ACUTE TOXICITY OF MOSQUITOCIDAL COMPOUNDS TO YOUNG MOSQUITOFISH, *GAMBUSIA AFFINIS*

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ABSTRACT. Toxicity of Florida mosquito larvicides and adulticides to 3–5 day old *Gambusia affinis* was determined in the laboratory. After 24-h exposure, the larvicides, temephos, fenoxycarb and petroleum distillates had LC_{50} values of 5.60, 1.05 and 593.4 ppm, respectively. After 24 h the adulticides resmethrin, fenthion, naled and malathion had LC_{50} values of 0.007, 2.94, 3.50 and 12.68 ppm, respectively. The only compound toxic to young mosquitofish at maximum field application rates was resmethrin. However, in the light of earlier tests, aerially applied adulticides generally reach the water surface at reduced concentrations and thus probably pose little or no risk to mosquitofish populations.

INTRODUCTION

The mosquitofish, *Gambusia affinis* (Baird and Girard), is the most common biological control agent in use against mosquitoes today (Meisch 1985) and has been integrated successfully into mosquito abatement programs worldwide. Mosquito control operations frequently apply larvicides and adulticides in or proximal to aquatic habitats containing mosquitofish. Integrated pest management (IPM) of mosquitoes using *Gambusia affinis* and conventional chemical compounds assumes compatibility between these agents. To develop sound IPM strategies, toxicological tests must be performed to assess the impact of mosquitocidal compounds on beneficial organisms.

During 1989 in Florida, 50 mosquito control districts applied the larvicides: temephos, methoprene, petroleum distillates and *Bacillus thuringiensis* (H-14); and the adulticides: malathion, naled, fenthion and resmethrin. This study investigates the effect of these insecticides on survival of young mosquitofish under laboratory conditions.

MATERIALS AND METHODS

To assess whether mosquitofish are susceptible to selected compounds, a "worst case scenario" was created; 3–5 day old mosquitofish were used in bioassays. *Gambusia affinis* were cultured in ponds at the John A. Mulrennan Sr. Research Laboratory. Gravid females were collected and individually placed in spawning chambers in the laboratory. Spawning chambers were constructed from sections of polyvinylchloride pipe 78 mm ID and 65 mm in length. One end of the chamber was covered with a 3-mm mesh plastic screen through which the young exited into the lower area of the beaker. The chamber was placed in a 1-liter plastic cup filled with aerated well water and covered with a Plexiglas lid to prevent the fish from escaping. Prior to testing, newly hatched fish were held from 3 to 5 days in 37.8-liter aquaria and fed newly hatched brine shrimp, *Artemia salina.*

Static toxicity tests were conducted in the laboratory to determine the acute effects of mosquito larvicides and adulticides on young mosquitofish. These tests utilized methods recommended by the American Society for Testing and Materials (ASTM 1980). The larvicides used were temephos (Abate® 4-E), fenoxycarb (Pictyl® EC), methoprene (A.L.L.®), *Bacillus thuringiensis* (H-14) (Bactimos®) and an oil (GB-1111®). The adulticides used were resmethrin (Scourge®), fenthion (Baytex®), naled (Dibrom® 14) and malathion (Cythion®).

Bioassays were conducted in 600-ml Pyrex® beakers containing 500 ml of well water (alkalinity and hardness of ca. 150 ppm). Prior to each test, the diluent water was placed in a 95-liter container and aerated, and treated with ultraviolet irradiation for 24 h to saturate the water with oxygen and reduce bacterial contamination.

Test beakers were suspended in a water bath (water temperature = 27 ± 0.5°C) circulated by an Haake® immersion heater/circulator. A photoperiod of 16 h light and 8 h darkness was maintained using fluorescent lights.

To obtain appropriate concentrations, adulticides were diluted with acetone and larvicides with deionized water, except for GB-1111 which was not diluted. One ml of the test dilution was pipetted into each beaker to obtain the desired dosages. These solutions were added to the beakers and stirred prior to the introduction of fish. The GB-1111 was pipetted as a concentrate onto the water surface and not stirred because it is a surface active agent. Five fish were placed in each beaker, and beakers were replicated 6 times per dosage (30 fish per dosage). A test consisted of a minimum of 7 dosages including a control.

Mortality was determined at 24 and 48 h post-treatment. The criterion for mortality was the cessation of all movement. Dead fish were removed to prevent fouling of the test water. When control mortality occurred, the treatment mortality was corrected by Abbott's formula (Abbott 1925). A test was considered invalid if check mortality exceeded 5% or dissolved oxygen in the beaker water was below 40% of saturation. Tests were used only if the data were homogeneous with the theoretical line determined by chi-square analysis (SAS Institute 1985). A minimum of 7 valid tests were conducted for each insecticide and the results combined and analyzed using a Probit procedure (SAS Institute 1985) to determine the LC values and confidence limits.

RESULTS AND DISCUSSION

The mosquito larvicides, temephos, fenoxycarb, methoprene, *Bacillus thuringiensis* (H-14) and oil (GB-1111) were found to be nontoxic to young mosquitofish at recommended field application rates (calculated for a water depth of 15.2 cm) (Fig. 1). Dose-response curves were not determined for methoprene or *B. thuringiensis* (H-14) because acute toxicities were not detected at concentrations less than 30,000 ppb and 100,000 ppb, respectively. Of the adulticides, resmethrin tested at recommended ground-ULV rates (0.007 AI/acre or 5.2 ppb) caused mosquitofish mortality, whereas fenthion, naled and malathion did not (Fig. 2). The dose-response curve for resmethrin suggests 20–38% mortality occurred at standard ground ultra-low volume application rates (Fig. 2).

Twenty-four- and 48-h exposures of mosquitofish to naled or fenoxycarb resulted in similar ranges in toxicity due to hydrolysis or degradation of the active compound (Figs. 1 and 2; Table 1). Naled is readily hydrolyzed in aqueous solutions above pH 7 (Chen 1984), having a half-life of less than 17 h. These compounds were generally inactive after 24 h. In contrast, compounds with residual activity after 24 h, such as malathion, resmethrin and temephos (Figs. 1 and 2; Table 1), were much more toxic after 48 h of exposure. Similarly, the toxicity of fenthion to young mosquitofish increased distinctly after 48 h of exposure (Table 1). This was expected since fenthion was found to have an half-life of 10.9 days in a natural freshwater lake (Wang et al. 1989).

Although larvicidal treatments may reach the aquatic habitat at intended rates, unintentional drift of adulticides over water may produce only slight contamination. Aerially applied fenthion as a thermal fog was found to reach the water

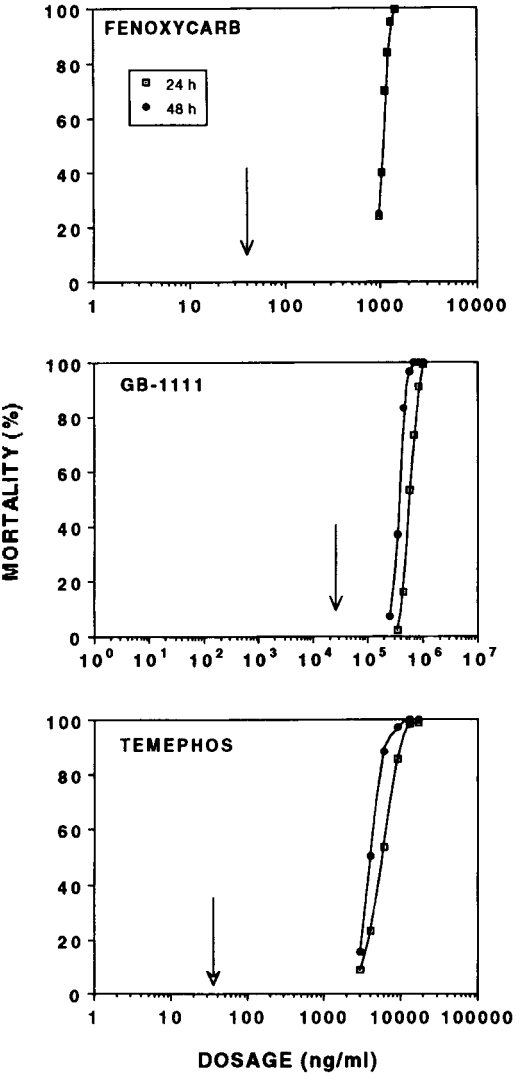

Fig. 1. Dose-response curves for 3–5 day old *Gambusia affinis* exposed to the mosquito larvicides: fenoxycarb, GB-1111 and Temephos for 24 and 48 h (arrows indicate maximum labeled field application rates in a water depth of 15.2 cm).

surface at 5.5% of the original concentration or 18.6 ng/cm^2 (Wang et al. 1987). Tagatz et al. (1974) applied malathion to a salt marsh in Florida by thermal fogging once at 420 g/ha and 3 biweekly ULV aerosol sprays at 57 g/ha. The former method resulted in malathion residues in the water at a concentration of 5.2 ppb, whereas the latter treatments resulted in 0.5 ppb. Temephos was aerially applied at 28.35 g/acre over a mangrove swamp, yet concentrations reaching intertidal waters of the swamp were undetectable 4 h post-treatment (Pierce et al.

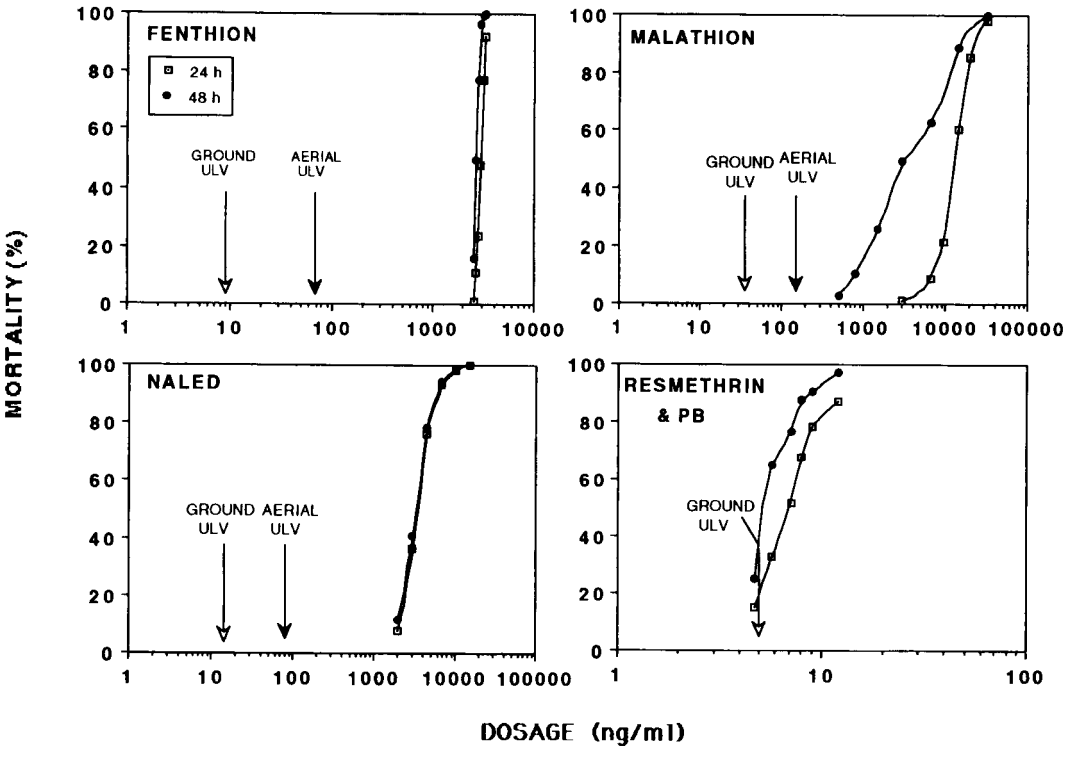

Fig. 2. Dose-response curves for 3–5 day old *Gambusia affinis* exposed to the mosquito adulticides: fenthion, malathion, naled and resmethrin for 24 and 48 h (arrows indicate maximum labeled field application rates in a water depth of 15.2 cm).

1989). The concentrations of such compounds reaching the water surface are further diminished by vertical mixing, tidal dilution (in estuaries), flushing and degradation (Wang et al. 1987, Borthwick et al. 1985). Assuming resmethrin (Scourge) behaves as do the adulticides mentioned above, field applications of this pyrethroid may have little or no impact on mosquitofish, but definitive tests are in order.

Adult mosquitofish were not killed when treated with fenthion at 1 ppm for 48 h (Darwazeh and Mulla 1974) or 5 ppm for 48 h (Patterson and von Windeguth 1964). In another test, von Windeguth and Patterson (1966) noted that after 24 h of exposure, fenthion had an LC_{50} of 2.0 ppm for adult mosquitofish. In the present study, fenthion had an LC_{50} of 2.94 ppm for young mosquitofish after 24-h exposure. While these values are comparable, further tests are needed to determine whether susceptibility changes with the age of the fish. Temephos was reported to be sublethal to adult mosquitofish at 5 ppm after 48 h (Darwazeh and Mulla 1974), whereas von Windeguth and Patterson (1966) determined the LC_{50} to be 200 ppm after 24 h. In our study, temephos had an LC_{50} of 5.60 ppm for young mosquitofish after 24 h, suggesting large changes in age-specific susceptibility to this compound. Finally, Lewallen (1959) found that malathion and naled tested at rates of 0.05 and 0.03 ppm, yielded average mortalities of adult *Gambusia affinis* of 40% and 3%, respectively. The LC_{50} of malathion after 24 h was 12.68 ppm, and LC_{50} of naled was 3.50 (Table 1). Both values suggest greater tolerance of mosquitofish to these compounds than was reported by Lewallen (1959).

Whereas the above tested compounds seem to have little acute toxicity to mosquitofish, sublethal effects may weaken their survival and/or reproductive capacity. Reduction of the abundance of highly susceptible prey items (i.e., crustaceans, mosquitoes and other aquatic insects) may also cause secondary impacts on mosquitofish survival and merit future investigation.

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Table 1. Toxicity of Florida mosquito adulticides and larvicides to 3–5 day old *Gambusia affinis*.

Compound[1]	Test length (h)	No. tests	Lethal concentration μg AI/ml LC_{50}	95% CL
		Larvicides		
Fenoxycarb	24	7	1.05	1.04–1.06
(Pictyl®)	48	7	1.05	1.04–1.06
Petroleum	24	9	593.35	580.36–606.39
distillates	48	8	372.44	363.49–381.41
(GB-1111®)				
Temephos	24	7	5.60	5.36–5.84
(Abate® 4-E)	48	7	4.11	3.79–4.43
		Adulticides		
Fenthion	24	10	2.94	2.92–2.96
(Baytex®)	48	7	2.65	2.63–2.66
Malathion	24	7	12.68	12.11–13.20
(Cythion®)	48	7	3.44	2.72–4.37
Naled	24	7	3.50	3.35–3.65
(Dibrom® 14)	48	8	3.31	3.17–3.45
Resmethrin[2]	24	9	6.93	6.45–7.39
(Scourge®)	48	9	5.24	5.02–5.44

[1] Methoprene (A.L.L.®) and *Bacillus thuringiensis* (H-14) (Bactimos® FC) acute dosage responses gretaer than those tested in range finding test.

[2] Concentrations in ng AI/ml.

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