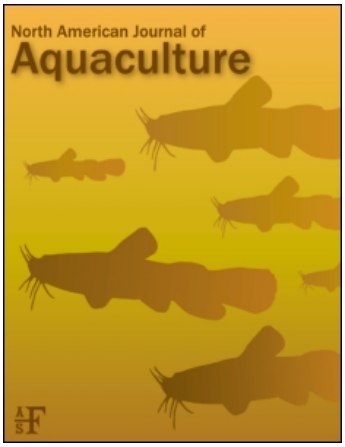

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[Article]

The Acute Toxicity of Praziquantel to Grass Carp and Golden Shiners

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Abstract.—The acute toxicity and highest nonlethal concentration of praziquantel (LC0) were determined in the laboratory for grass carp *Ctenopharyngodon idella* and golden shiner *Notemigonus crysoleucas*, two cyprinids known to harbor the Asian tapeworm *Bothriocephalus acheilognathi*. Praziquantel is an anthelmintic used to treat fish with tapeworms. The 24-h and 96-h LC50 values were 55.1 and 49.7 mg/L for golden shiners (1.3 g) and 63.4 and 60.6 mg/L for grass carp (9.1 g). The 24-h and 96-h LC0 values were 50.0 and 45.0 mg/L for golden shiners and 60.0 and 60.0 mg/L for grass carp.

Praziquantel (Droncit) is an anthelmintic that was developed to treat trematodes and tapeworms in humans and in dogs by the Bayer Corporation, Leverkusen, Germany (Andrews et al. 1983). Tests in fish have shown praziquantel to be effective against both tapeworms and trematodes when injected, fed (naturally or forced), or applied in a water bath (Andrews and Riley 1982; Pool et al. 1984; Moser et al. 1986; Sanmartin-Duran et al. 1989; Lewbart and Gratzek 1990; Rogstad et al. 1993; Flores-Crespo et al. 1994; Heckmann 1995; Mitchell 1995, 2004). Injection and force-feeding methods are labor intensive, and medicated feeds are often not consumed by all fish in a targeted population. Bath treatments, however, treat every fish, are less labor intensive, and when performed properly, should eradicate worms from treated fish. Previous work by Mitchell (2004) showed that treatment with praziquantel in water at 1.5 mg/L for 24 h removed all worms from heavily infected grass carp *Ctenopharyngodon idella* at fish densities of 6 g/L.

Little information is available on the toxicity of praziquantel to fish, and the safety margin between a treatment rate and toxic doses is unknown for most fish species. In fry of the African sharptooth catfish (also known as the sharptooth catfish) *Clarias gariepinus*, the 24-h concentration of praziquantel that would kill 50% of the fish present (LC50 value) was 13.4 mg/L (Obiekezie and Okafor 1995). Mitchell (2004) reported that five grass carp appeared to be healthy after exposure to praziquantel at 10 mg/L for 72 h in a static water bath. The purpose of the present study was to estimate the LC50 of praziquantel for golden shiner *Notemigonus crysoleucas* and grass carp in 24-h and 96-h exposures and to determine the highest nonlethal concentration (LC0) for these two species of fish.

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Methods

Praziquantel toxicity studies were carried out at the Harry K. Dupree–Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, on grass carp and golden shiners obtained from commercial production facilities within 80 km of the center. The praziquantel used in this study was a powdered 100% formulation (Sigma Chemical Co., St. Louis, Missouri). Because of its limited solubility in water, praziquantel was first dissolved in 70% ethanol—12.5 mg of praziquantel per milliliter of 70% ethanol—and this solution added to the water to give the appropriate chemical concentration. For every 1 mg of praziquantel applied, 44.24 mg of ethanol was also applied.

Preliminary tests were performed to find the approximate praziquantel concentrations needed for LC50 tests. Using these preliminary results, we set up replicated studies (10 fish per tank and three replicates per treatment) to determine the 24-h and 96-h LC50 estimates for golden shiners and grass carp. Golden shiners (average weight, 1.3 g) were exposed to 0, 45, 50, 55, 60, 65, and 70 mg/L praziquantel for 24 h in a static tank with 4 L of water and to 0, 40, 45, 50, 55, and 60 mg/L praziquantel for 96 h in a static tank with 10 L of water. Grass carp (9.1 g) were exposed to 0, 60, 62.5, 65, 67.5, and 70 mg/L praziquantel for 24 h in a static tank with 10 L of water and to 0, 57.5, 60, 62.5, 65, and 67.5 mg/L praziquantel for 96 h in a static tank with 40 L of water. The 0-mg/L praziquantel treatment in each of the four tests above contained the same concentration of ethanol as was used in the highest praziquantel treatment of that test (60, 67.5, and 70 mg/L praziquantel treatments had ethanol concentrations of 2,655, 2,986 and 3,097 mg/L, respectively).

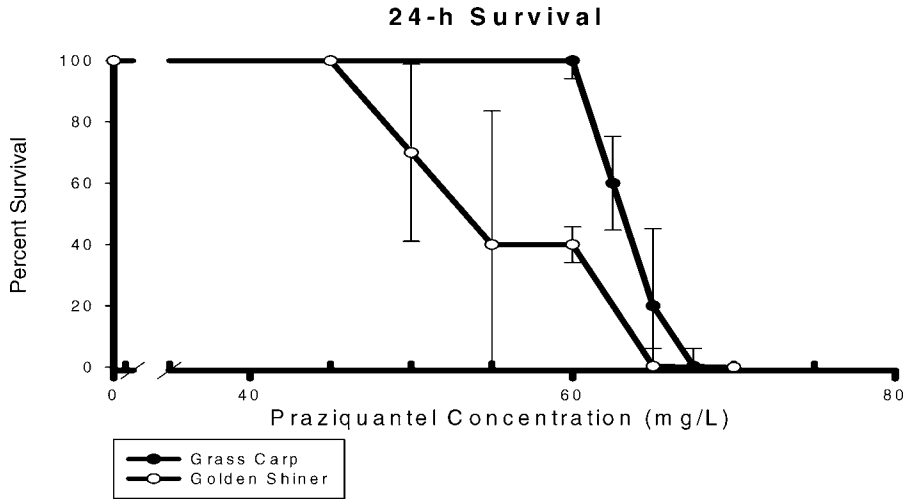

FIGURE 1.—Mean survival of grass carp and golden shiners after a 24-h exposure to praziquantel; vertical lines indicate SDs.

Dead fish were counted and removed from containers, starting at 1-h posttreatment and then once daily for 4 d after treatment. They were also removed during the 96-h treatments, starting at 24 h and once daily thereafter to help prevent fouling of the water.

Water quality was measured with a Hach Chemical Company Model FF-1A test kit (Loveland, Colorado), Hach DR/2010 portable datalogging spectrophotometer, and a Wissenschaftlich-Technische Werkstätten pH/Oxi 340i/SET meter (Weilheim, Germany). Temperature (19–24.5°C) was measured daily and at the beginning and end of each test with standardized thermometers to the closest 0.5°C. Total alkalinity (as $CaCO_3$), total hardness (as $CaCO_3$), pH, total ammonia nitrogen, and chloride measured for all well water used in the study just before the application of treatments were 257 mg/L, 103 mg/L, 7.5–8.0, 0–0.1 mg/L, and 45–60 mg/L, respectively. Total ammonia nitrogen and pH readings were recorded daily and at the end of the 96-h toxicity test. Total ammonia nitrogen readings were recorded at the end of the 96-h golden shiner toxicity test.

Values for survival from toxicity tests were analyzed with Toxcalc version 5.0.25. Data were tested for normality and equal variances by using Shapiro–Wilk's and Bartlett's test, respectively. Point estimates of the median lethal concentration (LC50) were calculated by using either Probit for normally distributed data or trimmed Spearman–Karber for nonparametric analyses. Analysis of variance (ANOVA) and Dunnett's test for normally distributed data or Wilcoxon rank-sum test with Bonferroni's adjustment for data requiring nonparametric analysis were used to calculate the highest nonlethal concentrations (LC0) by determining significant differences between control and treatment survival. Nonparametric analyses were used to calculate the toxicological endpoint values for the 24-h golden shiner and 96-h grass carp tests. All data were tested with $\alpha = 0.05$.

Results and Discussion

The mean ± SD 24-h LC50 values were 55.1 ± 1.5 and 63.4 ± 1.3 mg/L for the golden shiners and grass carp, respectively (Figure 1); the 24-h highest nonlethal concentrations (LC0) were 50.0 and 60.0 mg/L. The 96-h LC50 values were 49.7 ± 1.3 and 60.6 ± 0.5 mg/L for the golden shiners and grass carp, respectively (Figure 2); the 96-h LC0 values were 45.0 and 60.0 mg/L. In the preliminary 24-h trials, grass carp and golden shiners appeared somewhat lethargic after about 2 h of exposure to praziquantel at 20 and 30 mg/L and they were observed to lose equilibrium at 40 mg/L. The toxicity values were calculated from the 1-h posttreatment results because only two fish deaths (both in the golden shiner 96-h test) were reported after this sampling for all four tests; one control and one treatment fish (40 mg/L) were affected. These two fish deaths were probably not the result of praziquantel toxicity.

In our studies, the 70% ethanol solvent was observed to have no effect on the survival of either grass carp or golden shiners. In a preliminary test, grass carp were exposed for 24 h to about 7,078 mg/L ethanol and no mortality resulted. In the final LC50 tests for grass carp and golden shiners, the highest rates of ethanol used were 3,097 mg/L for a 24-h exposure and 2,986 mg/L for a 96-h exposure; these two levels were applied to the control fish with no resulting mortalities. Johnson

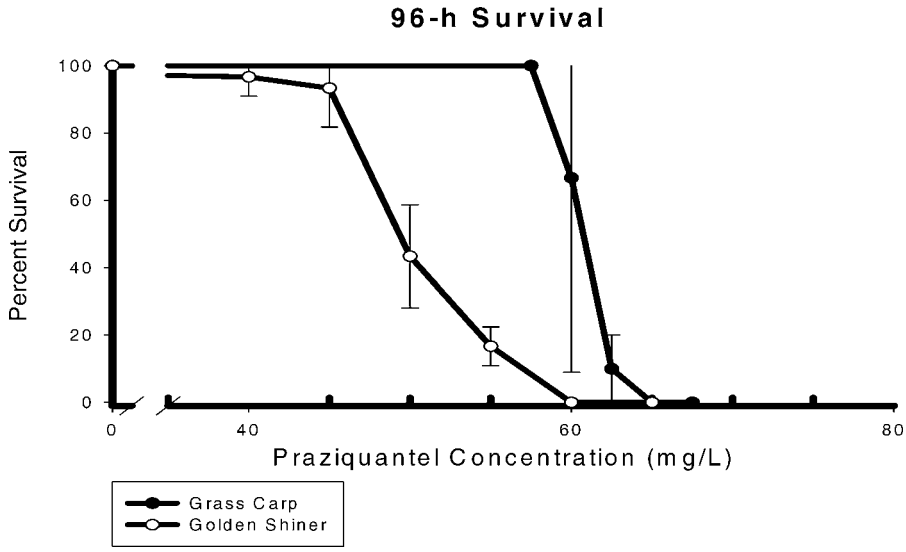

FIGURE 2.—Mean survival of grass carp and golden shiners after a 96-h exposure to praziquantel; vertical lines indicate SDs.

and Finley (1980) found the 96-h LC50 value for rainbow trout *Oncorhynchus mykiss* to be 13,000 mg/L ethanol. Mattson et al. (1976) found that the 96-h LC50 value for fathead minnow *Pimephales promelas* was 13,480 mg/L. Mortalities reported in the praziquantel acute toxicity tests probably were not attributable to ethanol.

The water in these studies would be considered hard water, with total alkalinity and hardness (measured as $CaCO_3$) of about 250 and 100 mg/L, respectively. Ammonia nitrogen levels remained relatively low even in treated static waters for 96 h. Total ammonia, temperature, and pH recorded in preliminary 24-h (high readings of 0.4 mg/L, 19.5°C, and 8.8, respectively) and 96-h (high readings of 0.9 mg/L, 20.0°C, and 8.8, respectively) tests with grass carp resulted in unionized ammonia (UI) levels of 0.02–0.18 mg/L. At the conclusion of the 96-h LC50 test for golden shiners, the two highest total ammonia readings recorded in any tank were 1.2 and 2.3 mg/L; because the pH was not taken at the time of these ammonia readings, the UI was not determined, but no fish died in either of these tanks. In the 96-h LC50 test for grass carp the highest total ammonia nitrogen measured was 0.6 mg/L and the UI never exceeded 0.08 mg/L in any container. We could not find ammonia toxicity values for golden shiners and grass carp but the UI 96-h LC50 values for four other cyprinids—bighead carp *Hypophthalmichthys nobilis*, silver carp *H. molitrix*, common carp *Cyprinus carpio*, and fathead minnow—were 0.30, 0.38, 0.66, and 1.0–2.5 mg/L, respectively (Thurston et al. 1983; Zu et al. 1994). The grass carp in our studies were subjected to UI levels that were much lower than the LC50 values reported for the other cyprinids, and the time during which they were exposed to the levels recorded at the end of our treatment period would certainly be much less than 96 h.

Obiekezie and Okafor (1995) found that the 24-h LC50 estimate for African sharptooth catfish exposed to praziquantel was 13.4 mg/L; this indicates that praziquantel is about four times more toxic to this clariid than to the cyprinids tested in our study. In unpublished studies, D. L. Ward (Arizona Game and Fish Department, Phoenix, Arizona) found that a 36-mg/L treatment of praziquantel did not affect the survival or growth of another cyprinid, the bonytail chub *Gila elegans*.

Praziquantel bath treatments as low as 0.5 mg/L for 24 h significantly reduced Asian tapeworm numbers in fish; tapeworm eradication was reported at 1.5 mg/L for 24 h (Mitchell 2004; Ward, unpublished studies). With a treatment concentration of 1.5 mg/L, there is a large safety margin (more than 30-fold) between the effective and acutely toxic doses and about a 13-fold margin from the earliest signs of effect (lethargy) on the fish used in our study.

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