Journal of the American Mosquito Control Association, 15(3):321–329, 1999

EFFECTS OF TEMEPHOS (ABATE® 4E) ON FIDDLER CRABS (*UCA PUGNAX* AND *UCA MINAX*) ON A DELAWARE SALT MARSH

ALFRED E. PINKNEY,[1] PETER C. MCGOWAN,[1] DANIEL R. MURPHY,[1] T. PETER LOWE,[2] DONALD W. SPARLING[2] and WILLIAM H. MEREDITH[3]

ABSTRACT. The nontarget effects of temephos (as Abate® 4E, 44.6% active ingredient) on fiddler crabs were examined on the salt marsh at Bombay Hook National Wildlife Refuge, near Dover, DE. Six 170 × 170-m plots were established; 3 were sprayed on 4 occasions at a rate of 1.5 fl oz/acre (0.054 kg active ingredient/ha) and 3 were controls. On each plot, marsh fiddler crab (*Uca pugnax*) populations were monitored by repeatedly counting the number of burrow holes in 2 counting areas marked out along tidal guts. One half of each counting area was covered with bird netting to evaluate sublethal toxic effects, which, if present, could result in increased susceptibility to bird predation. A statistically significant linear association was established between the number of holes and the number of crabs. No significant differences were found in the numbers of holes (or crabs) in the sprayed vs. control plots and in the covered vs. uncovered sections. However, survival of juvenile crabs in *in situ* bioassays was significantly reduced (16% lower) by the spraying. Median acetylcholinesterase activity in claw muscle of red-jointed fiddler crabs (*Uca minax*) collected 2 days after an operational spray with Abate 4E was significantly reduced (28% lower) compared to unsprayed crabs. In view of the toxicity to juvenile crabs and the cholinesterase inhibition, we recommend continued monitoring and research for nontarget impacts of Abate 4E on fiddler crabs to establish whether the reported level of cholinesterase inhibition results in acute or chronic toxicity.

KEY WORDS Fiddler crabs, temephos, cholinesterase, nontarget effects

INTRODUCTION

The insecticide temephos (*O,O'*-(thiodi-4,1-phenylene)*O,O,O',O'*-tetramethyl phosphorothioate) has been regularly applied to control mosquito production on the salt marsh at Bombay Hook National Wildlife Refuge (NWR), 13 km from Dover, DE (Fig. 1). Temephos, an organophosphorus insecticide, acts through inhibition of cholinesterase enzymes, which play a critical role in the nervous system. On the refuge, the emulsified liquid larvicide Abate® 4E (44.6% active ingredient [AI]; American Cyanamid, Princeton, NJ) is sprayed at a rate of 1.5 fl oz/acre (0.048 lb AI/acre = 0.054 kg AI/hectare). Airplanes and, to a lesser extent, helicopters are used for spraying, which is done by the Delaware Department of Natural Resources and Environmental Control (DNREC), Division of Fish and Wildlife, Mosquito Control Section.

Fiddler crabs are the most abundant and conspicuous macroinvertebrates on many salt marshes (Montague 1980). Their extensive burrowing activities aerate the marsh, enhancing decomposition through increasing surface area and stimulating aerobic bacteria. They are a vital component of the diet of birds such as clapper rails, herons, and willets (Burger et al. 1991). At Bombay Hook NWR, 2 species of fiddler crabs, the red-jointed fiddler crab (*Uca minax* Le Conte) and the marsh fiddler crab (*Uca pugnax* Smith), are found on the salt marsh. Large populations of fiddler crabs (primarily *U. pugnax*), which live in the intertidal zone along small tidal creeks (guts), are exposed to insecticides when the marsh is sprayed.

In a field test on a New Jersey salt marsh, Ward et al. (1976) demonstrated that application of granular Abate (2% temephos), at the 0.1 lb AI/acre (0.112 kg AI/ha) rate used for operational control, reduced the population of marsh fiddler crabs by about 30%. These investigators tested both open (uncovered) field plots as well as plots in which bird predators were excluded (covered). Because the reduction only occurred in uncovered plots, the authors concluded that temephos increased the crabs' susceptibility to bird predation. Laboratory experiments indicated that temephos interfered with the crabs' escape behavior (Ward and Busch 1976). In the field study, although crabs were observed eating Abate granules, temephos tissue concentrations did not increase through the growing season, presumably because the compound is metabolized rapidly.

The primary objective of the present study was to examine the nontarget impacts of Abate 4E on fiddler crabs. Effects on *U. pugnax* were analyzed using the caged and uncaged plot design of Ward et al. (1976) and through in situ bioassays. In addition, we investigated possible effects on cholinesterase activity in *U. minax*, and evaluated the persistence of temephos in pothole water and marsh sediments.

[1] U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, 177 Admiral Cochrane Drive, Annapolis, MD 21401.

[2] U.S. Geological Survey, Patuxent Wildlife Research Center, 11510 American Holly Drive, Laurel, MD 20708.

[3] Delaware Department of Natural Resources and Environmental Control, Division of Fish and Wildlife, Mosquito Control Section, 89 Kings Highway, Dover, DE 19903.

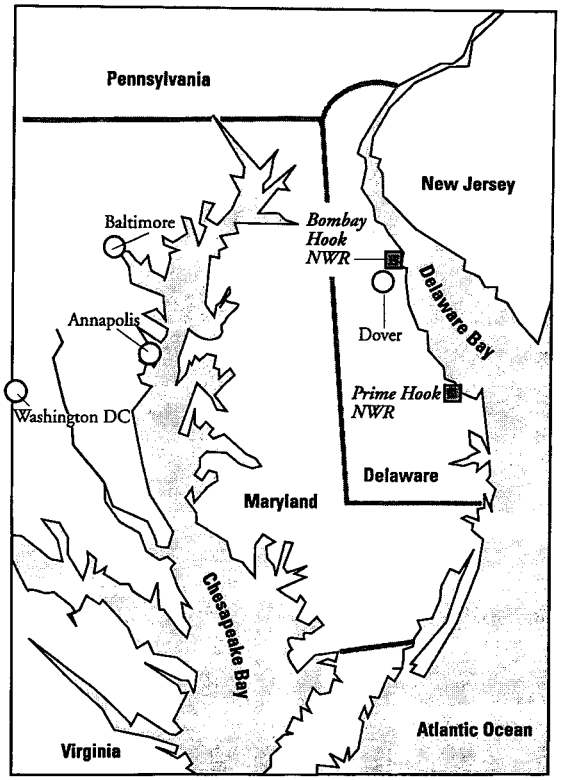

Fig. 1. Location of Bombay Hook National Wildlife Refuge and Prime Hook National Wildlife Refuge.

MATERIALS AND METHODS

Study plots: In the spring of 1994, 6 approximately 170 × 170-m study plots were delineated along Duck Creek and Shearness Gut on Bombay Hook NWR. Each plot was traversed by a small tidal gut with an intertidal zone populated with fiddler crabs. The corners of the plots were marked with 2-m-tall stakes covered with orange-painted bottles to increase visibility. The plots were numbered and randomly assigned to be spray (2, 3, and 5) or control (1, 4, and 6) plots. Adjacent plots were separated by at least 500 m to minimize the effect of any drift that may have occurred.

Spray application and chemical monitoring: In the summer of 1994, Abate 4E was applied by helicopter to 3 spray plots at 0.054 kg AI/ha on 4 occasions: July 6, July 27, August 24, and September 21. The spraying rate and 3- to 4-wk interval between spraying corresponds to typical operational spraying procedures (Meredith, personal communication). Spray was applied early in the morning (before 0800 h) when the air was still. Temephos concentrations in water and sediments were measured in samples collected before and after the 3rd spraying.

Spraying efficacy was determined for the 2nd, 3rd, and 4th sprays by collecting the sprayed larvicide from 9 equal-sized subdivisions within each sprayed plot and 4 subdivisions in each control plot. Sprayed larvicide was collected on 10 × 20-cm pieces of glass fiber filter paper clipped to aluminum foil-covered horizontal wooden platforms attached to vertical wooden stakes. Approximately 18 h before the scheduled spray, the stakes were pushed into the ground as close as practicable to the center of each of 9 equal-sized subdivisions until each stake's platform was 10 cm above the surface of the vegetation. Filter papers in treated plots were removed from the platforms within 1 h after spraying and placed immediately in 125-ml glass containers filled with methylene chloride (CH_2Cl_2) to begin the extraction. Filter papers in the control plots were removed within 1 h after spraying was completed at the nearest sprayed plot.

Five potholes within each sprayed plot were sampled 24 h before and 6, 24, and 48 h after the 3rd spraying to determine the rate of temephos degradation. Approximately 0.8 liters of water was collected, using a dipper made with an 0.8-liter beaker attached to a 2-m length of dowel. The sampling depth was a few centimeters below the water surface. The average water depth of the potholes was difficult to measure due to the soft mud bottoms, but can be estimated as 15–30 cm. Water was transferred into 1-liter amber-colored glass bottles containing 125 ml of CH_2Cl_2. Immediately after each sample was collected, the bottle was shaken vigorously for about 2 min to mix the CH_2Cl_2 and extract the temephos, then put in iced coolers. One duplicate and 1 spiked sample were collected each time water was sampled. Spiked samples were injected with 160 μg of temephos (U.S. Environmental Protection Agency [U.S. EPA] standard, 98.5% purity, U.S. EPA, Beltsville, MD).

Sediment was collected from upstream and downstream intertidal locations of the tidal guts passing though the sprayed study plots on the same schedule that water was collected. Each composite sediment sample (100 g) was composed of 5–10 individual samples scraped from the top 1 cm of sediment, using a 50-ml glass beaker attached to a length of dowel. The composite samples were placed immediately into chemically clean glass jars, packed into iced coolers, and stored in a freezer at −20°C.

The samples were analyzed for temephos by the U.S. Fish and Wildlife Service, Patuxent Analytical Control Facility (PACF), Laurel, MD, based on the methods of Belisle and Swineford (1988). Water samples were extracted 3 times with CH_2Cl_2, extracts were pooled, cleaned by florisil column chromatography, and analyzed by gas chromatography on a DB-1 capillary column using a flame photometric detector. Detection limits were 0.3 μg/liter. Sediment samples were mixed with sand and sodium sulfate, placed in a chromatography column and eluted with 1:1 acetone: CH_2Cl_2, then concentrated and analyzed by gas chromatography using the same column and detector. Detection limits

were 0.10–0.16 mg/kg dry weight. Quality control procedures included the analysis of blanks, field and laboratory duplicates, and matrix spikes.

Chemical data were analyzed using analysis of variance (ANOVA) within the framework of general linear models (Proc GLM) because the data did not fit into a balanced design (SAS Institute Inc. 1989). Duncan's multiple range test was used to separate means of main effects that were significantly different in the ANOVA. Data from samples in which temephos concentrations were below detection limits were represented as one half of the limit of detection. All statistical analyses were performed on log-transformed values to avoid heteroscedasticity.

Fiddler crab monitoring: The numbers of marsh fiddler crabs were estimated by counting the number of burrow holes in locations that were visited repeatedly. A linear relationship between the number of holes and number of marsh fiddler crabs (≥0.5-cm carapace diameter) was established on a Massachusetts salt marsh by Krebs and Valiela (1978) as number of crabs = 2.233 + 0.9712(number of holes); $r = 0.95$, $P < 0.01$, $n = 13$. This relationship was determined by sinking a 0.5 × 0.5-m square wooden frame into the mud, counting the holes, and then digging to a depth of 0.5 m, sieving through a 4-mm mesh, and counting the crabs. Using this same technique, the correlation between holes and crabs at Bombay Hook NWR was established at unsprayed intertidal habitats (separate from the locations that were visited repeatedly for hole counting). A total of 46 counts of the number of holes vs. crabs were made, with counts occurring on June 23, 25, and 29; July 22; August 19; and September 19.

Covered and uncovered areas were established so that both lethal and sublethal effects (increased susceptibility to bird predation) could be examined. On each of the 6 study plots in the intertidal zone along a tidal gut, 2 30 × 1-m areas were marked out. These areas were covered by approximately 10 cm of water at high tide and were out of the water at low tide. One half of each area was covered with plastic bird netting (1.9 × 1.6-cm mesh) to exclude avian predators. The netting was dug into the mud and fixed with horseshoe-shaped metal stakes. Within each area 2 1 × 1-m square counting areas were marked with fluorescent pink cord. Within the counting area, all vegetation was kept to a height of less than 2 cm by frequent clipping with hand shears. The number of burrow holes in each counting area was determined on the day before each spray (day −1), and on days +1, +2, and +7. Only holes at least 1 cm in diameter were counted, because this is the minimum size for *U. pugnax* burrow holes (Montague 1980).

A repeated-measures ANOVA (SAS Institute Inc. 1989) was used to test for the effects of spraying on the number of holes. The following model variables were identified and included in the analysis: treatment (spray or control), plot number, cover (covered or uncovered), and spray (whether the counting was performed prespray or postspray). Interactions between these variables were also evaluated. Correlation analysis was used to relate the numbers of holes with the numbers of crabs.

In situ bioassays: Juvenile *U. pugnax* (approximately 2- to 4-mm carapace width) were collected along Petersfield Ditch, an area of Prime Hook NWR (Fig. 1) that had not been sprayed with mosquito-control chemicals in 1994 (Meredith, personal communication). The crabs were kept in plastic trays containing collection site mud and held overnight in the laboratory. The next day crabs were transported to the study plots, where 10 crabs each were placed in 1-liter polyethylene jars with 5 × 7.5-cm windows on 2 sides covered with 1-mm mesh polyethylene screen. Each jar was tied to a galvanized minnow trap such that 1 window was facing upwards. One tablespoon of study plot mud was placed in each jar to provide moisture for the crabs. At each study plot on the day before the 3rd and 4th sprays, 2 traps were placed in an unvegetated area just above the high tide line The crabs were collected 2 days after spraying and the numbers of living and dead crabs were counted. Data were analyzed using 2-way ANOVA on arc sine square root-transformed survival data to test for effects of the spray, the spray event (3rd or 4th spray), and the interaction (SAS Institute Inc. 1989).

Uca minax *cholinesterase and residue analysis:* In 1995, the investigators were given several days notice by the DNREC of an upcoming operational spray with Abate 4E at 0.054 kg AI/ha. *Uca minax* were collected for cholinesterase assay and residue analysis because they are larger than *U. pugnax.* The crabs were collected from an area of Bombay Hook NWR along the Leipsig River that was to be operationally sprayed with Abate 4E and from a control area (Marshall Island) that has never been sprayed (Meredith, Delaware DNREC, personal communication). The spray occurred on July 18. Crabs were collected from Marshall Island on July 14 and July 20 and from the spray area on July 17 and July 20. On each date, male crabs were collected for residue analysis (25 per site per collection date) and cholinesterase assays (10 per site per date). Crabs were kept on ice after collection and stored at −20°C until analysis.

Uca minax samples were analyzed for temephos by the PACF, based on Belisle and Swineford (1988). Carapaces were removed and composites of 5 crabs were analyzed as a single sample. The weight of the samples ranged from 14.9 to 26.4 g. The soft tissues were homogenized with acetone and CH_2Cl_2. The organic extract was filtered and adjusted to volume for gas chromatography on a megabore capillary column using a flame photometric detector. The detection limit was 0.019–0.023 mg/kg wet weight.

For the cholinesterase assays, the large claw muscle tissues from individual male crabs were analyzed. Acetylcholinesterase (AChE) assays were conducted according to the methods of Hill and Fleming (1982), at a buffer : tissue (volume : weight) ratio of 20:1 and a homogenate volume of 0.1 ml. Butyrylcholine esterase (BChE) assays were conducted according to the same method, using the substrate butyrylthiocholine iodide instead of acetylthiocholine iodide. The buffer : tissue ratio was 20:1 and the homogenate volume was 0.3 ml.

Statistical differences in cholinesterase activity before and after spraying were analyzed by Mann–Whitney *U*-tests because parametric assumptions were not met, even after log transformation. Data from the Marshall Island control plot were used to analyze for differences in time unrelated to the spray and to evaluate variability between the sprayed and control plots.

RESULTS

Fiddler crab population monitoring

A significant linear relationship was found between the number of holes and the number of crabs with a carapace diameter ≥0.5 cm: number of crabs = 13.945 + 0.535(number of holes); $r = 0.50$, $P < 0.001$; $n = 46$. A similar result was obtained when the relationship was calculated for crabs with a carapace diameter ≥1.0 cm: number of crabs = 13.365 + 0.521(number of holes); $r = 0.49$, $P < 0.001$, $n = 46$).

The number of holes fluctuated considerably through the course of the monitoring (examples given in Figs. 2a, 2b). Based on the repeated-measures ANOVA, no significant effect of spraying was found on the mean number of holes ($P = 0.51$). The effect of cover ($P = 0.28$); the interactive effects of spraying and cover ($P = 0.91$); and the interaction of spraying, cover, and pre- vs. postspray ($P = 0.55$) were also not significant. The mean number of holes was similar in the sprayed (53.4 holes/m^2) vs. control (49.1 holes/m^2) areas, and in the covered (46.6 holes/m^2) vs. uncovered (55.9 holes/m^2) sections. Using the linear equation for crabs with a carapace width ≥ 0.5 cm, the mean numbers of crabs were 42.5 (sprayed), 40.2 (control), 38.9 (covered), and 43.9 (uncovered).

In situ bioassay

The mean survival of juvenile fiddler crabs in the sprayed jars (77.5%) was significantly less than that in the control jars (93.3%; 2-way ANOVA on arc sine square root-transformed data; $P = 0.01$, Table 1). This represents a 15.8% decrease in survival of juvenile crabs exposed to a temephos application.

Chemical monitoring

The application of temephos was uneven among sprays, and highly variable within sprays (e.g., spray 4 range was 75–11,500 μg/m^2; Table 2). Mean temephos concentrations were significantly different among sprays (ANOVA; $P < 0.001$) with the 2nd spray significantly lower than the 3rd and 4th sprays. The average per hectare application of temephos during sprays 2, 3, and 4 was 0.0105, 0.0633, and 0.1047 kg AI/ha, respectively (Table 2), compared to the desired 0.054 kg AI/ha application rate. Thus, sprays 3 and 4 appear to be within a factor of 2 of the desired rate, whereas spray 2 appears to be only about 20% of the desired rate. Concentrations on filter paper collected from unsprayed plots were always below the limit of detection (<0.50 μg/m^2; $n = 28$).

Temephos concentrations in pothole water at 6 h after spray 3 ranged from 7.69 to 132 μg/liter, with an average of 22.6 μg/liter. Mean temephos concentrations in pothole water were significantly different for all collection times, with the lowest concentrations occurring before spraying and the highest concentration occurring 6 h after spraying (Table 3). Concentrations decreased rapidly over the 1st 24 h. The mean concentration in water collected 24 and 48 h after spraying was 7.7 and 3.2%, respectively, of the mean concentration in water collected 6 h after spraying.

For the sediment concentrations after spray 3, the results of the ANOVA indicated that study plot and collection times were both significant ($P = 0.013$ for both variables; Table 4). Changes in temephos concentrations after the spray were not consistent among plots. Concentrations declined slightly at plot 2, decreased and then increased at plot 3, and increased and then decreased at plot 5. Mean values for the 3 plots indicate that approximately 63% of the temephos measured in sediments 6 h postspray was still present 48 h postspray.

Analysis of the blank samples did not indicate detectable temephos. Mean spike recovery from Bombay Hook NWR water (82.7%) was nearly equal to that of reverse osmosis water (81.6%; range 70–94%); however, the variability among samples was greater in the Bombay Hook water. In the 5 spiked samples with Bombay Hook water, recovery in 3 was 78–81%; however, 1 sample had 41% recovery and 1 had 134% recovery. Recoveries of spiked sediment samples were higher (91 and 114%) and more consistent than those for water samples. Duplicate sediment samples were more consistent (relative percent differences of 6 and 12%) than duplicate water samples (relative percent differences of 0, 19, 49, and 171%).

Uca minax cholinesterase and residue analysis

A significant decrease occurred in the AChE activity of the crabs collected after spraying compared

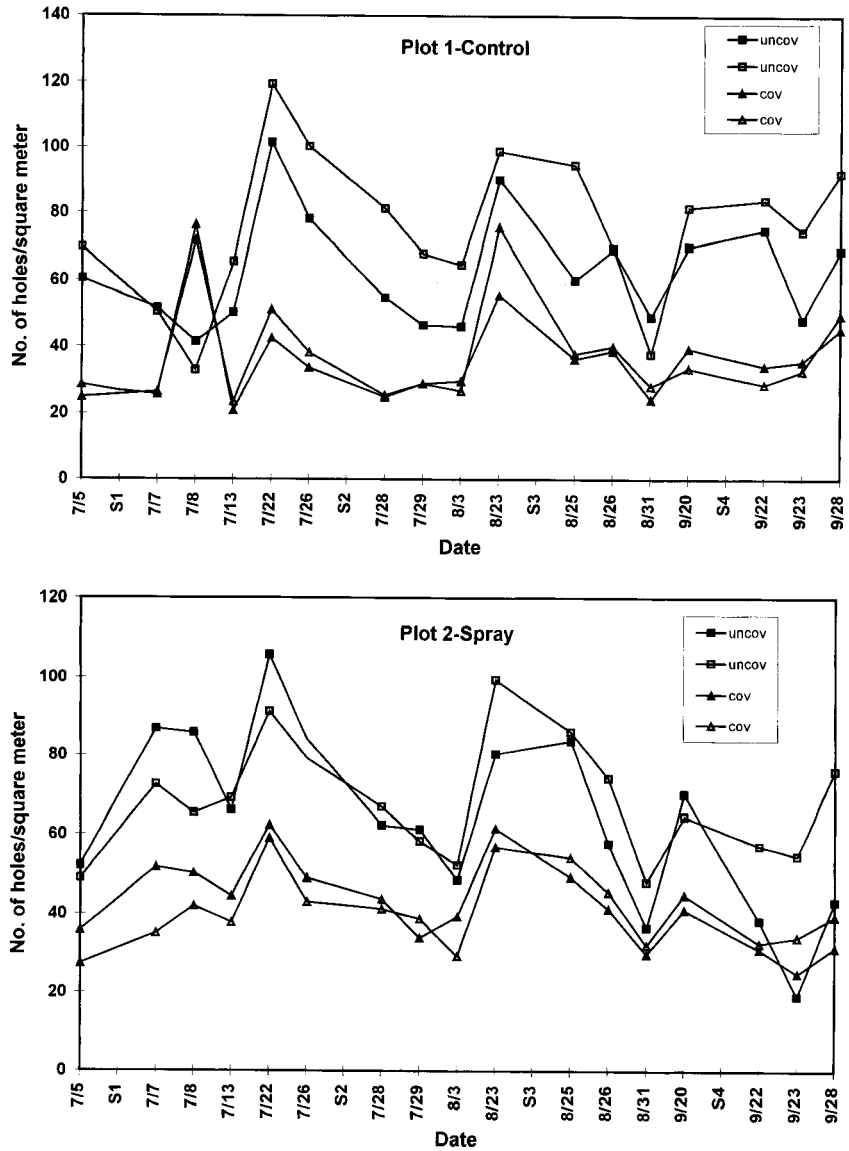

Fig. 2. Numbers of burrow holes counted in covered (cov) and uncovered (uncov) areas on study plots at Bombay Hook National Wildlife Refuge. S1, S2, S3, and S4 are the 4 sprays that occurred on July 6 and 27, August 24, and September 21. (a) Plot 1 (control) and (b) plot 2 (spray) are shown; other plots had similar results.

to those collected before spraying (Table 5). Median activity was 2.18 μmoles/min/g tissue at the spray site on the day before the operational spray (July 17) vs. 1.37 μmoles/min/g tissue 2 days postspray (July 20). At the control site, median activity was nearly identical on July 14 (1.83 μmoles/min/g tissue) and July 20 (1.85 μmoles/min/g tissue). The activities in the crabs collected postspray on the spray site were also compared with the pooled data from the prespray collection at the spray site and the 2 collections from the control site. These data were pooled after an analysis of variance indicated no significant differences between the 3 collections ($P = 0.058$). The postspray median activity (1.37 μmoles/min/g tissue) was significantly lower than the median of the pooled data (1.90 μmoles/min/g tissue). A 28% inhibition of AChE activity was calculated against this pooled median.

The BChE activity was not significantly inhibited at the spray site (Table 5). Median activity was 0.245 μmoles/min/g tissue on July 17 and 0.191 μmoles/min/g tissue on July 20 (Mann–Whitney *U*-test, $P = 0.10$). At the control site, activities were nearly identical—0.200 μmoles/min/g tissue on July 14 and 0.198 μmoles/min/g tissue on July 20.

No residues of temephos were detected in any of

Table 1. Results of 48-h in situ bioassays with juvenile fiddler crabs (*Uca pugnax*).

	% survival[1]	
	Sprayed plots	Control plots
Spray 3	68.3 (80, 65, 60)	93.3 (90, 100,[2] 90)
Spray 4	86.7 (80, 85, 95)	93.3 (95, 90, 95)
Overall[3]	77.5	93.3

[1] Mean survival followed by survival per plot (based on 2 jars per plot with 10 crabs per jar).

[2] Survival for 1 jar; in the other jar the mud was dried and all crabs were dead.

[3] Results of 2-way analysis of variance on arc sine square root-transformed data: treatment (spray vs. control), $P = 0.01$; spray event (3 vs. 4), $P = 0.24$; treatment × spray, $P = 0.10$.

Table 3. Concentrations of temephos in water (μg/liter) from sprayed potholes on Bombay Hook National Wildlife Refuge before and after spray 3.

Collection time	Frequency of detection	Geometric mean[1]	Minimum	Maximum
24 h prespray	4/13	0.23 A	0.15[2]	0.74
6 h postspray	15/15	22.6 B	7.69	132.3
24 h postspray	15/15	1.74 C	0.44	28.0
48 h postspray	11/15	0.72 D	0.15[2]	59.6

[1] Geometric mean values followed by different letters are significantly different using analysis of variance ($P = 0.013$) and Duncan's multiple range test ($P < 0.05$).

[2] Equal to one half of the limit of detection for all samples.

the *U. minax* samples. Detection limits were 0.019–0.023 mg/kg wet weight.

DISCUSSION

Effects on fiddler crab populations

The lack of effect of Abate 4E on fiddler crab populations, as evidenced by the burrow hole monitoring, contrasts with the studies of Ward et al. (1976) with granular Abate. This may be a result of the much lower exposure in the present study. First, the application of Abate 4E (0.054 kg temephos/ha) was less than one half of the 0.1125 kg temephos/ha rate of application used by Ward et al. (1976). Second, because Ward et al. (1976) observed crabs eating the granules, it is likely that some crabs obtained much higher concentrations of the compound than would be expected by broadcast of an emulsified insecticide. The Abate 4E would be expected to be rapidly diluted by the incoming and outgoing tides in the tidal gut, whereas the granular Abate might adhere to mud or vegetation and remain available for consumption for longer periods. In a study with the coffee bean snail (*Melampus bidentatus* Say), Fitzpatrick and Sutherland (1976) reported substantially higher uptake of granular vs. emulsified temephos, and attributed the increase to the snails' ingestion of whole granules.

The different results in the present study vs. that of Ward et al. (1976) may result from a less accurate method of estimating populations, which may have decreased the ability to detect an effect. Ward et al. (1976) observed and counted crabs, whereas we counted burrow holes as an indicator of the presence of crabs. The 0.50 correlation coefficient for the association between the number of holes and number of crabs was considerably weaker than the 0.95 coefficient reported by Krebs and Valiela (1978) on a Massachusetts salt marsh. However, the present study was based on 46 counts, whereas Krebs and Valiela based their relation on only 13 counts. Further investigation of the relationship between the number of burrow holes and the number of crabs is recommended. Alternate census methods for fiddler crabs, such as enclosing the crabs within frames at high tide and capturing and counting at low tide (Cammen et al. 1984), although more time-consuming, might be more accurate.

Pierce (1993) reported that application of Abate 4E to the south Florida salt marsh and fringing mangrove forest communities raised concerns for lethality to the salt-marsh fiddler crab, *Uca rapax* Smith.His laboratory test reported an estimated toxic threshold to *U. rapax* larvae (concentration at which no significant increase in mortality occurred through 2 days past the 1st molt) of 5 μg/liter. In the field, spraying Abate 4E at 0.5 oz temephos/acre (0.018 kg/ha) on the mid- and lower marsh killed fiddler crab larvae residing there. Spraying only the upper marsh area (where no crab larvae were observed) did not cause detectable temephos concentrations to move with the tide to the mid- and lower marsh.

Nontarget effects of mosquito larvicides on lar-

Table 2. Concentrations per unit area of temephos on sprayed study plots of Bombay Hook National Wildlife Refuge based on filter paper samples. Per hectare figures are equivalents of the mean temephos concentrations per square meter.

Spray number	Number of samples	Temephos		
		Mean ± SD[1] (μg/m²)	Range (μg/m²)	Mean (kg/ha)
2	24	1,083 ± 1,000 A	2.5–4,250	0.0047
3	27	4,911 ± 3,471 B	2.5–11,500	0.0283
4	23	6,023 ± 2,801 B	75–11,000	0.0468

[1] Mean values followed by different letters are significantly different using Duncan's multiple range test ($P < 0.05$).

Table 4. Geometric mean temephos concentrations in sprayed sediments (μg/g dry weight) at Bombay Hook National Wildlife Refuge before and after spray 3.

Collection time	Study plot 2	Study plot 3	Study plot 5	Number of samples	Overall geometric mean[1]
24 h prespray	0.067[2]	NA[3]	0.075[2]	4	0.071 A
6 h postspray	NA[3]	0.570	1.392	4	0.890 B
24 h postspray	0.216	0.485	1.613	6	0.553 B
48 h postspray	0.169	0.726	1.452	6	0.563 B

[1] Mean values followed by different letters are significantly different using analysis of variance ($P = 0.013$) and Duncan's multiple range test ($P < 0.05$).
[2] Mean of one half of the limits of detection for all samples in a group.
[3] Not analyzed.

val fiddler crabs may not be as great a concern in the Delaware salt marsh as in the mangrove marsh. In the mangrove marsh, the larval fiddler crabs may be present in the mid-marsh habitats where mosquito larvae are breeding and where spraying may occur (Pierce 1993). In the Atlantic coast salt marshes, *U. pugnax* larvae are released on a semilunar cycle coinciding with the high tides associated with new and full moons, which results in maximum dispersal from the marsh to the estuary (Wheeler 1978). Thus, except for those trapped on the high marsh in potholes, where according to our study, lethal concentrations may occur, larval fiddler crabs on the Delaware marsh would be unlikely to experience substantial exposure to larvicides.

Juvenile fiddler crabs may be the life stage most likely to be affected by Abate 4E. As shown in the in situ bioassays, exposure can result in a 16% decrease in survival. Juveniles may be more sensitive than adults to the pesticide because they are receiving a higher exposure per unit body weight. However, the caged exposure that occurred just above the high tide line may overestimate exposure in the intertidal zone, where dilution would occur with each tidal cycle.

Chemical monitoring

Temephos concentrations on the filter paper ranged over several orders of magnitude within sprays 2, 3, and 4 (Table 2). The concentration at the high end of the range might reflect a temephos buildup in the overlap zones of adjacent spraying swaths. The concentration at the low end of the range may reflect an area that received low temephos exposure, as might occur between spraying swaths.

The range in temephos concentrations was greater than the results that have been reported in another study using a similar technique. Temephos concentrations measured during two sprayings of a Florida mangrove swamp varied from 1,315 to 3,130 μg/m² (Pierce et al. 1989). The lower concentrations measured on the filter papers from the 2nd spray probably reflect the severe weather conditions that preceded spraying, rather than real differences in coverage. Heavy rains the night before spraying left the filter paper saturated with water, which displaced large amounts of the CH_2Cl_2 in the storage vessels. Because the storage vessels were just large enough to accommodate the filter papers, much of the CH_2Cl_2 with the dissolved temephos could not be salvaged.

The mean temephos concentration in pothole water at 6 h after spraying (22.6 μg/liter) is in reasonable agreement with expected concentrations. For the 0.054-kg/ha application rate, the theoretical concentration immediately after spraying would be 63 μg/liter for an average depth of 15 cm and 31.5 μg/liter for an average depth of 30 cm (based on Lores et al. 1985). A lower measured concentration can be expected because of the degradation that would have occurred over the 6 h after the spray and binding to suspended particles or sediments.

Degradation rates over the 1st 24 h in this study seem to have been similar to the rates reported in

Table 5. Fiddler crab acetylcholinesterase (AChE) and butyrylcholine esterase (BChE) activities.[1]

Enzyme	Area	Prespray[2] median (range)	Postspray[2] median (range)	Statistics[3]
AChE	Spray	2.18 (1.66–3.11)	1.37 (1.27–2.46)	Pre > post; $P = 0.009$
	Control	1.83 (1.28–3.33)	1.85 (1.58–2.03)	NS; $P = 0.70$
BChE	Spray	0.245 (0.199–0.290)	0.191 (0.141–1.125)	NS; $P = 0.10$
	Control	0.200 (0.139–0.256)	0.198 (0.179–0.273)	NS; $P = 0.90$

[1] Units: μmoles/min/g tissue; all data based on $n = 10$.
[2] Prespray sampling on July 14, 1995 (control) and July 17, 1995 (spray); spraying occurred July 18, 1995; postspray sampling on July 20, 1995.
[3] Mann-Whitney *U*-tests because data did not meet assumptions for parametric statistics; NS, not significant ($P > 0.05$).

some other studies. Geometric mean temephos concentrations in water collected 24 h post spray were 7.7% of those measured at 6 h postspray (Table 3). Hughes et al. (1980) reported that 90% of temephos had disappeared by 1.05 d postspray in a study of 2 freshwater ponds that were sprayed at a per square meter area rate resulting in an initial concentration of 10.0 μg/liter. The 24-h postspray concentrations reported by Lores et al. (1985) were about 4% of the concentrations reported 1 h postspray. Degradation rates in some studies seem to have been slower than those in this study or given above. Helgen et al. (1988) reported mean 24-h postspray concentrations for 5 experimental sites that were 21% of the mean 1-h postspray concentrations, and Pierce et al. (1989) reported 24-h postspray concentrations that ranged from 19 to 46% of 1-h postspray concentrations. However, in a later study, Pierce et al. (1996) reported 24-h postspray concentrations more in line with those reported by the earlier mentioned investigators, which were 12.5 and 4.6% of the 1-h postspray concentrations.

Four out of 13 pothole water samples collected 24 h before spray 3 contained detectable concentrations of temephos (mean of 0.23 μg/liter or about 1% of the 6-h postspray concentration). Thus, some traces of temephos from spray 2, which occurred 27 days earlier, were still evident in pothole water.

Mean temephos concentrations did not decline as rapidly in sediments as in water. Overall mean temephos concentrations in sediments 24 and 48 h postspray had declined to 62 and 63%, respectively, of 6-h postspray concentrations (Table 4), whereas water concentrations during the same period had declined to 7.7 and 3.2%, respectively (Table 3). Pierce et al. (1989) also indicated that temephos degradation in sediment samples proceeded more slowly than in water. Concentrations 24 h postspray were 44.4, 35.8, and 92.5% of 1-h post-spray concentrations at 3 sprayed sampling sites. Concentrations 48 h post pray were 68% of 1-h postspray concentrations at 1 sprayed sampling site. The high variability in sediment concentrations among sites and at different sampling times observed in the present study was also observed by Pierce et al. (1989).

No studies have been conducted to determine the persistence and fate of temephos applied to sediment; however, an investigation may be advisable because of the implications that slower degradation might pose when managing lands that experience regular tidal flooding. For example, if temephos was applied to recently exposed intertidal sediments, very little degradation of temephos would take place before the sediments were flooded again several hours later by the next high tide. If temephos was released from the sediments during flooding, it could be carried into aquatic areas where nontarget organisms could be exposed.

Cholinesterase inhibition

The 28% inhibition observed in the present study is only slightly below the 30–50% inhibition range suggested by Edwards and Fisher (1991) as an indicator of exposure in terrestrial and aquatic invertebrates, and the 30% threshold for freshwater mussels suggested by Moulton et al. (1996). No values for "normal" fiddler crab muscle tissues were identified in the literature, nor has an inhibition threshold been proposed. It is reasonable to conclude that the 28% level of inhibition is indicative of exposure, and that laboratory studies would be needed to establish the relationship between this level of inhibition and lethal or sublethal effects.

Conclusions

Although spraying with Abate 4E at an operational rate of 0.054 kg AI/ha did not affect populations of adult fiddler crabs (*U. pugnax*), as indicated by burrow hole counting, it caused a significant increase in mortality in juveniles in in situ bioassays. Acetylcholinesterase activity was significantly inhibited in *U. minax* exposed to Abate 4E at the same rate. In view of the toxicity to juvenile crabs and the cholinesterase inhibition, we recommend continued monitoring for nontarget impacts of Abate 4E on fiddler crabs, especially population-level effects, and research to establish whether the reported level of cholinesterase inhibition results in acute or chronic toxicity.

ACKNOWLEDGMENTS

This project was funded by the U.S. Fish and Wildlife Service, Division of Environmental Contaminants. Roger Wolfe, formerly with the DNREC, Mosquito Control Section, directed the spray applications. Mary Gustafson, Patuxent Wildlife Research Center, provided guidance on the cholinesterase assays and Andre Belisle performed the temephos analyses. The following present and former U.S. Fish and Wildlife Service personnel contributed to the study design, field work, data analysis, and illustrations: Robert Foley, Amy Derosier, Lisa Domico, and Laurie Hewitt.

REFERENCES CITED

Belisle, A. A. and D. M. Swineford. 1988. Simple, specific analysis of organophosphate and carbamate pesticides in sediments using column extraction and gas chromatography. Environ. Toxicol. Chem. 7:749–752.

Burger, J., J. Brzorad and M. Gochfeld. 1991. Immediate effects of an oil spill on behavior of fiddler crabs (*Uca pugnax*). Arch. Environ. Contam. Toxicol. 20:404–409.

Cammen, L. M, E. D. Seneca and L. M. Stroud. 1984. Long-term variation of fiddler crab populations in North Carolina salt marshes. Estuaries 7:171–175.

Edwards, C. A. and S. W. Fisher. 1991. The use of cholinesterase measurements in assessing the impacts of pesticides on terrestrial and aquatic invertebrates, pp.

255–275. *In:* P. Mineau (ed.). Cholinesterase-inhibiting insecticides. Their impact on wildlife and the environment. Elsevier Science Publishers, Amsterdam, The Netherlands.

Fitzpatrick, G. and D. J. Sutherland. 1976. Uptake of the mosquito larvicide temefos by the salt marsh snail, New Jersey—1973–74. Pestic. Monit. J. 10:4–6.

Helgen, J. C., N. J. Larson and R. L. Anderson. 1988. Responses of zooplankton and *Chaoborus* to temephos in a natural pond and in the laboratory. Arch. Environ. Contam. Toxicol. 17:459–471.

Hill, E. F. and W. J. Fleming. 1982. Acetylcholinesterase poisoning of birds: field monitoring and diagnosis of acute poisoning. Environ. Toxicol. Chem. 1:27–38.

Hughes, D. N., M. G. Boyer, M. H. Papst, C. D. Fowle, G. A. V. Rees and P. Baulu. 1980. Persistence of three organophosphorus insecticides in artificial ponds and some biological implications. Arch. Environ. Contam. Toxicol. 9:269–279.

Krebs, C. T. and I. Valiela. 1978. Effect of experimentally applied chlorinated hydrocarbons on the biomass of the fiddler crab, *Uca pugnax* (Smith). Estuarine Coastal Mar. Sci. 6:375–386.

Lores, E. M., J. C. Moore, P. Moody, J. Clark, J. Forester and J. Knight. 1985. Temephos residues in stagnant ponds after mosquito larvicide applications by helicopter. Bull. Environ. Contam. Toxicol. 35:308–313.

Montague, C. L. 1980. A natural history of temperate western Atlantic fiddler crabs (genus *Uca*) with reference to their impact on the salt marsh. Contrib. Mar. Sci. 23:25–55.

Moulton, C. A., W. J. Fleming and C. E. Purnell. 1996. Effects of two cholinesterase-inhibiting pesticides on freshwater mussels. Environ. Toxicol. Chem. 15:131–137.

Pierce, R. H. 1993. Temephos distribution and toxicity in a South Florida saltmarsh community. Mote Marine Laboratory Technical Report 333. Sarasota, FL.

Pierce, R. H., R. C. Brown, K. R. Hardman, M. S. Henry, C. L. P. Palmer, T. W. Miller and G. Wichterman. 1989. Fate and toxicity of temephos applied to a intertidal mangrove community. J. Am. Mosq. Control Assoc. 5: 569–578.

Pierce, R., M. Henry, D. Kelly, P. Sherblom, W. Kozlowsky, G. Wichterman and T. W. Miller. 1996. Temephos distribution and persistence in a southwest Florida salt marsh community. J. Am. Mosq. Control Assoc. 12: 637–646.

SAS Institute Inc. 1989. SAS/STAT user's guide version 6, 4th ed. SAS Institute Inc., Cary, NC.

Ward, D. V., B. L. Howes and D. F. Ludwig. 1976. Interactive effects of predation pressure and insecticide (temefos) on populations of the marsh fiddler crab *Uca pugnax*. Mar. Biol. 35:119–126.

Ward, D. V. and D. A. Busch. 1976. Effects of temefos, an organophosphate insecticide, on survival and escape behavior of the marsh fiddler crab *Uca pugnax*. Oikos 27:331–335.

Wheeler, D. E. 1978. Semilunar hatching periodicity in the mud fiddler crab *Uca pugnax* (Smith). Estuaries 1: 268–269.