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Toxicity of Organophosphorous Insecticides to Estuarine Copepods and Young Fish after Field Applications

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ABSTRACT

Ten field tests were conducted in east central Florida to investigate the acute toxicity of fenthion thermal fog, fenthion ultra low volume spray, malathion ultra low volume spray, and naled ultra low volume spray to non-target estuarine organisms. Animals tested included adults of the calanoid copepod *Acartia tonsa*, eggs of three species of fish, and juveniles of three species of fish. Significant mortality occurred only for copepods exposed to fenthion thermal fog in one test and naled ULV in one test. Environmental factors appear to influence the sensitivity of copepods to the insecticides.

INTRODUCTION

Because of proximity to adult mosquito habitats, non-target estuarine organisms may be exposed to insecticides that accidentally drift into the water. Because they are small, lack mobility, and often occur near the surface, zooplankton and young fish may be particularly vulnerable. Three insecticides commonly sprayed in coastal areas for adult mosquitoes are fenthion (Baytex), malathion, and naled (Dibrom). Fenthion usually is applied as an aerial thermal fog with oil adjuvants. Malathion and naled are usually applied by airplane or truck as ultra low volume (ULV) sprays. We investigated the occurrence and persistence of these three insecticides in estuarine water and their toxicity to copepods and young fish in ten field tests. Applications were performed according to standard procedures except that the insecticides were sprayed over water to simulate worst-case accidental drift.

MATERIALS AND METHODS

The field tests included observations of mortality of caged animals placed at each of two sites. Observations at the treated site assessed the effect of insecticide exposure, and those at the control site assessed the health of test animals, including possible effects of capture and handling.

Four insecticide mixtures were used in the tests: thermal fog—1.5% Baytex, 65% no. 2 diesel oil, and 33.5% fog oil; Baytex liquid concentrate—93% fenthion, 9.67 lb fenthion/gal; Cythion liquid concentrate—91% malathion, 9.33 lb malathion/gal; Dibrom liquid concentrate—85% naled, 14 lb naled/gal.

Fish eggs were exposed to aerial fenthion thermal fog in tests 2, 3, and 4 and to aerial naled ULV in test 6. Gulf menhaden (*Brevoortia patronus*) eggs for test 2 were obtained by hormone-induced ovulation followed by voluntary tank spawning. Spotted seatrout (*Cynoscion nebulosus*) eggs for test 3 and common snook (*Centropomus undecimalis*) eggs for test 4 were obtained by hormone-induced ovulation and strip-spawning. Spotted seatrout eggs for test 6 were obtained by voluntary tank spawning. Both snook and trout eggs were used in test 5 (malathion ULV by truck), but sudden heavy rains struck the egg chambers before they could be covered and mortality data could not be used.

Snook seined from the Sebastian River and sheepshead minnows (*Cyprinodon variegatus*) raised in a pond at HBOI were used for three truck field tests. Prior to tests, juvenile fish were held for 24-48 h in Indian River water. Common snook (5-12 individuals, 30-70 mm FL) and sheepshead minnows (12-15 individuals, 25-50 mm FL) were exposed to fenthion ULV in test

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8, malathion ULV in test 9, and naled ULV in test 10. Tarpon snook (*C. pectinatus,* 2-4 individuals, 41-65 mm FL) also were used in tests 9 and 10.

Adult calanoid copepods (*Acartia tonsa*) were exposed to aerial fenthion thermal fog in tests 1, 2, and 3, to aerial naled ULV in test 6, to aerial malathion ULV in test 7, to ground malathion ULV in test 9, and to ground naled ULV in test 10. They were collected from Little Jim Bridge, in the Indian River estuary near Fort Pierce Inlet, with a 200-μm mesh plankton net. Copepods were held overnight in the laboratory in Indian River water before testing.

The field study area was in St. Lucie mosquito impoundment no. 23, located on the west side of North Hutchinson Island, in northern St. Lucie County. Two test platforms were built in the perimeter ditch directly adjacent to the upland fringe of the impoundment. The platforms were about 8 m from the dike on which trucks were driven while spraying. The fringe consisted of a revegetating black mangrove-saltwort-glasswort marsh. The ditch opened into and had continuous water exchange with the Indian River. The platforms were 3,200 m apart and equidistant from the inlet. Spraying was conducted at the downwind (test) site, to minimize the chance of drift to the other (control) site.

Control and exposed cages of mosquitoes were deployed to determine effectiveness of the spray.

Fish egg and copepod cages were constructed of pyrex crystallizing dishes (100 mm in diameter and 50 mm high for fish, 80 mm x 40 mm for copepods) with attached cylinders of 183-μm mesh polyester screening to contain the animals but also allow water exchange. Beginning with the third field test, 80-μm mesh was used for the fish cages. Cages were suspended in the surface water layer (to about 20 cm deep) from small rafts. Each fish cage contained 20 eggs. Each copepod cage contained about 50 copepods; exact counts were made after retrieval. Five cages of each species were placed at each site just before spraying. After 24 h, live and dead animals were counted.

To test toxicity of the spray without effects of variation in impoundment water quality, additional tests were conducted on the docks. Menhaden eggs were used in test 2, seatrout eggs in tests 3 and 6, and snook eggs in test 4. Ten 200 ml dishes were stocked with 20 fish eggs each. Five were sealed in a styrofoam cooler. Five were left out on the dock during spraying and for 15 min after, then were sealed in a cooler. Both coolers were covered with insulation and shadecloth and were left on the dock for 48 h. Mortality was checked at 24 and 48 h.

For tests 2, 6, 7, 9, and 10, additional copepod controls were used. Copepods were held for 24 h at both sites without exposure to insecticide. For test 2, this was done 3 wk later when water quality was similar. For tests 6, 7, 9, and 10, pretreatment controls were conducted during the 24 h prior to spraying. Post-spray mortality in tests 1 and 3 was compared with overall mean control mortality observed at that site. Day-to-day differences in mortality at the spray site were compared with differences at the site not sprayed. In this way, uncertainty resulting from consistent site-specific differences in control mortality was reduced. Exposure-related percent mortality with 95% confidence intervals was computed as the difference between means (Sokal and Rohlf, 1981).

Juvenile fish were held just under the water surface in five-gallon plastic buckets with holes drilled in them. The fish were checked at least every 12 h up to 72 h for mortality.

Two or more no. 44 Whatman filter papers, 24 cm in diameter, were placed on the animal rafts or dock to determine the amount of insecticide deposited on the water surface. After the spray had drifted past, the filter papers were removed and extracted.

Two 200 ml dishes with water but no eggs were placed next to the egg dishes on the dock (beginning with test 2). Fifteen minutes after the spray, the water was removed and extracted.

At the test site, one pair of water samples was collected before and 12 after the spray, up to 48 h. At the control site, one pair was collected before and two after. One-liter amber glass bottles were used to collect two simultaneous 900 mL samples 2.5 cm below the water surface. Before analysis, each sample was acidified with 0.15 mL concentrated hydrochloric acid,

Table 1. Data from field tests. Fenthion thermal fog was applied by DC-3 aircraft. ULV applications were by Cessna 336 aircraft or truck. C = control; S = spray.

Test No.	Chemical Method Month	Site	Temp. (°C)	Salinity (°/oo)	pH	Dissolved Oxygen (mg/L)	Peak Concent. (μg/L)	Peak Concent. (min)	Persist. (h)	Mosquito Mortality (%)
Thermal Fog Tests										
1	Fenthion	C	24.8-32.7	22-25	7.8	1.6-13.2				2
	Aerial; Sep	S	25.2-29.2	20-27	7.2	0.9-5.8	1.69	45	24	54
2	Fenthion	C	21.8-27.5	34-36	6.8-7.4	2.8-11.2				3
	Aerial; Mar	S	22.7-26.0	35-38	6.5-7.2	1.5-12.4	≤0.01	19	24	94
3	Fenthion	C	27.3-34.4	36-39	7.3-8.0	0.4-10.7				2
	Aerial; Jun	S	28.2-33.1	35-40	7.3-7.8	0.4-5.9	0.16	46	18	96
4	Fenthion	C	27.4-33.9	30-34	5.9-8.0	0.5-9.1				3
	Aerial; Jun	S	29.1-32.4	30-34	6.5-8.1	2.3-9.3	0.16	45	24	96
ULV Spray Tests										
8	Fenthion	C	27.1-29.6	15-18	6.3-7.6	0.3-3.2				2
	Truck; Oct	S	27.2-29.2	20-22	6.6-8.0	0.4-6.1	0.49	30	4	2[a]
7	Malathion	C	23.8-26.0	19-26	7.5-8.1	1.7-6.2				5
	Aerial; Sep	S	23.9-26.8	24-30	7.2-8.3	1.3-7.5	5.00	84	48	86
5	Malathion	C	26.2-30.5	30-34	7.3-7.8	0.5-4.1				2
	Truck; Jul	S	25.7-29.6	26-35	7.5-7.8	0.5-3.3	0.09	243	4	2[b]
9	Malathion	C	25.6-30.5	24-28	6.8-8.5	1.0-10.5				2
	Truck; Oct	S	26.1-28.6	27-32	6.5-8.2	1.5-6.3	1.31	15	18	97
6	Naled	C	27.2-30.2	32-34	7.2-8.1	0.5-6.2				1
	Aerial; Sep	S	28.0-30.2	31-33	7.5-7.9	0.7-4.0	20.15	27	9	100
10	Naled	C	20.6-24.1	23-27	6.3-7.4	1.8-7.5				0
	Truck; Dec	S	21.2-24.0	27-29	5.9-7.4	1.4-6.7	0.71	15	4	94

[a]Although caged mosquitoes were not affected, the spraying operation was judged to be typical by all persons present.
[b]As spraying began, the wind shifted and blew the spray away from the test area.

reducing pH to 6.0 (Dale and Miles, 1969). The sample was immediately extracted with 50 mL methylene chloride (fenthion) or petrolum ether (malathion and naled) with three one-minute shakes. Samples were placed on ice and taken to the laboratory for two more extractions with hexane. The extract from each sample was passed through a Na_2SO_4 column and then placed in a K-D evaporator with a 5 mL ampule for concentration to 0.5 mL with a gentle stream of nitrogen. Concentrated extracts were analyzed with a Hewlett-Packard 5730A gas chromatograph equipped with a nitrogen-phosphorous detector.

Insecticide standards were obtained from Ultra Scientific. To determine deposition on filter papers, a calibration curve was constructed by analyzing known amounts of working standard solutions spiked to filter papers. During field tests, 900-mL water samples from the test site were spiked with known amounts of insecticide and were analyzed periodically for a routine quality control check. All results were corrected for recovery efficiency, which was 90-112% for fenthion, 98-114% for malathion, and 73-103% for naled. Minimum detectable concentrations were 0.01 μg/L for fenthion and 0.05 μg/L for malathion and naled.

RESULTS AND DISCUSSION

Conditions for the field tests varied with season, with the most stressful conditions (i.e., high temperature and low dissolved oxygen) occurring in the warmer months (Table 1).

In most cases, less insecticide reached ground level than would be expected from the application rate (Table 2). In fenthion thermal fogging, deposited fenthion was 0.1-5.4% and deposited oils were 46.3-78.7% of what was predicted from application rates given by operators. Fenthion may have been degraded more easily than the oils by the high temperature of the spraying device. Other losses occurred through drift. The fogging device was not tuned as well for test 1 as for later tests and produced a noticeable proportion of larger droplets that fell rather than

Table 2. Insecticide deposited on filter papers at ground level. Observed values are means of two. Samples were taken 12 (a), 24 (b), 30 (c), 36 (d), and 60 min (e) after spraying.

Test	Application Rate	Amount Deposited		
		Expected (ng/cm²)	Observed (ng/cm²)	Obs/Exp (%)
Thermal Fog Tests				
1 Fenthion (aerial)	0.031 lb fen/acre	342	18.6[a]	5.4
	0.2 gal oils/acre	16,218		
2 Fenthion (aerial)	0.031 lb fen/acre	342	0.88[a]	0.3
	0.2 gal oils/acre	16,218		
3 Fenthion (aerial)	0.031 lb fen/acre	342	1.24[a]	0.4
	0.2 gal oils/acre	16,218	7,516	46.3
4 Fenthion (aerial)	0.031 lb fen/acre	342	0.39[a]	0.1
	0.2 gal oils/acre	16,218	12,762	78.7
ULV Spray Tests				
8 Fenthion (truck)	0.013 lb fen/acre	146	2.92[a]	2.0
			2.12[b]	1.4
7 Malathion (aerial)	0.241 lb mal/acre	2,456	381[b]	15.5
			492[d]	20.0
9 Malathion (truck)	0.050 lb mal/acre	510	79.3[a]	15.5
			85.8[b]	16.8
6 Naled (aerial)	0.128 lb nal/acre	1,320	900[c]	68.2
			622[e]	47.1
10 Naled (truck)	0.025 lb nal/acre	257	57.3[a]	22.3
			53.8[b]	20.9

drifted. The fraction of aerially-applied naled reaching the ground was greater than that sprayed by truck. The fractions of fenthion and malathion deposited were similar, whether by air or ground. Vegetation and drift direction are important factors governing distribution of insecticide. Larger differences might have occurred if the test platforms were farther from the dike. Efficacy tests (Alan Curtis, pers. comm.) have shown that the amount of insecticide declines rapidly with distance from the truck.

In the three worst cases, insecticide concentrations in the ditch water peaked at 1.69 μg fenthion, 5.00 μg/L malathion, and 20.15 μg/L naled (Figure 1). Naled peaked earliest (first sample after spray) and dropped to non-detectable levels within 9 h. The fenthion concentration also dropped quickly, but to low detectable levels (0.1-0.2 μg/L) that persisted for up to a day. In other field tests (Table 1), peak concentrations ranged from 0.16 to 1.3 μg/L. For naled, the peak occurred within 0.5 h, and for fenthion and malathion, it occurred within 0.75 h. Naled was less persistent, becoming non-detectable within 12 h. In most tests, fenthion and malathion could be detected for more than 18 h. In two tests (5 and 8) disappearance was rapid. Low efficacy and low malathion concentration were observed in test 5 because of a reversal in wind direction during spraying. Coverage may have been low in test 8.

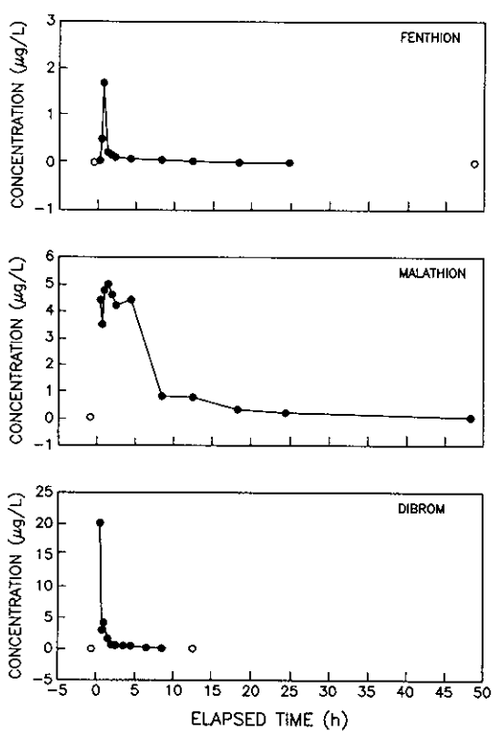

Fig. 1. Persistence of insecticides during three field tests (fenthion, test 1; malathion, test 7; Dibrom, or naled, test 6). Open circles indicate insecticide was not detected.

Statistically significant mortality occurred only for copepods exposed to fenthion thermal fog in test 2 and naled ULV in test 6 (Table 3). Both of these tests were at the north site where control mortality averaged 19.7% (95% CI = 3.4). Control mortality at the south site was consistently higher (37.4 ± 6%). The occurrence of significant mortality among controls in the field tests indicates that environmental factors (i.e., water quality) played an important role in the sensitivity of copepods to the insecticides. Although fenthion concentration in test 2 was low because of differences in spraying, degradation, or settling, a large amount of oil could have settled on the water. Significant mortality of fish was not observed, even in the dishes on the dock, where insecticide concentrations were higher than in the ditch: test 2, 0.40 μg/L fenthion; test 3, 1.06 μg/L fenthion; test 4, 1.62 μg/L fenthion; test 6, 158.2 μg/L naled. Laboratory acute toxicity tests with adult copepods and fish eggs and juveniles (Tucker et al. 1985, 1986) predicted that only copepods would be sensitive to the concentrations of fenthion in thermal fog and naled found in these field tests, especially at high temperatures and salinities (30°C, 35‰). Safety margins for fish were not necessarily wide. The 48-hour LC_{50} for spotted seatrout eggs exposed to fenthion thermal fog mixture was 4.7 μg/L fenthion, which is close to that found in field test 1 (1.69 μg/L).

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Table 3. Exposure-related mortality among organisms in field tests expressed as the difference between mortality at the sprayed site and that observed at the control site. In some, mortality at the spray site was ≤ mortality at the control site (n.s.) and in others there was no mortality at either site (0).

Test	Animals	Exposure-related Mortality (mean % ± 95% CI)
Thermal Fog Tests		
1 Fenthion (aerial)	Copepod adults	n.s.
2 Fenthion (aerial)	Copepod adults	52.3 ± 12.8
	Gulf menhaden eggs	n.s.
3 Fethion (aerial)	Copepod adults	n.s.
	Spotted seatrout eggs	n.s.
4 Fenthion (aerial)	Common snook eggs	n.s.
ULV Spray Tests		
8 Fenthion (truck)	Common snook juveniles	0
	Sheepshead minnow juveniles	0
7 Malathion (aerial)	Copepod adults	n.s.
9 Malathion (truck)	Common snook juveniles	0
	Tarpon snook juveniles	0
	Sheepshead minnow juveniles	0
	Copepod adults	n.s.
6 Naled (aerial)	Copepod adults	65.5 ± 10.8
	Spotted seatrout eggs	n.s.
10 Naled (truck)	Common snook juveniles	0
	Tarpon snook juveniles	0
	Sheepshead minnow juveniles	0
	Copepod adults	n.s.

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