Bull. Environ. Contam. Toxicol. (1984) 32:674–681

Bulletin of Environmental Contamination and Toxicology

# Nitrite-Induced Methemoglobin Formation and Recovery in Channel Catfish (*Ictalurus punctatus*) at Three Acclimation Temperatures

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Water-borne nitrite can interfere with the intensive culture of fish such as channel catfish, *Ictalurus punctatus* (Lovell 1979). Nitrite ($NO_2^-$) is an intermediate compound formed during the nitrification of ammonia which is the major nitrogenous waste product of aquatic animals. Lethal or limiting concentrations of nitrite develop owing to imbalances in the relative abundances of the bacteria *Nitrosomonas* and *Nitrobacter* in biofiltered systems. Also nitrite from denitrification can accumulate in anaerobic sediments of ponds used in the culture of channel catfish (Boyd and Hollerman 1980).

In vertebrates, nitrite imparts a distinctive brown color to blood by oxidizing hemoglobin to methemoglobin, a derivative incapable of binding with oxygen (Bodansky 1951). Methemoglobinemia has been observed in fish and larval amphibians (Smith and Williams 1974; Russo et al. 1974; Huey et al. 1980, Huey and Beitinger 1980 a, b). Catfish with moderate nitrite-induced methemoglobinemia recover within 24 h when placed in nitrite-free water (Huey et al. 1980). Recovery appears to be aided by a methemoglobin-reductase system hypothesized by Cameron (1971) and confirmed in channel catfish by Huey and Beitinger (1982) and in several other species of fish by Freeman et al. (1983). Since this enzyme system uses NADH to convert oxidized methemoglobin to reduced hemoglobin, it is known in mammals as the NADH-methemoglobin reductase system.

The relationship between nitrite concentration and degree of methemoglobinemia has been documented in catfish (Huey et al. 1980); however, the influence of temperature on this process has not been examined. In light of the effects of temperature on the movement of chemicals, it is tempting to hypothesize that higher temperatures always increase toxicity. Nevertheless, Cairns et al. (1975) conclude that temperature-toxicity interactions are complex. A temperature change may result in an increase, decrease or no change in the toxicity of a chemical pollutant. We conducted research to measure the effect of temperature on methemoglobin formation and recovery in nitrite-exposed channel catfish.

## MATERIALS AND METHODS

Fingerling channel catfish, *Ictalurus punctatus*, (12.5 ± 3.2 g, $\bar{X} \pm SD$) obtained from a local hatchery were brought into the laboratory and held at 24 C for five days. Then fish were randomly separated into three groups and acclimated to 10, 20 or 30 C (± 0.5 C) at a rate of 1 C per day. Once at their acclimation temperature, fish were held at that temperature for 10 days prior to testing. Temperature control was provided by Blue M cold fingers and Haake circulating thermoregulators. Catfish were fed 1 % body weight per day except for the 10 C group which was fed approximately one-half of this ration. Feeding was discontinued four days prior to tests. Biofiltration units were employed in addition to charcoal to filter holding water. Both ammonia and nitrite concentrations were monitored daily during acclimation with an Orion specific ion probe and an azo-dye method (APHA 1975), respectively. Neither ammonia nor nitrite concentrations exceeded 0.2 mg/L.

Nitrite exposure and recovery trials were conducted in 30 L all glass aquaria containing hard (160 mg/L total hardness), low chloride (5.0 mg/L), oxygen saturated water which was prepared by reconstituting distilled water according to E.P.A. (1975). Water pH was maintained near 7.0 by a 0.02 M phosphate buffer. Chemicals added to each aquarium were mixed with a mechanical stirrer. In exposure trials nitrite was introduced as reagent grade sodium nitrite. Nitrite was measured initially and at 12 h intervals. Any decay was corrected by additional sodium nitrite. Exposure and recovery aquaria were placed in a large constant temperature bath regulated at the desired test temperature. Temperatures were measured with a Digitex model 510 digital thermometer.

Methemoglobin formation and recovery were determined at each group's acclimation temperature. Six control, 10 exposure and 10 recovery fish from each group were used. Experiments began by exposing 20 fish to 3.0 mg/L nitrite ($NO_2^-$) for 24 h at their acclimation temperature. Immediately after 24 h of nitrite exposure, 10 of the 20 fish were bled by removing their tails at the caudal peduncle and methemoglobin was determined for each by a method modified from that of Evelyn and Malloy (1938). Recovery data were collected from the other 10 nitrite-exposed fish after they were placed in nitrite-free water for 12 h (at acclimation temperature) and then sampled for methemoglobin. Controls for each acclimation temperature were handled in the same manner with methemoglobin quantified in 3 fish after 24 h in nitrite-free water and in three others after handling and a 12 h "sham" recovery period.

A two-way analysis of variance was employed to survey the data for significant interaction between temperature and treatment group. Since significant interaction was noted ( $p < 0.05$), a quasi F ratio test was used to subdivide variation within the data matrix. All possible column and row combinations were tested using Duncan's multiple range test with an $\alpha$ equal to 0.05.

## RESULTS AND DISCUSSION

Water quality data collected during nitrite exposure and recovery for all control and experimental groups are presented in Table I. Nitrite concentration and pH were similar at the three experimental temperatures. Mean methemoglobin concentrations in the three control groups ranged from 0.12 to 0.20 g % and were not significantly different (Fig. 1).

Following exposure to 3.0 mg/L nitrite for 24 h, methemoglobin levels of catfish at all three acclimation temperatures were significantly greater than those of the controls. Mean methemoglobin concentrations equalled 1.88, 2.18 and 3.42 g% in the 10, 20 and 30 C groups, respectively. These progressively increasing values suggest that methemoglobin formation is directly related to temperature; however, only the 30 C group had methemoglobin levels which were significantly higher. An average methemoglobin formation rate of 0.13 g% / h occurred at 30 C, relative to 0.09 and 0.07 g% / h at 20 and 10 C, respectively. None of the 60 catfish died during nitrite exposure.

Methemoglobin levels did not decrease significantly in either the 10 or 20 C groups following 12 h in nitrite-free water. In fact at 20 C, mean methemoglobin levels in the recovery groups was about 10% higher than those of the fish examined immediately prior to nitrite exposure (Fig. 1). At 20 C, a 17.5% decrease in methemoglobin levels was found between the exposure and recovery groups; however, this difference was not statistically significant.

In contrast, substantial recovery from methemoglobinemia was observed at 30 C. Mean methemoglobin values decreased from 3.42 g% following exposure to 0.92 g% after 12 h of recovery. This 73% difference in mean methemoglobin levels between these two groups was statistically significant. Although the 30 C acclimation group had significantly larger amounts of methemoglobin following nitrite exposure (45 and 36% more than the 10 and 20 C groups), after 12 h of recovery, catfish at 30 C had significantly lower (ca. 50%) methemoglobin than fish at the other two temperatures. One fish in the 30 C recovery group was moribund and was not sampled.

Temperature has multiple effects on aquatic ectotherms such as fish (Fry 1947). Not only can temperature directly cause death (i.e. act as a lethal agent), nonlethal temperatures may influence the toxicity of chemicals, or alternately the ability of a fish to resist a particular chemical.

Elevated temperature increases the diffusion of molecules through water, gill permeability and metabolic rates. The latter increases oxygen demand and hence gill lamellar blood flow.

Figure 1. Methemoglobin in catfish. The 3 adjacent vertical bars give X ± 1 S.D. for the control, exposure and recovery groups at each temperature. Sample sizes are listed in parentheses.

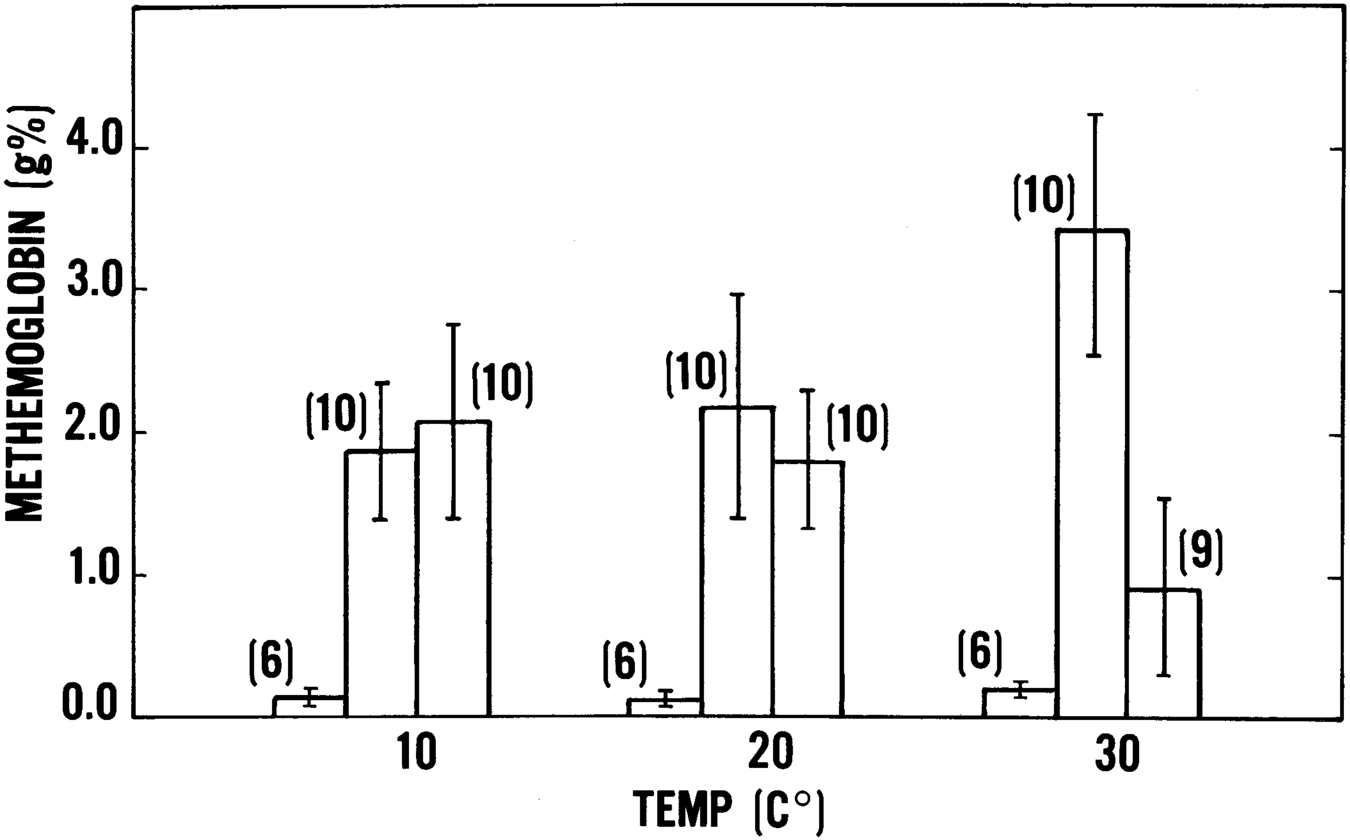

Table 1. Nitrite ($NO_2^-$ in mg/L) concentrations, pH and temperature during nitrite exposure and recovery trials.

| | | 30°C | | | 20°C | | | 10°C | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | | $NO_2^*$ | pH | C | $NO_2^*$ | pH | C | $NO_2^*$ | pH | C |
| EXPOSURE | 0h | 2.92 | 7.15 | 30.4 | 3.10 | 6.94 | 20.2 | 3.01 | 7.12 | 10.2 |
| | 12h | 2.74 | 7.38 | 30.0 | 2.60 | 7.20 | 20.3 | 2.79 | 7.30 | 10.2 |
| | 24h | 2.60 | 7.37 | 30.0 | 3.00 | 7.25 | 20.0 | 2.71 | 7.41 | 10.4 |
| RECOVERY | 0h | 0.01 | 7.40 | 30.5 | 0.00 | 6.98 | 20.1 | 0.00 | 7.30 | 10.2 |
| | 12h | 0.01 | 7.41 | 30.0 | 0.05 | 7.37 | 20.0 | 0.01 | 7.40 | 10.3 |
| CONTROL | 0h | 0.00 | 7.30 | 30.5 | 0.00 | 7.01 | 20.2 | 0.00 | 7.15 | 10.2 |
| | 12h | 0.00 | 7.43 | 30.0 | 0.00 | 7.47 | 20.3 | 0.00 | 7.19 | 10.2 |
| | 24h | 0.01 | 7.70 | 30.0 | 0.03 | 7.45 | 20.0 | 0.00 | 7.20 | 10.3 |
| | 36h | 0.03 | 7.71 | 30.0 | 0.05 | 7.43 | 20.0 | 0.01 | 7.26 | 10.4 |

*When necessary, nitrite was added at 12h intervals in exposure groups to replace decay, $NH_3$ was below 0.1 mg/L in all groups.

Increases in the rate of these internal and external processes enhance chemical uptake, particularly in chemicals such as nitrite whose primary port-of-entry is the gill surfaces. Also Bath and Eddy (1980) suggest that fish can transport nitrite against a concentration gradient via a branchial anion exchange mechanism.

Although we did not directly measure either nitrite uptake rates or plasma concentrations (See Bath and Eddy 1980), our methemoglobin formation data support a hypothesis of increasing chemical uptake at higher temperature. A trend of increasing mean methemoglobin levels with temperature was observed. Catfish at 30 C had nearly twice the methemoglobin levels of those at 10 and 20 C.

Differences in methemoglobin levels in the three acclimation groups following nitrite exposure do not necessarily indicate that greater lethality would occur at 30 C. Following 12 h in nitrite-free water, catfish acclimated to and tested at 30 C had significantly lower methemoglobin levels than fish at the other temperatures. These data suggest that increases in internal responses such as biochemical transformation and depuration may serve to compensate for increased uptake rates at higher temperatures. Data have accumulated which demonstrate a close correspondence between a species' final temperature preferendum and the temperature at which key physiological/biochemical processes are optimized (Brett 1971; Beitinger and Fitzpatrick 1979; McCauley and Casselman 1981; Jobling 1981). It is possible that activity of the methemoglobin-reductase system is optimized and resulted in the greater loss of methemoglobin in the 30 C acclimation group. This temperature is similar to the channel catfish's reported final preferendum, 30.0 to 30.5 C (Cherry et al. 1975; Cheetam et al. 1976) and temperature for maximum growth, 28.0 to 30.0 C (West 1965; Andrews and Stickney 1972; Andrews et al. 1972).

By stimulating both methemoglobin formation during exposure and methemoglobin conversion during recovery, temperature could be interpreted as having a paradoxical effect. An alternate interpretation is that the higher methemoglobin concentrations at 30 C during exposure is an unavoidable effect of high temperature, whereas the lower methemoglobin in this group following recovery is an adaptive response to high levels of methemoglobin.

Some intriguing questions are raised by this research. First, does methemoglobin cause death in nitrite exposed fish? No direct evidence has tied methemoglobinemia to death in nitrite exposed fish. Huey et al. (1980) proposed that nitrite-exposed channel catfish die owing to tissue anoxia induced by methemoglobin. Conversely, Margiocco et al. (1983) conclude that death in rainbow trout (*Salmo gairdneri*) results from the toxic action of nitrite upon vital organs and not methemoglobin. Another series of questions concerns the role of methemoglobin reductase in combating methemoglobinemia. For example, is this an inducable enzyme system and hence requires a methemoglobin threshold to develop prior to functioning? Our results indicate that this system was not functioning in either the 10 or 20 C acclimation groups.

In summary, our results corroborate Cairns' et al. (1975) conclusion that temperature toxicity relationships are complex. This complexity exists because temperature can affect any or all of the separate environmental, biochemical and physiological processes such as chemical availability, uptake, internal biochemical processing and depuration which influence toxicity. Each of these processes may have its own unique relationship with temperature. Since fish species appear to possess different temperature optima, it is possible that temperature-toxicity relationships may need to be studied on a chemical-by-chemical and species-by-species bases.

Acknowledgements. We thank J Nelson for her assistance in preparing the manuscript. Funds for this research were provided by a North Texas State University faculty research grant to the second author.

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Received October 27, 1983; accepted November 2, 1983