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TEMEPHOS DISTRIBUTION AND PERSISTENCE IN A SOUTHWEST FLORIDA SALT MARSH COMMUNITY

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ABSTRACT. The distribution and persistence of the mosquito larvicide temephos was monitored throughout an intertidal salt marsh community in southwest Florida following routine aerial applications of Abate 4-E (43% temephos) from 1988 through 1993. Temephos was found to be more highly concentrated in the surface water microlayer than in mid-depth water, exhibiting a mean of 330 μg/liter at the surface and 12 μg/liter at mid-depth from 1 fl. oz./acre applications and 120 μg/liter in the surface and 4.5 μg/liter in mid-depth water for 0.5 fl. oz./acre applications. Concentrations at both surface and mid-depth diminished rapidly within the first 24 hours. Mangrove leaves provided the most persistent reservoir for temephos, remaining more than 7 days. Temephos residues also were observed in select salt marsh organisms, including the sheepshead minnow (*Cyprinodon variegatus*), adult fiddler crabs (*Uca rapax*) and the ribbed mussel (*Geukensia* sp.).

INTRODUCTION

This investigation was undertaken to assess the distribution and persistence of the organophosphate insecticide, temephos (0,0,0,′0′-tetramethyl 0,0′-thiodi-p-phenylene phosphorothioate), (Abate® 4-E, American Cyanamid, Princeton, NJ) in a southwest Florida salt marsh community, in an attempt to establish the expected environmental concentrations (EEC) to which mosquito larvae and nontarget salt marsh organisms were exposed (Suter 1993). Coastal marshes flood with saltwater during lunar spring tides, initiating development of mosquito larvae in flooded areas of the salt marsh where the water is retained for several days. Several species of nontarget salt marsh organisms also are present during this period, raising concern about exposure to temephos. A previous study of temephos distribution in an intertidal mangrove community found rapid reduction of temephos in ambient water due to tidal flushing, although residues were observed to persist in mangrove leaves and oysters (Pierce et al. 1989). The study presented here investigated the potential for longer-term persistence in salt marsh area that remained flooded for several days, which might provide greater exposure to nontarget salt marsh organisms.

MATERIALS AND METHODS

Study sites: Temephos applications were investigated in the St. Jude salt marsh, a mangrove-fringed salt marsh community at the southern end of Pine Island in Lee County, FL. The lower marsh mangrove fringe area routinely experiences diurnal tidal flooding and ebbing. The middle marsh area experiences diurnal tidal flooding only during bimonthly lunar spring tides. Because the mid-marsh is dry at low tide, an artificial tidal pool (25 cm deep by 1 m^2) was prepared from which water samples could be collected during low tide. The upper marsh remains flooded (15 to 25 cm depth) for several days during lunar spring tides and following heavy rain, providing stagnant water conducive for mosquito larvae development. After the lunar spring tides have subsided, the upper marsh becomes dry, unable to sustain aquatic life.

A salt marsh control area was established adjacent to the test area, in a section of the marsh not receiving the aerial application of Abate. Samples of the same type of matrices were collected and analyzed from both the test and control areas during each application episode.

Larvicide applications: Investigations were carried out during routine larvicide applications by the Lee County Mosquito Control District (MCD) when salt marsh mosquito larvae were present and treatment was required. Temephos was applied by helicopter as Abate 4-E (43% AI, American Cyanamid Co.) at the calibrated rate of 1 fl. oz. Abate 4-E/acre (theoretical temephos deposition rate of 3,200 μg/m^2) from 1988 through 1991, and subsequently at 0.5 fl. oz. Abate 4-E/acre (1,600 μg/m^2) for applications during 1992 and 1993. Each test was considered valid only if adequate mosquito larvae mortality was observed by the Lee County MCD field inspectors.

Eighteen field larvicide applications were monitored over a 6-year period (1988–93) at the St. Jude salt marsh. Three applications were monitored in 1988 (August 27, September 10, and October 28). Samples of water, mangrove tree leaves (*Rhizophora mangle, Langularia racemosa* and *Arecennia germinans*), fiddler crabs (*Uca rapax*), ribbed mussels (*Geukensia* sp.) and (sediment/leaf litter were collected from 3 sites before and at 1, 2, 4, 24, 48, and 96 hours after applications for the first 2 episodes. Following the third application (October 28) samples were collected before and at 1, 2, 4, 7, 48, 72, 120, and 168 hours after application.

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Table 1. Temephos concentrations in salt marsh samples: 1988 Abate 4-E applications, 1 fl. oz. temephos/acre (3,200 μg/m²).

			Sample type		
	Application date	Time (h)	Filter (μg/m²)	Water (μg/liter)	Mangrove leaves (μg/m²)
Std. rec.[2]			103 ± 18	95 ± 2	95 ± 10
LOD[4]			1.0	0.05	5
	August 27	Preapplication	<[5]	<	<
		1	2,315 ± 1,800	25 ± 14	916 ± 275
		4		9 ± 3	1,294 ± 525
		24		<	215 ± 144
		48		<	276 ± 106
		96		<	218 ± 100
	September 10	Preapplication	<	<	<
		1	462 ± 154	14 ± 14	527 ± 100
		4	—	1.6 ± 1.6	—
		24	—	<	448 ± 356
		48	—	0.1 ± 0.1	95 ± 14
		96	—	<	127 ± 77
	October 28	Preapplication	<	<	
		1	2,678 ± 1,806	38 ± 14	541 ± 144
		2	—	29 ± 5	545 ± 236
		4	—	4 ± 3	712 ± 474
		7	—	3 ± 3	622 ± 105
		24	—	<	629 ± 275
		72	—	<	578 ± 134
		120	—	<	342 ± 38
		168	—	1 ± 2	266 ± 90

[1] Rounded to nearest 0.1.
[2] % recovery of standard temephos added with CPS to environmental samples, $\bar{x} \pm SD$, $n = 3$.
[3] Not analyzed.
[4] = Lower limit of detection for temephos in each matrix.
[5] < = Less than lower limit of detection.

Studies during 1989 focused on changes in temephos concentration over the first 24-hour tidal cycle after each application, in conjunction with field toxicity studies for crab larvae. Results of the toxicity tests are being prepared for presentation under separate publication. Three field larviciding applications were monitored (July 21, August 18, and September 14), collecting filter pads, bulk water, and mangrove leaves during all applications. Representative mussels, fiddler crabs, mangrove tree crabs (*Aratus pesonii*), snails (*Melampus coffeus*), and sheepshead minnows (*Cyprinodon variegatus*) were also collected for residue analyses during the 2nd and 3rd applications.

Two applications were studied during 1990. During the first (August 7, 1990) composite water samples were collected, whereas during the second (September 9, 1990) both surface water microlayer and bulk water samples were collected. This information was used to assess the EEC and persistence of temephos in the bulk water relative to temephos concentrations at the water surface microlayer. Representative mosquito fish, marsh fiddler crabs, and snails were also collected to determined the temephos body burdens so as to provide replication for the 1989 studies.

Field applications during 1991 and 1992 focused primarily on temephos distribution and persistence in the water in conjunction with *in situ* toxicity tests for crab and mosquito larvae. Temephos concentrations in adult fiddler crabs also were investigated in 1991 to assess internal bioaccumulation through ingestion relative to adsorption on external surfaces. To reduce the concentration of temephos in the salt marsh, the Lee County MCD reduced the rate of Abate 4-E application from 1 fl. oz./acre to 0.5 fl. oz./acre starting with the 1992 larviciding operations. Sites were established in the upper, middle, and lower marsh to provide information on the spatial distribution and persistence of temephos relative to the distribution of mosquito and nontarget larvae.

During the final year of this project, 1993, selective applications of Abate 4-E to specific areas within the salt marsh were investigated. The first application (September 9) was to the upper and middle marsh areas. This was to monitor temephos distribution within and between marsh areas, and to determine the EEC of temephos in marsh water to which Abate 4-E was directly applied. The second application (September 17) was restricted to the upper marsh only, to investigate the EEC of temephos

Table 1. Extended.

Sample type			
Leaf litter[1] (μg/g)	Sediment (μg/g)	Ribbed mussel (μg/g)	Fiddler crabs[1] (μg/g)
83 ± 9	—[3]	89 ± 17	79 ± 10
0.1	0.1	0.1	0.1
<[2]	—	<	<
0.1 ± 0.1	—	<	0.3 ± 0.1
0.5 ± 0.6	—	<	0.4 ± 0.1
1.8 ± 0.3	—	<	0.2 ± 0.1
0.9 ± 0.6	—	0.2 ± 0.4	0.2 ± 0.1
1.2 ± 0.1	—	<	0.1 ± 0.1
<	—	<	<
1.7 ± 0.9	—	0.3 ± 0.4	0.3 ± 0.3
2.0 ± 1.4	—	<	0.5 ± 0.2
1.0 ± 0.6	—	<	0.5 ± 0.3
<	—	<	0.6 ± 0.3
<	—	<	0.4 ± 0.1
<		<	<
—	—	—	—
—	—	—	0.4 ± 0.3
—	0.1 ± 0.02	—	0.6 ± 0.3
—	—	—	—
—	0.2 ± 0.1	—	0.1 ± 0.1
—	0.1 ± 0.01	—	<
—	0.1 ± 0.02	—	<
—	0.1 ± 0.03	—	<

transported from the upper to the middle and lower marsh areas in an attempt to assess the role of application conditions on temephos distribution and persistence in the different areas of the marsh.

Temephos residue monitoring: Various matrices were monitored for temephos concentration during the first 3 years of the study (1988–90), including water, sediment and leaf litter, adult fiddler crabs, adult mangrove tree crabs, ribbed mussel, coffee bean snail, sheepshead minnow, and mangrove tree leaves. The last 3 years (1991–93) focused primarily on temephos concentrations in salt marsh water as a function of different environmental and application conditions.

The amount of temephos deposited to the marsh surface was monitored by collecting aerial fallout on glass-fiber filter pads placed on styrofoam floats at the water or ground surface. Glass-fiber filter pads were set out before application and retrieved 1 h after application. Each filter was placed in a glass jar and an internal standard, chlorophenol sulfone (CPS, ChemService, West Chester, PA) was added, followed by 100 ml of dichloromethane (DCM, B&J, Muskegan, MI), to initiate extraction and to halt microbial action. The samples were stored on ice and returned to the lab for processing. Water quality parameters (salinity, dissolved oxygen, pH, and temperature) also were monitored.

Of primary concern for nontarget exposure was determination of the concentration and persistence of temephos in the salt marsh water. During the first 2 years (1988–89), 1-liter water samples were collected at 5 cm depth by submersing a glass bottle into the water 1 h before and at various intervals ranging from 1 to 168 h after the application. Because the intertidal portions of the marsh had no standing water during low tide (as opposed to the flooded upper portion of the marsh), a 25-cm-deep by 1-m^2 pool was dug in the marsh floor, from which water samples could be collected as described above.

In subsequent years (1990–93) a distinction was made between the water surface microlayer and bulk water collected at mid-depth. Water surface microlayer samples were obtained by submersing a 0.1-m^2 aluminum screen and raising it horizontally back through the surface, collecting the surface microlayer (*ca.* 0.5 mm) by capillary action on the screen. Approximately 50 ml was obtained from each dip by tilting the screen to one corner and collecting the water in a glass bottle. The mid-depth water was collected through a plastic tube by hand-operated vacuum pump into a 1-liter glass bottle. Water samples were initially treated in the field by the addition of internal standard (CPS) followed by 100 ml DCM; the samples were stored on ice and returned to the lab for processing.

Mangrove leaves were collected as sets of 20 leaves placed into a glass jar. An aliquot of internal standard (CPS) was then added to the leaves, which were then rinsed in 100 ml DCM and removed, to obtain surface temephos while minimizing extraction of leaf waxes. The samples were stored on ice and returned to the lab for processing.

Sediment and leaf litter were collected from the top 0.5 cm of the salt marsh floor, placed in a glass jar, and stored on ice during transport to the lab for processing. Samples of crabs, snails, and mussels were collected by hand (wearing latex gloves), placed into plastic bags, and stored on ice during transport to the lab for processing.

Temephos analysis: Filters were placed in Soxhlet extraction apparatuses (along with the initial DCM) and extracted with DCM for 12 hours. The DCM was evaporated from the extract using a rotary evaporation apparatus, and the residue was dissolved in methanol for HPLC analysis.

Water samples containing DCM were filtered through glass wool (to remove debris) into glass separatory funnels. Temephos was recovered by liquid/liquid extracted into DCM. The DCM was evaporated and the residue was dissolved in methanol for HPLC analysis as described above.

Sediment and leaf litter samples were weighed (*ca.* 20 g) into a Soxhlet thimble. Internal standard (CPS) was added and the samples were extracted with methanol for 4 hours and then with DCM until no more color emerged from the sample. The methanol/DCM extracts were combined in a separatory funnel and the methanol was separated by the addition of water. The DCM (bottom) layer was re-

Table 2. Temephos concentrations in salt marsh samples: 1989 Abate 4-E applications.

			Sample type	
	Application date	Time (h)	Filters (μg/g)	Water (μg/liter)
Std. rec.[1]			99 ± 7%	103 ± 10%
	July 21	Preapplication	<[3]	<
		1	1,585±	23 ± 20
		3	690	6 ± 6
		24	—	<
	August 18	Preapplication	<	<
		1	3,345±	57 ± 45
		2	2,100	38 ± 51
		4	—	10 ± 9
		8	—	2 ± 0.1
		24	—	<
	September 14	Preapplication	<	<
		1a	1,618 ± 1,240	8 ± 12
		1b	2,817 ± 1,640	—
		3	—	2 ± 1
		6	—	1 ± 0.5
		24	—	1 ± 1

[1] % recovery of temephos added to environmental samples, $\bar{x} \pm SD$, $n = 6$; fish $n = 1$.
[2] Whole body of crab.
[3] Less than lower limit of detection (See Table 1 for LOD).
[4] Not analyzed.
[5] Visc = Crab viscera; Exo = Crab exoskeleton.

covered, evaporated, and brought up in 1 ml hexane for silica column clean-up as follows:

1. column: 3 g silica (Davidson 923, Aldrich, Milwaukee, WI) 20% deactivated and 2 g dry sodium sulfate (Mallinckrodt, Paris, KY) in a jumbo pipett with glass wool plug;
2. rinse column with 10 ml 20% diethyl ether in hexane followed by 30 ml hexane;
3. add sample in 1 ml hexane, wash into column with 1 ml hexane and elute column with 20 ml hexane to remove pigments and other interfering lipoidal compounds, discard eluant, elute temephos with 12 ml of 20% diethyl ether in hexane; and
4. evaporate hexane, dissolve residue in 1 ml methanol and filter through 0.2 μm Gelma PVDF Acrodisc® for HPLC analysis.

The DCM rinse of mangrove leaves was filtered through glass wool to remove debris, evaporated to 1 ml volume and prepared for HPLC analysis as described above for sediment.

Crabs were either extracted whole or divided into exoskeleton and viscera samples. Sets of 5 crabs each were processed by homogenization (Virtis Tissue Homogenizer, Virtis Co., Gardinier, NY) in DCM, followed by maceration using an ultrasonic probe (Sonics and Materials, Danberry, CT). The DCM was evaporated and the residue was dissolved in hexane for silica column clean-up and HPLC analysis as described above for sediment.

Whole snails were crushed in sets of 10, internal standard and 100 ml DCM were added, and the entire sample was extracted and processed as described above for crabs.

Sets of 3–5 mussels were shucked to obtain about 5 g wet weight and the internal standard, CPS, was added. About 15 g of sodium sulfate was added to form a fryable powder and the mixture was homogenized in DCM. The sample was filtered through a glass-fiber filter and re-extracted with DCM, and the extracts were processed as above for the sediment extracts.

Quantitative and qualitative analyses were performed with a Varian 5000 high-performance liquid chromatograph (HPLC) (Varian Instruments, Sugar Land, TX) with a 25-cm by 4.6-mm DB-5, C-18 reverse phase column (Burdik and Jackson, Muskegon, MI), methanol/water, 85/15 mobile phase at a flow rate of 1.0 ml/min. Detection of temephos was by ultraviolet absorption at 254 nm, compared to internal as well as external standards.

Quality assurance program: A strict quality assurance/quality control program was maintained to assure accuracy, precision, and completeness of sample analyses and data integrity. Extraction and analysis procedures for each environmental matrix studied were verified by the addition and recovery

Table 2. Extended.

Sample type							
	Crabs						
	Uca (μg/g dry wt.)		*Aratus* (μg/g dry wt.)				
Leaves (μg/m²)					Mussels (μg/g)	Snails (μg/g)	Fish (μg/g)
118 ± 27%	103 ± 15%[2]		106 ± 24%[2]		105 ± 7%	108 ± 26%	61%
	Visc[5]	Exo[5]	Visc	Exo			
—[4]	<	<	—	—	—	—	—
787 ± 270	<	<					
901 ± 455	—	—					
478 ± 302	<	<					
<	<	<	<	<	—	—	—
1,140 ± 566	—	—	—	—			
1,114 ± 220	—	—	—	—			
1,173 ± 460	1.7	1.2	<	<			
996 ± 260	—	—	—	—			
952 ± 78	3.1	<	<	<			
<	4.9	<	<	<	<	0.3	<
1,558 ± 894	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	<	0.3	1.1	1.2	2.5	<	2.7
—	1.1	<	<	<	0.4	<	11.5

Table 3. Temephos concentrations in salt marsh samples: 1990 Abate 4-E applications, 1 fl. oz. temephos/acre (3,200 μg/m²): A—August 7, 1990; B—September 7, 1990.

A. August 7, 1990

		Sample application area #1		Sample application area #2[1]
	Time (h)	Water (μg/liter)	Crabs (*Uca*) (μg/g)	Water (μg/liter)
Std. rec.[2]		109 ± 1%	90 ± 10%	—[3]
	Preapplication	0.1 ± 0.05	0.14 ± 0.01	<[4]
	1	24 ± 2	—	18 ± 7
	3	2.6 ± 2	—	2.4 ± 1.8
	6	2.5 ± 1	0.15 ± 0.01	0.5 ± 0.1
	24	0.7 ± 0.8	0.17 ± 0.02	0.7 ± 0.4

B. September 7, 1990

		Sample application area #1		
	Time (h)	Filter (μg/m²)	(Water μg/liter) Surface	Mid-depth
	Preapplication	<	<	<
	1	6,445 ± 370	60 ± 8	7 ± 2
	2		11 ± 1	—
	4		5 ± 0.3	2 ± 2
	24		0.8 ± 0.1	—

[1] Sample application area #2 did not receive a temephos treatment.
[2] % recovery of temephos added to environmental samples, $\bar{x} \pm SD$, $n = 3$.
[3] Not analyzed.
[4] < = Less than lower limit of detection (See Table 1 for LOD).

Table 4. Temephos concentrations in salt marsh water and crab samples: summary of five 1991 Abate 4-E Applications, 1 fl. oz. temephos/acre (1,600 μg/m²), $\bar{x} \pm SD$, $n = 6$.

Time (h)	Sample type and application area		
	Upper marsh (open, flooded)		
		Water	
	Filter	Surface	Mid-depth
Std. rec.[3]		100 ± 10%	
Preapplication	<[5]	<	<
1	3,200 ± 1,000	403 ± 258	13 ± 16
4	—	71 ± 42	2 ± 1
8	—	103	0.4 ± 0.2
24	—	57 ± 110	0.6 ± 0.7
96	—	<	—

[1] Crab internal organs.
[2] Crab external surface.
[3] % recovery of temephos added to environmental samples, $\bar{x} \pm SD$, $n = 5$.
[4] Not analyzed.
[5] < = Less than lower limit of detection (See Table 1 for LOD).

of temephos standards from each sample matrix. A surrogate internal standard (CPS) was added to each sample for verification of quantitative and qualitative analyses. Field blanks and reagent blanks were obtained with each set of samples collected. Analytical instruments were calibrated daily, with periodic checks for linearity with external standards. All solvents were HPLC grade (B&J, Muskegon, MI). Temephos and CPS standards were obtained from ChemService, West Chester, PA.

The precision and accuracy of temephos extraction and analysis was established separately for each environmental matrix sampled. Known concentrations of temephos, along with the internal standard CPS, were added to field samples of each matrix prior to initiation of the study, and to a set of samples from the control site for each application.

RESULTS AND DISCUSSION

The first year of the study focused on distribution and persistence of temephos in representative components of the salt marsh community including mangrove tree canopy, leaf litter at the marsh surface, salt marsh water, and 2 nontarget invertebrates, the marsh fiddler crab and ribbed mussel. Results of the 3 applications of Abate 4-E monitored during 1988 are given in Table 1. The precision and accuracy of the extraction method is indicated as "Std. rec.," and the lower limit of detection (LOD) for temephos in each environmental matrix is given in Table 1. The standard recovery results show that use of CPS internal standard provided sufficient recovery of temephos such that results were not normalized for percent recovery. The LOD was verified periodically and the concentrations reported in Table 1 remained consistent throughout the study.

Field concentrations are reported as the mean and standard deviation of concentrations from 3 different sites within the application area (Table 1). Temephos accumulation on filter pads was less than 20% of the calculated (theoretical) application rate following the September 10, 1988 application. The subsequent application (October 10, 1988) was greatly improved with an average 84% of the calculated delivery rate. The difference in temephos concentrations collected on the filter pads reflects different depositional rates of temephos to the salt marsh surface, which could be caused by differences in wind patterns, application flight paths, equipment calibration, or larvicide formulation.

Water samples from the different application dates contained similar amounts of temephos with an average of 26 μg/liter at 1 hour after application, diminishing to below detectable limits within 24 hours. Mangrove leaves also exhibited similar amounts of temephos for all 3 applications, showing that temephos persisted on mangrove leaves, a possible source for exposure to mangrove tree crabs. The amount of temephos found in leaf litter and fiddler crabs was similar, suggesting adsorption to the crabs' external surfaces. Composite samples of ribbed mussels contained no detectable amounts of temephos, except in 1 out of the 3 samples collected at 24 hours.

Three applications also were monitored during 1989 and the results, shown in Table 2, are consistent with those from 1988 for water, mangrove leaves, and aerial deposition on filter pads, indicating consistent exposure concentrations in the salt marsh in subsequent years. Representative salt marsh invertebrates (fiddler crab, mangrove tree crab, ribbed mussel, and coffee bean snail) as well as a salt marsh fish (sheepshead minnow) also were collected to observe temephos bioaccumulation. Of the 2 crab species monitored, only the fiddler crab exhibited temephos accumulation after both the 2nd and 3rd applications. The tree crab did not contain

Table 4. Extended.

Sample type and application area				
Middle marsh (mangroves, intertidal)				
	Water		Crabs	
Filter	Surface	Mid-depth	Int[1]	Ext[2]
	—[4]		71 ± 3%	98 ± 3%
<	<	<	<	<
2,146 ± 2,082	263 ± 368	10 ± 2	0.3	<
—	43 ± 32	1.7 ± 1.6	3.2	0.1
—	14	<	—	—
—	6 ± 7	1 ± 1.6	0.6 ± 0.4	<
<	<	<	0.3	<

detectable levels after one application and was found to have low levels 6 hours after the 3rd application, but none at 24 hours. The internal organs of the crabs were extracted separately from the exoskeleton, and the results indicated internal accumulation as well as external adsorption. Mussels collected from the intertidal marsh area contained low concentrations, whereas snails did not contain detectable levels of temephos through 24 hours exposure. Fish collected from the static, flooded marsh area had the highest concentration of temephos at 24 hours after application.

This difference in temephos body burdens in salt marsh organisms may result from behavior differences in species considered. The snails graze at low tide, climbing tree trunks to avoid the rising tide, thus reducing exposure to temephos in the water. Mussels filter minute organisms from the intertidal water, remaining dormant during low tide; thus, the potential for temephos exposure varied with time and position of application. Fish living in the flooded upper marsh, however, were continuously exposed and fed upon organisms exposed to temephos concentrations in the water.

The 1990 studies focused initially on temephos concentrations in water and fiddler crabs over the first 24 hours after application. During the first application (August 7, 1990), the intended no-application (control) area also was treated with temephos, so this area was used as a second application study site. These results (shown in Table 3) were similar to those of previous years except that preapplication samples exhibited temephos residues, reflecting contamination from a mosquito larviciding application that occurred the previous week. Temephos concentration in fiddler crabs was consistent from pre- through 24 hours after application, indicating possible steady-state accumulation with environmental concentrations. No distinction was made between internal and external accumulation for this application study.

The second application (September 7, 1990) was designed to observe the distribution of temephos within the water column, distinguishing the surface microlayer from mid-depth bulk water. During this application, the control site did not receive any Abate 4-E, providing a valid control sampling area. No temephos was detected in any of the control site samples. Results (shown in Table 3) show a considerable difference in the amount of temephos at the surface microlayer relative to that in bulk water. This is a significant finding regarding temephos concentrations available to mosquito larvae that come in contact with the water surface relative to other aquatic invertebrates that generally stay within the bulk water column. Recognition of the high concentrations at the water surface microlayer prompted redesign of sampling protocol to provide accurate assessment of temephos concentrations in the bulk water.

Temephos application studies in 1991 focused on the distribution and persistence in the salt marsh water column and different areas of the salt marsh. A summary of results from 5 applications is given in Table 4, showing temephos in surface water and mid-depth water in the flooded (upper) marsh and the intertidal (middle) marsh areas. Filter pads reflect the amount of temephos reaching the mangrove floor and water surface in both the open flooded marsh and the mangrove-canopied middle marsh areas. The difference in temephos concentrations on the surface vs. bulk water and the subsequent longer persistence at the surface microlayer are evident from these samples. Fiddler crabs also were collected from the intertidal middle marsh area to distinguish between internal accumulation vs. external adsorption of temephos in crabs. These results show that the fiddler crabs did accumulate the temephos internally, and that concentrations persisted through 96 hours after application. Surface water samples in the upper marsh exhibited higher concentrations of temephos than did surface water from the middle marsh area; however, no significant difference in bulk water concentrations was exhibited for upper vs. middle marsh water through 24 hours after application. Therefore, even with tidal flushing of the middle marsh, nontarget organisms in bulk water were exposed to about the same

Table 5. Temephos concentrations in salt marsh water samples, μg/liter: summary of three 1992 Abate 4-E applications, 0.5 fl. oz. temephos/acre (1,600 μg/m²).

	Lower marsh intertidal		Middle marsh intertidal		Upper marsh static water		Standard recovery %[1]
Filter (1 h)	530 ± 417 (n = 3)		900 ± 400 (n = 7)		1,660 ± 560 (n = 4)		89 ± 4
Water samples	Surface	Mid-	Surface	Mid-	Surface	Mid-	108 ± 11
	(n = 3)		(n = 10)		(n = 6)		
Preapplication	<[2]	<	<	<	<	<	
Application (low tide)							
1 h	5 ± 3	0.2 ± 0.1	50 ± 30	3 ± 2	76 ± 35	5 ± 3	
3 h	7 ± 3	<	26 ± 18	1 ± 1	28 ± 12	3 ± 2	
High tide							
6 h	3 ± 3	0.1 ± 0.1	16 ± 16	0.3 ± 0.3	11 ± 2	0.7 ± 0.3	
24 h	<	<	4 ± 4	0.1 ± 0.1[3]	<	<	
48 h	<	<	<	<	<	<	
96 h	<	<	<	<	<	<	

[1] % recovery of temephos added to environmental samples, $\bar{x}$ ± SD, n = 6.
[2] < = Less than lower limit of detection (See Table 1 for LOD).
[3] Temephos detected in 1 out of 10 samples at 24 hours.

temephos concentrations in both upper and middle marsh areas, averaging about 12 μg/liter at 1 hour and 0.8 μg/liter at 24 hours after application. An important distinction, however, is that invertebrate larvae and other zooplankton are carried out of the mid-marsh into the adjacent estuary with each ebb tide, while zooplankton in the upper marsh remain in the static water until it dries up.

The final 2 years of the investigation (1992–93) focused on the distribution and persistence of temephos in 3 different areas of the salt marsh, operationally defined as the upper marsh (static water, flooded area), the middle marsh (intertidal during lunar high tides), and the lower marsh (daily tidal influence). This was done to compare intensity and duration of temephos exposure to susceptible nontarget organisms inhabiting the different marsh areas during larvicide applications. To reduce the amount of environmental exposure from temephos, the Lee County MCD reduced the rate of Abate 4-E application to the salt marsh from 1 fl. oz. temephos/acre to 0.5 fl. oz./acre. A summary of results from 3 applications in 1992 is given in Table 5. These data show the anticipated reduction in environmental concentrations of temephos in water (10–13 μg/liter reduced to 3–5 μg/liter) resulting from the reduction in application rate from 1 to 0.5 fl. oz./acre, respectively. Adequate mosquito larvae mortality was still attained with the lower dose, but over a longer period of time (6 hours as opposed to 3 hours for the higher rate). The results of temephos in surface vs. mid-depth water show that nontarget organisms in the upper and middle marsh areas were exposed to similar concentrations throughout the first tidal cycle, with no detectable levels remaining in most samples at 24 hours after application, even in the static, flooded upper marsh area. Organisms in the lower marsh area were exposed to relatively very low concentrations of temephos.

Results of the final year of the St. Jude salt marsh study (1993) are given in Table 6, showing the distribution of temephos resulting from applications to specific areas of the marsh. Replicating the 1992 applications over both middle and upper marsh areas resulted in temephos concentrations in the middle marsh bulk water similar to that observed in the 1992 study. When application was restricted to the upper (flooded) marsh only, temephos residues in the intertidal middle and lower marsh areas were below detectable levels, greatly reducing the environmental concentration to which intertidal, nontarget organisms were exposed. Organisms in the upper flooded marsh area, however, were exposed to 4 ± 3 μg/liter in the bulk water at 1 hour after application, reducing to about 1 μg/liter within 5 hours.

Temephos persistence in aquatic environments is affected by the rate of degradation (hydrolysis, photo and microbial), evaporation, dissolution, dispersion, and adsorption to suspended particles, sediments, and other surfaces such as plants (Fogash 1976, Hughes et al. 1980, Opong-Mensah 1984). Previous studies of temephos persistence in freshwater ponds indicated similar concentrations in water 1 hour after application; however, the temephos did not diminish as rapidly in the freshwater ponds (Lores et al. 1985). Applications of Abate 4-E (1 fl. oz./acre) exhibited 30 and 10 μg/liter 1 h after application, and 6 and 3 μg/liter at 24 hours. Applications of Abate 4-E designed to provide 10 μg/liter in artificial freshwater ponds resulted in 9 and

Table 6. Temephos distribution in salt march water samples: 1993 Abate 4-E applications, 0.5 fl. oz. temephos/acre (1,600 μg/m²).

Date	Water sample collection marsh areas					
	Lower marsh		Middle marsh		Upper marsh	
	Surface	Mid-depth	Surface	Mid-depth	Surface	Mid-depth
	Mid- and upper marsh					
September 2						
Preapplication	<[1]	<	<	<	—[2]	—
1 h (high tide)	<	<	253 ± 103	6.5 ± 3.2	—	—
5 h	<	<	16 ± 5	1.4 ± 0.4	—	—
	Upper marsh only					
September 17						
Preapplication	<	<	<	<	<	<
1 h (high tide)	<	<	<	<	90 ± 30	4 ± 3
5 h	<	<	<	<	24 ± 7	1.2 ± 0.2

[1] < = Less than lower limit of detection (See Table 1 for LOD).

6 μg/liter at 30 min, with 1.2 and 2.5 μg/liter at 24 hours (Hughes et al. 1980). Temephos concentrations in ponds following 3 monthly applications of Abate 4-E at the normal application rate (18 g/ha), and 10× normal (180 g/ha), 1 h after application ranged from 0.2 to 0.8 μg/liter and from 4.9 to 8.5 μg/liter for the normal and 10× rate of application respectively (Sanders et al. 1981).

Previous studies of temephos in estuarine and salt marsh environments have revealed about the same concentrations and persistence observed in freshwater pools and ponds; however, areas of tidal influence exhibited more rapid reduction in temephos concentrations (Henry et al. 1971, Fogash 1976, Pierce et al. 1989). As observed in the present study, previous studies indicated that tidal movement of the water rapidly decreased temephos concentrations in the application area.

CONCLUSIONS

Temephos concentrations at the surface water microlayer were found to be much higher than the amounts within the bulk water column following aerial applications of Abate 4-E. The expected environmental concentration (EEC) of temephos at the surface microlayer was 333 ± 90 μg/liter for applications of 1 fl. oz./acre and 120 ± 96 μg/liter for 0.5 fl. oz./acre. The EEC within the bulk water column was 12 ± 10 μg/liter for 1 fl. oz./acre, and 4 ± 3 μg/liter for 0.5 fl. oz./acre. Although concentrations varied among applications and sites, temephos concentrations in the mid-depth bulk water generally diminished to less than 1 μg/liter within 6 hours after application, and to less than 0.1 μg/liter within 24 hours. Selective application to specific areas of the salt marsh provided control of the amount of temephos within those areas, allowing for selective control of mosquito larvae while reducing temephos exposure to nontarget organisms in other areas of the marsh.

Of the salt marsh inhabitants investigated, the sheepshead minnow (*Cyprinodon variegatus*), inhabiting the flooded upper marsh exhibited the highest accumulation of temephos through 24 hours after application. Mussels (*Geukensia* sp.) within the mid- to lower marsh contained low levels, as did adult fiddler crabs (*Uca rapax*), which exhibited internal accumulation as well as external adsorption of temephos. Coffee bean snails (*Melampus coffeus*), did not accumulate detectable levels of temephos for up to 24 hours after application, probably resulting from their avoidance of water by climbing trees during high tide.

Leaves of the mangrove trees were found to contain high levels of temephos for up to 7 days after application, providing a reservoir for exposure to invertebrates inhabiting and feeding on the mangrove canopy. However, mangrove tree crabs inhabiting the mangrove canopy did not exhibit persistent accumulation of temephos. Leaf liter and associated sediment from the salt marsh floor exhibited low yet consistent levels of temephos throughout a 96-hour monitoring period. Similar concentrations of temephos associated with fiddler crabs throughout the 96-hour period would suggest steady-state interaction of crabs with the contaminated leaf litter/sediment environment, resulting from contact absorption onto outer surfaces and ingestion of temephos associated with food particles.

Temephos concentrations varied drastically from surface water to bulk water and from one area of the salt marsh to another, showing the importance of selective sampling techniques for accurate assessment of the EEC to which nontarget organisms may be exposed. The next step in this study is the comparison of the EEC with toxicity of temephos to nontarget salt marsh organisms to estimate the

environmental hazard associated with larvicide applications to salt marsh communities.

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