Efficacy and Chemical Persistence of Two Highly Active Carbamate Developmental Inhibitors

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Ro16-1294, 2-[*p*-(*m*-chlorophenoxy)phenoxy]ethyl ethylcarbamate, and Ro16-1295, 2-[*p*-(*m*-fluorophenoxy)phenoxy]ethyl ethylcarbamate, show very high efficacy against immature mosquitoes in laboratory and field trials. These compounds are stable in water over a wide range of temperature and pH. Ro16-1294 appears to be lost from water by the effects of sunlight and adsorption onto organic matter. Fish accumulate Ro16-1294 from water into their tissues more than 300-fold the water concentration, but the residues steadily decline when exposed fish are placed in untreated water. The efficacy and chemical persistence properties of Ro16-1294 offer good potential for its use as a mosquito control agent.

Numerous *N*-methyl-substituted carbamates have been developed as insecticides, but none have shown operational effectiveness for mosquito larviciding. Although some experimental carbamates have shown high larviciding efficacy (Schaefer and Wilder, 1970), none have been developed commercially. Due to the high degrees of insecticide resistance in numerous economically important mosquito species, the need for new larvicides, especially ones with new or different modes of action, continues as a high priority in mosquito control research. Recently several new carbamates, which have juvenile hormone-type activity (no direct toxicity but produce death or abnormalities at later stages of the life cycle), have shown high promise against several insects, e.g., termites (Jones, 1984). Because these experimental compounds, 2-[*p*-(*m*-fluorophenoxy)phenoxy]ethyl ethylcarbamate (Ro16-1295) and 2-[*p*-(*m*-chlorophenoxy)phenoxy]ethyl ethylcarbamate (Ro16-1924), show very high activity against mosquitoes in laboratory and field tests, studies on their efficacy and chemical persistence were conducted. Initial studies (1983) were conducted on Ro16-1295, but after Ro16-1294 became available (1984) emphasis was shifted to it.

MATERIALS AND METHODS

Chemicals. Technical standards for laboratory studies and EC formulations (125 g/L) for field trials were provided by MAAG Agrochemicals. Solvents used for extractions were reagent grade and were redistilled in all glass.

Laboratory Bioassays. To determine biological activities, 25 mosquito larvae were placed in 250 mL of tap water in Pyrex storage jars (80 × 100 mm). A series of concentrations of acetone solutions of the technical standards were applied to the water surfaces; each concentration was run in duplicate, and each test was replicated as specified. The test containers were held at 27 °C under a photoperiod of LD 14:10. After adult emergence was complete, the mortality counts were made; the mortalities of treatments were adjusted for those of controls. The volume of acetone used for treating each container (10 μL) was also applied to each control, and entire tests were discarded if control mortality exceeded 10%.

Preliminary tests showed that the fourth-instar larva is the stage that is affected by these compounds; to determine the critical age for sensitivity, a wide age mixture of fourth-instar larvae was placed in a series of glass aquaria and treated with a range of concentrations of Ro16-1295. Treated larvae were exposed 2 h, then removed, rinsed with untreated water, and then placed in tap water in rearing pans; pupae that formed during this process were removed and discarded. At intervals of 3 and 6 and at 6-h intervals thereafter up to 72 h newly formed pupae were removed and reared by age groups. After emergence or death was complete, the final mortalities of each group were determined. The LC_{90} for each age group was then determined by computer, probit analysis.

Field Tests. Pasture plots (0.02 ha each) 32 km northwest of Bakersfield, CA, were flood irrigated to obtain natural populations of mixed *Aedes nigromaculis* and *Aedes melanimon* larvae. These plots were hand sprayed with given doses of Ro16-1295 in 1983 and Ro16-1924, after it became available in 1984, in 6 L of water/plot. Water samples (600 mL) from some of the treated plots were collected and analyzed in the laboratory. Immediately after treatment and at 24 h thereafter, larval samples were collected from each plot and transported to the laboratory for rearing; mortalities of these samples were determined along with field observations to determine the final mortality of each treatment.

ANALYTICAL METHODS

High-Performance Liquid Chromatography (HPLC). A Varian Model 8500 HPLC having a UV photometer (254 nm) was used for all quantitative determinations. A MicroPak-CH column (octadecylsilane bonded on 10-μm particles), 8 mm × 25 cm, provided separations using acetonitrile as the mobile phase at 100 mL/h. At an ambient temperature of 27 °C Ro16-1294 has a retention time of 4.2 min and Ro16-1295 of 4.5 min.

Extraction from Water. Tap and field water samples (600 mL each) were fortified with 0.005, 0.01, and 0.02 ppm of either Ro16-1294 or Ro16-1295, in triplicate. Each was partioned two times against 100 mL of methylene chloride. The methylene chloride aliquots were dried over Na_2SO_4, combined, and brought to dryness in a rotary evaporator. The residue was redissolved in acetonitrile and subjected to HPLC. Sewage water was obtained from the secondary effluent of a local sewage disposal plant.

Water Solubility. Each compound (200 mg) was added to separate 500-mL flasks containing 300 mL of distilled water, the resultant mixture placed on a shaker for 1 h, and the liquid extract then filtered through Whatman No. 42 filter paper. The filtrate was then extracted, as described above, and analyzed by HPLC. Each compound was run in quadruplicate.

Combined Effect of Temperature and pH on Aqueous Stability. Tap water was buffered at pH 6.5

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Table I. Biological Activities[a] of Ro16-1294 and Ro16-1295 against Fourth-Instar Mosquito Larvae

compd	concn, ppm	*Culex quinquefasciatus*		*Culex tarsalis*		*Aedes taeniorhynchus*
		OP-S	OP-R	OP-S	OP-R	OP-S
Ro16-1294	0.000001	0	0	6	7	0
	0.000004	8	14	22	40	34
	0.00001	40	73	46	53	54
	0.00004	54	98	58	70	82
	0.0001	66	99	61	82	100
	0.0004	84	100	74	90	99
	0.001	92	100	81	94	100
	0.004	99	100	97	99	100
	0.01	100	100	99	100	100
no. tests[b]		5	2	3	3	2
Ro16-1295	0.00001	24		73		71
	0.00004	32	73	32		83
	0.0001	37	77	44		92
	0.0004	56	83	44		98
	0.001	59	90	65		99
	0.004	75	98	80		100
	0.01	100	100	100		100
no. tests		6	2	4	0[c]	4

[a] Values represent average percent inhibition of normal adult emergence. [b] Each concentration run in duplicate in each test. [c] Strain permanently lost.

Table II. Sensitivity of Various Gases of Fourth-Instar *Culex quinquefasciatus* Larvae Exposed for 2 h to Ro16-1295[a]

concn, ppm	posttreatment time[b]									
	0–3	3–6	6–12	12–18	18–24	24–30	30–36	36–42	42–48	48–54
0.00001	0	0	0	0	0	0	0	0	0	0
0.00002	0	12	0	0	0	0	0	0	0	0
0.00005	32	11	0	0	0	0	0	0	0	0
0.0001	69	47	53	36	0	0	0	0	0	0
0.0002	84	62	29	31	0	0	0	0	0	0
0.001	98	65	57	27	18	0	0	0	0	0
0.002	100	86	83	82	19	0	0	0	0	0
0.005	100	92	100	93	44	5	0	0	0	0
0.01	100	100	100	98	87	12	2	0	0	0
0.02	100	100	100	98	100	52	6	9	0	0
0.05	100	100	100	100	100	98	81	18	0	0
0.1	100	100	100	100	100	100	96	73	31	38
LC_{90}	0.00024	0.0027	0.0034	0.0057	0.0138	0.039	0.069			

[a] In percent inhibition of normal adult emergence. [b] Number of hours following 2-h treatment period before pupation.

(KH_2PO_4 + NaOH), 7.7 [tris(hydroxymethyl)aminomethane + HCl], and 10.0 ($NaHCO_3$ + NaOH). Thirty samples, 600 mL each, were fortified with 0.02 ppm Ro16-1294 and a second set with 0.02 ppm Ro16-1295. These were held in capped 1-L bottles in the dark in temperature-controlled incubators at 10, 24, and 38 °C. After exposure of 1, 2, 3, 6, and 9 days, duplicate samples from each temperature–pH combination were removed, extracted, and analyzed, as above. The initial concentration, temperature, and pH range were chosen to reflect those that would be expected in actual mosquito habitat conditions.

Aqueous Stability of Ro16-1294 in Sunlight. Samples (600 mL each) of tap water were treated with 0.02 ppm Ro16-1294 and either held in temperature-controlled cabinets in the dark at 10, 24, and 38 °C or placed outside in direct sunlight in temperature-controlled water baths and held at the same temperatures for 1, 2, or 3 days. After these intervals triplicate water samples were analyzed for Ro16-1294. This study was done in midsummer, during clear weather conditions in Aug 1984.

Adsorption of Ro16-1294 from Water into Fish Tissues. A 55-L tank containing 80 bluegill sunfish (average length 7.5 cm, average weight 8.0 g) was treated with a continuous flow at the maximum water solubility (0.93 ppm) of Ro16-1294; the stock solution was metered at a flow rate of 330 mL/min, and a constant depth of 30 cm was maintained. Fish were exposed for 24, 48, or 72 h and then were either sacrificed or placed in a continuous flow–rinse treatment with untreated tap water for 24, 48, and 72 h. After each of these intervals, samples of viscera, edible tissues, or whole bodies were analyzed for Ro16-1294. These protocols were followed in order to comply with EPA standards for estimating bioaccumulation in fish tissues (anonymous, 1978).

To fish samples (10 g each) was added 40 g of anhydrous Na_2SO_4, and the mixture was homogenized in 100 mL of methylene chloride and then filtered through Reeve Angel 934AH paper; the filter cake was rehomogenized in another 100 mL of methylene chloride and filtered again. The combined methylene chloride was reduced to ca. 2 mL with a rotary evaporator and transferred to a 10-g silica gel (Woelm, activity grade 1) column (15-mm i.d.). The active ingredient was eluted with 3% ethyl acetate in methylene chloride in the 25–125-mL fraction, reduced to dryness, and dissolved in acetonitrile for HPLC analysis, as described earlier.

To determine recovery data for fish tissues, triplicate samples were fortified at 1, 10, and 100 ppm with Ro16-1294 and then extracted and analyzed as above.

RESULTS AND DISCUSSION

Laboratory Tests. When mosquito larvae are exposed to either compound, there is no direct toxicity but mortality occurs in the pupal stage; at even lower doses, adults emerge from the pupal cases but are unable to leave the

Table III. Biological Activities of Ro16-1294 and Ro16-1295 against Mixed Larval[a] Populations of *Aedes melanimon* and *Aedes nigromaculis* on 0.02-ha Pasture Plots

test date	no. tests	rate, kg/ha	% inhibn[b]
		Ro16-1295	
8/3/83	1	0.05	100
8/3/83	1	0.03	100
8/3/83	2	0.01	100
8/10/83	2	0.005	100
8/23/83	2	0.003	99
8/30/83	2	0.002	92
8/23/83	2	0.001	85
8/30/83	1	0.0005	90
		Ro16-1294	
6/15/84[c]	3	0.01	100
6/15/84	2	0.005	100
7/11/84	2	0.0025	95
6/15/84	1	0.001	90
6/22/84	3	0.0075	100
6/15/84	1	0.0005	95

[a] Third- and fourth-instar larvae. [b] Percent inhibition of normal adult emergence. [c] For water residues, see Table VIII.

water surface and die there. Both Ro16-1294 and Ro16-1295 show very high activity against fourth-instar mosquito larvae (Table I); treatment of earlier instar larvae shows lower levels of activity and are not presented. Biological activities are presented in percent inhibition of normal adult emergence since some adults do develop but are abnormal and cannot leave the water surface. Ro16-1294 is slightly more active against *Culex* species than Ro16-1295. There was no indication of cross resistance to either of these compounds. Unfortunately, prior to completion of the testing several strains were permanently lost and therefore data for the resistant strain of *Culex tarsalis* were not obtained for Ro16-1295.

Mortality from a 2-h exposure of the youngest age groups (54 h or more prepupation) to Ro16-1295 was relative insignificant. Sensitivity of the fourt-instar larvae increased as they approached pupation (Table II).

Field Tests. Both compounds showed remarkable activity in the field trials (Table III); Ro16-1294 appeared to be slightly more active at the lowest dosages, but at rates of 0.001 kg/ha and below, results were variable but still very active. As most organophosphorus larvicides, e.g. parathion and fenthion, are applied at 0.1 kg/ha, these new compounds were ca. 100× more active in these field tests. Methoprene and diflubenzuron were effective in the field at ca. 0.03 kg/ha (Schaefer and Wilder, 1972; Schaefer et al., 1975) which is still ca. 10-fold less active than for compounds shown in Table III.

Recovery from Water. Table IV shows the recoveries of both compounds from tap water and also for pond and sewage water for Ro16-1294. Recoveries were better for Ro16-1294 than for Ro16-1295. Using the 600-mL sample sizes, it was possible to estimate the concentration of either compound to a detection limit of 0.001 ppm (minimum peak twice background). Reduced recoveries occurred from water sources having higher organic contents, presumably due to adsorption.

Water Solubility. At ambient temperature (25 °C) quadruple samples of Ro16-1295 showed an average water solubility of 4.4 ppm while that of Ro16-1294 averaged 0.93 ppm.

Combined Effect of Temperature and pH. Ro16-1294 shows a high degree of hydrolytic stability under this range of temperature and pH and in darkness (Table V). Ro16-1295 was less stable, but generally 50% or more of the initial concentration was present after 9 days in all of the conditions.

Table IV. Percent Recoveries of Ro16-1294 and Ro16-1295 from Water

H_2O type		concn, ppm 0.02	0.01	0.005
		Ro16-1295		
tap		91.5	82.0	83.8
		91.0	94.3	89.2
		96.2	90.5	81.1
	av	92.2	89.3	84.7
		Ro16-1294		
tap		98.5	98.2	94.2
		100.5	96.5	98.0
		96.1	97.3	90.6
	av	98.4	97.3	94.3
pond		97.1	95.7	82.9
		98.5	98.6	75.7
		94.8	91.4	80.0
	av	96.8	95.2	79.5
sewage		74.8	66.7	78.4
		61.1	73.7	76.4
		68.3	74.7	74.6
	av	67.4	71.7	76.4

Table V. Effects of Temperature and pH on the Aqueous Stability of Ro16-1294 and Ro16-1295 Held in Darkness (ppm)[a]

pH	temp, °C	days after treatment 0	1	2	3	6	9
			Ro16-1294				
6.5	10	0.020	0.021	0.020	0.020	0.020	0.018
7.7	10	0.021	0.020	0.020	0.020	0.018	0.018
10.0	10	0.020	0.021	0.020	0.020	0.019	0.019
6.5	24		0.020	0.020	0.019	0.019	0.018
7.7	24		0.019	0.020	0.019	0.018	0.017
10.0	24		0.020	0.019	0.018	0.018	0.016
6.5	38		0.020	0.020	0.020	0.018	0.017
7.7	38		0.019	0.019	0.018	0.017	0.014
10.0	38		0.019	0.019	0.018	0.018	0.016
			Ro16-1295				
6.5	10	0.022	0.021	0.020	0.019	0.017	0.014
7.7	10	0.023	0.021	0.020	0.020	0.017	0.014
10.0	10	0.023	0.022	0.021	0.020	0.018	0.015
6.5	24		0.019	0.018	0.018	0.016	0.010
7.7	24		0.019	0.019	0.019	0.014	0.011
10.0	24		0.020	0.019	0.018	0.016	0.012
6.5	38		0.018	0.018	0.017	0.014	0.011
7.7	38		0.019	0.018	0.017	0.013	0.011
10.0	38		0.017	0.017	0.017	0.012	0.010

[a] Average values of triplicate samples.

Stability of Aqueous Solutions of Ro16-1294 in Sunlight. Ro16-1294 shows an increasing loss as temperature and exposure to sunlight increase (Table VI). Only ca. 25% of the active ingredient remained at 38 °C after 3 successive days of natural sunlight.

Adsorption of Ro16-1294 onto Straw. The loss of Ro16-1294 from water onto straw is affected by both the quantity of organic material and the standing time (Table VII); thus, the loss of this compound from water in mosquito breeding habitats may result due to adsorption. The results are similar to those obtained for loss of a benzoylurea larvicide from water onto straw (Schaefer and Dupras, 1979).

Water Residues of Ro16-1294 from Field Tests. The residues in water from ponds treated with 0.01 kg/ha (Table III) are given in Table VIII. At 1-h posttreatment the highest residues were found on the south edges of treated ponds; this is the lee area (predominant winds are

Table VI. Stability of Aqueous Solutions of Ro16-1294 to Sunlight at Controlled Temperatures[a]

temp, °C	days indoors in dk				days outdoors in sunlt			
	0	1	2	3	0	1	2	3
10	0.020	0.019	0.019	0.019	0.020	0.019	0.019	0.018
24	0.020	0.020	0.020	0.020	0.020	0.018	0.016	0.013
38	0.020	0.019	0.020	0.019	0.020	0.014	0.012	0.005

[a] In ppm, average of triplicate samples.

Table VII. Adsorption of Ro16-1294 from Water onto Straw (ppm)[a]

amt straw: g/600 mL of H_2O	holding time, h		amt straw: g/600 mL of H_2O	holding time, h	
	24	48		24	48
0.0	0.017	0.017	1.5	0.003	0.001
0.5	0.007	0.002	2.0	0.002	ND[b]
1.0	0.002	0.002	3.0	0.001	ND

[a] Average of duplicate samples. [b] ND, not detected (<0.001 ppm).

Table VIII. Residues of Ro16-1294 in Pond Water Treated with 0.01 kg/ha (ppm)[a]

treatment no.	sampling time	sampling place in pond		
		north edge	middle	south edge
1	1 h	0.002	0.003	0.013
	1 day	0.001	0.002	0.002
	2 days	0.001	0.002	0.001
	3 days	0.001	0.002	0.002
2	1 h	0.002	0.002	0.004
	1 day	ND[b]	ND	ND
	2 days	ND	ND	ND
	3 days	ND	ND	ND
3	1 h	0.002	0.003	0.005
	1 day	0.002	0.002	0.003
	2 days	ND	ND	ND
	3 days	ND	ND	ND

[a] Single 600-mL water samples. [b] ND, not detected (<0.001 ppm).

Table IX. Residues of Ro16-1294 in Fish after Various Treatment and Rinse Periods (ppm)

rinse time, h	fish sample	treatment time, h		
		24	48	72
0	viscera	189.6	262.5	324.6
	edible tissue	69.9	41.4	62.0
	whole body	133.7	182.0	275.4
24	viscera	93.6	188.1	216.4
	edible tissue	40.4	24.1	88.5
	whole body	55.6	83.7	141.3
48	viscera	48.7	113.1	154.6
	edible tissue	25.1	37.4	68.9
	whole body	45.3	61.1	116.7
72	viscera	23.1	90.5	102.4
	edible tissue	9.4	15.2	19.2
	whole body	24.6	38.8	42.7

northwest), and we speculate that this is due to wind effect as similar results in these ponds have been experienced previously with another compounds (Schaefer et al., 1984). The lack of measurable residues after 1 h on test 2 cannot be explained but may be due to loss by adsorption following "stirring" by the wind. It is clear (Table VIII) that residues in field waters will be barely measurable by existing procedures following applications of rates that are effective against mosquito immatures.

Accumulation of Ro16-1294 from Water by Fish. Fish tissues fortified at 1, 10, and 100 ppm Ro16-1294 and then extracted and analyzed gave recoveries of 98.7%, 97.9%, and 95.1%, respectively. Using 10-g samples in these procedures the lowest detectable limit was 0.02 ppm (minimum peak twice background).

Bluegill sunfish accumulate Ro16-1294 from water into their tissues following a continuous exposure to levels above 300× that of the treated water (Table IX). However, after fish are placed in an untreated water rinse period, the residues show a steady decline. The accumulation of organic compounds from water by fish is expected (Schaefer and Miura, 1985), and the subsequent post-treatment decline indicates favorable environmental properties since long-term persistence is not likely.

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