Nitrogen Pollution: An Assessment of Its Threat to Amphibian Survival

Jeremy David Rouse,[1] Christine A. Bishop,[1] and John Struger[2]

[1]Canadian Wildlife Service, Environment Canada, Burlington, Ontario, Canada; [2]Ecosystem Health Division, Environment Canada, Burlington, Ontario, Canada

The potential for nitrate to affect amphibian survival was evaluated by examining the areas in North America where concentrations of nitrate in water occur above amphibian toxicity thresholds. Nitrogen pollution from anthropogenic sources enters bodies of water through agricultural runoff or percolation associated with nitrogen fertilization, livestock, precipitation, and effluents from industrial and human wastes. Environmental concentrations of nitrate in watersheds throughout North America range from < 1 to > 100 mg/L. Of the 8,545 water quality samples collected from states and provinces bordering the Great Lakes, 19.8% contained nitrate concentrations exceeding those which can cause sublethal effects in amphibians. In the laboratory lethal and sublethal effects in amphibians are detected at nitrate concentrations between 2.5 and 100 mg/L. Furthermore, amphibian prey such as insects and predators of amphibians such as fish are also sensitive to these elevated levels of nitrate. From this we conclude that nitrate concentrations in some watersheds in North America are high enough to cause death and developmental anomalies in amphibians and impact other animals in aquatic ecosystems. In some situations, the use of vegetated buffer strips adjacent to water courses can reduce nitrogen contamination of surface waters. Ultimately, there is a need to reduce runoff, sewage effluent discharge, and the use of fertilizers, and to establish and enforce water quality guidelines for nitrate for the protection of aquatic organisms. *Key words*: amphibians, nitrate, toxicity, water quality. *Environ Health Perspect* 107:799–803 (1999). [Online 31 August 1999]
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Several regional and global reviews have reported extinctions, extirpations, and serious declines of a number of amphibian species and their populations (*1–6*). The detrimental effect that habitat destruction has on amphibian populations is undeniable (*7*). Because anthropogenic pollution has had population level impacts on other vertebrates such as colonial waterbirds (*8*), mammals (*9*), reptiles (*10*), and fish (*11*), the lack of long-term population information on amphibians (*12–14*) should not prevent scientists from hypothesizing and studying the impacts of pollution on amphibian health and populations. Chemical stressors such as acid deposition, industrial chemicals, pesticides, heavy metals, salts, and nitrogen fertilizers are possible anthropogenic causes for the decline of some amphibian populations around the world, but the impact of these factors is poorly understood (*15*). This evaluation focuses on potential effects in North America of one pervasive pollutant, nitrate-nitrogen, which is highly toxic to amphibians.

Different forms of nitrogen are found globally in aquatic ecosystems. Nitrogen in the aquatic environment occurs in four forms (ammonium ion, ammonia, nitrite, and nitrate). All nitrate values in this review are reported as nitrogen in nitrate-nitrogen. The most toxic nitrogen to biota is ammonia, followed by nitrite and nitrate (*16*). Because ammonia and nitrite are quickly oxidized to nitrate by bacteria and algae in the aquatic environment, they are mainly problems when they originate in large volumes from point sources such as industrial effluents and livestock feed lots and slaughterhouses or areas that lack nitrification treatment of urban sewage. Although nitrate is the least toxic of the three forms, it occurs at the highest concentrations and is the most stable form of nitrogen in the aquatic environment (*16*). Natural background concentrations of nitrate in groundwater in temperate regions range from trace amounts to 3 mg/L (*17–19*), whereas concentrations above 3 mg/L reflect anthropogenic contamination (*18*).

Nitrogen contamination occurs in both agricultural and urban areas. The primary anthropogenic sources of nitrogen contamination of water in agricultural areas are nitrogen-based fertilizers and animal waste (*20*). In urban areas the main sources of nitrogen contamination are effluents from industrial and wastewater treatment plants, lawn fertilizers, and atmospheric deposition from the burning of fossil fuels. Concentrations of nitrate in aquatic ecosystems affected by agricultural and urban activities around the world can exceed 100 mg/L (*21–24*).

Sources

Nitrogen use in agricultural areas. Around the world, the amount of nitrogen applied to agricultural land has increased since the early 1960s (*25*). The estimated amount of nitrogen fertilizer used globally in 1991 was 72 million tons (*26*). In the United States, nitrogen-based fertilizer use increased from approximately 2.5 million tons in 1960 to almost 11.9 million tons/year in 1985 (*27*). The use of nitrogen fertilizers in North America is concentrated in Indiana, Illinois, Iowa, Ohio, and other intensely cultivated areas (*28*) (Figure 1).

Seasonal variation. Nitrate is soluble in water and can be transported both overland and underground (*29*). In temperate areas of North America, environmental nitrate concentrations in the water are usually highest during late fall, winter, and spring. The high levels are attributed to several factors, the most prominent being the reduced assimilation of nitrate by row crops and other plants during the dormant periods of plant growth such as in the fall, winter, and spring. Because the amount of nitrogen uptake by the plants decreases, the amount of nitrate available to be leached from the ground increases (*20*). For example, tributaries of the inner bay of Rondeau Provincial Park on the north shore of Lake Erie, Canada, contained concentrations of nitrate between 7 and 13 mg/L for the winter and spring months of February through May 1983. During July and August, the concentration in these tributaries was 4 mg/L (*30*). These tributaries drain intensive row-cropped agriculture. Increased frequency of storm events during this time period may also lead to increased transport of nitrate from runoff and tile drains to streams and rivers in North America (*20*).

Concentrations in streams. Average nitrate concentrations in streams traversing agricultural landscapes in North America typically range between 2 and 40 mg/L (*20,25*). Nitrate concentrations > 10 mg/L in the water can persist for several weeks (*25*). Hooda et al. (*31*) demonstrated that stream nitrate concentrations increased as land use changed from woodland through grassland to arable farming. Many studies from North America, Europe, and Australia show the same relationships with land use change and

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Address correspondence to C.A. Bishop, Canadian Wildlife Service, Environment Canada, Box 5050, Burlington, Ontario, Canada L7R 4A6. Telephone: (905) 336-4843. Fax: (905) 336-6434. E-mail: cab.bishop@ec.gc.ca

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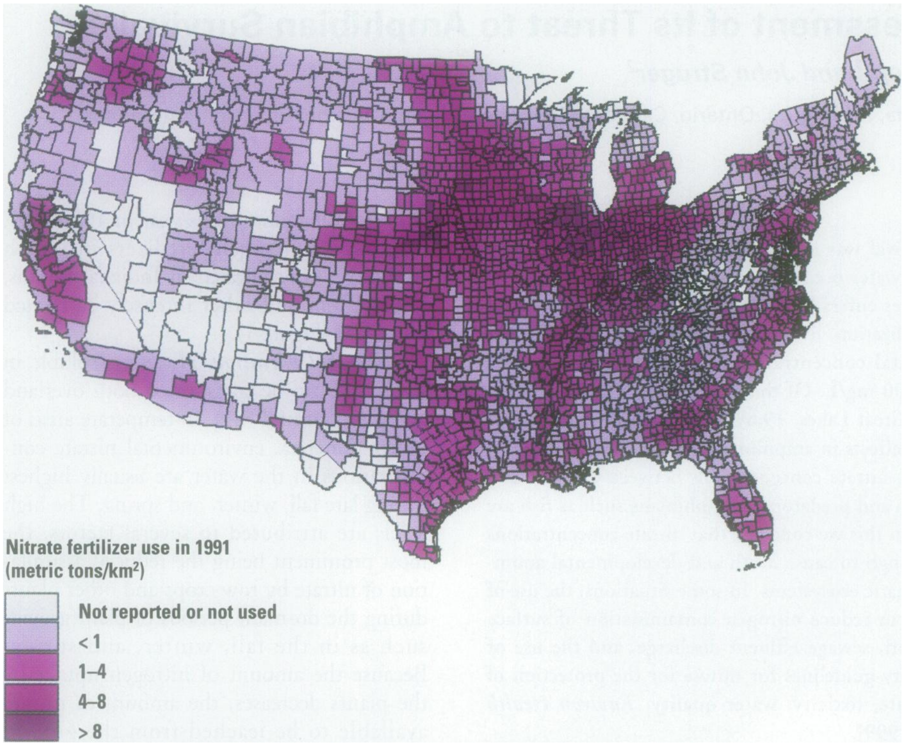

Figure 1. Nitrogen fertilizer use in the continental United States, 1991 (*28*).

nitrate concentrations (*18,32–34*). Small streams draining intensively row-cropped agricultural land have higher concentrations of nitrate than do larger streams.

Nitrogen in urban areas. Industrial effluents and wastewater-treatment plant discharges are a substantial source of nitrogen to aquatic ecosystems. Although these point sources contribute only a small percentage of the total nitrogen released to the environment, long-term direct discharge to a watercourse could have a significant detrimental effect on stream ecosystems downstream of the discharge site. Elevated nitrate concentrations were found in Coote's Paradise, a wetland complex in Dundas, Ontario, Canada (*35*). The nitrate originated from the Dundas sewage treatment plant effluent pipe, which discharges approximately 700 m upstream of this wetland. Nitrate concentrations in the water increased as the distance to the discharge pipe decreased (*35*). In 1997, concentrations of nitrate in the wetland ranged from 4.2 mg/L on 2 July to 9.5 mg/L on 4 June, with an average concentration between 7 May and 3 September of 6.3 mg/L (*36*). Similar concentrations of nitrate have been recorded in the Coote's Paradise wetland since the sewage treatment plant installed a nitrification system in 1978 that reduces the amount of ammonia but increases the amount of nitrate in the effluent (*35*).

Another source of nitrate contamination in urban areas is precipitation. Rain, snow, and fog contain various amounts of nitrogen depending on geographic location. Motor vehicle and industrial exhausts contribute nitrogen oxides to the atmosphere that are deposited into the aquatic ecosystem through precipitation. In heavily populated industrialized areas the concentration of nitrogen in precipitation can be elevated. For example, concentrations of nitrate > 2 mg/L are found in precipitation in the Great Lakes area (*37*). Atmospheric nitrogen can enter aquatic ecosystems through direct precipitation on the watercourse or through runoff into the watershed via storm sewers. In forested or heavily vegetated areas that are not artificially fertilized, the land has a natural ability to absorb and utilize the nitrogen in the precipitation. Atmospheric deposition may be a problem in watersheds that do not have an extensive ground cover of natural vegetation such as in urban areas.

Effects on amphibians. Nitrate at concentrations detected in surface waters has both acute and chronic toxic effects on several species of amphibians (*38–41*). Berger (*41*) attributed a 20-year decline of amphibian numbers in an agricultural landscape near Turew, Poland, to high levels of nitrate in surface waters resulting from nitrogen fertilizers. Bishop et al. (*42*) studied an intensive vegetable-growing area in the Holland Marsh in Ontario, Canada, and concluded that habitat loss and nitrate levels in wetlands were more important than pesticide use in affecting amphibian survival and species diversity. Although nitrate levels were elevated, pesticide concentrations were low and often not detectable in the agricultural area.

Short-term experiments that determine the lethal concentration of nitrate to 50% of test individuals (96-hr LC$_{50}$) and 100-day chronic toxicity experiments have been conducted on tadpoles of the western chorus frog (*Pseudacris triseriata*), northern leopard frog (*Rana pipiens*), and green frog (*Rana clamitans*) (*38*). For these species, eggs were collected in the wild, hatched in captivity, and exposed to nitrate as tadpoles. Western chorus frog tadpoles were the most susceptible to nitrate, followed by northern leopard frog and green frog (*38*) (Table 1). Hecnar (*38*) also showed that physical and behavioral abnormalities developed at concentrations as low as 3 mg/L in 96-hr LC$_{50}$ tests. These effects included reduced feeding and mobility resulting in severe weight loss and high mortality of the individuals. In addition to reduced swimming and feeding, developmental deformities including bent tails, body swelling and bulging, head deformities, and digestive-system deformities occurred. The severity of the effects was positively correlated with increasing concentrations of nitrate. The effects observed in the chronic experiments were similar to those in the 96-hr LC$_{50}$ experiments.

Baker and Waights (*39,40*) found that concentrations of nitrate at 9 and 22.6 mg/L caused reduced growth, behavioral changes, and increased mortality in the common toad (*Bufo bufo*) and White's tree frog (*Litoria caerulea*) (Table 1). The effects of nitrate on the tadpoles were similar in the low and high concentrations. Approximately half of the tadpoles died within 8 days of being exposed to the lower concentration; however, a large percentage of the mortality occurred within the first 96 hr (*39,40*).

Hecnar (*38*) also performed 96-hr LC$_{50}$ nitrate determinations for American toads (*Bufo americanus*) collected in the wild and exposed as tadpoles in captivity. The 96 hr-LC$_{50}$ values for two samples of American toad tadpoles were 13.6 and 39.3 mg/L. The sample of toads showing a 96-hr-LC$_{50}$ of 39.3 mg/L was collected at a later time of year from a pond in an agricultural area. These samples of tadpoles could have been differentially exposed to nitrate contamination or the sample from the agricultural area could represent a resistant population.

Baker and Waights (*39*) and Xu and Oldham (*43*) conducted studies to determine the toxicity of nitrate for the common toad. The effective concentrations were strikingly different (Table 1). This was probably due to differences in the test species and in experimental design. The studies used two populations of common toads as well as tadpoles of different ages. Baker and Waights (*39*) collected their test sample from the wild as eggs and allowed them to hatch in captivity, whereas Xu and Oldham (*43*) collected their test sample as tadpoles. They also used different rearing media: Baker and Waights used distilled water and Xu and Oldham used artificial pond water.

Hecnar (*38*) suggested that a possible mechanism for reduced feeding was a nitrate-caused disturbance of a symbiosis between the tadpole and gut bacteria involved in digestion. The probable mechanism causing the reduced activity in the tadpoles is a result of the development of methemoglobinemia. In humans, this is known as blue-baby syndrome and occurs in infants younger than 6 months of age that are exposed to nitrate-contaminated water (*32*). The condition results from gut bacteria converting nitrate to nitrite, which is absorbed and then oxidizes iron in hemoglobin to form methemoglobin that is unable to bind oxygen (*32*). Young children and possibly young tadpoles have an inadequate number of bacteria required to efficiently reduce the available nitrite and are not capable of proper nitrate metabolism. Conversely, adults have a more diverse population of gut bacteria and are capable of effectively reducing the amount of available nitrite (*32*). Methemoglobin has been detected in bullfrog (*Rana catesbeiana*) tadpoles exposed to nitrites (*46*). If the development of methemoglobin is the mechanism responsible for reduced activity in amphibians, then it may also be responsible for reduced feeding activity. Marco and Blaustein (*6*) found that tadpoles exposed to low levels of nitrite transformed more slowly than did control tadpoles. They also found that the exposed tadpoles occupied shallow water more often, and speculated that the tadpoles were trying to get more oxygen. The water quality guideline for the protection of human health for drinking water for nitrates is a concentration of 10 mg/L (*47*); however, water quality criteria for nitrate for the protection of wildlife do not exist.

Most reports on the effect of nitrate on amphibians have examined the effects on tadpoles, not on adults. However, one study examined the effect of ammonium nitrate on adult common frogs (*Rana temporaria*) (*44*). Oldham et al. (*44*) spread ammonium nitrate granules on moist chromatography paper at concentrations of 0 (control), 1.5, 3.1, 6.2, and 12.4 g/m^2 and on soil at the same concentrations with the addition of a 24.8-g/m^2 exposure. They placed three adult male frogs on each of the substrates at each exposure level except the control, where 12 frogs were used, and observed them for toxic symptoms. The frogs were considered affected and were removed from the substrate when they exhibited clinical signs of acute toxicosis, which was predicted would lead to death. At the 3.1 and 6.2 g/m^2 paper concentrations, two of three frogs were affected within 300 min exposure. At the 12.4-g/m^2 paper exposure, three of three frogs exhibited clinical signs of acute toxicosis and were removed by 120 min. On the soil substrate, one frog was affected at 6.2 g/m^2 ammonium nitrate by 60 min, three frogs were affected at 12.4 g/m^2 by 360 min, and three frogs were affected at 24.8 g/m^2 by 15 min exposure. Oldham et al. (*44*) also conducted a field study in which they exposed common frogs to concentrations of 10.8 and 19.9 g/m^2 ammonium nitrate existing in a wheat field and a grass field, respectively. In both treatments (three individuals per field), 100% of the frogs exposed were affected by the exposure concentrations. However, they also found that persistence of the toxic effect of granular ammonium nitrate decreased quickly once the granules dissolved, which under normal field conditions usually takes 1 hr. A similar field study in Germany found that nitrate fertilizers seriously harmed and killed amphibians as they migrated over recently fertilized fields (*48*).

Few other studies examining the sublethal impacts of nitrate on amphibians exist. However, one study revealed that nitrate stress may depress immune response and blood hemoglobin in amphibian tadpoles. Dappen (*49*) found decreased levels of circulating white cells and decreased hemoglobin values in bullfrogs and leopard frogs exposed to 9–26 mg/L of nitrate for 3 weeks.

Because data are lacking for most species, definitive conclusions on all anuran species cannot be made. However, environmental nitrate concentrations overlap with concentrations that have direct lethal and sublethal effects on amphibians in the laboratory.

Effects on amphibian prey and predators. Tadpole diets consist mainly of plant matter, plankton, and bacteria, whereas diets of adult frogs consist mainly of insects and small vertebrates. Tadpoles are prey to many predators including mammals, birds, snakes, turtles, salamanders, other frogs, insects, and spiders (*50*).

The limited data on the toxicity of nitrate to prey and predators of amphibians seem to suggest that amphibian survival can be impacted. Nitrate toxicity experiments using caddisfly larvae show 96-hr LC$_{50}$ values above 90 mg/L for *Cheumatopsyche pettiti* and *Hydropsyche occidentalis* (*16*) (Table 1). Adult fish have higher 96-hr LC$_{50}$ values for nitrate, ranging from 800 to 12,000 mg/L (*51,52*). Nitrate concentrations in the range of 1–10 mg/L are lethal to the eggs and, to a lesser extent, the fry of two salmonid species (*45*) (Table 1). A large percentage (31%) of rainbow trout (*Salmo gairdneri*) eggs and 15% of the fry died when exposed to 2.3 mg/L nitrate (*45*) (Table 1).

Ecological implications. Although nitrate toxicity negatively affects the physiology and behavior of amphibians and other aquatic organisms, few studies have examined the resulting influences these effects may have on the ecology of the exposed species or populations (*38*). Therefore, the consequences of nitrate pollution on amphibian populations are hard to quantify. However, the data suggest that the problem of nitrate pollution is extensive and that the compound is toxic enough to represent one of the most pervasive contaminant threats to amphibian survival in

Table 1. The toxicity of nitrate to amphibians and their prey and predators.

Species	Stage	End point	Concentration of nitrate (mg/L)	Reference
Amphibian				
Bufo americanus	Tadpole	96 hr-LC$_{50}$	13.6, 39.3	(*38*)
Pseudacris triseriata	Tadpole	96 hr-LC$_{50}$	17	(*38*)
Rana pipiens	Tadpole	96 hr-LC$_{50}$	22.6	(*38*)
Rana clamitans	Tadpole	96 hr-LC$_{50}$	32.4	(*38*)
P. triseriata	Tadpole	Developmental	2.5–10	(*38*)
R. pipiens	Tadpole	Developmental	2.5–10	(*38*)
R. clamitans	Tadpole	Developmental	2.5–10	(*38*)
Bufo bufo	Tadpole	96 hr-LC$_{50}$	385	(*43*)
B. bufo^a	Tadpole	Developmental	9	(*39*)
B. bufo^a	Tadpole	Death	22.6	(*39*)
Litoria caerulea^a	Tadpole	Developmental	9	(*40*)
L. caerulea^a	Tadpole	Death	22.6	(*40*)
Rana temporaria^b	Adult	EC$_{50}$, paper	3.6 g/m^2	(*44*)
R. temporaria^b	Adult	EC$_{50}$, soil	6.9 g/m^2	(*44*)
Amphibian prey and predators				
Cheumatopsyche pettiti	Larvae	96 hr-LC$_{50}$	113.5	(*16*)
Hydropsyche occidentalis	Larvae	96 hr-LC$_{50}$	97.3	(*16*)
Salmo gairdneri	Egg and fry	46% mortality	2.3	(*45*)
Salmo clarki	Egg and fry	41% mortality	4.5	(*45*)

Abbreviations: 96-hr LC$_{50}$, lethal concentration of nitrate to 50% of test individuals; EC$_{50}$, median effective concentration. ^{a}Tadpoles were exposed to two concentrations of nitrate; therefore, the 96-hr LC$_{50}$ was not determined. However, significant effects were found on tadpoles as compared to controls in an 8-day test. ^{b}Frogs were placed on moist paper or soil spread with ammonium nitrate granules. Symptoms of acute toxicity, which Oldham et al. (*44*) predicted would lead to death, were considered the effect.

North America and perhaps elsewhere. Lethal concentrations of nitrate for a number of anuran species are in the range of 13–40 mg/L, with chronic effects occurring at concentrations below 10 mg/L. Water quality data for the agricultural and urban areas that have been sampled in North America show that the nitrate concentrations in surface waters exceed these critical toxicity levels for amphibians for extended periods of time and during sensitive times of anuran development, such as the egg and tadpole stages (Figure 2). The average concentration shown in Figure 2 is conservative and does not represent the maximum concentration that could possibly occur. Because these are the only data available, Figure 2 shows sample results of larger streams. We suggest that these habitats will typically have lower average concentrations of nitrate than small ponds, ditches, and watercourses near point sources simply because of high dilution factors. Smaller ponds and ditches currently represent a large portion of the available amphibian habitat in agricultural areas (*55*). Despite these conservative nitrate concentrations, even these average values in surface waters in North America indicate many areas that may be directly toxic to amphibians (Figure 2). Of the 8,545 water quality samples collected from the states and provinces bordering the Great Lakes, 19.8% contained nitrate concentrations that exceeded 3 mg/L and 3.1% of the samples exceeded 10 mg/L.

Because adult fish are less susceptible to nitrate than amphibians, nitrate-resistant adult fish may increase the predation pressure on eggs and tadpoles. However, the susceptibility of fish eggs and fry to nitrate may also reduce some fish populations. The same can be said about invertebrates: the toxicity data imply that some invertebrates may be more resistant to nitrate than some amphibians. This may increase a tadpole's chance of predation if it is exposed to levels of nitrate that alter behavior. Birds, reptiles, and mammals may also find it easier to catch amphibian tadpoles that have been compromised by nitrate exposure.

What can be done about this problem? The use of vegetated buffer zones around watercourses can drastically reduce the amount of nitrate entering the surface water through runoff (*56,57*). Effective buffer strips can range from mixed woodland to grassland that varies in size from a few meters to hundreds of meters. A 24-m grass buffer in southern England reduced nitrate concentrations in a watercourse from 12 to < 1 mg/L (*58*). Similarly, a 19-m mixed woodland buffer in the state of Maryland reduced concentrations in a stream from approximately 7 to < 0.5 mg/L during the spring and summer (*56*). Buffer zones are easy to construct and can be effective within 1 year (*56,57*). Fences along watercourses exclude grazing cattle and assist vegetation regeneration, which protects the watercourse and increases the habitat for amphibians and other aquatic organisms (*59*). Unfortunately, buffer strips will not help to reduce nitrate that enters the streams through tile drainage. However, the key to minimizing the agricultural input of nitrate to surface water is the efficient use of fertilizers as indicated by the proportion of added nitrogen that is removed by the harvested portion of the crop. The time of application is also important; if the fertilizer is applied as a pre-emergent or at the postseason stage, crop uptake, the major utilizer of nitrogen, will be absent. Therefore, the use of the best management practices developed for nitrogen fertilization along with buffer zones around watercourses can reduce or virtually eliminate the impacts of nitrogen contamination on wildlife.

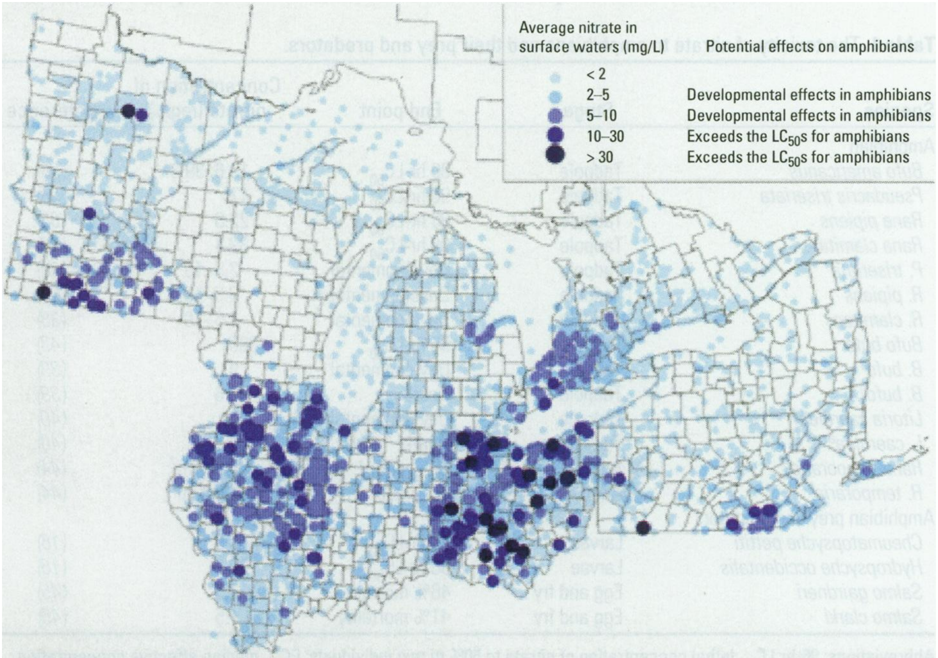

Figure 2. Average nitrate concentrations in surface water from the U.S. states and the Canadian province that border the Great Lakes. LC$_{50}$, median lethal concentration. Canadian information from the Ontario Ministry of Environment and Energy (*53*). U.S. information from the U.S. Environmental Protection Agency (*54*).

Conclusion

Nitrate levels in many agricultural ecosystems of North America exceed 1 mg/L, i.e., concentrations that are toxic to amphibians and/or other aquatic organisms. The benefits of increased productivity due to nitrate are likely outweighed by the impact on wildlife health and survival (*38,41*). We conclude that it is highly probable that nitrate concentrations in surface waters in North America are adversely affecting amphibian survival. Nitrogen pollution will undoubtedly become an even larger global problem if agricultural and urban development continues in developed and developing countries without the incorporation of safeguards to reduce the amount of nitrogen that enters aquatic environments. Increasing the number of species studied, testing with environmentally relevant concentrations (2–100 mg/L), and examining the impact on wild populations are vital to a better understanding of the effects of nitrates on amphibian ecology. Because high levels of nitrates and other agricultural chemicals such as pesticides occur in the spring and early summer months in North America, additive or synergistic toxicity of these chemicals also needs to be determined. Although there is a need to conduct more field experiments on nitrate toxicity to amphibians, the known information suggests a serious potential for toxicosis that must be addressed immediately.

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