TOXICITY OF HERBICIDES AND MOSQUITOE LARVICIDES TO THE MOSQUITO FISH *GAMBUSIA AFFINIS*

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ABSTRACT. No acute mortality of mosquito fish *Gambusia affinis* (Baird and Girard) was observed within 48 hr when treated with six soil sterilant type herbicides at 10 ppm in the laboratory, or within 5 days with 5 lb/A a.i. in the field.

In 20-gallon tubs, Abate® (*o,o',o'*-tetramethyl-*o, o'*-thio-di-p-phenylene phosphorothiate) produced no fish kill at 5 ppm within 48 hr, while chloropyrifos, Bay 69047 (*o,o*-dimethyl-*o*-[4-nitro-3-isopropyl-mercaptophenyl]-thionophosphate), and Bay 77488(*o,o*-diethyl thiophosphoryl *o*-α-cyanobenzol-doxine) at 5 ppm produced complete mortality. In the field, chlorphyrifos, Bay 77488 and Bay 69047, at larvicidal rates caused negligible fish mortality. At 0.1 lb/A, Akton, (*o,o*-diethyl *o*-[2-chloro-1-(2-5-dichlorophenyl)vinyl] phosphorothioate) was highly toxic.

The toxicity of aliphatic amines to mosquito fish varied according to type of solvents used. At the larvicidal rates (1-5 ppm), most formulations were highly toxic in the laboratory, but no acute toxicity was observed at larvicidal rate (0.25-1.0 lb/A) in the field. Surface breathing insects, and water skimming spiders, however, were adversely affected.

The use of mosquito fish *Gambusia affinis* (Baird and Girard) for the control of mosquito larvae in California has recently attracted increased attention. Some of the factors leading to the increased utilization of these fish include development of resistance in mosquito larvae to most available chlorinated hydrocarbon and organophosphorus larvicides (Froli 1968, Kauffman 1968), and the efficiency of fish to control larvae of *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken in rice fields (Hoy *et. al.*, 1971). To meet the increasing demand by mosquito control agencies for predaceous fish in California, efforts have been recently extended to mass produce these fish in various parts of the state.

Weed control in aquatic environments such as dairy drains, irrigation ditches, ponds, and drainage sumps is used to prevent mosquito larval breeding and to facilitate water flow in irrigation and drainage structures. According to Witten (1968), McFarland (1968) and others, routine weed control in certain mosquito breeding sources is an essential part of mosquito abatement programs in California. Weed control for increasing yield and saving labor costs is also practiced in rice fields and other crops. In order to minimize adverse effects of herbicides and mosquito larvicides on mosquito fish, the herbicides and mosquito larvicides used should be harmless to fish and other mosquito predators and nontarget aquatic organisms. The current studies were initiated to determine the toxicity of various mosquito larvicides and herbicides to the mosquito fish *Gambusia affinis* and some nontarget organisms.

METHODS AND MATERIALS. Materials listed in Table 1 were evaluated in the laboratory against the mosquito fish *Gambusia affinis* (Baird and Girard). Evaluation methods employed are described elsewhere (Mulla *et al.* 1967). In summary, 20 fish were placed in a polyethylene tub containing 20 gallons of canal water. Formulations used were either emulsifiable concentrates, solutions of technical materials, or wettable powders suspended in water. The organonitrogen compounds were prepared in Toxisol TB or Toxisol FLC petroleum oils (Atlantic-Richfield Corp.) with one percent Polytergent B-200 surfactant (Olin Mathieson Chemical Corp.). The required amounts of toxicants in ppm were calculated and applied with a pipet to the water surface. The maximum amount of solvent utilized in the treatments was also applied to several tubs used as checks. Each concentration tested

TABLE 1. Chemical description of compounds studied.

Compound	Chemical description
Abate	o,o',o'-tetramethyl-o,o'-thio-di-p-phenylene phosphorothioate
Akton	o,o-diethyl o-[2-chloro-1-(2-5-dichlorophenyl)vinyl] phosphorothioate
Bay 69047	o,o-dimethyl-o-(4-nitro-3-isoprophyl-mercaptophenyl)-thionophosphate
Bay 77488 (Baythion)	o,o-diethyl thiophosphoryl o-α-cyanobenzol-doxine
Chlorpyrifos	o,o-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate
Fenthion	o,o-dimethyl o-[4-(methylthio)-m-toyl] phosphorothioate
Alamine-11	Oleyl (primary) amine
Alamine-21	Coco oil (Lauryl 48%) primary amine
Aliquat-204	dimethyl dilauryl ammonium chloride
Aliquat-206	dimethyl dipalmityl ammonium chloride
Aliquat-221	dimethyl dicoco ammonium chloride
Aliquat-336	methyl tricapryl ammonium chloride
Armeen L-15	primary beta-amine 15 carbons
Diam-21	N-coco-1,3-propylene diamine
Diam-26	N-tallow-1,3-propylene diamine
Duomeen L-15	beta-diamine 15 carbons
Isothan DL-1	dimethyl dialkyl ammonium bromide
G-27692 (simazine)	2-chloro-4,6-*bis*[ethylamino]-s-triazine
G-30027 (Atrazine)	2-chloro-4-ethylamino-6-isopropylamino-s-triazine
G-30028 (Propazine)	2-chloro-4,6-*bis*[isopropylamino]-s-triazine
G-34161 (caparol)	2,4-*bis*[isopropylamino]-6-methylthio-s-triazine
G-34162 (Ametryne)	2,-ethylamino-4-isopropylamino-6-methylthio-s-triazine
Pramitol	2-methoxy-4,6-*bis*[isopropylamino]-s-triazine

was replicated twice, amd mortality reading was obtained 24 and 48 hours after treatment. Fish utilized in these studies were collected from the Santa Ana river bottom in Riverside, California. Fish used were uniform in size and measured about an inch in length. Tests were conducted outdoors, and the test units placed in a tree-shaded area.

Field experiments were conducted in experimental ponds in Bakersfield, California. These facilities are described elsewhere (Hurlbert *et al.* 1970). Two screened wire cages were placed in every pond, containing 25 fish per cage. Emulsifiable concentrates and wettable powder formulations were mixed with 1000 ml of water and applied with a handsprayer. Ten dips per pond were taken prior to and 24 hours after treatment. All organisms recovered by dipping were recorded and observations on fish mortality were made 1, 2, 3, 4 and 5 days after application. When all the fish died in a given treatment, 25 new fish were added. Each concentration and the checks were replicated twice in every test.

RESULTS AND DISCUSSION. In laboratory studies the herbicides, atrazine, (G-27692), G-30027, G-30028, G-34162, and G-34161, were evaluated as technical materials in acetone, and showed no acute toxicity to the mosquito fish up to 10 ppm concentration for 48 hr (data not presented). However, complete mortality was obtained with EC formulation of the organophosphorus larvicides chlorpyrifos, Bay 69047 at the rate of 5 ppm and Bay 77488 at the rate of 1 ppm. The OP technical materials were less toxic than the EC formulations. The rates used here are much higher than the larvicidal rates needed for mosquito control. The effective larvicidal rate is lower than 0.1 ppm for these three materials. No acute toxic effects were observed at 5 ppm of Abate® and 1 ppm of

TABLE 2. Toxicity as determined by mortality of various pesticides against the mosquito fish *Gambusia affinis* maintained in 20-gallon water tubs.

Chemical and formulation	Conc. ppm	Average Per Cent mortality 24	Hours after treatment 48
Abate EC 4	1.0	0	0
	5.0	0	0
Chlorpyrifos (Tech.)	0.1	0	0
	0.5	0	0
Chlorpyrifos EC 4	1.0	85	92
	5.0	100	—
Bay 77488 (Tech.)	0.1	3	3
	0.5	13	18
Bay 77488 EC 4	1.0	100	—
	5.0	100	—
Bay 69047 EC 2	1.0	0	0
	5.0	100	—
Fenthion EC 4	1.0	0	0
	5.0	40	40
Check	—	0	0

TABLE 3. Toxicity of various organonitrogen chemicals against the mosquito fish *Gambusia affinis* exposed in 20-gallon water tubs.

Chemical	Conc. ppm	Avg. (%) mortality after treatment (hrs) Toxisol FLC 24	48	*Toxisol* TB 24	48
Duomeen L-15	1.0	3	4	50	63
	5.0	52	70	100	—
	10.0	—	—	100	—
Armeen L-15	1.0	0	0	0	0
	5.0	0	12	0	34
Diam-21	1.0	—	—	100	—
	5.0	—	—	100	—
Diam-26	5.0	—	—	80	100
	5.0	—	—	100	—
Aliquat 204	0.1	—	—	0	0
	0.5	—	—	0	0
	1.0	—	—	100	—
	5.0	—	—	100	—
Aliquat 336	0.1	—	—	0	0
	0.5	—	—	100	—
	1.0	100	—	100	—
	5.0	100	—	100	—
Isothan DL-1	1.0	—	—	18	25
	5.0	—	—	100	—
Aliquat 206	1.0	—	—	0	0
	5.0	—	—	25	100
Aliquat 221	1.0	0	0	10	13
	5.0	0	0	100	—
Alamine 11	1.0	0	0	0	0
	5.0	0	0	0	0
Alamine 21	1.0	0	0	0	0
	5.0	100	—	100	—
Check	5.0	0	0	0	0

fenthion and Bay 69047. Chlorpyrifos caused 85 percent mortality at 1 ppm and no mortality at 0.5 ppm (Table 2).

Most of the organonitrogen compounds studied produced high or complete mortality at mosquito larvicidal rates. These rates for most of the compounds range from 1-5 ppm. Type of solvents utilized in the formulation of the organonitrogen chemicals had a bearing on the toxicity of some of the materials to the fish (Table 3). For example, materials such as Duomeen L-15, Armeen L-15, and Aliquat 221 in Toxisol TB showed higher toxicity than their formulations in Toxisol FLC. Aliquat 336 was equally toxic with both formulations at 1.0-5.0 ppm. Alamine 11 was not toxic in either one of its formulations and Armeen L-15 also showed a low level of activity. Increased biological activity of some of the chemicals formulated in Toxisol TB may be attributed to the higher spreading ability of this petroleum oil than Toxisol FLC. The latter oil breaks into lenses and patches soon after treatment.

The aliphatic amines studied in the field at mosquito larvicidal rates (0.25-1.0 lb/A) showed little or no acute toxicity against *Gambusia affinis* (Table 4). Surface breathing insects as well as surface skimming spiders were adversely affected. The trends here agree with those found in the treatments with larvicidal oils by us and many other workers.

The herbicides tested in the field were harmless to the fish and the various aquatic organisms in the ponds at the rates of 1 and 5 lb/A (Table 5). No marked level of mortality was observed up to 4 days post-treatment.

The four organophosphorus larvicides (Table 6) evaluated in the ponds were quite toxic to fish at the higher rates. At the rate of 0.5 lb/A Akton and chlorpyrifos produced complete fish mortality 1-2 days after

TABLE 4. Toxicity of organonitrogen compounds (aliphatic amines) against the mosquito fish *Gambusia affinis* exposed in wire cages in experimental ponds.

Chemical[a]	Dosage lb/acre	(%) cumulative mort. (days) aft trt. 2	5	Nontarget organisms[b]
Isothan DL-1	0.25	0	0	DBA, C dead
	0.50	0	0	
	1.00	0	10	
Aliquat 336	0.25	0	0	DBA, B, C dead
	0.50	0	0	
	1.00	18	18	
Duomeen L-15	0.50	0	0	DBA, B, C dead
	1.00	2	2	
Alamine 21	0.50	2	2	Pardosa spiders drowning
	1.00	4	4	
Aliquat 204	0.50	0	0	DBA, C dead
	1.00	0	0	
Aliquat 221	0.50	0	0	Pardosa spiders drowning
	1.00	0	2	
Alamine 11	0.50	4	4	Chemical solidified
	1.00	0	0	
Check	—	0	0	C, DBA dead
Check	—	0	0	

[a] All formulated in Toxisol TB. Checks treated with the solvent and emulsifying agent at 1.0 gal/A.

[b] Nontarget organisms' mortality occurred at the rates of 0.5 and 1.0 lb/A. B = Belostomatidae, C = Corixidae, DBA = Diving beetle adult.

TABLE 5. Field toxicity of herbicides against the mosquito fish *Gambusia affinis* in field ponds.

Chemical and formulation	Dosage lb/acre	Avg. cumul. % mortality (days after treatment) 2	4	Nontarget organisms[a]
Simazine (80w)	1	16	16	DBA, Young FNS alive
	5	6	6	
Atrazine (80w)	1	26	26	TPS, DBA
	5	8	8	alive
Propazine (80w)	1	6	6	C, DBA DBL
	5	12	12	alive
Caparol (80w)	1	22	22	TPS, DBA, FNS
	5	8	16	alive
Ametryne (80w)	1	6	6	DBA dead, FNS
	5	16	16	MFN alive
Ametryne (EC 2)	1	26	26	FNS DBA, TPS
	5	4	16	alive
Pramitol (EC 2)	1	4	4	FNS DBA, TPS
	5	14	14	alive
Check	—	2	4	DBA, TPS FNS alive
Check	—	8	8	B, DBL alive

[a] B = Belostomatidae; C = Corixidae; DBA = Diving beetle adult; DBL = Diving beetle larvae; FNS = Finger nail shrimp; MFN = May fly naiad; TPS = Tadpole shrimp.

Nontarget effects assessed at both concentrations.

treatment. Bay 77488 and Bay 69047 at 0.5 lb/A also produced high mortality of the fish. At the larvicidal rates (0.1 lb/A for Bay 77488 and Bay 69047 and 0.05 lb/A for chlorpyrifos) fish mortality was negligible. Akton, at larvicidal rate (0.1 lb/A), however, caused high mortality of the fish.

From the data presented here it seems that the herbicides studied caused no marked acute toxicity at the 1 and 5 lb/A rates. Similarly, the organonitrogen chemicals applied at larvicidal rates caused slight or no mortality of the fish, but these compounds caused mortality or stress in some of

TABLE 6. Field toxicity of organophosphates against the mosquito fish *Gambusia affinis* in ponds.

Chemical and formulation	Dosage lb/acre	(%) cumulative mortality after treatment (days) 1	2	3	4	5
Bay 77488 EC 4	0.1	8	10	12	—	—
	0.5	60	62	64	—	—
Bay 69047 EC 2	0.1	6	6	6	—	—
	0.5	28	74	09	—	—
Akton EC 2	0.1	78	82	82	—	—
	0.5	100[a]	100[a]	98	—	—
Chlorpyrifos EC 4	0.05	0	0	0	0	0
	0.10	12	40	54	56	56
	0.50	80	100[a]	28	56	56
Check	—	0	0	0	0	0

[a] 25 new fish were added.

the invertebrate nontarget organisms. Of the organophosphorus insecticides, Akton showed considerable toxicity to the fish at mosquito larvicidal rates. Although chlorpyrifos showed considerable toxicity against mosquito fish, it is unlikely that a high level of mortality can be caused by mosquito larvicidal rates (0.025-0.05 lb/A of this material. Caution, however, must be exercised in the application of this material to avoid overdosing in areas where fish are present.

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