

Acute Toxicity of Mosquito Control Compounds to *Cyprinodon variegatus* and *Menidia beryllina*: Laboratory and Field Tests

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ABSTRACT

Young sheepshead minnows (*Cyprinodon variegatus*) were exposed to mosquito larvicides and adulticides to determine acute toxicity in the laboratory. Bioassays using adulticides resulted in 24 h LC50s of 3.5, 61, 78 and 1400 ppb for resmethrin, malathion, naled and fenitrothion; larvicides resulted in 24 h LC50s of 8.08, 1322, and 2387 ppm for temephos, fenoxycarb and petroleum distillate (GB-1111), respectively. Field tests were conducted in replicated marshland ponds by exposing *C. variegatus* and *Menidia beryllina* within floating cages during 7 day intervals. Malathion and resmethrin were applied at rates simulating pond water concentrations resulting from drift from mosquito adulticide applications. Resmethrin resulted in greater field survival than predicted by laboratory bioassays, perhaps due to exposure to sunlight. In contrast, malathion applications resulted in significant mortality at 168.8 ppb when compared to 39.9 ppb and controls. Malathion was detected in water up to 7 days post-application when treated at 39.9 ppb and up to 14 days at 168.8 ppb. Even though resmethrin had the highest acute toxicity to minnows compared with the other compounds tested in the laboratory, malathion concentrations calculated from labeled aerial application rates caused higher mortality in field tests. Based on other studies, however, concentrations of malathion deposited in water during normal mosquito control operations may be no greater than 6% of these simulated rates producing little or no acute toxicity to these minnows.

INTRODUCTION

Acute toxicity as measured by laboratory bioassay is an accepted technique to assess an organism's susceptibility to a pesticide. Toxicity tests are most useful for initial assessments of an animal's susceptibility, indicating whether further testing is warranted. As a general rule, laboratory based results should never be the sole basis for environmental risk assessments (Stern and Walker 1978). The rationale for this statement is that field effects may be radically different from those of the laboratory due to factors such as degree of exposure as dictated by a variety of factors such as stability of the compound, (Lichtenstein 1972), physical distribution and transport of the compound relative to the organism (Mulla et al. 1981), as well as others too numerous to list here. Most organophosphates and pyrethroids labeled for mosquito control are characterized by having relatively short half-lives due to rapid chemical and biological degradation into nontoxic moieties. Rapid degradation may be due to hydrolysis (e.g., naled [dimethyl 1, 2-dibromo-2,2-

dichloroethyl phosphate]) (Chen 1984), photodecomposition, (e.g., methoprene [isopropyl (2E,4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate], resmethrin [(5-benzyl-3-furyl) methyl, 2,2-dimethyl-3-(2-methylpropenyl) cyclopropanecarboxylate] and temephos [0, 0, 0', 0'-tetramethyl 0, 0-thiodi-*p*-phenylene phosphorothioate]) (Rosen 1972), or microbial breakdown as measured for malathion [0, 0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate] (for review see Mulla et al. 1981). The most stable mosquito control compound used in the United States appears to be permethrin, which was reported to degrade 32% of its original activity after 12 weeks in lake sediment (Sharon and Solomon 1981). Compounds lacking persistence and having minimal nontarget effects have been selectively labeled for mosquito control in order to reduce prolonged environmental impacts as observed for DDT (dichlorodiphenyl-trichloroethane) and other chlorinated hydrocarbons (Lichtenstein et al. 1960).

This study was designed to assess the toxicity of "major-use" mosquito control pesticides to the sheepshead minnow, *Cyprinodon*

variegatus Lacepede in the laboratory. The compounds exhibiting greatest toxicity to *C. variegatus* and the inland silverside, *Menidia beryllina* (Cope) (Tietze et al. 1992) in the laboratory were subsequently evaluated for these two species under simulated field conditions.

MATERIALS AND METHODS

During the spring of 1993, wild *C. variegatus* were collected by cast netting in shallow margins of local bays, particularly in and near tidal tributaries in North Bay, Panama City, Florida. These fish augmented populations cultivated at the John A. Mulrennan, Sr. Research Laboratory (JAMSRL) in Panama City, Florida. Beginning in late April, fish eggs were collected on substrata as described for *M. beryllina* (Tietze et al. 1992). Unlike *M. beryllina*, *C. variegatus* eggs were hatched and reared in 5-gallon buckets in the laboratory. The fry were fed newly hatched *Artemia* twice daily and after 5 days powdered Tetramin was added to their diet.

Six to 11 day old sheepshead minnows were exposed to the mosquito adulticides, fenthion [0, 0-dimethyl-0-(3-methyl-4-(methylthio) phenyl) phosphorothioate], malathion, naled and resmethrin and larvicides, petroleum distillate (GB-1111), temephos, methoprene and *Bacillus thuringiensis* var. *israelensis*. Bioassay methods were identical to that for *M. beryllina* (Tietze et al. 1992). A minimum of 5 tests were combined by compound and analyzed using a probit procedure (SAS Institute Inc. 1990) to determine LC50 and 95% confidence limits.

To further determine the toxicity of resmethrin and malathion to minnows, field tests (7 day durations) were conducted in replicated ponds that averaged 6.1 and 3.7 m in length and width, respectively. Ponds were flooded with brackish water by raising the water level in an adjacent "retention pond" that spilled into each pond via 10.2 cm polyvinyl chloride pipes. Due to differences in water volume between ponds, it was necessary to calculate the average depth of each pond by sampling depth 10 times at selected locations. Estimated pond volumes were factored into application rate determination. Resmethrin (0, 2.6 and 5.2 ppb) and malathion (0, 39.9 and 168.8 ppb) treatments were based on maximum labeled field rates for ground and air applications

assuming 100% deposition within the target swath (i.e., 0.40 ha where the dimensions are, 44.2 m parallel to the line of application by 91.4 m swath width). Treatments were made by direct application of the compound diluted in acetone using squirt bottles. Controls received acetone only.

Young *C. variegatus* (8 to 10 days old) and *M. beryllina* (15 to 17 days old) were placed in submerged test cages suspended near the water surface using Styrofoam rings. Each pond had three replicate cages for both species with 10 individuals per cage. For acclimation, test fish were kept in cages in the ponds 24 h pre-treatment and any dead fish were replaced with healthy individuals just prior to treatment. Caged fish were fed newly hatched *Artemia* twice daily. The two test concentrations and controls were conducted in triplicate (i.e., three ponds per treatment). Fish mortality was assessed 1, 2, 4 and 7 days post-treatment. Physical factors, water temperature, salinity and oxygen content were determined pre-treatment, and on days 1, 2, 3, 5 and 7 post-treatment. Salinity was measured using a salinity refractometer. Pond water was collected for malathion residue determinations one day pre-treatment and 1, 2, 7 and 14 days post-treatment. Rainfall was recorded during each test period.

Malathion residues in test ponds were assessed by means of gas chromatography. Malathion was extracted from 1 liter of sample water three times within a 2-liter separatory funnel, each time using 200 ml methylene chloride. The extract was then passed through a drying column filled with anhydrous sodium sulfate and reduced to a volume of about 10 ml in an evaporator connected to a 3-ball Snyder column. The sample was then exchanged into 50 ml of hexane and again evaporated to a volume of about 10 ml. Concentration of malathion was determined using a Varian 3400 gas chromatograph equipped with an Inboard Data Handling option, split/splitless injector, DB-5 capillary column (30 m, id = 0.25 mm, dI = 0.1 μ m) connected to a thermionic sensitive detector. Carrier gas was helium at 25 ml/min and detector gases were hydrogen and air at 4.5 and 175 ml/min, respectively. Temperatures were set at 230° C for the injector; the column was held at 80° C for 1 minute then increased at 20°

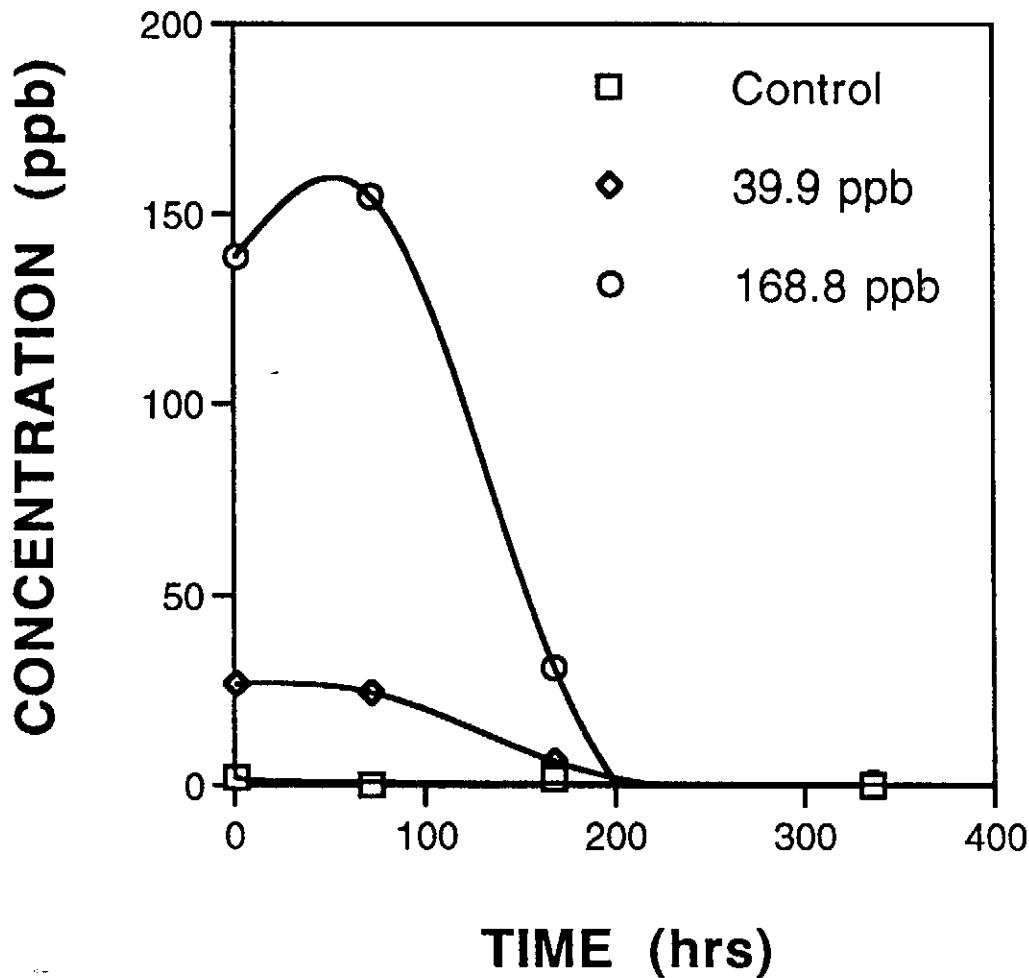


Fig. 3. Mean concentration of malathion recovered from pond water for 3 treatment rates.

malathion. Higher mortality was observed during the first of the two tests perhaps due to depressed oxygen levels and warmer temperatures. In field tests exposing *C. variegatus* to malathion, mortalities more closely resembled those predicted in the laboratory. Probit analysis predicted 39.9 ppb malathion (solid arrow Figure 2) to cross the dose-mortality line at less than 5% mortality, while 168.8 ppb (open arrow, Figure 2) crossed the dose-mortality line at nearly 100%.

Mosquito control adulticides applied as ultra-low volume sprays that drift over water may never create predicted worst-case scenario concentrations. Adulticide concentrations resulting from ULV sprays depositing on water may amount to 5-6% of expected

concentration (Tietze et al. 1994, Wang et al. 1987). Deposition studies recovered a maximum of 5.8% of expected mass of malathion when applied from a truck-mounted Leco (Lowndes Engineering, Valdosta, GA) (Tietze et al. 1994). Based on these figures, malathion may be expected to have a maximum concentration of 2.2 ppb in 15.2 cm of water; a concentration well below the level toxic to minnows.

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Table 1. Toxicity of mosquito larvicides and adulticides to 6-11 day old *Cyprinodon variegatus* derived from laboratory bioassays.

Compound	Exposure Duration (hrs)	No. Tests	Lethal Concentration ($\mu\text{g Al/ml}$)	
			LC ₅₀	(95% C.L.)
<i>Larvicides</i> ¹				
Fenoxycarb (Pictyl)	24	5	1322	(1313-1331)
	48	5	1319	(1309-1328)
Petroleum Distillates (GB-1111)	24	6	2387	(2318-2468)
	48	5	1150	(1034-1265)
Temephos (Abate 4E)	24	8	8.17	(8.08-8.25)
	48	7	8.12	(8.05-8.24)
<i>Adulticides</i>				
Fenthion (Baytex)	24	6	1.438	(1.408-1.467)
	48	5	1.307	(1.269-1.343)
Malathion (Cythion)	24	5	0.061	(0.054-0.066)
	48	5	0.050	(0.045-0.055)
Naled (Dibrom 14)	24	7	0.783	(0.737-0.831)
	48	7	0.728	(0.684-0.775)
Resmethrin ² (Scourge)	24	9	3.563	(3.328-3.826)
	48	7	2.973	(2.887-3.061)

¹Methoprene (A.L.L.) LC₅₀ > 2591× the maximum recommended field application rate and *Bacillus thuringiensis* var. *israelensis* (Bactimos FC) had no effect 108× the maximum field application rate.

²Concentrations in ng Al/ml.

C/min to 200°C, and held for 6 min.; detector temperature was 300°C. Calibration standards (i.e., 10, 25 and 50 ppb malathion) (Chem Service Inc., Westchester, PA) and an acetone blank were run during each test.

Field results of resmethrin and malathion tests were analyzed by comparing the dependent variable "number of fish dead within 7 days" between insecticide treatments and fish species using general linear analysis of variance and Student-Newman-Keuls mean separations test.

RESULTS AND DISCUSSION

The mosquito larvicides, *Bacillus thuringiensis* var. *israelensis*, fenoxycarb, methoprene, petroleum distillates and temephos, were found to be nontoxic at expected field concentrations (Table 1, Fig. 1). When comparing 24 h LC₅₀s with other minnow species, *C. variegatus* was 17X more tolerant to petroleum distillates than *M. beryllina* and 4X more tolerant than the mosquitofish (Tietze et al. 1991, 1992). Tolerance to temephos in the sheepshead was only slightly greater than the other two minnow species.

The mosquito adulticides, in order of decreasing toxicity to *C. variegatus*, were resmethrin, malathion, naled and fenthion (Table 1, Fig. 2). Sheepshead susceptibility to resmethrin and fenthion was very similar to that of the *M. beryllina* (Tietze et al. 1992). Sheepshead minnows were about 58X more susceptible to malathion than inland silversides and 207X more susceptible to malathion compared to mosquitofish (Tietze et al. 1991).

Two, 7-day tests exposing minnows to resmethrin yielded no significant ($P > 0.05$) mortality for either fish species. All *C. variegatus* survived the tests in control and resmethrin (2.6 and 5.2 ppb) treated ponds. During the August 1992 test, inland silversides had 7-day mortalities of 10.0, 5.4 and 1.1% in controls, 2.6, and 5.2 ppb treatments, while September 1992 test mortalities were 5.6, 5.6 and 8.9% in 2.6 and 5.2 ppb treatments and controls, respectively.

Field tests using malathion yielded significant ($P < 0.05$) mortality for both minnow species tested. After 7 days, *C. variegatus* had significantly greater mortality in ponds treated at 168.8 ppb compared with 39.9 ppb or controls ($df = 2$; F Value = 12.15;

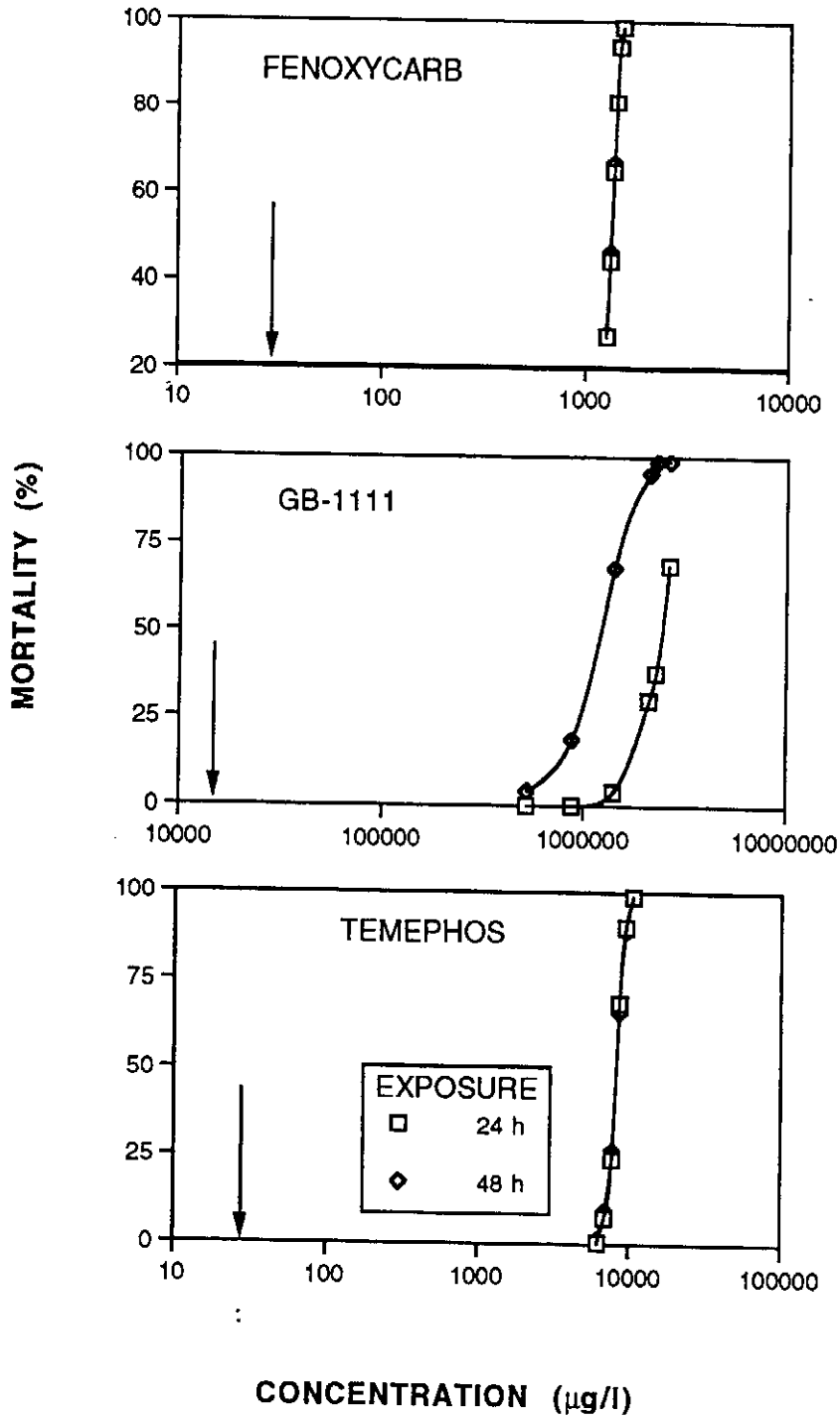


Fig. 1. Dose response curves for *Cyprinodon variegatus* exposed to larvicides during 24 and 48 h intervals. Arrows indicate expected maximum concentration based on 100% deposition in 15.4 cm water.

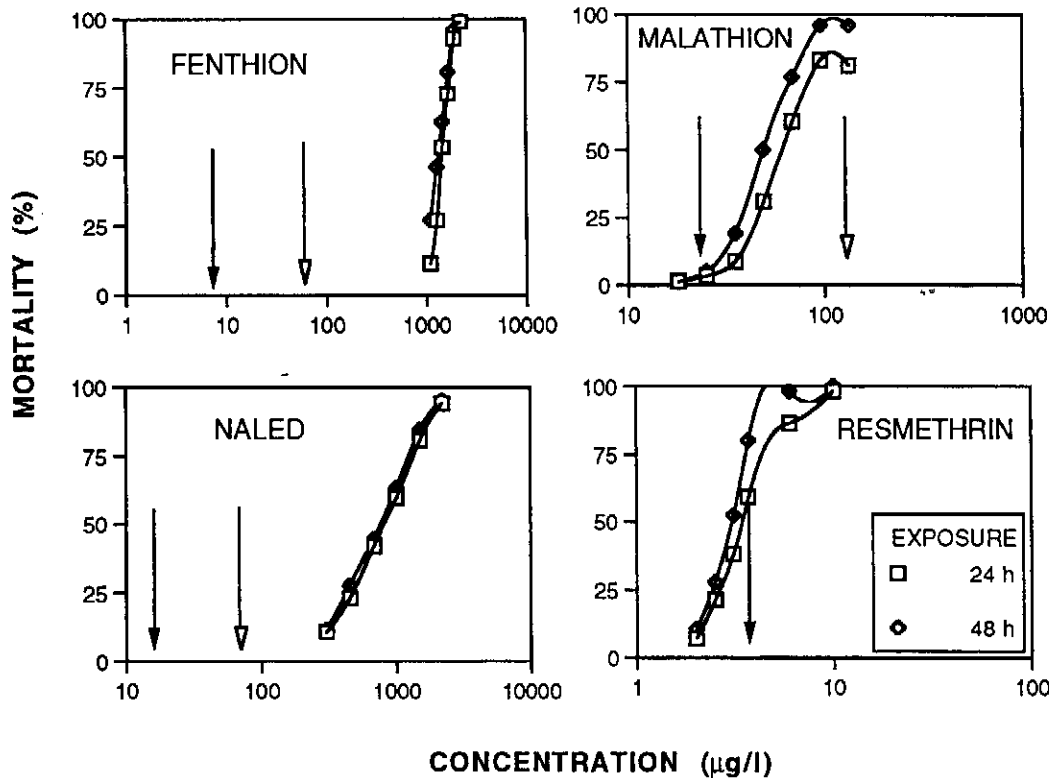


Fig. 2. Dose response curves for *Cyprinodon variegatus* exposed to adulticides during 24 and 48 h intervals. Solid arrows indicate expected maximum allowable ground-based concentration in 15.4 cm water; open arrows indicate maximum labeled rate for aerial applications on 15.4 cm water.

$P < 0.001$) (Table 2). Similar results were obtained for *M. beryllina* ($df = 2$; F Value = 21.41; $P < 0.001$). There were no significant differences ($P > 0.05$) between species at treatment rates of 39.9 or 168.8 ppb. Control mortality was significantly greater for *M. beryllina* compared with *C. variegatus* ($df = 1$; F Value = 8.44; $P = 0.016$) (Table 2).

Field conditions varied between, as well as among, test intervals. Oxygen content and salinity in ponds decreased during the test interval due to rainfall (Table 3). Rainfall during the August 1992 test was 0.84, 0.56, 1.75, 1.35 and 5.08 cm for days -1, 0, 1, 2 and 5 post-treatment, respectively. During the September 1992 test, 1.24 and 0.89 cm of rainfall was recorded on post-treatment day 5 and 7, respectively. The August 1993 test received 0.30, 1.27, 1.42, 2.89 cm rain on post-treatment day -1, 0, 2 and 4, respectively. Negligible rainfall was detected during the September 1993 test. Water temperatures during the resmethrin tests in

1992 ranged from 26 to 28.2° C during the first test and 25 to 29° C during the second. In 1993 tests, water temperature ranged from 28 to 36° C and 26 to 35° C during the first and second tests, respectively.

In ponds treated to create a malathion concentration of 39.9 ppb, residue in pond water diminished from 26.7 ppb one hour after application to negligible levels by about 7 days post-treatment (Fig. 3). When ponds were treated at 168.8 ppb, maximum concentrations of 154.4 ppb were detected 1 day post-treatment, by two weeks post-treatment malathion residue in pond water had greatly diminished (0.8 ppb). Malathion residue in control ponds was always negligible.

In laboratory bioassays, resmethrin was by far the most toxic compound to *C. variegatus* yielding a 24 h, LC50 of 3.6 ppb. Field tests, however, caused no mortality to young *C. variegatus* when the operational mosquito control rate of 5.2 ppb was applied. An explanation for this disparity is that in the

Table 2. Mortality of *Cyprinodon variegatus* and *Menidia beryllina* exposed to malathion during two, 7-day field tests.

Species/Concentration (ppb)	Cumulative mortality (%) by day post-treatment			
	1	2	4	7
<i>August 24-31, 1993</i>				
<i>C. variegatus</i>				
0.0	0.0	0.0	0.0	0.0
39.9	38.9	38.9	38.9	38.9
168.8	66.7	66.7	66.7	66.7
<i>M. beryllina</i>				
0.0	15.6	15.6	15.6	16.7
39.9	23.3	25.6	26.7	26.7
168.8	52.2	81.1	81.1	82.2
<i>September 22-28, 1993</i>				
<i>C. variegatus</i>				
0.0	0.0	0.0	0.0	0.0
39.9	0.0	0.0	0.0	0.0
168.8	0.0	2.2	13.2	30.0
<i>M. beryllina</i>				
0.0	3.3	3.3	3.3	3.3
39.9	3.3	3.3	3.3	4.4
168.8	33.3	38.9	42.2	43.3

field, resmethrin is quickly photo-degraded by sunlight (Rosen 1972) thus greatly reducing exposure time. In contrast, fluorescent lights in the laboratory may not produce wavelengths that degrade resmethrin. Photo-degradation, due to exposure to sunlight, has been reported to be an important factor in reducing persistence of resmethrin (Rosen 1972). Rosen found resmethrin was completely photolyzed within a 5 h exposure to sunlight. The same disparity held for

M. beryllina, when exposed to resmethrin in the laboratory, with a LC50 of 3.87 ppb at 24 h (Tietze et al. 1992): while our current study field assessments indicated no significant ($P>0.05$) mortality at 2.6 and 5.2 ppb.

Compared to the more labile pyrethroid, malathion caused significant minnow mortality at 168.8 ppb, and was detected in water samples up to 7 days post-application. Mortality of *C. variegatus* varied markedly between the two field tests using

Table 3. Mean (SD) oxygen content and salinity of pond water (n = 4) during field tests exposing *Cyprinodon variegatus* and *Menidia beryllina* to adulticides, resmethrin and malathion.

Adulticide/Parameter	Days Post-Treatment					
	-1	0	1	2	5	7
<i>August 18-26, 1992</i>						
Resmethrin						
Oxygen (ppm)	4.7 (1.3)	5.4 (1.0)	7.7 (0.4)	3.7 (1.1)	1.2 (1.1)	2.2 (2.2)
Salinity (ppt)	16.9 (2.0)	15.9 (1.0)	15.0 (1.6)	12.7 (2.5)	6.6 (3.7)	9.1 (3.5)
<i>September 9-17, 1992</i>						
Resmethrin						
Oxygen (ppm)	6.8 (2.1)	7.4 (1.4)	6.4 (2.7)	5.2 (2.3)	6.4 (2.3)	5.7 (1.2)
Salinity (ppt)	17.7 (1.2)	16.6 (1.8)	16.3 (1.6)	16.0 (1.6)	13.8 (1.8)	14.1 (1.5)
<i>August 24 - September 1, 1993</i>						
Malathion						
Oxygen (ppm)	7.1 (1.7)	4.1 (3.0)	2.1 (2.7)	3.0 (3.6)	2.8 (1.6)	2.8 (2.3)
Salinity (ppt)	20.6 (2.3)	18.9 (2.1)	16.2 (2.7)	16.7 (2.1)	8.7 (4.0)	10.4 (3.6)
<i>September 21-29, 1993</i>						
Malathion						
Oxygen (ppm)	7.9 (1.8)	6.5 (2.1)	6.1 (2.2)	4.7 (2.5)	5.2 (2.9)	4.0 (2.1)
Salinity (ppt)	18.8 (0.6)	18.1 (0.9)	17.9 (1.0)	17.1 (1.2)	14.4 (1.3)	13.2 (1.0)

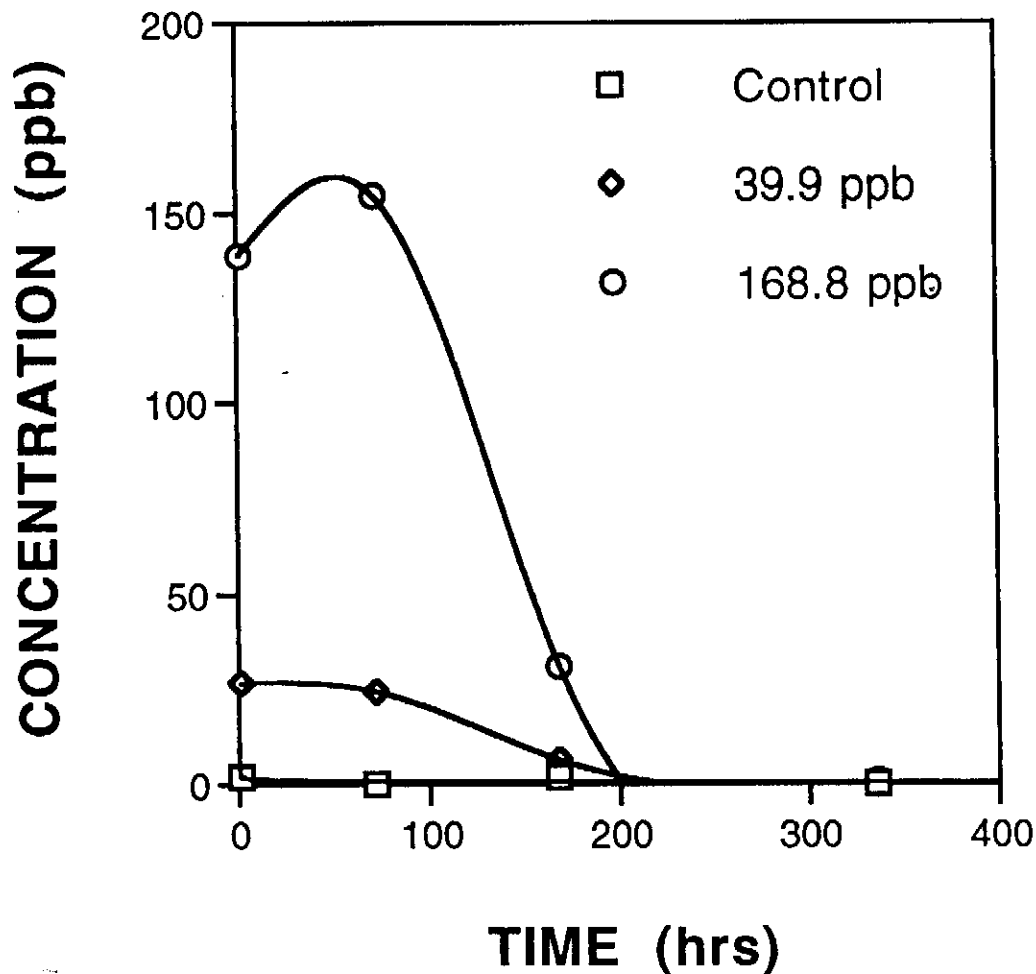


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