

## **Acute Toxicity Effects of Simazine on *Daphnia Pulex* and Larval Striped Bass**

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*Abstract:* Tests were performed to determine the acute toxicity of simazine, a herbicide commonly used in pond aquaculture, to the cladoceran *Daphnia pulex* and to 3- and 7-day old larval striped bass (*Morone saxatilis*). The 48-hour LC<sub>50</sub> for 3-day-old striped bass was 18 mg/liter in hard water (220 mg/liter as CaCO<sub>3</sub>) and 16 mg/liter in soft water (120 mg/liter as CaCO<sub>3</sub>); corresponding estimates for 7-day-old larvae exceeded 100 mg/liter. The estimated 48-hour LC<sub>50</sub> for *D. pulex* exceeded 50 mg/liter in both hard and soft water. These estimates were considerably higher than recommended pond application rates of simazine, but abnormal behavioral responses of both test organisms to sublethal concentrations suggested that long-term exposure could be harmful. Inasmuch as 7-day-old larvae were far less sensitive than 3-day-old larvae, the timing of simazine applications in relation to age of fish could have pronounced effects on survival of striped bass in ponds.

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Many federal and state fish hatcheries use ponds to rear striped bass from prolarvae to fingerlings (Stevens 1974, Westin and Rogers 1978); survival rates range from 10.7 to 34.1% (Reeves and Germann 1971, McBay

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1972). Efforts to improve survival have included application of organic fertilizers to stimulate zooplankton production, herbicides to control algae, and insecticides to control predaceous insects. Except for the work by Hughes (1969, 1971, 1975), evaluations of the toxicity of many commonly used chemicals to larval striped bass have been lacking, and additional research is needed to understand factors that stress or kill food organisms as well as larvae.

This study was designed to examine the acute toxicity effects of simazine, 2-chloro-4, 6-bis(ethylamino)-s-triazine, on larval striped bass and a representative prey organism, *Daphnia pulex*. Simazine is the active ingredient of Aquazine<sup>®</sup>, which is a registered, non-persistent, non-volatile herbicide used to eliminate undesirable algal blooms that may occur when fertilizers are added to enhance zooplankton production in rearing ponds (Tucker and Boyd 1978).

The acute toxicity of simazine to neonate *D. pulex* and to 3- and 7-day-old larval striped bass was quantified as the 48 hour LC<sub>50</sub> (the calculated concentration lethal to 50% of the test organisms in 48 hours). Effects of simazine on behavior and the influence of water hardness on simazine toxicity were also evaluated.

## Methods

### Daphnia Toxicity Tests

Early instar *D. pulex* (24 ± 12-hours-old) were used for all toxicity tests (APHA et al. 1975, Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Stock cultures of daphnids were acclimated to the test temperature (20 ± 1 C) and photoperiod (16L:8D) and fed *Chlamydomonas reinhardi*, a unicellular green alga, ad libitum daily. An extract of "trout-chow," cerophyll, and yeast was also fed 3 times per week during the acclimation period (Buikema 1970).

Simazine solutions were prepared by mixing 0.125 g Aquazine<sup>®</sup> (Ciba-Geigy Corp., Greensboro, NC; 80% wettable powder formulation of simazine) with 1000 ml of distilled, deionized water to make a 100 mg/liter stock concentration. Stock solutions were mixed thoroughly, stored in the dark at room temperature, and used within 24 hours to prepare the test solutions. Two hardnesses of dilution water were used: soft well water (120 ± 15 mg/liter total hardness as CaCO<sub>3</sub> and pH 7.7 ± 0.1 units) that was passed through a 0.45-μm millipore filter, and hard water (220 ± 11 mg/liter as CaCO<sub>3</sub> and pH 8.1 ± 0.1 units) that was reconstituted from some of the soft water filtrate (Committee on Methods for Toxicity Tests with Aquatic Or-

<sup>1</sup> Reference to a trade name does not imply U.S. Government endorsement.

ganisms 1975). Dilution water was aerated for 24 hours and then mixed with the stock solution to produce test concentrations ranging from 1 to 50 mg/liter. Because we wished to closely simulate pond application methods and eliminate possible synergistic effects, we did not use organic solvents as carriers of the herbicides.

Five randomly selected daphnids were placed in each test container (400-ml glass beakers containing 300 ml of solution), and 48-hour static toxicity tests were conducted separately for 2 series of simazine concentrations (1.0, 1.8, 3.2, 5.0 mg/liter; 10, 18, 32, and 50 mg/liter). Concentrations higher than 50 mg/liter were not tested because it was difficult to observe *Daphnia* in turbid solutions. Each test series included 10 beakers: 2 replicates at each of 4 simazine concentrations and 2 control containers. Tests for each series were repeated if any mortality in the controls occurred, and until at least 30 *D. pulex* had been exposed to each of the 8 simazine concentrations in both hard and soft water.

Daphnids were checked for changes in behavior, and the number of organisms surviving was recorded after 3, 6, 12, 24, 36, and 48 hours. Dead or immobile organisms were not removed from the beakers until the end of each test, to avoid removal of organisms possibly only narcotized by the herbicide. Death was defined as the absence of visible movement of the antennae, thoracic appendages, and postabdomen when the organisms were exposed to light or other external stimuli (Buikema et al. 1980). Daphnids were not fed and solutions were not aerated during the tests.

Dissolved oxygen, hardness, alkalinity, and pH were measured (APHA et al. 1975) in control solutions and in the highest concentrations of simazine at the beginning and end of each test series.

### Striped Bass Toxicity Tests

We used 3- and 7-day-old striped bass, and all larvae in a particular test were from the same broodfish. Larvae were protected from direct sunlight and transported in fresh water approximately 1 km from the hatchery to our laboratory. No tranquilizers or anesthetics were used during transportation, and larvae were tempered to test conditions for at least 3 hours before a test was started. Test containers, environmental conditions, and water hardnesses were similar to those used for the *Daphnia* tests.

Concentrations of simazine ranging from 10 to 100 mg/liter were used to determine the 48-hour LC<sub>50</sub> for 3- and 7-day-old larvae in hard and soft water. Static toxicity tests were conducted separately for 2 series of concentrations: 10, 18, 32, and 50 mg/liter; and 65, 75, 87, and 100 mg/liter. Each test series consisted of 2 replicates for each of 4 simazine concentrations and 2 control containers. Each series was repeated at least 6 times in soft and hard water.

Five larvae were placed in each test container with a modified Gilson Pipetman. The inside diameter of the pipette was increased to 3.5 mm and the volume was adjusted to 3 ml. Larvae were gently drawn into the pipette and then allowed to swim out into the test containers. Behavioral characteristics and numbers of larvae surviving after 8, 12, 24, 36, and 48 hours were recorded. Larvae were not fed during the tests and the containers were not aerated. We initially specified that control mortality should not exceed 10%, but persistently higher losses led us to accept the results of tests having up to 30% mortality in the control containers.

Chemical analyses were run on the initial and final control water, on the highest concentrations of simazine in each test series, and on the rearing water at the hatchery. Variables tested were the same as in the daphnid tests.

### Data Analysis

We calculated concentrations and 95% fiducial limits by using Finney's (1971) probit analysis procedure on the Statistical Analysis System (Barr et al. 1979) with  $\log_{10}$  transformations (Stephan 1977). Data for daphnids were pooled within each water hardness category before statistical analysis. The LC<sub>50</sub> values for striped bass were determined after 8, 12, 24, 36, and 48 hours by pooling all data within each water hardness and age-group combination.

The validity of the log-probit model for the striped bass data was evaluated by determining whether survival after 48 hours was linearly related to simazine concentration. Arcsin transformations of percent survival were regressed on the  $\log_{10}$  of the simazine concentration by using a Linear model for each age group of larvae in hard and soft water (Sokal and Rohlf 1969). Statements of statistical significance in the text refer to  $P \leq 0.05$ .

### Results

Water analyses indicated that chemical conditions in controls remained relatively stable throughout the 48-hour test periods. Dissolved oxygen ranged from 8.7 to 10.1 mg/liter; pH from 7.4 (soft water) to 8.2 (hard water); alkalinity from 100 mg/liter (soft water) to 180 mg/liter (hard water); and total hardness from 125 mg/liter (soft water) to 200 mg/liter (hard water). The addition of simazine caused immediate decreases of all variables tested, but conditions then remained stable throughout the tests. Simazine solutions were 0.1 to 0.2 pH units lower than the controls and total hardness and alkalinity were usually reduced about 25% after simazine was added.

Simazine concentrations greater than 10 mg/liter were slightly cloudy and some material settled out during the tests. Filtering of stock solutions be-

fore dilution reduced turbidity and precipitation, but filtering was not routinely used because preliminary tests indicated no effects on daphnid survival. Chemical characteristics of well water used to prepare simazine solutions were not different from those of the water used at the hatchery.

The estimated 48-hour LC<sub>50</sub> values of simazine for *D. pulex* (in both hard and soft water) exceeded 50 mg/liter. Extrapolation suggested LC<sub>50</sub>'s of 92 mg/liter in soft water and 424 mg/liter in hard water, but these estimates were beyond the range of tested concentrations and should be treated with caution. We did not observe 100% mortality in any *Daphnia* tests. Mortality usually was greatest at 50 mg/liter in soft water, but at 10 or 18 mg/liter in hard water. The calculated 48-hour LC<sub>2</sub> in soft water and the 48-hour LC<sub>6</sub> in hard water (concentrations predicted to kill 2 and 6% of the population, respectively) were about 2.5 mg/liter, which is the maximum application rate recommended by the manufacturer (Table 1).

All concentrations of simazine produced abnormal daphnid behavior. Control organisms constantly swam throughout the beakers, but daphnids exposed to simazine remained near the bottom of the containers and the rates of movement of antennae and appendages were reduced. Immobilization and death occurred soon after normal swimming patterns ceased. Abnormal behavior was most evident at the 12th hour of exposure, and most mortality occurred between 12 and 24 hours. Some mortality, however, occurred as early as the third hour in concentrations of 32 and 50 mg/liter.

Water hardness had no significant effect on mortality of striped bass larvae (Table 2). The calculated 48-hour LC<sub>50</sub> for 3-day-old larvae was 16 mg/liter in soft water and 18 mg/liter in hard water, and corresponding estimates for 7-day-old larvae exceeded 100 mg/liter. Extrapolations of the probit models indicated that 48-hour LC<sub>50</sub>'s for 7-day-old larvae were about 127 mg/liter in soft water and 157 mg/liter in hard water. Mortality of 3-day-old fish was usually greatest in the highest concentrations, suggesting a true dose-induced response. Results from the 7-day-old larvae, however, were

**Table 1.** Estimated 48-Hour LC Values and 95% Fiducial Limits (in parentheses) for *Daphnia pulex* Exposed to Simazine in Hard and Soft Water

Percent Mortality	Water Hardness	48-Hour LC Values and 95% Fiducial Limits (mg/liter)
2	soft	2.6 ( 0.7 - 4.8)
20	soft	21.3 (14.8 - 31.9)
50	soft	92.1 (54.3 - 259.1) <sup>a</sup>
6	hard	2.1 ( 0.2 - 5.0)
20	hard	24.1 (11.9 - 108.0) <sup>a</sup>
50	hard	424.0 (99.1 - 8.2 × 10 <sup>4</sup> ) <sup>a</sup>

<sup>a</sup> Estimate was beyond the maximum tested concentration and should not be regarded as definitive.

**Table 2.** Calculated 48-Hour LC50 Values (mg/liter) and 95% Fiducial Limits (in parentheses) for Larval Striped Bass Exposed to Simazine in Hard and Soft Water

Larval Age	Water Hardness	N	48-Hour LC50 Values and 95% Fiducial Limits (mg/liter)
3-day-old	soft	380	15.9 ( 5.9 - 27.3)
3-day-old	hard	390	18.4 ( 0.2 - 34.5)
7-day-old	soft	410	157.2 <sup>a</sup> (77.3 - 8.2 × 10 <sup>7</sup> )
7-day-old	hard	400	127.0 <sup>a</sup> (62.3 - NC) <sup>b</sup>

<sup>a</sup> Estimate was beyond the maximum tested concentration and should not be regarded as a definite LC50 value.

<sup>b</sup> NC indicates that fiducial limits could not be calculated by probit analysis.

less consistent. Larvae from some broods were extremely sensitive to concentrations of 10 to 50 mg/liter; other groups showed no mortality at 100 mg/liter after 48 hours.

Regression analyses were performed to determine if striped bass survival (arcsin transformation) after 48 hours was linearly related to simazine concentration ( $\log_{10}$  transformation). The linear model was statistically valid in all tests except that of 7-day-old larvae in soft water. This validity indicated that the probit model was generally appropriate for estimating LC50's and that a dose-response relation existed for striped bass larvae.

Exposure to simazine at all of the concentrations tested also affected behavior of striped bass larvae. The 3-day-old control fish generally swam together throughout the beakers, but larvae exposed to the toxicant remained inactive near the bottom of the containers. Long periods of inactivity were interrupted by occasional bursts of swimming in small circular patterns. The 7-day-old control larvae schooled near the water surface or remained near the bottom, facing downward, and displayed characteristic feeding behavior even though food was not present. Larvae exposed to simazine, however, first developed tremors or swam in small circular patterns and later were inactive except for occasional movement of the caudal or pectoral fins. About 60% of the exposed 7-day-old larvae eventually developed a scoliotic curvature of the vertebral column that may have been caused by muscle spasms associated with the tremors.

The toxicity tests showed a pronounced age-related sensitivity of larval striped bass to simazine. The trend of sensitivity was apparently related to morphological or physiological development of larvae. Yolk-sac absorption and mouth part development were complete by the fifth day of age, when the larvae were least tolerant to simazine. Sensitivity increased as the yolk-sac was absorbed and then decreased as the larvae became more capable of feeding.

## Discussion

The maximum solubility of simazine in water has been reported to be 3.5 mg/liter (Herbicide Handbook 1979) and 5.4 mg/liter (McCann and Hitch 1980). McCann and Hitch (1980), however, reported that simazine could remain in suspension at concentrations greater than 5.4 mg/liter, and we observed complete suspension at concentrations up to 10 mg/liter. At higher concentrations, the solutions are cloudy and some material settled out during the tests. Simazine, however, is non-volatile, and precipitated material remained in the test containers and was available to the test organisms. Toxicity values that exceed the maximum solubility levels should be considered to be a measure of both the water soluble and insoluble fractions of simazine (Mauck 1974).

The LC<sub>50</sub> estimates in this study were considerably higher than those obtained by other investigators for s-triazine herbicides and similar test organisms. The majority of published work, however, has been done with technical grade materials that were dissolved in organic solvents such as ethanol or dimethyl sulfoxide, before test solutions were prepared. Schober and Lampert (1977) reported that 0.5% ethanol was synergistic with atrazine (an s-triazine herbicide) and significantly reduced survival of *D. pulex* in chronic toxicity studies. Sanders (1970) estimated the 48-hour LC<sub>50</sub>'s of atrazine for *D. magna* were reported to be 3.6 mg/liter (Federal Water Pollution Control Administration 1968) and 5.7 mg/liter (Macek et al. 1976). Sanders (1969), the only investigator known to us who tested toxicity of typical field formulations of simazine to aquatic invertebrates, reported that 24- and 48-hour LC<sub>50</sub>'s for the scud *Gammarus lacustris* were 30 mg/liter and 21 mg/liter, respectively.

Simazine concentrations as low as 1.0 mg/liter produced abnormal daphnid behavior. Behavioral patterns we observed were remarkably similar to those described by Schultz and Kennedy (1976) for *D. pulex* exposed to the herbicide 3-amino-1, 2, 4,-triazole. Simazine and triazole affect plants by interfering with electron transport and oxidative phosphorylation (Carter 1969, Bohlar-Nordenkamp 1979). The similarity of *D. pulex* behavior when exposed to simazine and triazole may indicate similar modes of toxicity.

No published studies of simazine toxicity to larval striped bass were located, but Wellborn (1969) evaluated the acute toxicity to fingerlings (60 mm long) and found 24-, 48-, and 96-hour LC<sub>50</sub>'s of 0.60, 0.44, and 0.25 mg/liter, respectively. McCann and Hitch (1980) attempted to duplicate Wellborn's experiment by exposing striped bass fingerlings (42 mm long) to Aquazine®, but no fish died at concentrations as high as 180 mg/liter. Alabaster (1969) reported that the 24-hour LC<sub>50</sub> for rainbow trout exposed to a wettable powder formulation of simazine was 95 mg/liter, but Dodson and

Mayfield (1979) observed no mortality of age I rainbow trout, *Salmo gairdneri*, exposed to a 200 mg/liter solution of simazine for 24 hours.

Our data and those of McCann and Hitch (1980) indicate an age-related response of striped bass to simazine. Sensitivity apparently was related to development of physical or physiological characteristics associated with reliance on an external food supply. Larvae were most sensitive prior to completion of yolk-sac absorption and mouth part development (3 to 5 days of age), but tolerance increased thereafter (7 to 9 days of age). Sanders (1970) and Tucker and Leitike (1979) also reported that toxicity of chemicals was related to the size and age of test organisms.

Buoyancy and equilibrium anomalies, aberrant swimming behavior, and deformities of the vertebral column that we observed are typical responses of larval fishes to stressors (Rosenthal and Alderdice 1976). Larvae that were stressed during transportation or handling before the tests were more susceptible to all concentrations of simazine, and mortality of the control fish was higher than usual. Behavior of the control groups was also affected by stress, but these fish did not display tremors, deformities of the vertebral column, or circular swimming patterns shown by larvae exposed to simazine. Most of these tests were excluded in the data analyses because mortality exceeded the 30% criterion.

Approximately 60% of the exposed 7-day-old larvae developed a scoliotic curvature of the vertebral column. These fish sank to the bottom of the test containers and eventually died. Couch et al. (1977) noted a similar occurrence in sheepshead minnows (*Cyprinodon variegatus*) exposed to low levels of Kepone. They postulated that tetanic convulsions, chronic muscular rigor, or both, were associated with scoliosis and produced the fractured vertebrae of severely affected fish; a similar mechanism may be responsible for the effects noticed in simazine-exposed 7-day-old larvae.

Our method of pooling striped bass data within age and water hardness categories produced LC<sub>50</sub>'s with wide fiducial limits. Responses of 3-day-old larvae were relatively uniform among broods and replicate tests, but 7-day-old larvae exhibited wide variations in response to different simazine concentrations. Larvae from some broodfish were very sensitive to low concentrations, whereas groups from other broodfish showed no mortality at 100 mg/liter. Precision of the estimates might have been improved by treating sibling groups separately, but larvae from individual broodfish are seldom reared apart, and the pooled data reflect genetic, physiological, and environmental variations between broodfish and their larvae.

The maximum pond application rate of simazine recommended by the manufacturer is 2.5 mg/liter, but repeated applications are often required to control some species of filamentous and net algae in ponds (Snow 1963, Sutton et al. 1966). The maximum recommended pond application rate is con-



siderably lower than the LC<sub>50</sub>'s we obtained for *D. pulex* and striped bass, but the pronounced behavioral responses of both organisms to simazine and the age-related sensitivity of striped bass indicate the need for studies of sublethal and indirect effects. Toxic effects of simazine on copepods, insects, and other cladocerans that are important prey for striped bass should also be evaluated. Control of undesirable plants or algal blooms may justify a slight reduction of zooplankton biomass in ponds, but prolonged exposure to sublethal concentrations could increase zooplankton vulnerability to predation, depress reproduction, or lead to increased competition or cannibalism among striped bass larvae. Chronic exposure of *D. pulex* to 4 mg/liter simazine interfered with molting, reduced growth rates, and significantly lowered reproductive rates (Fitzmayer et al. 1982).

Our study indicates that simazine could be applied to striped bass rearing ponds at the recommended rate with only minor effects on survival. Culturing practices, however, should minimize pre-stocking handling stress, account for the age-related sensitivity to simazine, and avoid sequential applications that would lead to prolonged, sublethal exposures. Direct effects of simazine could probably be reduced by preventing exposure until the yolk-sac is absorbed. Ponds could be treated at least 1 week before stocking larvae 7 or 8 days old or by treating ponds only after larvae are actively feeding. Stocking of older larvae may improve survival even if simazine is not used. Reeves and Germann (1971) found that larvae stocked after yolk-sac absorption had higher rates of survival than did larvae stocked at 2 to 4 days of age.

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