
Mouse	LD_{50}	50.0	SD	IP	Stoner et al. 1989
Mouse	LD_{50}	50.0	SD	IP	Fawell et al. 1994
Mouse	LD_{50}	70.0-120.0	SD	IP	Carmichael 1988
Mouse	LD_{50}	125.0	SD	IP	Brooks and Codd 1987
Mouse	LD_{50}	67.0	SD: 33, 50, 75, or 113 µg/kg toxin	IP	Tsujl et al. 1994
Mouse	LD_{50}	90.0	SD: 40, 60, 90, 135, or 203 µg/kg toxin	IP	Tsujl et al. 1994
Mouse	LD_{50}	5000.0	SD	Oral	Fawell et al. 1994

Table 5.11. Acute and chronic effects of cyanobacterial toxins on terrestrial organisms (concluded).

Organism	Endpoint	Conc. (mg/kg)	Exposure period	Comments	Reference
Rat	body weight; organ weight	50-150 µg/kg/d	28 d	Oral in drinking water	Heinze 1999
***Anabaena flos-aquae* - extract of unidentified toxins**					
Mouse	LD_{50}	26000.0	SD	IP	Falconer 1991
Mouse	LD_{50}	37.5	2 hour	IP	Cook et al. 1989
***Anabaena flos-aquae* - anatoxin-a**					
Mouse	LD_{50}	200	SD	IP	Carmichael 1988
Fish	heart rate	400 µg/L	SD	Oral to embryos. Effects were temporary; no chronic effects	Oberemm et al. 1999
Mouse	LD_{50}	50	SD	IP	Carmichael 1988
***Aphanizomenon flos-aquae* – aphanatoxin**					
mouse	LD_{50}	10	SD	IP	Carmichael 1988

Toxic blue-green algae have been found in lakes in Alberta, Saskatchewan, Manitoba, and Ontario (Gorham 1964; Carmichael et al. 1985; CCREM 1987; Jones et al. 1998). In one Saskatchewan lake, several calves were believed to be poisoned by algae (*Anabaena*) in 1965 and more than twenty dogs between 1960 and 1970 (Carmichael and Gorham 1978; Mahmood and Carmichael 1987). In June 1962, incidents of horse and cow poisonings from toxic algae occurred in Alberta (Gorham 1964). In Manitoba, animal deaths (1 horse; 9 dogs) from algae ingestion have been reported as early as 1951 (McLeod and Bondar 1952). More recent surveys for Microcystin toxins in several provinces showed that during summer 1998, only 28 of 279 raw water samples collected from various surface water sources (lakes, rivers, dugouts, etc.) tested positive (0.3 µg/L to 14 µg/L) using the protein phosphatase method for toxin analysis (Giddings 2000). In 1999, 27 of 92 samples collected tested positive (0.3 µg/L to 9.4 ug/L) for the toxin. The most common algal species identified in these surveys were *Anabaena*, *Aphanizomenon*, *Microcystis* and *Phomidium*.

Toxic algae have also been implicated for direct poisoning of waterfowl populations as well as contributing to outbreaks of avian botulism (Ted Leighton, Canadian Cooperative Wildlife Heath Centre, personal communication). In Saskatchewan, human illness has also been reported from drinking water contaminated with toxic algae; symptoms included stomach cramps, vomiting, diarrhoea, fever, headache, pains in muscles and joints, and weakness (Dillenberg and Dehenl 1960). Algal toxin poisonings of animals in Canadian prairie lakes have generally been associated with the genera *Microcystis*, *Anabaena*, and *Aphanizomenon*. Toxin-producing blue-green algae may also cause economic problems for the aquaculture industry because of fish deaths (Gorham and Carmichael 1980; Skulberg et al. 1984).

At present, the World Health Organization has a provisional guideline of 1 µg/L for microcystin-LR in drinking water (WHO 1997b). Health Canada has proposed a drinking water guideline of 1.5 µg/L for total microcystins based on the toxicity of microcystin-LR (Health Canada 1998a). This proposed guideline is higher than the toxin levels measured for raw water samples (< 0.1- 1.0 µg/L) and treated water samples (<0.1- 0.6 µg/L) in Manitoba in 1995 (Jones et al. 1998) and the rest of Canada (Health

Canada 1998a). As well, the Canadian Council of Ministers of the Environment advises against watering livestock from lakes, ponds, or streams that contain heavy growth of cyanobacteria (CCREM 1987).

The best way to avoid the problems associated with blue-green algal blooms is to prevent blooms from forming. Although there is no fail-safe method for doing so, bloom formation can be minimized by controlling nutrient inputs to surface waters and, in the case of reservoirs, ensuring mixing of the water column (Health Canada 1998b). Water supplies contaminated with toxic blue-green algae are often treated with copper-based algaecides (Edmonson 1969; Hodson et al. 1979). However, death of the algae may result in the release of toxins into the surrounding water (Carmichael 1994; Jones and Orr 1994). Human injuries by hepatotoxins have been reported immediately following treatment of drinking water supply with copper sulfate (Bourke et al. 1983).

Trihalomethanes

Chlorination by-products are the chemicals formed when the chlorine used to kill disease-causing micro-organisms reacts with naturally occurring organic matter (e.g., decay products of vegetation) in the water. Nutrient loading can affect the production of chlorination by-products because nutrient addition enhances production of organic matter, such as algae and aquatic weeds, in drinking water supplies. The most common chlorination by-products found in Canadian drinking water supplies are the trihalomethanes (THMs) (Health Canada 1993). The principal THMs are chloroform, bromodichloromethane, chlorodibromomethane and bromoform. Other less common chlorination by-products include the haloacetic acids and haloacetonitriles.

The amount of THMs formed in drinking water can be influenced by a number of factors, including the season and the source of the water. For example, THM concentrations are generally lower in winter than in summer, because concentrations of natural organic matter are lower and less chlorine is required to disinfect at colder temperatures. THM levels are also low when wells or large lakes are used as the drinking water source, because organic matter concentrations are generally low in these sources. The opposite -- high organic matter concentrations and high THM levels -- is true when rivers or other surface waters are used as a source of the drinking water. The preferred approach of controlling THMs is removal of organic matter (the precusors) from the source water before disinfection so that it cannot react with chlorine or other disinfectants to form by-products.

Laboratory animals exposed to very high levels of THMs have shown increased incidences of cancer (see Mills et al. 1998 for a review of health risks). Also, several studies of cancer incidence in human populations have reported associations between long-term exposure to high levels of chlorination by-products and an increased risk of certain types of cancer. For example, a recent study conducted in the Great Lakes basin reported an increased risk of bladder and possibly colon cancer in people who drank chlorinated surface water for 35 years or more. Possible relationships between exposure to high levels of THMs and adverse reproductive effects in humans have also been examined recently. In a California study, pregnant women who consumed large amounts of tap water containing elevated levels of THMs were found to have an increased risk of spontaneous abortion. The available studies on health effects do not provide conclusive proof of a relationship between exposure to THMs and cancer or reproductive effects, but indicate the need for further research to confirm their results and to assess the potential health effects of chlorination by-products other than THMs. Current evidence

indicates that the benefits of chlorinating our drinking water -- reduced incidence of water-borne diseases -- are much greater than the risks of health effects from THMs.

5.4. Conclusions

Effects of elevated concentrations of ammonia, nitrate, nitrogen dioxide and nitrite have been studied, with varying degrees of effort, on a diversity of aquatic and terrestrial biota. Ammonia, in the form of un-ionized ammonia, is toxic to aquatic animals (fish and benthic invertebrates) and in most surface waters, total ammonia concentrations greater than about 2 mg/L are toxic. Aquatic plants also show evidence of ammonia toxicity, although they appear more tolerant than aquatic animals. Ammonium (NH_4^+) and un-ionized ammonia (NH_3) in the atmosphere can be toxic to terrestrial plants, whereas inhalation of un-ionized ammonia can be dangerous to humans. In contrast to ammonia, nitrate is essentially non-toxic to aquatic and terrestrial plants. However ingestion of more than 10 mg/L nitrate-N can be lethal for humans; nitrate-N concentrations greater than 1% dry weight in feed can cause death of livestock. Nitrogen oxides in the atmosphere can be toxic to terrestrial vegetation. Comparatively little is known about the toxicity of nitrite because it rapidly oxidizes to nitrate and is rarely present, therefore, in high concentrations naturally. Phosphorus is not directly toxic in its naturally occurring states, even at high concentrations.

Acute lethalities caused by point-source loading of N are uncommon in Canada. This fact is due, in part, to restrictions imposed upon municipal authorities and industries to ensure that wastewater discharges are appropriately treated and diluted to safeguard aquatic ecosystems and the humans that use these waters. Industrial and sewage discharge exceedences are generally unusual events caused by equipment failure or, in the case of sewage discharges, high rainfall or snowmelt that exceeds the capacity of the treatment plant. In contrast to point-source inputs, spills of agricultural N-containing material (particularly manure) are common and often result in fish kills (on average, four reported fish kills per year in Southwestern Ontario, with likely many more unreported kills), suggesting the need for better management of farm nutrients.

In contrast with surface waters where toxic N concentrations are uncommon, concentrations of nitrate in ground water exceed the drinking-water guideline of 10 mg N/L in many parts of Canada. Lethalities are avoided as a result of public education programs advising parents who use ground water as their drinking-water source to make up formulas for infants with bottled water. Livestock lethalities resulting from consumption of nitrate-enriched plants are also avoided by education of livestock producers.

The chronic effects of elevated concentrations of ammonia, nitrate, nitrogen dioxide and nitrite are less well understood. Because the symptoms of chronic nitrogen toxicity may mimic or be masked by other environmental changes, the extent of chronic nitrogen toxicity in the environment is generally not known. However, recent evidence suggests that nitrate concentrations in runoff and surface waters in southern Ontario are sufficiently high to cause chronic (as well as acute) nitrate toxicity in amphibians. Evidence is accumulating that suggests the decline of amphibians, such as in southern Ontario, may be due, in part, to toxic concentrations of nitrate.

In addition to the direct toxic effects of certain N compounds, nutrient addition to fresh and coastal waters enhances the growth of algae, including species of algae that produce toxins. Consumption of algal species that produce toxins (in the case of fresh water) or the organisms that accumulate algal toxins (in the case of coastal waters) can affect the health of terrestrial animals, including humans. In

Canada, toxic algal blooms occur most frequently in lakes and potholes in the Prairies. Here, deaths of livestock and pets are reported each year. Toxic algal blooms have occurred on both the Pacific and Atlantic coasts of Canada; however, their frequency is sporadic in coastal waters. In response, in part, to the 105 cases of poisoning and three deaths caused in 1987 by consumption of mussels containing an algal toxin, the Government of Canada developed the Canadian Shellfish Sanitation Program and the Canadian Shellfish Water Quality Protection Program to ensure that growing areas for all bivalve molluscan shellfish meet approved water quality criteria.

Nutrient loading can also indirectly affect the quality production of treated drinking water supplies. Chlorine addition to disinfect water can lead to the formation of a number of chlorination by-products if the water supply contains organic matter such as algae or aquatic weeds. Among the many disinfection by-products, trihalomethanes (THMs) are most often present. The preferred approach of controlling THMs is by removing organic matter (the precusors) from the source water before disinfection.