Bull. Environm. Contam. Toxicol. 27, 588–595 (1981)

# Temperature Effects on Hydrazine Toxicity to Bluegills

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There is a wealth of data on the acute toxicity of xenobiotics in aquatic species, yet few studies have been conducted to relate chemical toxicity to water temperature (CAIRNS et al. 1975). The limited amount of available research indicates that water temperature alters toxicity but that the nature of the changes in toxicity is not consistent among substances and organisms (HODSON & SPRAGUE 1975, CAIRNS et al. 1975). Considering the wide range of water temperatures found in the natural environment and the lack of applicable information, a need exists to take a closer look at this aspect of aquatic toxicity.

Hydrazine is found in many Air Force weapon systems including certain aircraft and missile platforms. In addition, it is commonly used in industrial processes as an antioxidant and is very water soluble. Several investigators have found that hydrazine is highly toxic to aquatic species (KLEIN & JENKINS 1977, SLONIM 1977, FISHER et al. 1978, HENDERSON et al. 1980). Although previous research has generated a substantial amount of information on hydrazine toxicity, the effect of water temperature on the toxicity is not known. In this paper we describe the acute toxicity of hydrazine in the bluegill, Lepomis macrochirus, at three water temperatures.

## METHODS

Bluegills, obtained from Fenders Fish Hatchery, Baltic, Ohio, had an average length of 42 mm (range 26 to 58), and 8 to 10 fish were used in each 30-L exposure aquarium. Water quality parameters monitored included total hardness, dissolved oxygen, effluent hydrazine concentrations, pH and temperature. Throughout the testing the total hardness ranged from 160 to 190 mg/L (as $CaCO_3$), pH from 6.7 to 8.0 and dissolved oxygen from 5.8 to 11.4 ppm. Hydrazine of at least 95% purity was obtained from Eastman Chemical Company. Hydrazine concentrations were measured using a colorimetric procedure (REYNOLDS & THOMAS 1964).

Toxicity tests were conducted at 21°C, 15.5°C, and 10°C. These temperatures fall within the thermal tolerance zone for bluegills (FENDER, personal communication). At each temperature four exposure conditions were used: static exposure of 1, 6, and 24 h duration and a continuous-flow exposure of 96 h duration. The

0007-4861/81/0027-0588 $01.60

continuous-flow procedure ensured constant hydrazine concentrations over a 4 day period. Hydrazine does not degrade appreciably in relatively soft water (hardness of 120 mg/L as $CaCO_3$) during a 24 h period (SLONIM & GISCARD 1976). Because water used in our tests had only slightly higher hardness (see above), the 96 h exposures were the only tests requiring continuous-flow methods to maintain stable hydrazine levels.

Acclimation procedures were consistent for all exposures. Thermal changes can seriously damage organisms if they occur too abruptly or exceed the thermal tolerance zone (CAIRNS et al. 1975); therefore, acclimation was accomplished slowly. Fish were initially placed in a 450-L Living Stream® containing water at the same temperature as that of the water in which the fish were supplied. The water temperature was slowly adjusted over a 48 h period to the desired temperature. Fish were acclimated at this temperature for at least 5 days prior to experimentation.

Fish were considered dead when gill movement ceased or when no response resulted when the fish were prodded with a blunt rod. Median lethal concentrations (LC50s) were calculated for each exposure using a probit analysis (FINNEY 1971).

Static Studies. Static tests of 1, 6, and 24 h were completed at each temperature using at least five concentrations and an untreated control. The toxicant concentrations for exposures at all three temperatures ranged from 19.6 to 410 mg/L for the 1 h experiments, 0.54 to 31.1 mg/L for the 6 h studies, and 0.54 to 50.4 mg/L for the 24 h tests. Water quality parameters were measured at the beginning of each test. Additionally measurements, including dissolved oxygen, effluent hydrazine concentration, and temperature were taken at the end of each 24 h test.

The cooler temperatures (10°C and 15.5°C) were maintained by placing the test aquaria in a 450-L Living Stream. Circulating water in the Living Stream was cooled to the desired temperature and acted as a water bath to sustain stable temperatures during the experiments. Each test aquarium was aerated during the static exposures to ensure adequate levels of dissolved oxygen.

Continuous-Flow Studies. Continuous-flow conditions were maintained over a 96 h period using a proportional diluter. The diluter delivered the effluent at five hydrazine concentrations and maintained an untreated control. Each 30-L aquarium received 6.5 L of solution per hour or 5.2 volume changes in 24 h.

Hydrazine was dispensed using either a Hamilton® pneumatic syringe or a Cole-Parmer variable speed polystaltic pump. The toxicant concentrations for the three 96 h exposures ranged from 0.36 to 7.93 mg/L of hydrazine.

Constant temperatures were maintained during exposures by

using cooled water and insulation. Water was pumped into the diluter apparatus after it had been cooled to approximately 2°C below the desired temperature. Styrofoam insulation placed on the bottom and sides of the aquaria and plastic bubble insulation draped over the tanks insured that the water temperature was held at the desired temperature ± 1.5°C.

Water quality parameters were measured at the beginning of each test and once during each subsequent 24 h period for the remainder of the test. Temperature was monitored continuously using a Yellow Springs Instrument® temperature probe and a linear strip chart recorder. A gravity feed system added sodium hypochlorite (3%) to the effluent to neutralize the hydrazine.

## RESULTS AND DISCUSSION

Although final hydrazine concentrations were not measured in the 1 and 6 h exposures, results from the 24 h studies indicated that only minimal loss occurred in the static tests. For the three 24 h toxicity tests, the greatest hydrazine loss was 6%. The other two exposures showed much less hydrazine degradation over the 24 h. In the 96 h continuous-flow studies, toxicant concentration remained relatively constant throughout the exposure period.

The LC50 values are shown in TABLE 1. The 96 h LC50 at 21°C (1.17 mg/L) appears to compare favorably to the value of 1.08 mg/L obtained by FISHER et al. (1978). Their value, however, was obtained in static conditions in which significant hydrazine degradation occurred during the 96 h period. The difference in test conditions suggests that a larger difference should exist between the two methods and that the continuous-flow study should render the smaller of the two estimates. For each temperature, hydrazine toxicity increased as length of exposure increased. This is consistent with previous work on hydrazine toxicity to fish (FISHER et al. 1978). To examine the nature of the relationship between exposure duration and toxicity, the cross product of the two values was calculated at each temperature (TABLE 2). The cross products show no consistent trends. In fact, the LC50 times exposure duration values are almost constant within each temperature.

The dependence of the LC50 on exposure duration was examined mathematically using the following relationship:

$$(LC50)\ (t^m) = k$$

Logarithmic transformation yields a linear relationship, $\log (LC50) = \log k - m \log t$. This equation predicts that a plot log (LC50) vs log t will be linear with slope m and intercept log k. These plots are shown in Figure 1.

The slope of each regression line was negative (TABLE 3) indicating that the toxicity of hydrazine increased with duration of exposure at all temperatures. Furthermore, the slopes

are close to -1 suggesting a cumulative toxicity from 1 to 96 h. That is, the toxicity of hydrazine over the range of water temperature used appears to depend inversely upon the length of exposure according to a constant LC50 X exposure duration relationship.

TABLE 1. LC50s (mg/L) and 95% Confidence Intervals Calculated for Each Exposure at the Three Temperatures

| Length of Exposure (h) | 10°C | 15.5°C | 21°C |
|---|---|---|---|
| 1 | 265 (219-335) | 68 4 (60.9-79.7) | 37.7 (34.4-40.3) |
| 6 | 12.9 (8.3-19.7) | 12.4 (9.0-19.8) | 3.8 (2.5- 5.9) |
| 24 | 7.7 (5.3-11.0) | 3.8 (2.4- 5.6) | 1.7 (1.1- 2.6) |
| 96 | 1.6 (1.2-2.3) | 1.0 (0.7- 1.3) | 1.2 (0.9- 1.6) |

TABLE 2. Cross Products of LC50s and Exposure Durations with Respective 95% Confidence Intervals

| Length of Exposure (h) | WATER TEMPERATURE 10°C | 15.5°C | 21°C |
|---|---|---|---|
| 1 | 265 (219-335) | 68 (61- 80) | 38 (34- 40) |
| 6 | 77 ( 50-118) | 74 (54-119) | 23 (15- 35) |
| 24 | 185 (127-264) | 91 (58-134) | 41 (26- 62) |
| 96 | 154 (115-221) | 96 (67-125) | 115 (86-154) |

TABLE 3. Parameters of Best Fit Least Square Lines For Regression of Log (LC50) on Log (Exposure Duration) at Three Temperatures

| Temperature (°C) | Slope | Correlation Coefficient |
|---|---|---|
| 10 | -1.06 | 0.94 |
| 15.5 | -0.92 | 1.00 |
| 21 | -0.76 | 0.91 |

Water temperature also had a marked effect on toxicity. The role of temperature was analyzed mathematically by logarithmic transformation. In this case it was assumed that, for a given exposure duration, the LC50 was an exponential function of temperature:

$$LC50 = k(e^{-\alpha T})$$

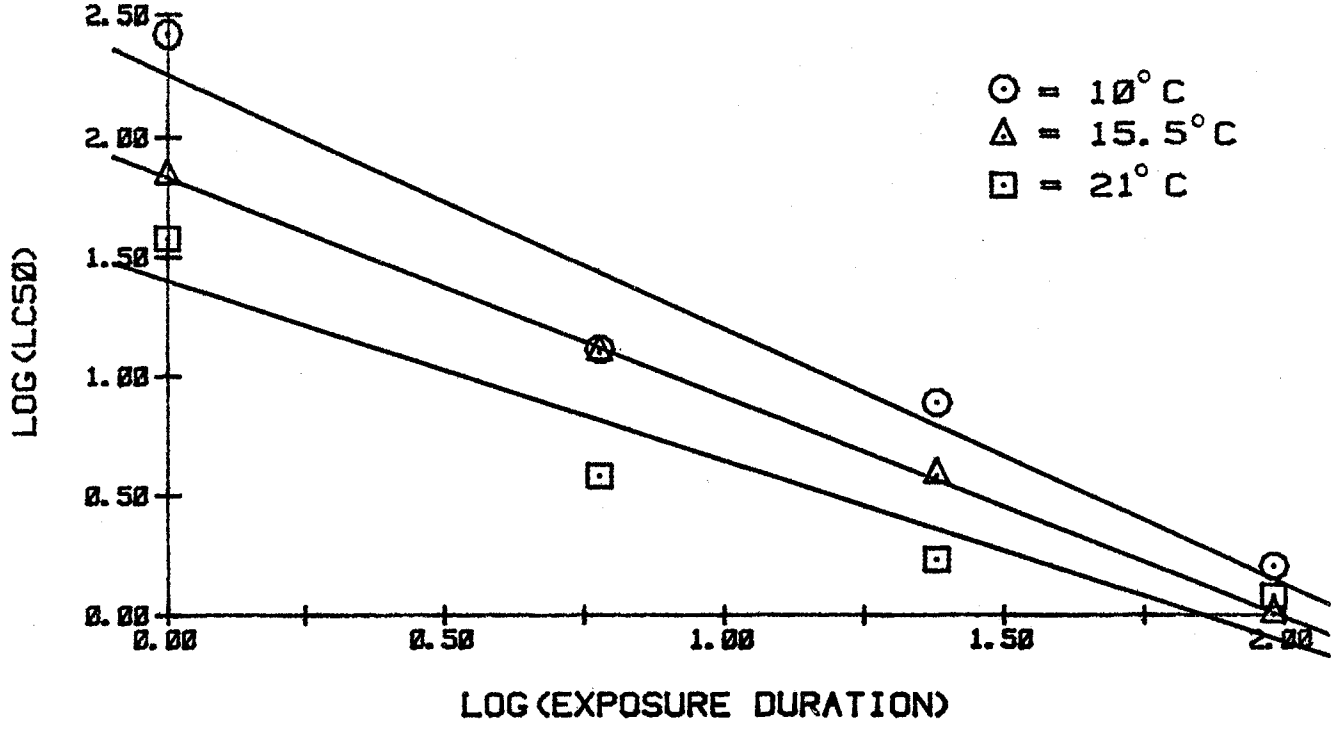


Fig. 1: Logarithmic transformation of LC50 data to illustrate the effects of exposure duration on hydrazine toxicity.

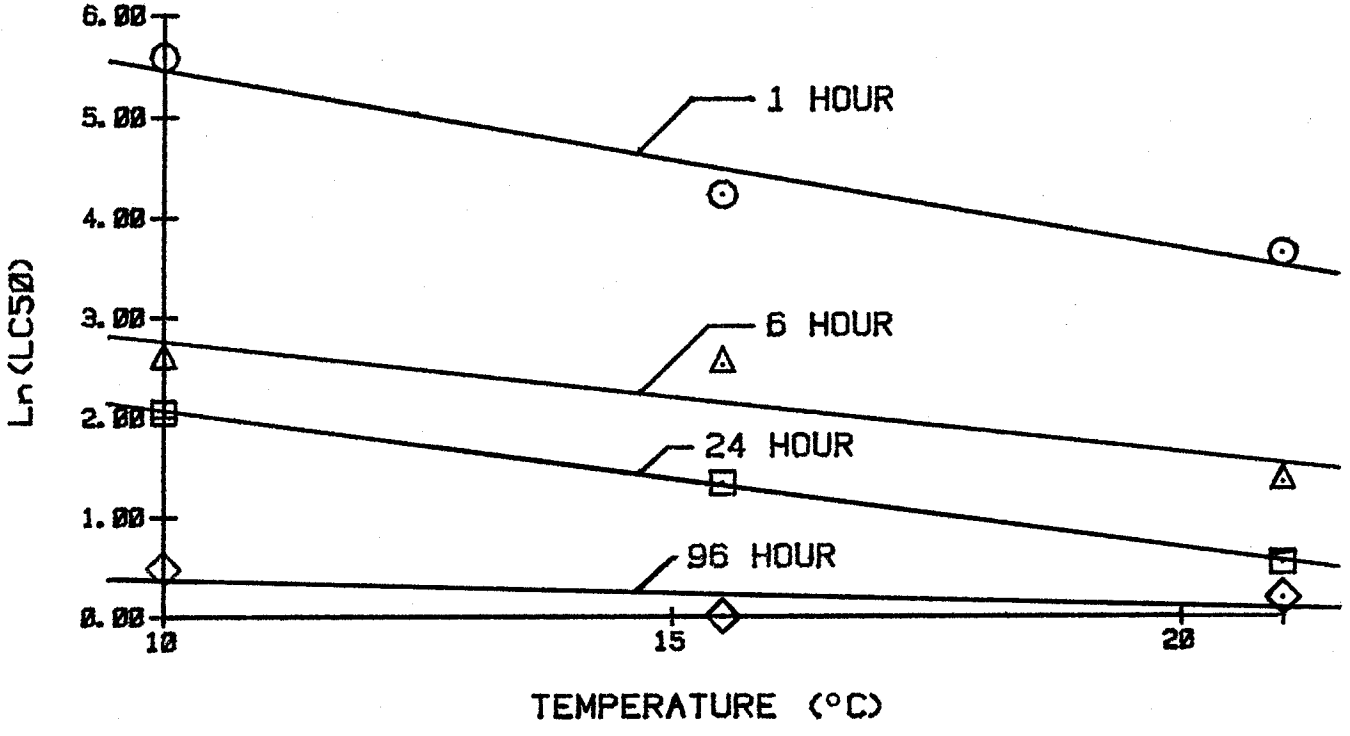


Fig. 2: Logarithmic transformation of LC50 data to illustrate the effect of water temperature on hydrazine toxicity.

Taking the natural logarithm of both sides and rearranging the terms yields:

$$\ln (LC50) = \ln k - \alpha T$$

A plot of ln (LC50) vs temperature should produce a straight line with a slope $-\alpha$ and an intercept ln k. This graph appears in Figure 2.

All slopes were again negative indicating that toxicity increased with water temperature. If the temperatures had the same effect on toxicity over different exposure periods, the slopes should all be equal; they are not (TABLE 4). The steeper slopes in the shorter tests suggest that water temperature plays a greater role for the 1, 6, and 24 h exposures than for the 96 h exposures.

TABLE 4. Parameters of Best Fit Least Square Lines For Regression of ln (LC50) on Water Temperature for Four Exposure Durations

| Exposure Duration (h) | Slope | Correlation Coefficient |
|---|---|---|
| 1 | -0.18 | 0.95 |
| 6 | -0.11 | 0.77 |
| 24 | -0.14 | 1.00 |
| 96 | -0.03 | 0.43 |

We can draw the following conclusions from this study: hydrazine toxicity increased with exposure duration, apparently in a cumulative manner, and it increased as water temperature increased. More definite conclusions cannot be offered due to the low correlation of several of the regression lines. This may have resulted from one or two aberrant points; namely 6 h LC50 at $10^{o}C$ and especially, 96 h LC50 at $21^{o}C$. Further experimentation is needed to confirm whether these two values are truly representative and to make more specific conclusions describing the effect of temperature on hydrazine toxicity.

ACKNOWLEDGEMENTS

This investigation was made possible through the assistance and support of R. D'Allessandris, L. Weaver, C. B. Harrah and C. Buchanan. A special thanks to M. E. Andersen for his invaluable suggestions during preparation of this manuscript.

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Accepted July 30, 1981