

Toxicity of Nitrite to Fish: A Review

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Abstract.—Nitrite, an intermediate in the oxidation of ammonium to nitrate, changes hemoglobin to methemoglobin, which does not carry oxygen; nitrite may thus cause anoxia in fish and other aquatic organisms. The published literature on nitrite toxicity to fish, which consists of about 40 papers, shows that the ratio of the 24-h LC50 (concentration lethal to half of the test organisms in 24 h) to the 96-h LC50 has a median value of 2.0 and is fairly uniform across species; toxicity tests of differing duration can therefore be standardized to a common duration. In general, chronic effects are difficult to detect at concentrations below one-fifth of the 96-h LC50. Most fish concentrate nitrite in fresh water; chloride in the external environment offsets the toxicity of nitrite by competing with nitrite for uptake through the chloride cells of the gills. The strength of the chloride effect is greatest for the least-sensitive species and smallest for the most-sensitive species. The addition of 1 mg/L chloride increases the 96-h LC50 by 0.29 to 2.0 mg/L nitrite-N, depending on the species. Bicarbonate also reduces the toxicity of nitrite, but it is less than 1% as effective as chloride. Calcium reduces the toxicity of nitrite, but much less than chloride; the effects of other metal cations have not been studied. Hydrogen ion concentration of the medium has not been shown to have a discrete effect on the toxicity of nitrite except at extreme concentrations uncharacteristic of the environments in which fish ordinarily live. Nitrite toxicity is exacerbated by low oxygen concentrations because nitrite reduces the oxygen-carrying capacity of the blood. The effects of temperature have not been adequately studied. Very small fish seem less sensitive to nitrite than fish of intermediate or large size. Present evidence suggests that salmonids are among the fishes most sensitive to nitrite; channel catfish *Ictalurus punctatus*, blue tilapia *Tilapia aurea*, logperch *Percina caprodes*, and brook stickleback *Culaea inconstans* are equally sensitive or slightly less sensitive. The fathead minnow *Pimephales promelas*, other cyprinids, catostomids, the mottled sculpin *Cottus bairdi*, and the black bullhead *Ictalurus melas* are considerably less sensitive. The least-sensitive species tested thus far are the largemouth bass *Micropterus salmoides* and bluegill *Lepomis macrochirus*; the largemouth bass does not concentrate nitrite.

Nitrite (NO_2^-) is a naturally occurring anion in fresh and saline waters. Nitrite is intermediate in oxidation state between ammonium and nitrate, and its concentration in oxygenated waters is typically less than 0.005 mg/L. Under some circumstances, however, concentrations of nitrite may be sufficiently high to alter hemoglobin and thus be toxic to aquatic organisms. Because two genera of bacteria are involved in the two-step conversion

of ammonium to nitrate, factors that affect species of these two genera differentially may lead to accumulation of nitrite. For example, *Nitrobacter* spp., which convert nitrite to nitrate, are more sensitive to un-ionized ammonia than *Nitrosomonas* spp., which convert ammonium to nitrite (Anthonisen et al. 1976). Acidity affects the amount of un-ionized ammonia. Thus changes in acidity can, in the presence of large amounts of the am-

TABLE 1.—Names of fish species mentioned in the text and tables.

Common name	Scientific name
Atlantic salmon	<i>Salmo salar</i>
Black bullhead	<i>Ictalurus melas</i>
Bluegill	<i>Lepomis macrochirus</i>
Blue tilapia	<i>Tilapia aurea</i>
Brook stickleback	<i>Culaea inconstans</i>
Channel catfish	<i>Ictalurus punctatus</i>
Chinook salmon	<i>Oncorhynchus tshawytscha</i>
Coho salmon	<i>Oncorhynchus kisutch</i>
Common carp	<i>Cyprinus carpio</i>
Creek chub	<i>Semotilus atromaculatus</i>
Cutthroat trout	<i>Salmo clarki</i>
European minnow	<i>Phoxinus laevis</i>
Fathead minnow	<i>Pimephales promelas</i>
Guppy	<i>Poecilia reticulata</i>
Largemouth bass	<i>Micropterus salmoides</i>
Loggerch	<i>Percina caprodes</i>
Mosquitofish	<i>Gambusia affinis</i>
Mottled sculpin	<i>Cottus bairdi</i>
Pink salmon	<i>Oncorhynchus gorbuscha</i>
Quillback	<i>Carpoides cyprinus</i>
Rainbow trout	<i>Salmo gairdneri</i>
White sucker	<i>Catostomus commersoni</i>

monium ion, inhibit nitrate formation and cause accumulation of nitrite. Other factors that differentially affect these bacteria could also cause accumulation of nitrite.

Nitrite is present at unusually high concentrations under some natural conditions. For example, McCoy (1972) found high concentrations of nitrite in several aquatic environments in Wisconsin. Nitrite may also sometimes accumulate in the deep layers of lakes during stratification and subsequently appear at the lake surface when the lake mixes (e.g., Infante et al. 1979).

Certain human activities increase the amount of nitrite in aquatic systems. According to Klingler (1957), high nitrite concentrations can be expected in the effluents from industries producing metals, dyes, and celluloids. Sewage effluents can contain high amounts of nitrite (Anthonisen et al. 1976; Alleman 1978). Nitrite can also be produced in quantity by some types of aquaculture (Liao and Mayo 1974; Schwedler and Tucker 1983).

Nitrite Uptake and Toxicity Mechanisms

Fish gain ions through the diet, and most are also able to accumulate ions through active uptake mechanisms associated with the chloride cells of the gills (Maetz 1971). The chloride cells are of two types: filament and lamellar (Laurent and Dunel 1980). The filament cells appear to excrete unwanted ions in seawater and the lamellar cells take up ions in dilute media. In fresh water, the lamellar cells can give off ammonium or hydrogen

ions in exchange for equal numbers of sodium ions and can give off bicarbonate ions in exchange for an equivalent number of chloride ions (Love 1980).

The nitrite ion is actively taken up by most freshwater fishes; nitrite concentrations in the blood plasma may reach 10 times the concentrations in the surrounding medium (Eddy et al. 1983). Thus it has been concluded that the nitrite ion is pumped into the body fluids by the chloride cells (e.g., Bath and Eddy 1980). The rate of nitrite uptake appears to be very similar to the rate of chloride uptake (Eddy et al. 1983). Presence of nitrite in fresh water causes enlargement and rapid turnover of chloride cells (Gaino et al. 1984). The fish apparently maintain a fixed internal chloride concentration even when nitrite is present in quantity; this may cause the chloride cells to do more work when nitrite is present.

A second explanation for nitrite uptake against a concentration gradient has been offered by Colt and Tchobanoglous (1976) and Wedemeyer and Yasutake (1978). Some of the nitrite in water combines with hydrogen ions to make nitrous acid (HNO_2). Within the pH range of most natural waters, the nitrite ion is 4 to 5 orders of magnitude more concentrated than the nitrous acid with which it is in equilibrium. Because nitrous acid has no electrical charge, it would not be pumped into the body fluids through the chloride cells, but it is soluble in lipids (Hunn and Allen 1974) and therefore might enter the gills through epithelial cells. Inside the fish, nitrous acid would dissociate, and the resulting nitrite ions would be retained efficiently by physiological mechanisms that prevent loss of ions. According to this explanation, the buildup of nitrite would be especially pronounced when the pH of the fish (about 8.0 at the gills) is higher than that of the surrounding medium.

The literature, although not conclusive on the role of nitrous acid, does strongly support the idea that the nitrite ion enters fish through the chloride cells (Tomasso et al. 1980; Krous et al. 1982). The additional significance of nitrous acid has to do largely with the importance of environmental pH as a regulator of nitrite toxicity. If nitrite enters fish primarily as nitrous acid, the relative internal and external pH levels are of great importance. If nitrite enters as ions primarily through the chloride cells, then the expected role of pH is considerably smaller. This matter will be considered below in connection with pH.

From the blood plasma, nitrite diffuses into red blood cells, where it oxidizes the iron in hemoglobin to the +3 oxidation state. Hemoglobin that

is changed in this way is called methemoglobin or ferrihemoglobin (Kiese 1974), which lacks the capacity to bind oxygen reversibly (Bodansky 1951). As nitrite raises the fraction of methemoglobin in the blood, it reduces the total oxygen-carrying capacity of the blood (e.g., Cameron 1971). A visible symptom of high methemoglobin levels is a brown color in the blood or gills.

Methemoglobin forms spontaneously, although slowly, in the absence of nitrite. Thus fish blood typically contains a measurable amount of methemoglobin even in the absence of nitrite: reported values include 0.9 to 3.6% for rainbow trout (Cameron 1971; Brown and McLeay 1975; Smith and Russo 1975), 10.9% for prespawning pink salmon (Cameron 1971), and 17.2% for channel catfish (from intercept of regression by Schwedler and Tucker 1983; see Table 1 for scientific names). It is evident that the presence of methemoglobin, even somewhat above 10%, should not be viewed as exceptional among fish. Fish differ in this respect from mammals, whose methemoglobin levels seldom exceed 1% (Beutler 1968).

Most fish species form methemoglobin readily if exposed to nitrite in waters of low chloride content. One exception is the largemouth bass, which forms large amounts of methemoglobin only in response to very high nitrite concentrations (Palachek and Tomasso 1984a). The largemouth bass, unlike other fishes whose blood nitrite concentrations have been studied, does not concentrate nitrite in the blood plasma, and thus appears to discriminate nitrite from chloride.

When the methemoglobin content of blood exceeds 70 to 80% of the total hemoglobin, fish become torpid. This behavioral response has been documented for a European minnow (Klingler 1957), for chinook salmon (Westin 1974), and for channel catfish (Konikoff 1975). As the methemoglobin content of the blood approaches 100%, fish typically become unresponsive and disoriented.

Inactive fish have a very low oxygen demand and thus may not be immediately threatened by severe methemoglobinemia (Crawford and Allen 1977). However, if a fish with methemoglobinemia is frightened, or is otherwise forced to become active, it may die of anoxia (Huey et al. 1980).

The amount of methemoglobin necessary to kill, to reduce growth of, or to prevent normal behavior of fish varies with the species and with the environmental conditions. As a rough rule of thumb, methemoglobin concentrations in excess of 50%

could be considered threatening to fish (e.g., Bowser et al. 1983), although healthy fish have been taken from nature under circumstances leading to methemoglobin concentrations above 50% (e.g., Schwedler and Tucker 1983). Channel catfish with 100% methemoglobin have survived for 2 d in warm water (25°C), although the fish were inactive (Tomasso et al. 1979). When methemoglobin concentrations are below 50%, there is usually no mortality.

The red blood cells of fish contain a reductase that reconverts methemoglobin to hemoglobin (Cameron 1971; Huey and Beitinger 1982). This occurs steadily and will typically restore the normal proportion of hemoglobin within 24–48 h if a fish is transferred to water that lacks nitrite (Huey et al. 1980). When nitrite is present, the ultimate level of methemoglobin in the blood is a result of the balance between methemoglobin formation and reversion to hemoglobin by the reductase.

Because fish are quite resistant to death from methemoglobinemia or from CO inactivation of hemoglobin (Anthony 1961; Cameron and Wohlschlag 1969; Cameron and Davis 1970; Holeyton 1971), several authors have suspected that nitrite toxicity involves mechanisms other than methemoglobinemia (Smith and Williams 1974). Alternative mechanisms of nitrite-induced mortality have as yet not been well demonstrated, however. Gills of fish exposed to nitrite show either little or no damage (channel catfish: Colt et al. 1981; rainbow trout: Wedemeyer and Yasutake 1978). Smith and Williams (1974) reported changes in the thymus of rainbow trout exposed to nitrite, but only at lethal concentrations. Arillo et al. (1984) considered general tissue hypoxia to be an unlikely cause of death; these authors believed that liver hypoxia causes fatal liver dysfunction. Quick death at high concentrations may simply be due to general hypoxia, however.

Toxicity for Exposures up to 96 h

Generally, a 24-h exposure is required for maximum accumulation of nitrite within a fish (Huey et al. 1980; Eddy et al. 1983). Thus the earliest meaningful LC₅₀ (concentration lethal to half the test organisms in a specified time) for acute exposures is probably 24 h, although mortality of fish can result from shorter exposures if the concentrations are extraordinarily high. As would be expected, the LC₅₀ declines beyond 24 h; the rate of decline is very low by the time the exposure has reached 96 h. Thus the relevant durations for test-

ing short-term toxicity are probably 24 to 96 h, as is the case for many toxicants.

The 96-h LC50 provides the most conservative estimate of the short-term nitrite tolerance of fish under specified conditions. The literature contains many LC50 determinations over shorter intervals. It is possible, however, to express all data in terms of 96-h LC50 by use of a conversion procedure derived from studies in which LC50 has been determined over several durations (Table 2). Table 2 gives the ratios of each one of the LC50s to the LC50 for 96 h. The ratios of the 96-h LC50 to the shorter-term LC50s for the same group of fish with the same water quality are, with the exception of one data set for channel catfish (Palachek and Tomasso 1984a), similar across fish species and across experimental conditions. From this information we conclude that it is reasonable to use the median numbers (given at the bottom of Table 2) to approximate a 96-h LC50 when the LC50 was determined over a shorter interval. This method of approximation greatly expands the information base on 96-h LC50.

Toxicity beyond 96 h

Toxicity after 96 h has been quantified in terms of mortality, growth suppression, and tissue damage. We shall consider these separately.

Mortality

Westin (1974) reported the relative 96-h and 7-d toxicity of nitrite to chinook salmon (1 and 11 g). According to the trend line she established for percent mortality, the 7-d LC50 was approximately two-thirds of the 96-h LC50. In similar experiments involving rainbow trout, Russo et al. (1974) showed that the LC50 became asymptotic after 8 d at concentrations approximately 60% of the 96-h LC50. The maximum concentration of nitrite for no mortality was essentially equal at 96 h and at 8 d, and varied between 30 and 50% of the 96-h LC50. Thurston et al. (1978) found that the 10-d LC50 for cutthroat trout (3 g) was equal to or greater than 75% of the 96-h LC50; the LC50 appeared to be asymptotic after about 5 d. The LC50 for small cutthroat trout (1 g) exposed for periods as long as 36 d converged on an asymptote close to that for the shorter exposures on slightly larger fish.

Although LC50s for periods longer than 96 h are unavailable for warmwater fishes, the work of Tucker and Schwedler (1983) with channel catfish demonstrated, on the basis of methemoglobin levels, a degree of acclimation over long intervals.

This supports the impression from bioassays on salmonids that few changes occur in mortality after 5 to 7 d, and that resistance might begin to develop within fish after this period of time, presumably due to improvement in the efficiency of the hemoglobin reductase system.

Growth Suppression

Wedemeyer and Yasutake (1978) found no statistically significant growth suppression of steelhead during 6-month exposures to nitrite concentrations as high as 10% of the 96-h LC50. Working with channel catfish, Colt et al. (1981) found that the minimum amount of nitrite capable of causing detectable growth suppression over 31 d was equal to 44% of the minimum nitrite concentration required to induce mortality. The work of Bowser et al. (1983) showed that the minimum nitrite required to cause mortality of channel catfish would equal approximately half of the 96-h LC50, which implies that the minimum amount of nitrite capable of causing detectable growth suppression in channel catfish under the conditions studied by Colt et al. would be approximately one-fifth of the 96-h LC50. The maximum growth suppression at such concentrations would be approximately 10%; the maximum growth suppression actually observed by Colt et al. at any nonlethal concentration was 21%.

Tissue Damage

Wedemeyer and Yasutake (1978) examined tissues of rainbow trout that had been held for 28 weeks at sublethal nitrite concentrations. The kidneys, blood, and thymus did not show damage; slight changes in the gill tissues, judged by the authors to be of minimal importance, were observed after 3 weeks but had disappeared after the fish had been held 7 weeks. Colt et al. (1981) found no gill damage in channel catfish, even at lethal concentrations of nitrite.

In general, literature on long-term effects of nitrite both for warmwater and coldwater fishes uniformly suggests that the 96-h LC50 is only a few times greater than concentrations showing very minimal or negligible nonlethal effects. There is as yet no evidence that a nitrite concentration equal to 10% or less of the 96-h LC50 would be detrimental to freshwater fishes.

Environmental Factors

Chloride

Prior to 1977, the literature on nitrite toxicity suggested great random variation in the toxicity

TABLE 2.—Nitrite toxicity tests on five species over intervals of 24 to 96 h. Ratios of the shorter-term LC50s (median lethal concentrations) to the 96-h LC50 are given and the medians of these ratios are shown at the bottom of the table. Water quality data are midrange values.

Species or statistic	Source ^a	Cl ⁻ (mg/L)	pH	Ca ⁺⁺ (mg/L)	Alkalinity (CaCO ₃ , mg/L)	Nitrite-N LC50 (ratio to 96-h value)			
						96 h	72 h	48 h	24 h
Coldwater									
Rainbow trout	(1)	0.35	7.9	60	176	0.24	0.30(1.25)	0.32(1.33)	0.49(2.04)
	(2)	10	7.7	52	171	3		5 (1.67)	8 (2.67)
		20	7.7	52	171	8		10 (1.25)	15 (1.88)
	(3)	40	7.7	52	171	11		20 (1.82)	28 (2.54)
		1	7.0	4		3.9	4.2 (1.07)	4.9 (1.25)	9.8 (2.50)
Warmwater									
Channel catfish	(4)		7.5		60	7.5	8.3 (1.10)	8.7 (1.16)	10.3 (1.39)
	(5)	22	7.9	80	190	7.1	13.5 (1.90)	17.5 (2.46)	30.0 (4.30)
Fathead minnow	(6)	22	7.9	80	190	70	79 (1.13)	85 (1.22)	182 (2.61)
	(6)	22	7.9	80	190	45	45 (1.00)	46 (1.01)	57 (1.25)
Blue tilapia	(5)	22	7.9	80	190	16	20 (1.25)	22 (1.38)	31 (1.94)
Largemouth bass	(5)	22	7.9	80	190	140	140 (1.00)	142 (1.01)	160 (1.14)
Median ratio ^b							1.10	1.25	1.99
Greatest ratio ^b							1.25	1.82	2.67

^a (1) Russo et al. (1974) for 96-h, 48-h, 24-h LC50s; Russo and Thurston (1977) for chloride and 72-h LC50; Russo et al. (1981) for calcium; average for fish heavier than 10 g.

(2) Russo (1980).

(3) Eddy et al. (1983).

(4) Konikoff (1975).

(5) Palachek and Tomasso (1984a); calcium estimated from hardness.

(6) Palachek and Tomasso (1984b); first row 0.3–0.8 g, second row 0.9–3.3 g.

^b Statistics exclude one set of outlying values for channel catfish (5).

of nitrite to fish, even within the same species. In 1977, however, Crawford and Allen showed that the toxicity of nitrite to small chinook salmon depended greatly on the salinity of the water in which the nitrite exposure occurred; mortality in seawater occurred at nitrite concentrations 50 to 100 times higher than in fresh water. Also in 1977, Perrone and Meade showed that chloride could protect coho salmon against nitrite toxicity and proposed that chloride competes with nitrite for transport across the gills (see also Russo and Thurston 1977). The effect of chloride on the toxicity of nitrite is now known to be so great that experiments in which chloride concentrations are not documented are of very little value because they cannot be meaningfully compared with the results of other studies.

Although several studies offer comparisons of toxicity at two or three chloride levels, very few are based on a sufficiently large number of chloride concentrations that the complete form of the relationship between chloride and toxicity can be discerned. One of the best studies on salmonids is that of Russo and Thurston (1977), who gave the

results of nitrite toxicity tests at six chloride concentrations for rainbow trout. The relationship between nitrite toxicity and chloride concentration is linear. Our regression on their data shows that the slope of the relationship between 96-h LC50 (Y , mg/L nitrite-N) and chloride concentration (X , mg/L) is 0.29 (SE, 0.0087; significant at $P = 0.01$; intercept = 0.53 mg/L nitrite-N): an increase of 1 mg/L in the concentration of chloride raises the 96-h LC50 by 0.29 mg/L nitrite-N; an increase of 1 mM in chloride raises the 96-h LC50 for nitrite-N by 0.73 mM.

Tomasso et al. (1979) compared methemoglobin levels of channel catfish over a range of chloride and nitrite concentrations. Although it is not possible to derive LC50 data from these experiments, it is clear from the experiments that chloride concentration is very important in governing methemoglobin formation. For example, channel catfish in water containing 0.1 mM chloride (3.54 mg/L) developed a 77% methemoglobin level in the presence of 1.5 mg/L nitrite-N. In contrast, in the presence of 1.7 mM chloride (60 mg/L) and the same amount of nitrite, the methemoglobin

level was indistinguishable from background (8.8%).

Schwedler and Tucker (1983) did a study similar to that of Tomasso et al. (1979). Combining their own data with those of Tomasso et al., they concluded that the relationship between percent methemoglobin (Y) and the ratio of nitrite to chloride (X) was $Y = 7.33 + 78.17X$. The 96-h LC50 for channel catfish corresponds approximately to 80% (70–90%) methemoglobin (Bowser et al. 1983), for which the molar ratio X is 0.94 according to the equation. The implication of this relationship is that the addition of 1 mM of chloride (35 mg/L) raises the 96-h LC50 for nitrite by approximately 0.94 mM (13 mg/L); 1 mg/L of Cl^- offsets 0.37 mg/L of nitrite-N.

Yet another relationship between chloride and nitrite can be derived from the combined data of Palachek and Tomasso (1984b), Russo and Thurston (1977), and McConnell (1985) for the fathead minnow. As shown by McConnell (1985) from regression analysis of this combined data set, 1 mg/L of chloride offsets 2.0 mg/L of nitrite-N; 1 mM of chloride raises the 96-h LC50 for nitrite by 5.08 mM.

Among the three species for which a chloride-toxicity relationship is available, rainbow trout and channel catfish are the most sensitive and the fathead minnow is considerably less sensitive. The effect of chloride on LC50 for nitrite appears to be inversely related to sensitivity of a fish species to nitrite: the most-sensitive species benefit least from chloride addition, although the benefit is large even for sensitive fish. Possibly the less-sensitive fish are able to discriminate to some degree against nitrite; this would explain why chloride is more potent in offsetting nitrite toxicity in these fish.

Other Anions

There is some information on the effects of anions other than chloride on nitrite toxicity. Bromide, which is similar chemically to chloride, was studied at Eddy et al. (1983), who found that 1 mM sodium bromide (80 mg/L Br^-) was enough to offset almost completely the presence of 0.7 mM nitrite (32 mg/L nitrite-N) for Atlantic salmon in fresh water. Although this shows that chloride is not the only ion that can offset nitrite toxicity, bromide is not normally a major constituent of fresh waters.

Bicarbonate, although chemically very different from chloride, is of interest because it accounts for a high fraction of the total anions in fresh waters. The studies by Eddy et al. on Atlantic salmon

included a test of the effect of sodium bicarbonate on nitrite toxicity. Sodium bicarbonate at 2.5 mM (152 mg/L HCO_3^-) did reduce toxicity significantly, but not nearly so much as sodium bromide at 1 mM.

Huey et al. (1980) showed for channel catfish that 840 mg/L of sodium bicarbonate (610 mg/L HCO_3^-) was sufficient to hold methemoglobin levels at 8%, the background level, whereas controls without sodium bicarbonate showed 60% methemoglobin at the experimental exposure of 0.76 mg/L nitrite-N. It is not possible to make direct comparisons between chloride and bicarbonate from Huey's work because sodium bicarbonate was added in excess of the minimum necessary to hold methemoglobin levels at background. Given the general relationship already described above for channel catfish, however, we estimate that 2 mg/L (0.057 mM) of chloride, which is the amount required to offset 0.76 mg/L (0.054 mM) of nitrite-N, would have been required to hold methemoglobin levels near background. In contrast, 610 mg/L bicarbonate (10 mM) was required to achieve almost complete suppression of methemoglobinemia. Thus, on a molar basis, the bicarbonate ion in this case was approximately 0.54% as effective as the chloride ion.

Bath and Eddy (1980) showed that rainbow trout experiencing 90% mortality at 9.8 mg/L nitrite-N (0.7 mM) after 72 h showed 50% survival under the same conditions in the presence of 152 mg/L (2.5 mM) bicarbonate. This experiment, like the two already mentioned, suggests that bicarbonate is effective, but not so effective as chloride, in offsetting nitrite toxicity. Bowser et al. (1983) also found that bicarbonate was less effective than chloride in preventing methemoglobinemia. As noted by Maetz (1971), chloride uptake from the medium is inhibited by bicarbonate; bicarbonate thus may repress nitrite uptake by a similar mechanism.

Russo et al. (1981) documented a small nitrate repression of nitrite toxicity. Divalent and trivalent anions that have been studied in connection with nitrite toxicity include sulfate, phosphate, and borate. Huey et al. (1980) found that 1.42 g/L of sodium sulfate failed to reduce the methemoglobin percentages of channel catfish exposed to 0.76 mg/L nitrite-N. Eddy et al. (1983) showed that magnesium sulfate and potassium sulfate at seawater concentrations had very little effect on the toxicity of nitrite to rainbow trout. Russo et al. (1981) showed minimal effects of sulfate and phosphate on the toxicity of nitrite to rainbow trout.

In summary, the literature presently shows that chloride and bromide are highly effective in reducing nitrite toxicity, that bicarbonate and nitrate have detectable effects but are not nearly so effective as chloride and bromide, and that divalent and trivalent anions have very little effect on nitrite toxicity.

Cations

Calcium, magnesium, sodium, and potassium are typically present in considerable quantities in fresh waters. The effects that these ions might have on nitrite toxicity are therefore of interest. The mechanism of any effects would obviously be different from that of chloride, however.

Crawford and Allen (1977) found that the addition of calcium sulfate to fresh water containing small chinook salmon decreased the toxicity of nitrite but did not reduce methemoglobinemia. Conversely, nitrite in water lacking calcium was highly toxic but did not induce appreciable methemoglobinemia. These findings should be interpreted with caution, however, because of the very high nitrite concentrations that were used (30 mg/L nitrite-N), which could induce toxic reactions of a different nature from those observed at lower, more realistic, concentrations. The dissociation of methemoglobinemia from mortality in the presence of calcium in these tests remains unexplained and has not yet been reported in other studies.

Although Crawford and Allen cited the work of Weber (1966) on the guppy as additional evidence for a possible connection between calcium and nitrite toxicity, Weber did not separate the effect of calcium from that of the associated anions or acidity. Likewise, Bath and Eddy (1980) did not separate the effect of calcium from that of anions (NO_3^-) added with calcium. For this reason, the results are inconclusive with regard to calcium.

Bowser et al. (1983) found that sodium chloride and calcium chloride provided equivalent protection against nitrite toxicity for channel catfish, suggesting that the identity of the metal cation was of little importance. Tomasso et al. (1980) provided similar information for channel catfish. Wedemeyer and Yasutake (1978) found nitrite to be markedly less toxic in the presence of calcium than of sodium, but their results are difficult to interpret because the LC50 values are very different from the others reported in their paper or elsewhere in the literature for similar chloride concentrations.

Krous et al. (1982) pointed out that high concentrations of calcium generally reduce the loss of

chloride through the gills. This, in turn, reduces the requirement for uptake of chloride, which decreases the uptake of nitrite. Thus there are theoretical reasons to expect that calcium ions will reduce the toxicity of nitrite, although experimental work that has been done thus far suggests that the effect is a weak one.

Acidity

The effect of hydrogen ion concentration on toxicity of nitrite is still very uncertain. Contributions to the literature on this subject frequently are not definitive because they fail to separate the possible effects of anions from those of acidity or they use pH ranges outside the normal adaptive range of the fish.

Bath and Eddy (1980) reported for rainbow trout that acidity of the water had no significant effect on nitrite toxicity except at extreme pH levels (below pH 5 and above pH 10), but they did not give details of their findings. Working with coho salmon at two different chloride levels, Meade and Perrone (1980) showed that pH, which they controlled by means of a tris buffer, affected the plasma nitrite concentrations and plasma methemoglobin. Within a pH range of 6.5 to 8.0, which could be considered representative of natural conditions for this species, both methemoglobin and plasma nitrite changed by approximately the same amount: they were about twice as high at pH 6.5 as at pH 8.0 (15 mg/L chloride and 10 mg/L nitrite-N). The use of a buffer for manipulation of pH in such an experiment, however, changes the anion background. Thus it is difficult to separate the effects of changing anion background from the effects of acidity.

For channel catfish, Huey et al. (1980) adjusted the holding water to a pH of 5.3 using a phosphate buffer and to a pH of 9.1 using a sodium bicarbonate buffer. Channel catfish held at 0.76 mg/L nitrite-N for 24 h showed methemoglobin levels of 11% at pH 9.1 and 76% at pH 5.3. Because experimental evidence suggests that bicarbonate reduces the uptake of nitrite, the addition of 1,220 mg/L (20 mM) bicarbonate, as in these experiments, might well result in reduced methemoglobin levels even in the absence of a pH change. Thus it is difficult to interpret the experiments from the viewpoint of pH alone.

In other experiments, Huey et al. (1982) used pH 4 as the lower end of a pH test range for bluegill. Although there was substantial interaction between pH and nitrite toxicity because of a lower LC50 at pH 4, the results are probably not very

meaningful because pH 4 is well outside the normal adaptive range of the fish.

The strongest case for an acidity effect is based on the work of Wedemeyer and Yasutake (1978) with steelhead. In these experiments, the pH of soft water was adjusted to 6, 7, and 8 with a weak phosphate buffer. If results for pH 6, which is extreme for steelhead in view of unlikelihood that such a pH would occur in its natural environment, are discounted, the results showed 96-h LC50s of 1.5 mg/L nitrite-N at pH 7 and 2.5 mg/L at pH 8 for fish weighing 5 g. The LC50s were 2.3 mg/L at pH 7 and 3.6 mg/L at pH 8 for fish weighing 10 g. Thus the acidity effect, although statistically significant, was small. Furthermore, it should be noted that pH adjustment of soft water changes the bicarbonate content of the water by influencing the atmospheric equilibrium of CO₂ with water.

Experiments by Russo et al. (1981) dealt with the effects of acidity on rainbow trout. Although the ionic background is better documented for this experiment than for most others, it is still difficult to separate the effects of acidity from other effects. The authors conclude that pH is quite important. In their experiments, pH values ranging from 6.44 to 8.10 were achieved by use of a phosphate buffer. Over this pH range, there was no pattern in the 96-h LC50 (correlation analysis; $P > 0.05$). Above pH 8.1, the authors added sodium hydroxide to maintain high pH, and the 96-h LC50 began to show a pH response. However, a pH in excess of 8.1 might be outside the adaptive range in view of its rarity in natural trout habitats; the experiments do not demonstrate an acidity effect within the most common environmental pH range.

In summary, there is no unequivocal evidence as yet for an effect of acidity on nitrite toxicity over the normal environmental acidity range of freshwater fishes. We reach this conclusion in spite of frequent references to the role of acidity in governing nitrite toxicity. The interest of investigators in the importance of pH has been very high since the suggestion, based on experiments by Colt and Tchobanoglous (1976), that the amount of nitrite in the blood plasma of fishes is governed by the entry of undissociated nitrous acid through the gills. For example, Wedemeyer and Yasutake (1978) concluded that pH must have an important effect because it controls the balance between nitrite and nitrous acid, both in the medium and inside the fish. As we have explained above, however, the proposition that nitrous acid is the main mode of entry of nitrite into fish is very much open to question. The recent discovery by Pala-

chek and Tomasso (1984a) that nitrite concentrations in largemouth bass do not exceed environmental concentrations is a particularly persuasive argument against the theory of passive nitrite accumulation through nitrous acid penetration of the gills. The weight of evidence at present favors the interpretation that the nitrite ion is pumped into the fish through the gills except in those fish that are able to discriminate against nitrite (largemouth bass), and that the role of pH in governing the entry of undissociated nitrous acid is unimportant. This does not rule out some other roles for the hydrogen ion, however. For example, as pointed out by Meade and Perrone (1980), a change in the pH could alter the uptake characteristics of the ion-concentrating mechanisms in the gills, leading to pH effects over very large pH ranges. Over the most likely natural pH ranges, once the effects of different ionic backgrounds have been taken into account, the effect of pH on nitrite toxicity appears to be small.

Oxygen and Temperature

Oxygen can affect the toxicity of nitrite because nitrite reduces the oxygen-carrying capacity of the blood; reduction of the oxygen supply in the external medium will exacerbate the oxygen supply problem within the fish. In the only published study that bears on this question, Bowser et al. (1983) showed that an oxygen concentration of 5 mg/L, in the presence of nitrite, was not sufficient for channel catfish, even though channel catfish normally tolerate oxygen concentrations below this.

Temperature, which affects tissue oxygen demand, could also be expected to affect nitrite toxicity. The only experimental study of the relationship between temperature and nitrite toxicity appears to be for channel catfish (Colt and Tchobanoglous 1976; Huey et al. 1984; Watenpaugh et al. 1985). Over a relatively small range (22–30°C), Colt and Tchobanoglous (1976) showed no significant relationship between nitrite toxicity and temperature. In the study of Huey et al. (1984), channel catfish held at 30°C in the presence of 0.91 mg/L nitrite-N over a period of 24 h developed methemoglobin concentrations almost twice as high as those of fish held at 10°C. It is not clear from these experiments whether the methemoglobin levels had reached equilibrium in the fish held at different temperatures. Huey et al. also found that the fish held at 30°C showed a more rapid return to background hemoglobin levels in the absence of nitrite. There were, however, no LC50 data with this study. Watenpaugh et al. (1985)

showed that the critical thermal maximum was slightly lower (35.9°C) for channel catfish exposed to 0.43 mg/L of nitrite-N than for controls (38.0°C); this is consistent with the concept that higher temperatures reduce the LC50 of nitrite by raising the tissue oxygen demand.

The higher amount of oxygen in water at lower temperatures and the lower metabolic rates of fish at lower temperatures might render nitrite a less potent toxin at lower temperatures. However, given that lower temperatures also reduce the efficiency of detoxification mechanisms, general conclusions should be approached with caution.

Fish Size

An early study by Smith and Williams (1974) on rainbow trout showed that small fish were less sensitive to 24-h exposures of nitrite than were larger ones. Russo et al. (1974) showed that rainbow trout larvae were slightly less susceptible to nitrite than larger fish, and subsequent studies by Russo and Thurston (1977) on rainbow trout also suggested that the larger fish are slightly more sensitive to nitrite. Thurston et al. (1978) reported no difference in the sensitivity of cutthroat trout weighing 1 and 3 g. This, however, could be explained by the very small size range of the fish. For rainbow trout over the range 2 to 387 g, Russo (1980) found no statistically significant relationship between size and mortality over 96 h. The statistical procedure used by Russo was not well suited for evaluation of the experiments, however. Russo's data show that the four highest LC50 values occurred in the first 5 of 20 size groups. It is possible to determine directly the probability that such an uneven distribution of LC50 values could occur by chance. The probability of the observed outcome or a more extreme one (all 5 of the smallest sizes being the 5 highest LC50s) by chance is $5 \cdot 5! \cdot 16! / 20!$, or 0.005. Thus the small fish had significantly higher LC50s than the large fish.

Perrone and Meade (1977) showed that very small coho salmon (0.65 g) were less susceptible to nitrite than yearlings (22 g). In the only study demonstrating more resistance in larger fish, Wedemeyer and Yasutake (1978) concluded that rainbow trout weighing 10 g were slightly less vulnerable to nitrite toxicity than rainbow trout weighing 5 g, although standard errors for the 96-h LC50 values overlapped.

For warmwater fish, the only extensive study of the relationship between size and nitrite toxicity is that of Palachek and Tomasso (1984b). These authors concluded that fathead minnows weighing

between 0.3 and 0.8 g were more tolerant of nitrite than fish weighing 0.9 to 3.3 g. The 96-h LC50 for the smaller fish was about 50% higher than that of the larger fish.

We conclude from the presently available literature that small fish, even larvae, are unlikely to be more sensitive to nitrite than larger fish of the same species. Furthermore, there is definite evidence for some species that very small fish are less vulnerable to toxicity than fish of intermediate or large size.

Species-Specific Toxicity

Table 3 summarizes nitrite bioassay data for fish and gives the experimental conditions insofar as possible. We have converted all of the LC50 data to a 96-h basis by use of the LC50 ratios shown in Table 2. We have also standardized the 96-h LC50s to a constant chloride concentration of 20 mg/L. Salmonids were corrected to 20 mg/L chloride by use of the slope relating LC50 to chloride as described previously (0.73 moles of NO_2^- per mole of Cl^-). Channel catfish were corrected with a slope of 0.94; the same slope was used for logperch, brook stickleback, and blue tilapia, which have broadly similar sensitivities to nitrite. The slope 5.08 was used for cyprinids, the black bullhead, the mottled sculpin, and catostomids because of the approximate match between the sensitivity of these taxa and that of the fathead minnow, for which the slope is 5.08. No correction was used for centrarchids, whose sensitivities are so low that chloride correction is not meaningful. Some amount of variation is caused by bicarbonate, calcium, fish size, and temperature, but corrections for these factors are not possible because their quantitative nature is poorly known.

Considerably more data will be required before the characteristics of individual species are known with confidence, but Table 3 does show evidence of much interspecific variation and some clustering of taxa. Salmonids are among the most sensitive of the taxa that have been studied, and show very little difference among species. There is considerable variation among the warmwater fish taxa: channel catfish are as sensitive as salmonids, and the logperch, brook stickleback, and blue tilapia seem to be similarly sensitive or only slightly less so. The cyprinids, catostomids, mottled sculpin, and black bullhead are considerably less sensitive. The centrarchids are especially insensitive to nitrite toxicity. Critical concentrations for the large-mouth bass are quite high because the blood plasma concentrations do not exceed those of the

TABLE 3.—Summary of nitrite LC50 data (median lethal concentrations) for all fish species on which there are published data.

Species	Source ^a	Cl ⁻ (mg/L)	Ca ⁺⁺ (mg/L)	Alka- linity (CaCO ₃ , mg/L)	Tem- per- ature °C	pH	LC50 Nitrite-N	Time (h) for LC50	LC50 adjusted to 96 h	LC50 adjusted to 96 h, 20 mg/L Cl ⁻	
Coldwater											
Rainbow trout	(1)	0.35	60	176	10	7.9	0.24	96	0.24	5.90	
	(2)	0.24	54	164	11	7.3	0.27	96	0.27	8.06	
		0.24	54	139	12	7.2	0.15	96	0.15	7.94	
		0.24	54	174	11	7.9	0.26	96	0.26	8.05	
		0.24	54	186	11	8.6	0.70	96	0.70	8.49	
		10	51	174	12	7.5	3.74	96	3.74	6.62	
		10	51	177	12	7.9	3.54	96	3.54	6.42	
		10	51	188	12	8.5	4.35	96	4.35	7.23	
		10	51	184	12	8.6	5.34	96	5.34	8.22	
		(3)	0.35	50	177	10	7.8	0.25	96	0.25	8.02
			1.2	50	177	10	7.9	0.46	96	0.46	5.88
			5.1	50	177	10	8.0	2.36	96	2.36	6.65
			10.4	50	177	10	7.9	3.54	96	3.54	6.31
			20.2	50	177	10	7.8	6.69	96	6.69	6.63
			40.9	50	177	10	7.7	12.20	96	12.20	6.18
			40.8	50	177	10	7.7	12.60	96	12.60	6.61
		(4)	<1.0	<4		10	7.0	3.7	96	3.7	9.4
			38	4		10	7.0	9.8	96	9.8	4.6
		(5)				10		1.6	24	0.8	
		(6)	1.4	8	25	10	6.2	0.5(0.9)	96	0.5(0.9)	5.9(6.3)
		1.9	16	50	10	6.8	0.5(1.9)	96	0.5(1.9)	5.7(7.1)	
		4.2	40	100	10	7.3	4.7(5.8)	96	4.7(5.8)	9.2(10.4)	
		8.4	70	300	10	7.8	10.3(12.1)	96	10.3(12.1)	13.6(15.4)	
		1.9	16	50	10	6.0	0.3(1.4)	96	0.3(1.4)	3.6(4.7)	
		1.9	16	50	10	7.0	1.5(2.3)	96	1.5(2.3)	4.8(5.6)	
		1.9	16	50	10	8.0	2.5(3.6)	96	2.5(3.6)	5.8(7.1)	
Chinook salmon	(7)				15		0.88	96	0.88		
	(8)	20		20	13	7.2	9.2	24	4.7	4.7	
	(9)		32		9		5.75	48	4.6		
Cutthroat trout	(10)	0.44	53	176	12	8.0	0.52	96	0.52	6.6	
Warmwater											
Ictaluridae											
Channel catfish	(11)			65	23	7.5	7.6	96	7.6		
	(12)			220	22	8.7	12.8	96	12.8		
	(13)	22	80	190	32	7.9	7.1	96	7.1	6.4	
Black bullhead	(14)	10					>40	>48	>32	>52	
Cyprinidae											
Fathead minnow	(3)	0.35	53	177	13	8.0	2.99	96	2.99	42	
		0.35	53	177	13	8.0	2.30	96	2.30	41	
	(15)	22	80	190	23	7.9	70	96	70	66	
		22	80	190	23	7.9	45	96	45	41	
European minnow	(16)				20		28	96	28		
Creek chub	(17)	9	27	98	18	8.3	81	24	>41	>63	
Common carp	(14)	10					>40	48	>32	>52	
Catostomidae											
White sucker	(14)	10					>100	48	>80	>100	
Quillback	(14)	10					>100	24	>80	>100	
Centrarchidae											
Largemouth bass	(15)	22	80	190	23	7.9	140	96	140	140	

TABLE 3.—Continued.

Species	Source ^a	Cl ⁻ (mg/L)	Ca ⁺⁺ (mg/L)	Alka- linity (CaCO ₃ , mg/L)	Tem- per- ature °C	pH	LC50 Nitrite-N	Time (h) for LC50	LC50 adjusted to 96 h	LC50 adjusted to 96 h, 20 mg/L Cl ⁻
Bluegill	(18)	60			30	4.0	4.4	24	2.4	
		60			30	7.2	211.3	24	108	108
		5			30	4.0	4.6	24	2.4	
		5			30	7.2	282.0	24	144	144
Other families										
Mosquitofish	(19)			<100	22	7.3	1.5	96	1.5	
Blue tilapia	(15)	22	80	190	23	7.9	16.0	96	16.0	15
Logperch	(14)	10					<5	24	<3	<9
Brook stickleback	(14)	10					<5	24	<3	<9
Mottled sculpin	(3)	0.35	53	177	13	8.1	>67	154	>67	>106

- ^a (1) Russo et al. (1974); chloride from Russo and Thurston (1977).
 (2) Russo et al. (1981); weighted means according to number of fish; pH treatments involve different anion treatments.
 (3) Russo and Thurston (1977); calcium estimated from Thurston et al. (1978).
 (4) Eddy et al. (1983).
 (5) Smith and Williams (1974).
 (6) Wedemeyer and Yasutake (1978); 5-g fish (10-g fish); chloride from Table 2, contradicted by text and Table 1.
 (7) Westin (1974).
 (8) Peronne and Meade (1977); based on observed 50% mortality, not a true LC50 analysis.
 (9) Crawford and Allen (1977).
 (10) Thurston et al. (1978).
 (11) Konikoff (1975).
 (12) Colt and Tchobanoglous (1976).
 (13) Palacheck and Tomasso (1984b); calcium estimated from hardness.
 (14) McCoy (1972); chloride values estimated from personal communication with city of Madison, Wisconsin.
 (15) Palacheck and Tomasso (1984a); higher LC50 is for fish of 0.30–0.83 g, lower for fish of 0.9–3.33 g; calcium estimated from hardness.
 (16) Klingler (1957); estimated from data table.
 (17) Gillette et al. (1952).
 (18) Huey et al. (1982); pH 4 probably unrealistic; 60 mg/L Cl⁻ calculated from Cl⁻ : NO₂⁻ ratio for 24-h LC50.
 (19) Wallen et al. (1957).

environment. Other insensitive taxa, including bluegill, mottled sculpin, catostomids, and some of the least sensitive cyprinids, should also be studied for blood plasma response to nitrite in the environment.

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