Toxicity of Fintrol® (Antimycin) and Prenfish® (Rotenone) to Three Amphibian Species

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Intermountain Journal of Sciences
Vol.13, No.1:1-8
March, 2007

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ABSTRACT

The toxicity of two piscicides, Fintrol® and Prenfish®, to Columbia spotted frogs (*Rana luteiventris*), long-toed salamanders (*Ambystoma macrodactylum*), and Rocky Mountain tailed frogs (*Ascaphus truei*) of varying life stages was determined from 96-hr tests. The 96-hr LC50 values for Fintrol ranged from 13.7 to 192 µg/L, and for Prenfish the range was 0.009 to 9.65 mg/L. Tailed frog larvae were the most sensitive to both piscicides, surviving exposure to Fintrol as low as 3.7 µg/L, and having 10-percent mortality to the lowest test concentration of Prenfish tested (0.005 mg/L). Spotted frog adults survived exposure to Fintrol at concentrations six times the label prescription and survived exposure to Prenfish up to 4.5 times the label prescription. Long-toed salamander larvae survived exposure to Fintrol at levels ~30 percent higher than the label prescription, but had a similar sensitivity to Prenfish as some species of fish. Comparing the results of these tests with tests on fish and other amphibians showed that when used in the field, Fintrol would likely not have an impact on any of the species or life stages tested, and Prenfish would not likely impact adult amphibians but could have an impact on larvae.

Key words: Antimycin, Fintrol®, rotenone, Prenfish®, Columbia spotted frog, long-toed salamander, Rocky Mountain tailed frog, salamander, frog, larvae.

INTRODUCTION

Antimycin and rotenone are active ingredients in piscicides commonly used for fisheries management and commercial aquaculture. However, despite increasing use nationally (Finlayson et al. 2002, McClay 2005), there is limited published information available that evaluates effects of piscicides on non-target organisms such as amphibians (Cutkomp 1943, Schnick 1974a, 1974b, Chandler and Marking 1982), and to our knowledge there is none for the Columbia spotted frog (*Rana luteiventris*), long-toed salamander (*Ambystoma macrodactylum*), and Rocky Mountain tailed frog (*Ascaphus truei*). It has been shown that piscicides generally do not affect amphibians at label-prescribed dosages for fish removal (Schnick 1974a, 1974b). For example, several antimycin field trials conducted by Berger (1964, 1965a, 1965b, 1966a, 1966b) showed no effect on adult and larval frogs, and evaluations of rotenone-treated lakes in northwest Montana showed in most cases that amphibians persisted after treatment (Grisak 2003). These three amphibian species commonly coexist with trout in lakes and streams in northwest Montana. What effect using piscicides for native trout conservation or to improve sport fisheries in northwest Montana could have on these specific amphibians remains unknown.

Piscicide applications in northwest Montana are typically conducted in the autumn or fall. During this time period, cool water temperature and lower intensity of sunlight help maximize piscicide performance, water flow is typically low and amphibians have generally metamorphosed and migrated to wintering habitats. Long-toed salamanders and Columbia spotted frogs use lakes in the spring for breeding and depositing eggs, then immediately return to forested areas and semi-aquatic habitat (Marnell 1997). Larvae of both species metamorphose by fall and then follow similar behavior patterns as adults. This life history cycle favors conducting piscicide treatments in the fall when theses species are in advanced metamorphic stages and moving away from targeted aquatic environments. Tailed frogs are dependant on streams for their three-year larval stage and adults remain close to streams or in nearby wetted areas for the majority of their life (Werner et al. 2004, Jones et al. 2005). Consequently, a larger portion of the tailed frog population is more likely to be subject to exposure to piscicide-treated water.

The goal of any piscicide treatment is to remove unwanted fish, while minimizing effects to non-target organisms. Here we tested the piscicide brands Fintrol® (antimycin) and Prenfish® (rotenone) on spotted frog adults, long toed salamander adults and larvae, and tailed frog larvae to (1) determine the acute effects of the piscicides on these species and (2) compare these results with toxicity tests on other amphibian and fish species.

Methods

We used the American Society for Testing and Materials – International Standard Guide for Conducting Toxicity Tests on Test Materials with Amphibians (ASTM 2002) as the basis for our methodology. Tests were conducted at the Montana Fish, Wildlife and Parks facility in Kalispell in 2003 and 2004. Field crews used dip nets to collect spotted frog adults and long-toed salamander adults and larvae from lakes (1750-1850 m elevation) in the Swan Mountain Range of northwest Montana in September. These individuals were segregated, quarantined and acclimated at the test facility in 1850-L tanks for at least 14 days. We fed them a diet of dipteran larvae and arthropods until 48 hr prior to the experiments. Field crews used dip nets to collect tailed frog larvae from Hungry Horse Creek in December. These were held for no longer than 24 hr in 1850-L tanks at the test facility. Tailed frog larvae feed primarily on epilithic periphyton, so prolonged quarantine and feeding in the test environment was not practical.

We used Flathead River water for all quarantine, acclimation, and testing (mean values were: dissolved oxygen, 9.2 mg/L (8.8-10.4); alkalinity, 87 mg/L (86-89); conductivity 179 µS/cm (174-186); hardness, 92 mg/L (88-96); pH, 8.1 (8.1-8.2); TDS, 98 mg/L (90-103)). The test chambers were 55-L glass aquaria. Because little is known about the sensitivity of these species to the piscicides, we first conducted test concentration range-finding evaluations, and chose the range of test concentrations that were separated by at least 0.6 of the next highest concentration (ASTM 2002). The number of individuals in the tests was constrained by their availability from wild sources. Tests on adult spotted frogs, larval salamanders, and adult salamanders were conducted with five individuals in 3 L of water (3.5, 2.6 and 2.6 g/L loading, respectively). Adult spotted frogs could remain submerged with their nostrils exposed while in a sitting position. Salamander larvae stayed completely submerged, but adults could keep their heads out of water. Tests with tailed frog larvae were conducted with 10 individuals in 6 L of water (1.7 g/L loading). We replicated each concentration of piscicide tested, and tests on each species and life stage included replicated controls. Antimycin testing of adult salamanders was limited to one concentration due to a limited number of test animals. The tests ran for 96-hr using the renewal technique (ASTM 2002) in which we completely replaced treated and control water every 24 hr.

Our target test water temperature was 10 °C to mimic fall water temperatures during a typical piscicide application in Montana. We maintained water temperature by regulating air temperature in the lab using a thermostatically controlled natural gas heater and monitored it (every 15 min) using Onset® Optic Stowaway thermographs that were housed in each control chamber. The mean water temperature during the tests was 9.6 °C (7.7-11.6). The photoperiod of the facility was similar to the natural photoperiod (10:14 hr light: darkness).

We made concentrated stock solutions of antimycin and rotenone daily. Antimycin consisted of equal proportions of Fintrol-concentrate 23-percent (w/w%) active ingredient and Fintrol dilutent, plus nonoxynol-9 (as recommended by the manufacturer to maintain solubility as a surfactant) at a volume of 15 percent that of the Fintrol mixture. Fintrol-concentrate was placed in a warm water bath before mixing to ensure the soy lipids were in solution before making the stock. The rotenone came from Prenfish 5 percent active ingredient liquid formulation. Piscicides were dissolved in distilled water to make stock solutions. We added the appropriate amount of stock solution to test water using Nichiryo® model 5000DG 200-1000µl and 10-50µl digital micro pipettes to achieve the desired concentrations. For reporting purposes, and to remain consistent with the product labels, references to Fintrol are of active ingredient, and references to Prenfish are of formulation.

Test organisms that were fed did so readily until they were removed from feeding 24 hr before testing and did not appear to be stressed during the quarantine and acclimation period. Over the 96-hr test period we recorded mortalities at scheduled intervals (hr 1, 2, 4, 8, 12, 24) daily. We defined mortality as no movement, no reaction to gentle prodding with a glass instrument and no visual signs of life. During the tests, we observed no large differences in the progression of mortality between each replicated test concentration and all control organisms survived. We combined results of replicated test concentrations to calculate mortality at each concentration and for each species. The median lethal concentration (LC50) for each test was calculated using the Probit Method (Finney 1978) and the Trimmed Spearman-Karber Method (Hamilton et al. 1977) with the U.S. Environmental Protection Agency's Probit Analysis Program, version 1.5 and the Trimmed Spearman-Karber Method Program, version 1.5.

Results

Fintrol was toxic to these three amphibian species over a wide rage of concentrations. The only behavioral change observed in any of the amphibians exposed to Fintrol was that adult spotted frogs relaxed their legs and lowered their heads below the water line. We observed no discoloration of any amphibians exposed to Fintrol, as is common in fish (Rosenlund and Stevens 1992). With tailed frog larvae, we first observed mortality after 8 hr of exposure to the highest concentration (300 µg/L; Fig. 1). Minimum concentration that caused mortality (15%) was 7.5 µg/L after 96 hr of exposure. The 96-hr LC50 for tailed frog larvae was 13.7 µg/L (Table 1). Salamander larvae mortality was first observed after 4 hr of exposure to concentrations of 300 µg/L and greater. Maximum concentration that caused no lethal effects on salamander larvae after 96 hr was 15 µg/L. The 96-hr LC50 for salamander larvae was 81.7 µg/L (Table 1). Adult salamanders were exposed to only 7.5 µg/L of Fintrol, following the same protocol, and all survived the 96-hr period. The first sign of mortality in adult spotted frogs occurred at 250 µg/L after 52-hr and 96-hr exposure to this concentration, only 70 percent of the specimens died. Maximum concentration level that had no observable effect to adult spotted frogs was 60 µg/L at 96 hr and the 96-hr LC50 was 192 µg/L (Table 1).

The amphibians we exposed to rotenone displayed no clinical signs of toxicity such as surface gulping or loss of equilibrium as is common in fish (Brown and Ball 1943, Parker 1970, Loeb and Engstrom-Heg 1971, Teixeira et al. 1984). With the tailed frog larvae, the first sign of mortality occurred at the 8-hr observation interval at Prenfish concentrations of ≥ 0.35 mg/L. The 96-hr LC50 for tailed frog larvae was 0.009 mg/L (Table 1). We first observed mortality in larval salamanders after 2 hr of exposure to ≥ 14.5 mg/L, and all concentrations tested caused complete mortality after 72 hr. Adult salamander mortality was first observed after 2 hr of exposure to 116 mg/L, and the minimum concentration tested (3.5 mg/L) caused 50 percent mortality after 96 hr of exposure. With spotted frog adults, we first observed mortality after 1 hr of exposure to concentrations of ≥ 73 mg/L. The maximum concentration level that had no observable effect on adult spotted frogs after 96 hr was 4.5 mg/L (Fig. 2) and the 96-hr LC50 was 9.65 mg/L (Table 1).

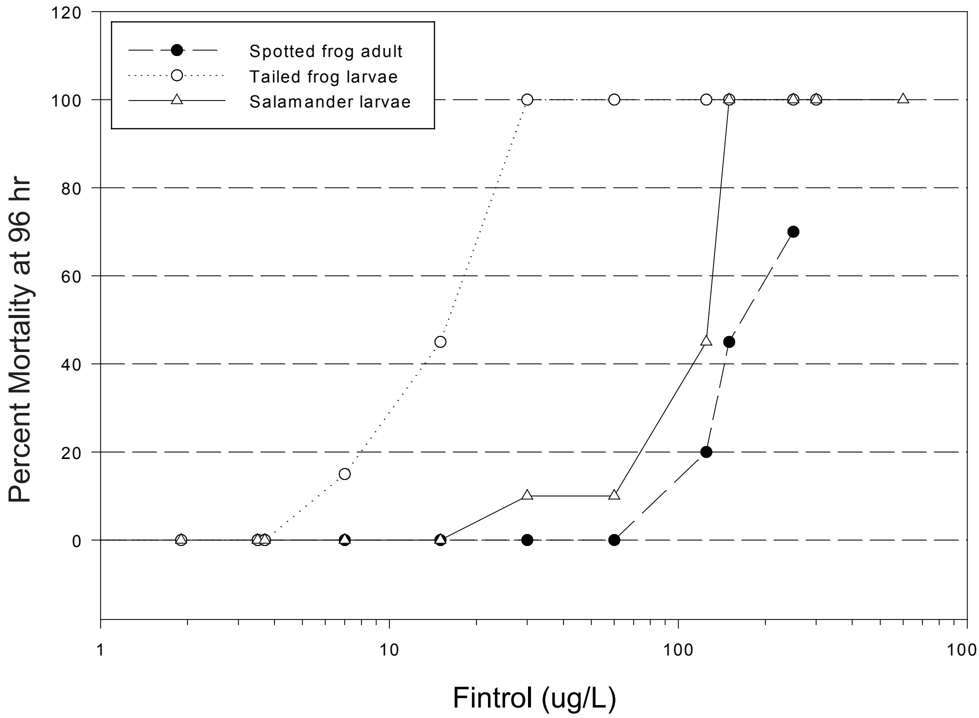

Figure 1. Toxicity of Fintrol (µg/L) to three amphibian species after 96 hours.

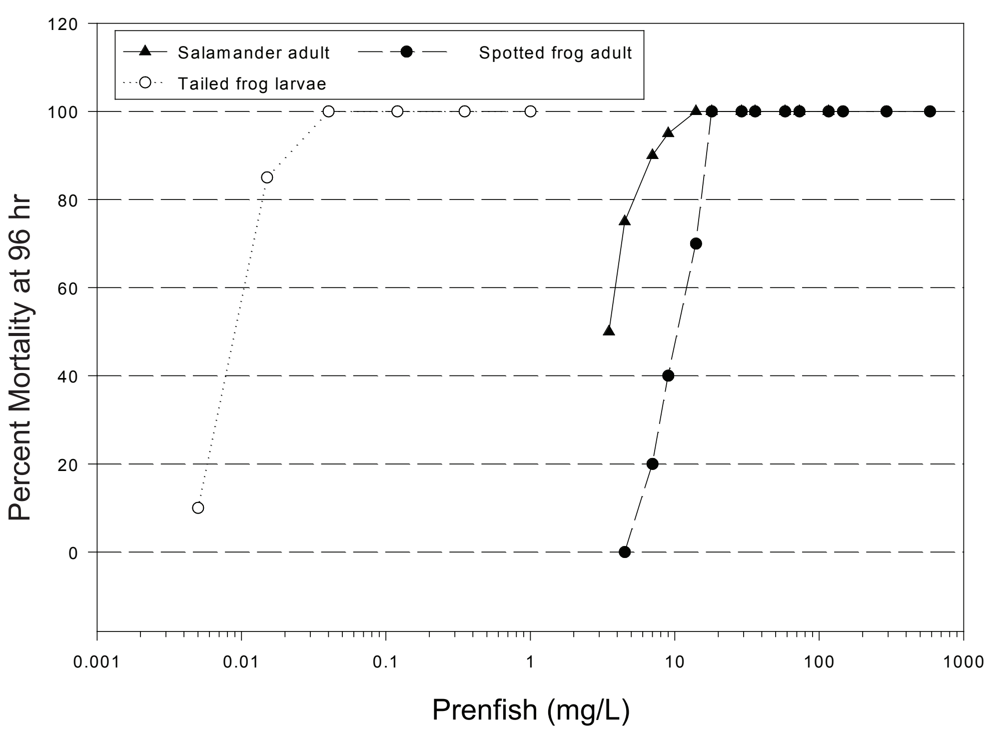

Figure 2. Toxicity of Prenfish (mg/L) to three amphibian species after 96 hours.

Table 1. The 24-hr and 96-hr LC50 values (± 95% CI) for Columbia spotted frogs, long-toed salamanders and tailed frogs exposed to Fintrol and Prenfish.

		Fintrol (µg/L)		Prenfish (mg/L)	
species	life stage	24 hr	96 hr	24 hr	96 hr
Columbia spotted frog	Adult	>250 a (---)	192 (141-313)	41.5 (33.9-50.9)	9.65 (7.8-12.0)
Long toed salamander	Adult	---	b	8.0 (6.6-9.8)	3.5 (0.8-4.9)
	Larvae	225 (150-300)	81.7 (66.8-99.1)	<0.23 c (---)	<0.23 d (---)
Tailed frog	Larvae	77.6 (66.3-90.7)	13.7 (11.1-16.7)	0.037 (0.030-0.047)	0.009 (0.007-0.012)

a – no effect at greatest exposure (250) within 24 hr
b – exposed to 7.5 µg/L for 96 hr with no mortality
c – lowest level (0.23) caused 70 percent mortality at 24 hr
d – lowest level caused 100 percent mortality at 72 hr

Discussion

Trout are considered among the most sensitive fish species to antimycin (Walker et al. 1964, Gilderhus et al. 1969) and are the target fish most likely to co-exist with the amphibians we tested. Fintrol label prescription for removing trout is 5-10 µg/L of antimycin. In our tests, spotted frog adults survived Fintrol up to six times that amount, and only 70 percent mortality occurred at 25 times the label prescription. These findings agree with the results of a field trial in Wisconsin that used 10 ug/L of Fintrol and had no effect on adult and larval frogs (*Ranidae* spp.) or salamanders (*Ambystomidae* spp.) (Gilderhus et al. 1969). Comparisons of 96-hr LC50 values for Fintrol showed that adult spotted frogs are 16 times more tolerant than leopard frogs (*Rana pipeins*) (Lesser 1970) and 3840 times more tolerant than rainbow trout (*Oncorhynchus mykiss*) (Marking and Dawson 1972). Long-toed salamander larvae had only 10 percent mortality at the amount prescribed for trout and to attain 100 percent mortality required 15 times that amount. Similarly, adult tiger salamanders (*Ambystoma tigrinum*) survived exposure to 80 µg/L antimycin over 96-hr (Walker et al. 1964), but succumbed to 600 µg/L in 24-hr (Berger 1966c). Tailed frog larvae were more susceptible to Fintrol than other species tested; even then, only 5 percent of tailed frog larvae died at concentrations specified for trout (5-10 µg/L), and this response occurred after 72 hr of exposure and three complete reconstitutions of the test material. By comparison, six species of fish tested by Gilderhus (1972) succumbed to this concentration within 0.5 to 6-hr. On the basis of the LC50 values, tailed frog larvae showed greater tolerance than 21 species of fish, with only the black bullhead being more resilient (Berger et al. 1969, Marking and Dawson 1972).

As with antimycin, trout are considered among the most sensitive fish species to rotenone (Gilderhus 1972). Prenfish label prescription for normal pond use is 0.5 to 1.0 mg/L of formulation, and this range is used in Montana when trout are the target species. Both salamander and spotted frog adults survived 96-hr exposure to these concentrations, but tailed frog larvae did not. Furthermore, the no observable effect concentration for spotted frog adults was 4.5 to nine times greater than the label prescription for normal pond use. Salamander adults were 15 times more tolerant of Prenfish than the larvae based on 96-hr LC50 (3.5 mg/L and < 0.23 mg/L, respectively) (Table 1). Although this study could not determine the Prenfish level that had no observable effect to larval salamanders or larval tailed frogs, under laboratory conditions apparent sensitivity of these life stages suggests that some mortality could occur during normal field use of Prenfish.

Comparing our findings with 96-hr LC50's of rainbow and brook trout (0.046 mg/L and 0.044 mg/L, respectively; Marking and Bills 1976) showed the respective tolerances of spotted frog adults and salamander adults are 210 and 76 times greater than those fish species. Larval l salamanders were about five times more tolerant than the trout, but larval tailed frogs were about five times more sensitive than the trout.

In conclusion, our findings indicated that when applied in the field according to the label prescription for trout Fintrol would not have an impact on any of the species or life stages tested, Prenfish would not have an impact on the adult amphibians we tested, but could impact larvae if present in the treatment zone. Some of our results showed that achieving an effect on amphibians would require prolonged exposure to Prenfish. Piscicide applicators should also consider that under field conditions, natural factors such as water chemistry, vegetation, and light intensity might greatly reduce persistence of piscicides, which could increase larval amphibian survival (Marking and Dawson 1972, Marking and Bills 1976, Chandler and Marking 1982, Dawson et al. 1991). Implementing piscicide treatments in the fall when adult amphibians typically are not in the treatment zones or after the larvae have metamorphosed will also lessen impacts to non-target amphibians.

Acknowledgements

The authors would like to express their gratitude to Jon Cavigli, Scott Hawxhurst, Aaron Rasmussen, Kristian Skybak, Lynda Fried and Kris Tempel who contributed their expertise and assisted with collections of test specimens and laboratory operations, Ron Morinaka, Joe DeHerrera and Cecelia Brown for administering the funding, Dave Yerk for contributing expertise with graphics, one anonymous reviewer and Sharon Johnston who edited and formatted the manuscript. This study was funded by Bonneville Power Administration through the Hungry Horse Dam Fisheries Mitigation Program.

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Received 23 June 2005
Accepted 26 January 2007