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Toxicity of Nitrite to Larvae of the Salamander *Ambystoma texanum*

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Ammonia the major nitrogenous waste product of aquatic animals is converted to nitrite by bacteria. In ponds, aquatic recirculating culture systems, laboratory holding systems, sewage plant receiving waters and natural systems when animal biomass is high, nitrite can reach lethal or limiting levels particularly if imbalances occur in the relative abundances of Nitrosomonas and Nitrobacter. These two genera of bacteria oxidize ammonia and nitrite, respectively. In ponds nitrite can accumulate from denitrification in anaerobic sediments (BOYD and HOLLERMAN 1980).

Nitrite toxicity has been studied in several fish species (eg. KLINGER 1959, SMITH and WILLIAMS 1974; WESTIN 1974; RUSSO and THURSTON 1977). The major toxic action of nitrite results from oxidation of hemoglobin to methemoglobin which is incapable of binding oxygen (BODANSKY 1952). Nitrite induced methemoglobinemia can result in tissue anoxia and death (CAMERON 1971). Lethal levels as 96 hr LC-50's for fishes range from 27.0 mgL^{-1} for channel catfish, Ictalurus punctatus, (KONIKOFF 1975) to 0.7 mgL^{-1} for rainbow trout, Salmo gairdneri (RUSSO et al. 1974) and are highly dependent upon water chemistry. Presence of monovalent anions greatly curtails nitrite toxicity (PERRONE and MEADE 1977; RUSSO and THURSTON 1977; WEDEMEYER and YASUTAKE 1978; TOMASSO et al. 1979; HUEY et al. 1980).

Little data exist concerning nitrite toxicity in non-fish vertebrates. In a previous study we (HUEY and BEITINGER in press) reported nitrite induced methemoglobinemia in bullfrog tadpoles, Rana catesbiana. For comparative purposes, we designed research to determine the 96 hr LC-50 for aquatic larvae of the salamander (Ambystoma texanum). In addition, environmental chloride levels were increased in one trial to test if this monovalent anion functions to suppress nitrite lethality as reported for fishes and crayfish.

MATERIALS AND METHODS

Aquatic larvae of the salamander, Ambystoma texanum, (0.45 $\pm$ 0.08 g, $\bar{x} \pm s$) obtained by seining a pond in Denton, County, Texas were placed in 190-L filtered aquaria and held post-absorptive for four days prior to testing. Standard methodology for static bioassay (E.P.A. 1975) was employed. Trials were conducted in 25°C, O_2 saturated, medium hardness (140 mgL^{-1}, total hardness), low chloride (5.0 mgL^{-1}) water at pH 7.0, except for one trial at 300

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mgL^{-1} chlorides. Reagent grade chemicals were added to 30-L test tanks and initially mixed with a mechanical stirrer. Appropriate quantities of nitrite, as sodium nitrite were added to yield 11 test concentrations ranging from 0.08 to 103.4 mgL^{-1} NO_2^-. A concentration of 0.01 mgL^{-1} NO_2^- served as a control. Nitrite concentrations were quantified using the azo-dye technique (Standard Methods 1971). Temperature, dissolved O_2, pH, nitrite and ammonia were monitored at 12 hr intervals. Total hardness and chlorides were measured at the beginning and termination of the experiment. Ten larvae were exposed to each concentration. Loss of equilibrium, i.e. ecological death, was the criterion for lethality. Lethal concentration, as 96 hr LC-50, was calculated by a program developed by Dr. C. E. Stephan, E.P.A. laboratory, Duluth, MN.

RESULTS AND DISCUSSION

The 96 hr LC-50 for (Ambystoma texanum) in low chloride (5.0 mgL^{-1}) water equalled 1.09 mgL^{-1} NO_2^- (Table 1) with conservative 95% confidence limits extending from 0.48 to 0.2 mgL^{-1}. Nearly 100%

Table 1. Lethality of nitrite to larval salamanders (Ambystoma texanum). All tests include 10 animals exposed for 96 hr at pH 7.0.

Nitrite mgL^{-1} as NO_2^-	% Dead	LC-50 Nitrite mgL^{-1} NO_2	NO_2-N
103.40	100		
51.80	100		
10.10	90		
6.60	100		
2.50	90		
0.98	10	1.09	0.33[A]
0.90	70		
0.48	10		
0.09	0		
0.08	0		
0.01	0		

[A] Computed as Binominal Test for LC-50.

mortality was observed in trials as low as 2.5 mgL^{-1} NO_2^-. At exposures in excess of 10 mgL^{-1} NO_2^- larvae immediately exhibited stress including excessive mucus production and air gulping. Differences in chemical composition of test water make relative nitrite toxicity comparisons tenuous; however, values for A. texanum indicate that this species is highly susceptible to NO_2^-. The 96 hr LC-50 approaches the lowest concentration causing lethality, 0.7 mgL^{-1}, reported for vertebrates, rainbow trout (RUSSO et al 1974).

The single group of larva exposed to 300 mgL^{-1} chlorides at 10 mgL^{-1} nitrite suffered no mortality in 96 hr. This increase in nitrite tolerance in the presence of chlorides was expected and corroborates results of CRAWFORD and ALLEN (1977) demonstrating reduced nitrite lethality for chinook salmon (Oncorhynchus tshawytscha) in seawater relative to freshwater. Results of this experiment support a monovalent ion-competitive uptake model hypothesized to occur in fish. In this model monovalent anions compete with nitrites for ionic uptake sites on the respiratory surfaces. We suggest that decreased nitrite mortality in the presence of chlorides is not an outcome of increased physiological tolerance but rather relates to lower NO_2^- uptake rates.

Salamander larvae of this genus often inhabit warm, stagnant pools with low dissolved oxygen and "gulp" atmospheric O_2 to support aerobic metabolism; surprisingly they are highly sensitive to nitrite toxicity. Results suggest that their tissue tolerance to reduced oxygen produced by nitrite oxidized hemoglobin is low. Tissue anoxia caused by methemoglobinemia may trigger air gulping in these animals; however, hemoglobin bound oxygen cannot be increased owing to the inability of methemoglobin to bind oxygen. Sensitivity to nitrites is probably a result of high ionic uptake of NO_2^- and a poorly developed or nonexistent methemoglobin reductase system.

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