EFFECTS OF GROUND APPLICATIONS OF MALATHION ON SALT-MARSH ENVIRONMENTS IN NORTHWESTERN FLORIDA [1]

M. E. TAGATZ, P. W. BORTHWICK, G. H. COOK AND D. L. COPPAGE

U. S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561

(Associate Laboratory, National Environmental Research Center, Corvallis, Oregon)

ABSTRACT. Effects of thermal fog {6 wt. oz/acre (420 g/ha)} and ULV aerosol spray {0.64 fl. oz/acre (57 g/ha)} applications of malathion 95 (o,o-dimethyl phosphorodithioate of diethyl mercaptosuccinate) on salt-marsh environments near Pensacola Beach, Florida, were investigated. Studies were conducted on selected plots after each of three treatments using a portable thermal fogger and three ultra low volume (ULV) sprays with a truck-mounted generator. The ULV sprays were typical of usual mosquito-control operations. The foggings were on a small scale and results should be considered as indicative of what may occur under usual conditions. Deaths due to malathion were not observed among confined blue crabs, *Callinectes sapidus;* grass shrimps, *Palaemonetes vulgaris* and *P. pugio;* pink shrimp, *Penaeus duorarum;* or sheepshead minnows, *Cyprinodon variegatus.* Brain acetylcholinesterase activity was not reduced in confined *C. variegatus* exposed to one or more treatments. Confined animals and the snail, *Littorina irrorata,* contained no measurable malathion at our limit of detectability. The chemical was not detected in sediment, but concentrations as high as 4.10 parts per million (ppm) were found in *Juncus* sp., trace amounts persisting as long as 14 days (>0.05 but <0.10 ppm). Highest concentration in marsh water after fogging was 5.2 parts per billion (ppb); after ULV spraying, 0.49 ppb. For each method of application, only trace amounts (>0.1 but <0.3 ppb) persisted in marsh water as long as 1 day.

INTRODUCTION

The organophosphate insecticide malathion (O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate), applied in thermal fogs or as ultra low volume (ULV) non-thermal mist sprays, is commonly used in marsh areas to control adult mosquitoes. Both methods are effective, but ULV mist sprays require less insecticide than fog applications and cause less environmental contamination (Taylor and Schoof, 1971; Fultz *et al.,* 1972).

Few field studies have been conducted on effects of malathion on aquatic environments, particularly estuaries. Malathion formulated in fuel oil and sprayed by airplane at the rate of 8 wt. oz/acre (560 g/ha) on tidal marshes in Delaware was toxic within 4 hours to ocellated killifish, *Fundulus ocellaris,* held in tubs (Darsie and Corriden, 1959). Aerial application of 3 fl. oz/acre (256 g/ha if technical grade chemical) to marsh embayments in Texas killed 14 to 80 percent of commercial shrimp, *Penaeus aztecus* and *P. setiferus,* held in live boxes, within 49 hours (Conte and Parker, 1971). Residues of malathion up to 48 hours after spraying ranged from 0.8 to 3.2 ppm (parts per million) in the water and from 0.28 to 2.67 ppm in tissues of living shrimp. Significant inhibition of brain acetylcholinesterase in fishes was associated with aerial ULV application of 3 fl. oz/acre of malathion in Louisiana (Coppage and Duke, 1971). ULV (3 fl. oz/acre) applications of technical malathion over towns in Hale County, Texas had no authenticated adverse effect on various freshwater fishes in reservoirs and ponds (Hill *et al.,* 1971).

The objectives of our study were to evaluate effects of thermal fog and ULV ground applications of malathion on some estuarine animals in saltwater marshes in northwestern Florida and to determine occurrence and persistence of the chemical in various components of the marshes.

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MATERIALS AND METHODS

Each thermal fog or ULV spray was applied near the time of low tide to permit maximum settling and retention in the marsh and near sunset, when sprays are usually applied to coincide with the greatest activity of mosquitoes and with optimum winds and temperatures which provide for the spray to remain close to the ground. Salinity, water temperature and pH were measured at the time of the second fogging and each of the ULV sprayings.

Water, grass, sediment and animals were analyzed by gas chromatography with a flame photometric detector in the phosphorus mode to determine concentrations of malathion. Based on our levels of detection, the terms nondetectable (N. D.) and trace (Tr.) amounts of malathion in the estuarine components sampled are defined as follows: 1.5l water sample, not above 0.1 parts per billion, ppb (N. D.) and >0.1 but <0.3 ppb (Tr.); 20 g sample of sediment or grass, not above 0.05 ppm (N. D.) and >0.05 but <0.10 ppm (Tr.); and 5 g animal sample, not above 0.2 ppm (N. D.) and >0.2 but <0.3 ppm (Tr.). Samples spiked with known amounts of malathion in the laboratory were recovered with efficiencies greater than 80%, but sample concentrations were not corrected for percentage recovery. Water was extracted in the field with petroleum ether; animals, grass and sediment were placed on ice or dry ice to inhibit degradation of malathion during transport from field to laboratory.

I. THERMAL FOG APPLICATIONS. Malathion (Cythion®[2] Technical 95% formulated in fuel oil) was applied to a *Juncus* sp.-dominated salt-marsh with a Sears, Roebuck and Co. portable gas-engine fogger, Model 71–14871. Maximum volume output of the hand-held fogger was 17,500 cu. ft. (495 m^3) per minute.

The treated and control plots were in a marsh at Range Point, about 1 mile (1.6 km) east of Pensacola Beach, Florida. The marsh was connected to Santa Rosa Sound by an inlet that allowed tidal exchange. One area, 85 ft. x 115 ft. (26 m x 35 m), was fogged with malathion in diesel fuel oil and served as the treated plot, and an area of the same size 100 yards (91.4 m) distant was fogged with diesel fuel oil and served as the control plot. Tidal canals, 10-ft. (3.0 m) wide and 1- to 4-feet (0.3- to 1.2 m) deep, bordered one side of the *Juncus* plots and were included in the treated areas.

Malathion was applied to the treated plot at approximately 2-week intervals (September 18, October 3 and 16, 1972) at a theoretical rate of 6 wt. oz/acre (420 g/ha). The maximum thermal fog recommendation is 8 wt. oz/gal (60 g/l) at 40 gph (151 l/hr) at 5 mph (8 km/hr), equivalent to 1.3 fl. oz/acre (111 g/ha).[3] An employee of the Escambia County Mosquito Control Department, Pensacola, Florida, walked the plot along fog-swath transects until the content of the insecticide tank {1.35 wt. oz (38.3 g) malathion and 3 qts. (2.8 l) fuel oil} was dispensed (about 15 minutes). The control plot was treated in a similar way with fuel oil carrier.

Prior to the first application, animals were placed in cages in the tidal canal portions of the plots, and all but shrimp were held for the three foggings. In each plot, 10 juvenile blue crabs, *Callinectes sapidus,* were held in each of 2 cages; adult or near-adult sheepshead minnows, *Cyprinodon variegatus,* in 3 cages (20 per cage); and adult grass shrimp, *Palaemonetes vulgaris,* in 2 cages (16 per cage). Shrimp were held near the surface of the water in small cages

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[2] ® Registered trademark of American Cyanamid Company, Princeton, N. J. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

[3] Personal communication, C. B. Rathburn, Jr., West Florida Arthropod Research Laboratory, Panama City, Florida 32401.

made of nylon screen over acrylic frames; crabs and fish, in large cages made of hardware cloth over wooden frames. To prevent cannibalism, shrimp and crabs were confined in individual compartments. After the first application, unaccountably high mortality of grass shrimp occurred in treated and control groups after 7 days; therefore, juvenile pink shrimp, *Penaeus duorarum,* were used in the second treatment. Shrimp studies were terminated 3 days after the second fogging because of vandalism of some pink shrimp cages. Data on mortalities of grass shrimp were obtained only during 7 days after the first fogging; and data for pink shrimp, 3 days after the second fogging. Mortalities among crabs and fish were recorded prior to the second and third treatments; survivors 6 hours after the third treatment were analyzed for malathion residues. Shrimp were not analyzed for residues.

Sheepshead minnows (300 in each of two cages) were held in the treated plot for a study on activity of brain acetylcholinesterase (AChE) in fish exposed to one or more applications of the chemical. Using procedures reported in Coppage (1972), 25 fish (representing five samples) were used to measure AChE activity at each of the following times: 1, 4 and 7 days after the first fogging, 12 hours and 3 days after the second fogging, and 6 and 12 hours after the third fogging. Coppage (1972) found that reduction of AChE activity in sheepshead minnows below a specific level (about 18% of normal) indicates death or impending death from organophosphate poisoning.

Samples of sediment, *Juncus,* marsh water (at base of *Juncus*), and canal water were analyzed for malathion at 6 hours, 12 hours, 1, 3, 7 and 14 days after each fogging operation or until malathion was not detected. Except for canal water, each type of sample was a composite of material from three locations. These locations were selected randomly from a grid pattern of 54 divisions per plot.

II. ULV Aerosol Sprays. ULV malathion (Cythion® Technical 95%) was applied to a *Juncus*-dominated salt-marsh by a truck-mounted Leco HD ULV cold aerosol generator.[4] Discharge was toward the rear at an upward angle of 45°. Actual discharge rate was 0.5 gallon per hour at 2½ mph (1.9 l/hr at 4 km/hr) which is equivalent to 2.0 gph at 10 mph (7.6 l/hr at 16 km/hr), the maximum allowable rate for ground ULV application in Florida. Pressure in the insecticide tank was 4 pounds/square inch (0.28 kg/cm ^{2}). Discharge rate for the flowmeter setting used was calibrated for temperature. Volume discharged was 160 ml and spray time was 5 minutes.

Three sprays were applied (May 15, June 11 and 25, 1973) to approximately 8½ acres (3.4 ha) of marsh by employees of the West Florida Arthropod Research Laboratory, Panama City, Florida. The rate was equivalent to 0.64 fl. oz/acre (57 g/ha) based on a swath of 330 feet (100.6 m). For the first and second treatments, the Range Point marsh served as the control plot and a similar *Juncus*-dominated marsh about 5 miles (8 km) east of Range Point was the treated plot. Range Point was selected as the treated area for the third spray because of a more favorable wind direction for chemical drift; the marsh east thereof was the control. Both sites were connected to Santa Rosa Sound by inlets that allowed tidal exchange. Wind velocities during the three sprayings averaged 6.3, 6.0 and 10.2 mph (10.1, 9.6 and 16.4 km/hr).

Prior to each spray, grass shrimp (adult *P. pugio*), blue crabs (15–25 mm wide) and sheepshead minnows (25–40 mm total length) in 18 in. (45.7 cm) diameter polyethylene tubs containing 25 liters of water were placed in the marshes. In each marsh, tubs were positioned in two rows of three adjacent tubs. The rows were 50 ft. (15.2 m) apart, and animals in each row consisted of 25 shrimp in

[4] Lowndes Engineering Company, Inc., Valdosta, Georgia.

one tub, 15 crabs in a second tub, and 20 fish in a third tub. Immediately after treatment, screens were placed over tubs to keep out predators. Mortalities were determined 1 and 3 days after spray. One day after treatment, duplicate living samples of 15 shrimp, 5 crabs or 10 fish were removed for chemical analyses.

Seventy sheepshead minnows in a 5.4 sq. ft. (0.5 m^2) polyethylene pool containing 91 liters of water were centered between the rows of tubs in the treated marsh. AChE activities in the fish were measured at 6 hours and 1 day after each spray.

At selected intervals after treatment samples of sediment, *Juncus,* water from the marsh, water from tubs, and snails (*Littorina irrorata* collected from *Juncus*) were analyzed for malathion. A composite water sample (1.5 l) was obtained from each of the two groups of tubs; composite samples of marsh water and other materials were obtained randomly in the vicinity of the tubs.

To determine the effectiveness of our ULV sprays for mosquito control, caged mosquitoes were placed on 5-ft. (1.5 m) poles in the marsh for the third spraying by personnel of the West Florida Arthropod Research Laboratory. One hundred forty *Aedes taeniorhynchus* in six cages and 131 *Culex nigripalpus* in six cages were used in the treated plot, and as a check, approximately the same numbers of mosquitoes were held in 12 cages away from the treated plot.

RESULTS

I. THERMAL FOG APPLICATIONS. Physical and chemical characteristics of canal water in the treated and control plots for the second fogging were: temperature, 24.5°–25.0° C; salinity, 27.5–28.0 ppt (parts per thousand); and pH, 7.2–7.6.

No effects of malathion on caged animals were observed. Mortality of crabs and fish did not differ greatly between control and treated groups after the first and second foggings (Table 1). Treated crabs (average width 83.2 mm, range 44–115) and control crabs (77.0 mm, 41–113 mm) each molted seven times in the 28-day period. In our limited shrimp studies, single deaths of grass shrimp occurred in each plot after 7 days, and no deaths of pink shrimp occurred after 3 days. Fish and crabs obtained 6 hours after the third treatment contained no detectable malathion. No decided inhibition of AChE activity in brains of sheepshead minnows was detected after any of the three treatments.

No deaths that could be attributed to the treatments were observed among resident populations of shrimp, crabs, and fish.

Malathion did not persist for long in sediment, *Juncus* or water after each application. The chemical was not detected in sediment after 6 hours. However, trace amounts occurred in samples of *Juncus* after 14 days (Table 2). Malathion was not detected in water after 1

TABLE 1. Mortality of confined animals in salt-marshes after thermal fog applications of malathion 95 at 6 wt. oz/acre (420 g/ha).

Animal	14 days after 1st treatment		14 days after 2nd treatment		Total	
	No. dead	%	No. dead	%	No. dead	%
Blue crabs						
Control	3	15	5	25	8	40
Treated	3	15	2	10	5	25
Sheepshead minnows						
Control	4	7	3	5	7	12
Treated	5	8	6	10	11	18

day. After 6 and 12 hours, residues of the toxicant ranged from <0.1 to 5.2 ppb in marsh water and from <0.1 to 0.42 ppb in canal water. Malathion was not found in sediment, *Juncus* or water from the control plot, except for trace amounts in the water 6 hours after the second application.

II. ULV AEROSOL SPRAYS. Ranges of various water properties in both marshes at the time of ULV sprayings were: water temperature in tubs and marsh, 24.5°–32.5° C; salinity of tub water, 14–16 ppt, of marsh water, 13.5–20.0 ppt; and pH, 7.5–7.8.

We observed no effects of malathion on

TABLE 2. Malathion residues (ppm) in samples of *Juncus* from a salt-marsh after thermal fog applications of malathion 95 at 6 wt. oz/acre (420 g/ha).

Treatment	Time elapsed after treatment					
	6 hrs.	12 hrs.	1 day	3 days	7 days	14 days
1	0.31	0.21	N.D.[1]	0.16	N.D.	N.D.
2	1.21	4.10	2.10	....	Tr.[2]	Tr.
3	0.45	1.20	N.D.	0.71	Tr.	Tr.

[1] N.D. (non detectable)=not above 0.05 ppm.
[2] Tr. (trace)=>0.05 but <0.10 ppm.

TABLE 3. Deaths of confined animals in salt-marshes after ULV sprays of malathion 95 at 0.64 fl. oz/acre (57 g/ha).

Spray	Animal	Number start	Deaths 0–1 day	Number 1 day[1]	Deaths 1–3 days
1	Blue crabs				
	Control	30	2	18	0
	Treated	30	2	18	1
	Sheepshead minnows				
	Control	40	0	20	0
	Treated	40	0	20	0
	Grass shrimp [2]				
	Control	50	1	19	8
	Treated	50	0	20	0
2	Blue crabs				
	Control	30	1	19	5
	Treated	30	0	20	0
	Sheepshead minnows				
	Control	40	0	20	0
	Treated	40	0	20	0
	Grass shrimp [2]				
	Control	50	0	20	6
	Treated	50	0	20	3
3	Blue crabs				
	Control	30	0	20	2
	Treated	30	4	16	2
	Sheepshead minnows				
	Control	40	0	20	0
	Treated	40	1	19	0
	Grass shrimp [2]				
	Control	50	0	20	2
	Treated	50	0	20	0

[1] Number of animals remaining after 1 day upon removal of dead animals and living samples (10 crabs, 20 minnows or 30 shrimp) for residue analyses.
[2] Most shrimp listed as dead were not found; some may have escaped or been eaten.

animals. Few deaths occurred among treated animals (Table 3). Some deaths of crabs in both marshes were due to cannibalism. The bodies of most shrimp listed as dead were not found; some may have escaped or been eaten. No deaths of resident crabs, fishes, and shrimps were noted after any treatment. Confined crabs, fish and shrimp and free-living snails obtained 1 day after each spray contained no detectable malathion. AChE activities in brains of sheepshead minnows were not altered by any of the three sprays.

Residues of malathion in sediment, water, or *Juncus* 3 days following ULV applications generally were low or not detected (Table 4). Malathion was not detected in sediment 1 or 6 hours after treatments. None was detected in water or from *Juncus* after the first spray. After the third spray, however, it persisted at least 3 days at concentrations up to 0.34 ppb in tub water and 0.28 ppm in *Juncus* samples. Malathion was not detected in control samples.

Deaths of caged mosquitoes after the third spraying were 100% treated and 0.1% check for *A. taeniorhynchus,* and 89.3% treated and 0% check for *C. nigripalpus.* Mortalities were within the range found in other field tests using caged mosquitoes and ULV ground equipment (Rathburn and Boike, 1972).

TABLE 4. Malathion in samples of tub water (from each of two rows of tubs), marsh water and *Juncus* from salt-marshes after ULV sprays of malathion 95 at 0.64 fl. oz/acre (57 g/ha).

Sample and time elapsed	Spray 1	Spray 2	Spray 3
Tub water 1			
1 hour	N.D.[1]	Tr.[1]	1.52 ppb
6 hours	N.D.	Tr.	0.58 ppb
12 hours	N.D.	Tr.	0.73 ppb
1 day	N.D.	Tr.	0.48 ppb
3 days	N.D.	N.D.	Tr.
Tub water 2			
1 hour	N.D.	Tr.	0.32 ppb
6 hours	N.D.	N.D.	Tr.
12 hours	N.D.	N.D.	0.36 ppb
1 day	N.D.	N.D.	0.32 ppb
3 days	N.D.	....	0.34 ppb
Marsh water			
6 hours	N.D.	N.D.	N.D.
12 hours	N.D.	0.49 ppb	N.D.
1 day	N.D.	Tr.	N.D.
3 days	N.D.	N.D.	N.D.
Juncus			
6 hours	N.D.[2]	Tr.[2]	N.D.
12 hours	N.D.	Tr.	N.D.
1 day	N.D.	N.D.	0.41 ppm
3 days	N.D.	N.D.	0.28 ppm

[1] N.D. (non detectable) = not above 0.10 ppb; Tr. (trace) = >0.10 but <0.30 ppb.

[2] N.D. (non detectable) = not above 0.05 ppm; Tr. (trace) = >0.05 but <0.10 ppm.

DISCUSSION

No adverse effects of malathion on confined animals or on the salt-marsh environment were observed under the conditions of these studies. In addition, no deaths were noted among resident crabs, fishes and shrimps after any of the treatments. Malathion was not detected in animals or sediment. In general, when found in plant samples or water, concentrations were low and did not persist. We found trace amounts of the chemical in *Juncus* samples for as long as 14 days after treatment. Although Bender (1969) found that carp, *Cyprinus carpio,* accumulated malathion when exposed to relatively high concentrations (1.0–7.5 ppm), he reported that the average half-life of the chemical, calculated from his data, was only 12 hours. The highest concentration we detected in marsh water was 5.2 ppb; only trace amounts persisted as long as 1 day. Guerrant *et al.* (1970) reported that 0.5 ppm malathion, the highest concentration found in waters of Hale County, Texas after ULV aerial spraying, was completely decomposed in 1 day.

In our field studies, the applications of thermal fog were on a small scale, but the ULV aerosol sprays were typical of usual operations for mosquito control. Even so, the fogging studies indicate what might occur in typical operations with truck-mounted equipment over larger areas.

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