FATE AND TOXICITY OF TEMEPHOS APPLIED TO AN INTERTIDAL MANGROVE COMMUNITY

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ABSTRACT. The distribution, persistence, and toxicity of the mosquito larvicide temephos was monitored following aerial applications to an intertidal mangrove community in Lee County, Florida. The amount of temephos penetrating to the mangrove floor ranged from 15 to 70% of the amount entering the upper leaf canopy, with 50–60% of that applied remaining on the mangrove leaves. Rainfall caused an additional influx of temephos from the leaves to the mangrove floor. Residues were detected in intertidal water at 2 h, but not 4 h after application. However, temephos was observed to persist in simulated tidal pools and on mangrove leaves for up to 72 h and in oysters for up to 48 h after application. Marine organisms placed in cages at 3 test sites and a control site were monitored for toxic effects. Mortality among natural mosquito larvae was simultaneously monitored. Mysids (*Mysidopsis bahia*)exhibited a significant mortality at one site during 1 of 3 applications monitored; however, no correlation was observed between mortality and temephos concentration in water. No significant mortality was observed for the other organisms, which included: brown shrimp (*Panaeus aztecus*), grass shrimp (*Palaemonetes pugio*), juvenile snook (*Centropomis undecimalis*) and sheepshead minnow (*Cyprinodon variegatus*).

INTRODUCTION

This investigation was undertaken to observe the distribution and persistence of the mosquito larvicide, temephos, (Abate® 4-E, American Cyanamid, Princeton, NJ) during routine aerial applications to an intertidal mangrove community, and to assess acute toxicity to select estuarine organisms under natural field conditions. Temephos is widely used as a larvicide against pest mosquitoes, including: *Aedes taeniorhynchus* (Wied), *Culex niqrinalpus* Theobald, and *Culex quinquefasciatus* Say (Boike et al. 1985, Lores et al. 1985).

Previous studies of temephos persistence in ponds in south Florida revealed 20–40 μg/liter in the water immediately after aerial application, which diminished to 2–5 μg/liter after 24 h (Lores et al. 1985). Concentrations reported by Sanders et al. (1981) were similar in treated ponds 24 h after application, and Henry et al. (1971) found 26 to 131 μg/liter afterapplications to a saltmarsh. Although well below acute toxicity levels to fish, these concentrations are within levels causing sublethal effects (Gehrke 1988, Sanders et al. 1981). The 48-h toxicity (LC_{50}-48) reported for brown shrimp (*Penaeus aztecus* and the pink shrimp (*Penaeus duorarum*) are 5 μg/liter and 10 μg/liter, respectively (American Cyanamid 1980), raising concern for aquatic invertebrates present in areas where temephos is applied (Christy 1982, Forward and Costlow 1978, Zucker 1978, Ruber and LaFrance 1983, Hughes et al. 1980). Of particular concern is that at normal application rates, temephos has been shown to have a significant effect on fiddler crab populations (Ward and Howes 1974, Ward et al. 1976, Ward and Busch 1976).

Adverse effects of pesticides on nontarget organisms depend not only on the concentration of chemicals applied, but also on persistence and availability to susceptible life stages of the organisms. The intent of this project was to determine the amount of temephos deposited within various components of a mangrove community (leaves, water, sediment, oysters) and the duration of chemical exposure within these matrices resulting from routine mosquito larvicide applications. In addition, specimens of 5 species of fish and crustaceans were held in cages within the intertidal fringes of the mangrove community to assess acute toxicity to representative estuarine organisms inhabiting these regions.

MATERIALS AND METHODS

Study sites: The study area consisted of intertidal red mangrove forests (*Rhizophora mangle*) at the mouth of St. James Creek, in the southeast corner of Pine Island in Lee County, FL. This area contains extensive mangrove and saltmarsh that are routinely treated with Abate® 4-E for control of mosquito larvae by the Lee County Mosquito Control District (MCD). Tidal influence from San Carlos Bay maintains a brackish water environment so that juveniles of estuarine species inhabit this area. Three separate test sites were established approximately 100 m apart within the application area, and a control sampling site was monitored outside of the larvicide application area. A schematic depiction of the various aspects of this study is given in Fig. 1. Mangrove community components investigated for temephos included: mangrove leaves, intertidal water, simulated tide

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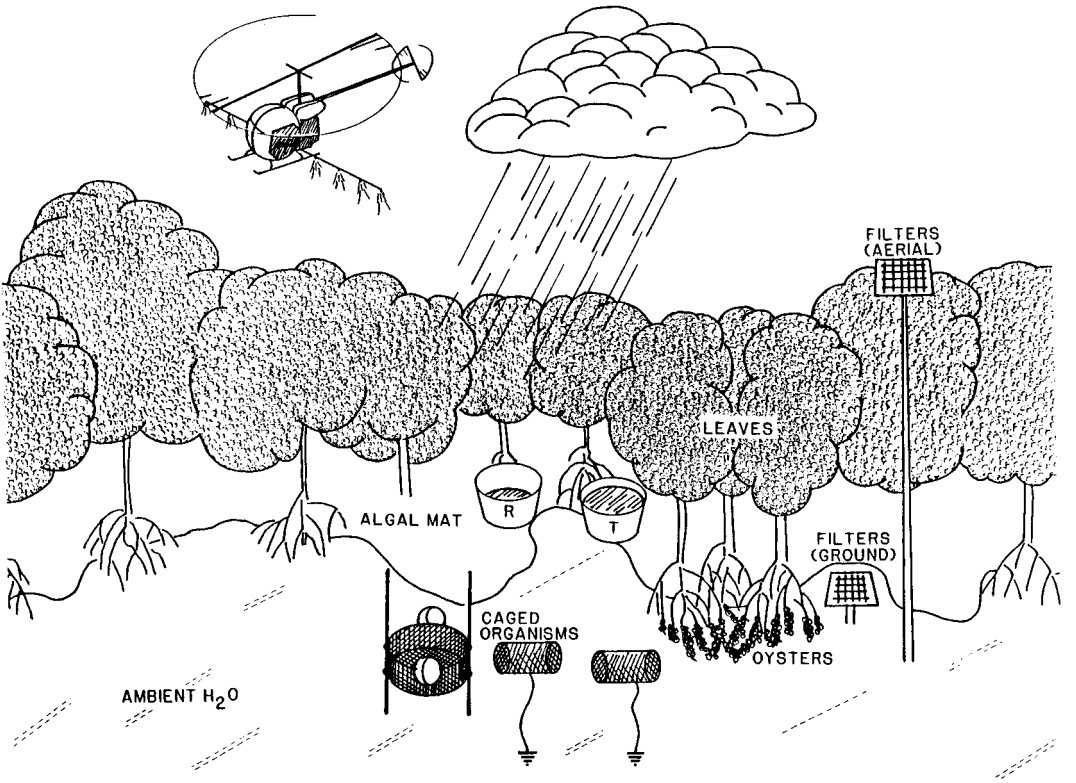

Fig. 1. Schematic of mangrove community components investigated for temephos residue distribution and persistence, and caged organisms for acute toxicity tests. Simulated tidal pool (T), collected rainwater (R).

pool water, rainwater runoff from mangrove leaves, sediment and oysters. The amount of temephos deposited per surface area was assessed by collecting aerial fallout on filter paper deployed at the water surface and at the mangrove canopy level. In addition to monitoring temephos distribution, various estuarine organisms were held in cages to assess acute toxicity; and water quality parameters (pH, DO, T°C, S°/oo) were monitored throughout each study.

Temephos residue monitoring: Temephos was applied as Abate® 4-E (43% active, American Cyanamid Co.) by the Lee County MCD, using a Bell Soloy helicopter. The application rate was 1 fl oz per acre, resulting in a theoretical deposition of 3,200 $\mu g/m^2$.

Three treatment episodes were monitored encompassing 5 temephos applications. Episode 1 encompassed a 148-h period including 2 applications (June 13 and June 17, 1987) with sample collection for temephos residue analysis and toxicity monitoring of caged organisms at intervals of 1 h before application and 1, 6, 24 and 48 h after each application. Although a considerable amount of new information was gained during episode 1 (Pierce et al. 1988), these data are considered preliminary, due to study design changes and methods development, therefore, the results are not presented here.

Results of applications monitored subsequent to episode 1 are reported. Episode 2 was a 24-h period following one temephos application on July 24, 1987, with sample collection and toxicity monitoring at intervals of 1 h before and 1, 3 and 24 h after application. The application occurred during an out-flowing tide. Episode 3 encompassed a 96-h period including 2 temephos applications (September 29 and October 2, 1987), with sample collection and toxicity monitoring at intervals of 1 h before and 1, 2, 4, 7, 24 and 72 h after the September 29 application and 1, 2, 4, 7 and 24 h after the October 2 application. The September 29 application occurred during an out-flowing tide (mangrove floor exposed), and the October 2 application occurred during a high tide (mangrove floor underwater). Episode 3 was designed to investigate the fate and toxicity of temephos during repeat applications 3 days apart.

Temephos surface impact: The amount of temephos impacting surface water under the mangroves was determined by placing duplicate glass fiber filters (Whatman, EPM-2000, 20 × 25 cm, Whatman Ltd., Maidstone, U.K.) on Styrofoam

floats in the water adjacent to caged animals under the mangrove canopy. During the third application episode, the amount of temephos falling onto the upper mangrove canopy also was determined by placing duplicate glass fiber filters on a Styrofoam platform on top of a 7-m length of polyvinyl chloride pipe (Fig. 1).

For both the aerial and the ground filters, samples were collected within 1 h after application, folded up-side-in and placed in a 1-pint glass jar. An internal standard, chlorophenylsulfone (CPS), was added followed by methylene chloride (CH_2Cl_2), HPLC-grade (Burdick and Jackson, Muskegon, MI), to initiate temephos extraction and to preserve the sample. The CPS was chosen as an internal standard due to chemical and physical properties similar to temephos. The samples were stored on ice the for transport to the laboratory for immediate processing.

The glass fiber filters were processed by blending in CH_2Cl_2, followed by filtration. The CH_2Cl_2 was evaporated, replaced with methanol (CH_3OH) and reduced to 1 ml volume for high performance liquid chromatographic (HPLC) analysis.

Mangrove leaves: The amount of temephos adhering to mangrove leaves was investigated during the second and third application episodes to assess the potential impact on herbivores and the influx of temephos to the mangrove floor during subsequent rainstorms. The temephos associated with leaves was determined as a composite of 30 leaves collected randomly from the upper canopy within each test site and from the control site at specific time intervals up to 72 h after application. The average leaf area was 25 cm^2 (1 side), providing 750 cm^2 of leaf area for each study site. The 30 leaves were placed in glass jars, and the internal standard was added. The leaves were then rinsed with CH_2Cl_2 and discarded. The jars containing the CH_2Cl_2 and the temephos residue were placed on ice in the dark for transport to the lab and processing for HPLC analysis, as described above.

Sediment: Surface sediment (top 2 cm) was collected at all designated times as a composite from several places, consisting primarily of the algal mat that covers the mangrove floor. The samples (ca. 50 g) were stored in glass jars and placed on ice in the dark for transport to the lab for processing.

Sediment samples were extracted using a Soxhlet apparatus. Approximately 20 g of wet sediment were placed in the extraction thimble. The internal standard was added and extracted after 4 h with CH_3OH. The CH_3OH was removed, replaced with 100 ml CH_2Cl_2, and extracted until the solvent around the thimble remained clear. The extracts were combined in a 1-liter separatory funnel, and water was added to separate the CH_3OH from the CH_2Cl_2. The CH_2Cl_2 layer (bottom) was collected, and the water/methanol fraction was again extracted with 100 ml of CH_2Cl_2. The CH_2Cl_2 fractions were combined, evaporated, and the residue was redissolved in 1 ml of hexane for subsequent silica column cleanup as follows:

3 g 20% deactivated silica + 2 g sodium sulfate (pack wet in hexane);

wash column with 10 ml of 20% ether in hexane followed by 30 ml hexane;

add sample to column in 1 ml hexane, wash into column with 1 ml hexane;

elute column with 20 ml hexane (f_1) to remove pigment interference;

elute pesticides with 12 ml of 20% ether in hexane (f_2);

evaporate f_2, redissolve residue in 1 ml methanol and filter for HPLC analysis.

Water: Water samples were collected from the top 5 cm of intertidal water during each sampling episode. During episode 3, water also was collected from simulated tidal pools (60-liter galvanized metal tubs) filled with 40 liters of ambient water. Rainwater was also collected after falling through the mangrove leaf canopy into empty 60-liter tubs. The rainwater-collection tubs were placed in the mangroves 1 h after temephos application. A 3-liter water sample was collected at each site in an amber glass jug. The internal standard was immediately added followed by approximately 100 ml of CH_2Cl_2 with vigorous shaking. These jugs were then transported to the lab for prompt extraction and processing as described above.

Oysters: Oysters (*Crassostrea virginica*) were collected at all designated times from the study sites. A composite of at least 12 oysters was taken at each site from the mangrove prop roots at the level of the surface water to assure recent exposure to temephos-containing water. The oysters were rinsed in ambient water, wrapped in aluminum foil, sealed in plastic bags and placed on ice in the dark for prompt transport to the lab.

For temephos extraction, oysters were shucked and homogenized in a 250 ml beaker. A 10-g sample was mixed with about 30 g sodium sulfate to produce a friable powder. To this was added the internal standard followed by approximately 100 ml CH_3OH. The oyster sample was then homogenized using a Virtis tissue homogenizer (Virtis Co., Gardinier, NY) followed by sonication using an ultrasonic probe (Sonics and Materials, Ultrasonic Processor, Danbury, CT). The sample was then filtered by suction through a glass fiber filter, reextracted with 100 ml of CH_2Cl_2 and processed as described above for sediment.

Instrumentation: All analyses were preformed

using a Perkin-Elmer (Norwalk, CT) Sigma 1 data system coupled with a Perkin-Elmer series 3B liquid chromatograph, using a Perkin-Elmer LC-95 uv/vis spectrophotometer detector. Analytical conditions for liquid chromatography were: column, 25 cm × 4.6 mm DB5 C-18 reverse phase (Burdick and Jackson, Muskegon, MI) mobile phase, 80/20, methanol/water; flow rate 1.5 ml/min; range 0.05; detector, UV at 254 nm. Temephos reference standards were obtained from Chem Service (West Chester, PA).

Field toxicity tests:

Test organisms: Five species endemic to shallow estuarine environments were observed for behavior and mortality. These organisms, representing teleost fishes and crustaceans, were as follows: a) mysid (*Mysidopsis bahia*, b) snook (*Centropomis undecimalis*), c) brown shrimp (*Panaeus aztecus*), d) grass shrimp (*Palaemonetes pugio*) and e) sheepshead minnow (*Cyprinodon variegatus*).

Juvenile snook (58 days after hatch), adult sheepshead minnows and adult mysids were obtained from Mote Marine Laboratory cultures. Adult brown shrimp were acquired through a local bait shop, and grass shrimp were caught locally.

The panaeid shrimp were held in cylindrical cages (0.5 m high, 1 m diam) that were floated with 2 Styrofoam floats such that three-fourths of the cage was underwater, as described by Pierce et al. (1988). Two 3.3 m poles were threaded through handles on opposite sides of the cage and hammered into the bottom to allow the cages to move vertically with the height of the tide (Fig. 1).

The mysids, grass shrimp, sheepshead minnows and juvenile snook were held in small cylindrical floating cages (16 cm high × 12 cm diam) that were tethered to weights sunk into the substrate, permitting movement with the tidal flux (Fig. 1).

Natural food was available to the panaeid shrimp. The mysids, sheepshead minnows and grass shrimp were fed freshly hatched *Artemia* nauplii, and the snook were fed a paste of Salmon Moist (Rangen, Inc., Buhl, ID) daily.

Mortality monitoring: In each of the 3 test areas, each species was contained in a separate cage. A duplicate set of the caged organisms was held at the control area with extra individuals of each species available at test sites to replace mortalities if needed during the acclimation period. Natural populations of mosquito larvae were present during each application episode and monitored by Lee County MCD personnel.

Organisms were placed in cages at test and control sites and allowed to acclimate for 72 h. During episode 2 (July 24, 1987) four species of test organisms were monitored. The number of individuals at each site was as follows: *M. bahia* (20 each at sites 1, 2 and control; 5 at site 3); *C. variegatus* (10 each at sites 1, 2 and 3; 20 at control); *P. pugio* (15 each at sites 1, 2 and 3; 14 at control); and *P. aztecus* (20 at each site). Episode 3 (encompassing temephos applications on September 29 and October 2, 1987) used one cage of each test organism at each of the 3 test sites and 2 cages of each at the control site. The organisms tested during episode 3 included: *M. bahia* (19–21 per cage); *C. undecimlais* (9–10 per cage); and *P. aztecus* (17–26 per cage) at test and control sites.

The condition of all test and control organisms was observed 24 h and 1 h prior to temephos application. Any dead were promptly removed and replaced with area-acclimated organisms. Following larvicide application, acute toxicity observations were performed at the designated times by noting all live and dead organisms in each cage, as well as behavior and natural functions. The G-test of independence (Sokal and Rohlf 1981) was used to analyze the field toxicity data to determine survivorship of treated relative to control organisms.

RESULTS

Temephos residues: The accuracy and precision of temephos recovery was verified for all matrices studied by spiking each sample type with a known amount of temephos and the internal standard. Accuracy of temephos content of field samples was ensured by adding the internal standard to each sample prior to extraction. Accuracy and precision of the standard recovery for each matrix is given in Table 1. Data for those matrices exhibiting less than 85% recovery were corrected to 100% recovery (sediment and oysters). Detection limits for each matrix are shown in Table 1. Data from field studies presented in Tables 2 through 4 represent the analysis of one sample from each site at each designated collection time. Samples of each matrix were collected 1 h before each larvicide application to detect any residues from previous applications.

Surface area impact to mangrove leaves and filters: No temephos was found in the filters or mangrove leaves at the control site during any of the application episodes. The amount of temephos recovered during episode 2 from leaves and filter paper under the leaf canopy is shown in Table 2. These data show a greater amount of temephos applied to site 3 (3,100 $\mu g/m^2$) than sites 1 and 2 (690 $\mu g/m^2$ and 650 $\mu g/m^2$, respectively), with the amount penetrating to the ground-level filters ranging from 140 to 115 $\mu g/m^2$ at sites 1 and 2, and 350 $\mu g/m^2$ at site 3.

Table 1. Temephos detection limits and percent recovery from environmental samples.

	Sample matrix				
	Filter $\mu g/m^2$	Leaves $\mu g/m^2$	Water μg/liter	Sediment $\mu g/g$	Oyster $\mu g/g$
Lower limit of detection	0.1	0.1	0.01	0.05	0.05
Replicates (n)	7	6	6	9	8
% recovery ± SE	87 ± 11	89 ± 20	91 ± 3	71 ± 20	64 ± 10

Table 2. Temephos concentrations in mangrove forest components following aerial application: episode 2, July 24, 1987.

Sample collection time (Site =)	Sample: Filter ($\mu g/m^2$) 1	2	3	Leaves ($\mu g/m^2$) 1	2	3
Pre-application	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Application						
1 h	140	115	350	690	650	3,100

Sample collection time (Site =)	Sample: Sediment ($\mu g/g$) 1	2	3	Oysters ($\mu g/g$) 1	2	3
Pre-application	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Application						
1 h	—	—	—	0.1	0.3	<
3 h	0.45	0.06	<	0.3	0.5	0.05
24 h	—	—	—	<	<	<

The amount of temephos collected on aerial and ground filters during episode 3 is shown in Table 3. For the September 29 application the 3 aerial filters contained 2,535 ± 860 $\mu g/m^2$, and ground filters contained 372 ± 125 $\mu g/m^2$, showing about 15% penetration through the leaf canopy. Results of the October 2 application (Table 3) show 2,302 ± 320 $\mu g/m^2$ in aerial filters, and 1,595 ± 590 $\mu g/m^2$ on ground filters, representing an average penetration of 70%.

The persistence of temephos on mangrove leaves was investigated at each site during episode 3 as shown in Table 3. One hour after the September 29 aerial application, the mean temephos concentration among the 3 sites was 1269 ± 466 $\mu g/m^2$. Temephos concentrations on the leaves gradually decreased with time, with a marked decrease between the 7 and 24-h sampling, coinciding with a light rain that occurred between these 2 samplings. After 72 h, the leaves still had an average temephos concentration of about 300 $\mu g/m^2$. This concentration was present on the leaves at the time of the October 2 application. Within 48 h after the October 2 application, the average concentration on leaves from all 3 test sites had diminished from 1,484 ± 90 μ/m^2 (at 1 h) to 1,120 ± 120 $\mu g/m^2$.

Sediment: No temephos was found in sediment at the control site during any of the application episodes. Sediment temephos levels after the July 24 application are given in Table 2 showing no detectable residues when the sediment was underwater (1 h), but some deposition as the tide receded (3 h). Small amounts of temephos also were found in the sediment samples following episode 3 (Table 3). These residues were also detected when the tide was out and after rain, ranging from 0.4 μ/g to 1.2 μ/g.

Ambient water: No temephos was found in control site water at any of the designated sampling times, and no residues were found in water during the episode 2 study. During episode 3, no temephos was detected in water after the first (September 29) application, however, after the subsequent application (October 2) temephos was recovered during the first 2 h but not after 4 h. As shown in Table 4, the concentrations ranged from 0.55 μg/liter to 35.14 μg/liter.

Simulated tidal pools: Simulated tidal pools were used only during episode 3. No temephos was found in simulated tidal pools from the control site. The average amount of temephos in the 3 simulated 40-liter tidal pools 1 h after the September 29 application was 3.5 ± 1.7 $\mu g/$

Table 3. Temephos concentrations in filters, leaves and sediment following aerial larviciding: episode 3, September 29 and October 2, 1987.

Sample collection time (Site =)	Sample[1]								
	Filter			Leaves			Sediment		
	($\mu g/m^2$)			($\mu g/m^2$)			($\mu g/g$)		
	1	2	3	1	2	3	1	2	3
Pre-application	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.05	<0.05	<0.05
Appl. no. 1 (Sept. 29)	Aerial								
1 h	1,315	3,160	3,130	1,397	1,765	645	1.24	1.06	0.47
	Ground								
	195	472	449						
4 h	—	—	—	490	869	257	<	<	<
7 h	—	—	—	500	582	793	<	<	<
Rain									
24 h	—	—	—	291	576	259	0.55	0.38	<
48 h	—	—	—	161	368	106	<	0.72	<
72 h	—	—	—	231	374	273	<	<	<
Appl. no. 2 (Oct. 2)	Aerial								
1 h	1,845	2,515	2,545	1,547	1,546	1,358	<0.05	<0.05	<0.05
	Ground								
	1,630	2,300	855						
4 h	—	—	—	1,915	1,019	1,292	<	<	0.40
7 h	—	—	—	1,959	1,201	1,308	<	<	<
24 h	—	—	—	987	1,272	1,107	0.68	<	0.37

[1] Control sites contained no detectable amounts of temephos.

Table 4. Temephos concentration in water and oysters following aerial larviciding: episode 3, September 29 and October 2, 1987.

Sample collection time (Site =)	Sample								
	Intertidal water[1] (μg/liter)			Simulated tidal pool[1] (μg/liter)			Oysters[1] ($\mu g/g$)		
	1	2	3	1	2	3	1	2	3
Pre-application	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
Appl. no. 1 (Sept. 29)									
1 h	<	<	<	5.39	1.28	3.79	0.38	<	0.32
2 h	<	<	<	3.44	1.50	1.06	—	—	—
4 h	<	<	<	2.84	1.19	2.46	0.53	0.61	0.55
7 h	<	<	<	1.17	0.96	0.68	0.79	0.87	<
Rain									
24 h (Rainwater)[2]	<	<	<	2.30 (17.3)	1.84 (29.0)	1.22 (29.5)	<	2.48	0.53
48 h	<	<	<	0.86	1.26	1.12	<	1.06	<
72 h	<	<	<	1.12	0.90	0.92	<	<	<
Appl. no. 2 (Oct. 2)									
1 h	8.40	0.58	35.14	14.52	7.04	2.94	3.28	2.44	0.99
2 h	16.95	0.55	11.84	—	—	—	—	—	—
4 h	<	<	<	5.84	5.47	—	3.52	4.58	3.21
7 h	<	<	<	3.24	4.04	3.04	2.75	2.87	<
24 h	<	<	<	2.74	1.64	1.34	<	1.71	1.72

[1] No temephos was detected at the control site.
[2] 2.5 μg/liter detected in control site rainwater.

liter (Table 4). This concentration steadily decreased through 7 h. At the 24-h sampling, a slight increase in temephos concentration had occurred due to influx with rainwater falling from mangrove leaves. After the rain, the concentration of temephos continued to decrease to an average value of 1.0 ± 0.1 μg/liter at 72 h.

For the October 2 application, an average of 8.2 ± 4.8 μ/liter of temephos was detected among the 3 sites. At 24 h, the average temephos concentration was reduced to 1.9 ± 0.6 μg/liter.

Rainwater: Rainwater was collected only during episode 3 (September 29 application). A small amount of temephos was recovered from the control site rainwater collection tub (0.8 μg). This was not considered significant relative to the test sites; however, it does suggest contamination warranting further study to evaluate the source. Approximately 300 ml of rainwater was collected in the tubs and had an average concentration of about 25 μg/liter temephos (Table 4) resulting in a total influx to the tub of about 8 μg temephos.

Oysters: No temephos was found in oyster samples taken from the control site during any of the applications. Temephos recovered from oysters during episode 2 is shown in Table 2. These results show temephos accumulation between 1 h and 3 h after application, with none detected after 24 h. Temephos was detectable in oysters within the first hour following both applications during episode 3. Concentrations rose to a maximum at 24 h after the September 29 application and between the 4 and 7 h after the October 2 application (Table 4) and then gradually decreased. Temephos was found in only one sample at 48 h with no temephos detected after 72 h.

Temephos concentrations during the October 2 application were consistently higher than the September 29 application. Oysters taken 1 h following the September 29 application showed about 0.35 ± 0.03 μg/g temephos, whereas those taken 1 h following the October 2 application averaged 2.24 ± 0.95 μg/g temephos (Table 4). Maximum concentrations were 2.48 and 4.58 μg/g temephos for the September 29 and October 2 applications, respectively. Oysters retained greater than 1 μg/g temephos 48 h after September 29, and for 24 h following the October 2 application.

Field toxicity tests: Four species were observed for mortality during episode 2 including: 60 *M. bahia*, 45 *P. pugio*, 30 *C. variegatus* and 60 *P. aztecus*, with additional cages of each organism placed at the control site (as described above in Methods). Observations for 24 h after application showed no mortality of any test or control organisms.

Episode 3 provided 96 h of mortality monitoring for 3 species (*M. bahia*, *C. undecimalis* and *P. aztecus*) encompassing 2 temephos applications. The number of individuals and results of episode 3 acute toxicity studies are given in Table 5. During this final episode, some mortality of the mysids was observed following the September 29 application. Out of a total of 59 mysids at the 3 test sites, 8 died within 48 h (14%), relative to 0 deaths out of 40 individuals at control sites. Considering the individual test sites, no mysid mortalities were observed at test site 1, whereas 6 of 19 (32%) and 2 of 19 (11%) died at test sites 2 and 3, respectively, indicating significant mortality at site 2, but not at sites 1 and 3 (Table 5). No significant mortality of mysids occurred during the subsequent 24-h period (48–72 h) or within the first 24 h after the second temephos application (October 2).

The snook and brown shrimp suffered no significant acute toxicity following either of the applications (Table 5). The cage at test site 3 did have 2 of 10 individuals die (20%) between 48 and 72 h; however, none died after the second temephos application. Deaths observed at 96 h (24 h after the October 2 application) were due to stress from being stranded in shallow, muddy water due to unusually low ebb tides.

DISCUSSION

The theoretical application rate of temephos to the mangrove community was 1 fl oz/acre (3,200 μg/m^2). The actual influx to the upper leaf canopy, collected on filters at each of the 3 test sites during episode 3, was 2,535 ± 860 μg/m^2 during the September 29 application, and 2,302 ± 323 μg/m^2 during the October 2 application, showing good agreement with the theoretical amount.

A considerable amount of temephos remained adsorbed to the leaves after each application. The average among the 3 sites was 1,500 ± 1,100 μg/m^2 for July 24; 1,269 ± 466 μg/m^2 for September 29; and 1,358 ± 89 μg/m^2 for October 2. When compared to the amount collected on the aerial filters, the leaves retained 50% and 60% of that applied during the September and October applications, respectively.

The amount of temephos applied to the mangrove community and that retained by the leaves was quite consistent throughout the 3 larviciding applications. The amount penetrating the leaf canopy, and collected on ground-level filters, however, varied among the sites and from one application to another. When compared to the amount collected on aerial filters (episode 3), the ground-level filters contained 15%, 15% and 14% (mean 15%) at sites 1, 2 and 3, respectively, for the September 29 application, and 88%, 91% and 34% (mean 70%) at sites 1, 2 and 3, respec-

Table 5. Organism mortality following each temephos application (episode 3: September 29 and October 2 applications).

	Application 1						Application 2	
	1–24 h		24–48 h		48–72 h		1–24 h	
Species replicate	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Mysidopsis bahia								
test (composite)	59	1	51	8	59[1]	2	60[1]	1
Mb-1	21	0	21	0	21	0	21	0
Mb-2	19	0	13	6	18[1]	2	19[1]	1
Mb-3	19	1	17	2	20[1]	0	20	0
Mb-C1	20	0	20	0	20	0	18	2
Mb-C2	20	0	20	0	20	0	20	0
Control composite	40	0	40	0	40	0	38	2
Centropomus undecimalis								
test (composite)	29	0	28	1	27[1]	2	22[1]	7
Cu-1	10	0	9	1	10[1]	0	3[2]	7
Cu-2	9	0	9	0	9	0	9	0
Cu-3	10	0	10	0	8	2	10[1]	0
Cu-C1	10	0	10	0	10	0	8[2]	2
Cu-C2	10	0	10	0	10	0	1[2]	9
Control composite	20	0	20	0	20	0	9	11
Panaeus aztecus								
test (composite)	63	0	62	1	66[1]	0	66	0
Pa-1	20	0	20	0	20	0	20	0
Pa-2	17	0	16	1	20[1]	0	20	0
Pa-3	26	0	26	0	26	0	26	0
Pa-C1	26	0	26	0	26	0	26	0
Pa-C2					22[3]	0	22	0
Control composite	26	0	26	0	48	0	48	0

[1] Dead organisms replaced with acclimated organisms.
[2] Stranded cages.
[3] Added to system.

tively, for the October 2 application. This variation most probably resulted from changes in placement of the collection filters relative to canopy density, as well as helicopter positioning, wind and other variables associated with field operations.

Temephos was found to persist in mangrove leaves, up to 72 h after application. The concentration diminished to about 30% of the 1-h concentration at 72 h after the September 29 application, and to about 75% of the 1-h amount at 24 h after the October 2 application. The rate at which leaf temephos concentrations decreased was greater following the first application (893 $\mu g/m^2/24$ h) than after the second (187 $\mu g/m^2/24$ h), possibly influenced by the rain. At the latter rate, temephos on mangrove leaves had a half-life of about 48 h. Temephos in association with mangrove leaves represents a reservoir for impact on herbivorous animals and their predators, as well as a source for continued influx to the mangrove forest floor and to tidal pools with falling leaves and rain.

Sediment appeared not to be a reliable indicator of temephos fate in the intertidal region. Interestingly, the amount of temephos observed to collect on the mangrove forest floor (sediment) at each site showed a negative correlation with the amount collected on ground-level filters. The amount of temephos recovered from sediment varied with mangrove canopy density, the ratio of surface to subsurface sediment collected, and the tidal status at the time of application and sample collection.

Intertidal water around the mangrove fringes contained no delectable amounts of temephos after episode 2 (July 24). None was detected after the September 29 application; however, considerable amounts were found after the October 2 application (ranging from 0.6 to 35 μg/liter and 0.6 to 17 μg/liter at 1 and 2 h, respectively) with none detected at 4 h or more. These results reflect rapid dispersion of temephos due to tidal flow during the July and September applications which occurred during out-flowing tides. The October 2 application occurred at high tide resulting in detectable amounts of temephos in the water for at least 2 h, with subsequent dispersion of temephos with the outgoing tide.

These results agree with concentrations reported after aerial applications to ponds (Lores et al. 1985) and after application to a saltmarsh

(Henry et al. 1971). Fortin et al. (1987) reported temephos activity to mosquito larvae up to 7 days in treated ponds. After the October 2 application, some water samples contained temephos concentrations above the 48-h LC_{50} for pink shrimp, *P. duorarum* (10 μg/liter), and brown shrimp, *P. aztecus* (5 μg/liter) (American Cyanamid 1980). Although the short exposure time (2 h) reduced the potential for acute toxicity. These data indicate sufficient concentrations for potential sublethal effects.

Simulated tidal pools showed an average of 140 ± 68 μg temephos entering the 40-liters of water in tubs after the September 29 application, and an average of 330 ± 190 μg temephos entering the tubs after the October 2 application. The larger influx during the October application is consistent with the greater amount of temephos recovered from the ground filters. The concentration steadily decreased to about 1 μg/liter (40 μg/tub) at 72 h after the September application, and to 2 μg/liter (80 μg/tub) at 24 h after the October application.

Rainwater collected after the September application contained an average of 25 μg/liter temephos. This same quantity would have produced an increase of 0.75 ug/liter in the 40-liters of tub water. The average increase for the simulated tidal pools was about 0.67 μ/liter, which is consistent with that entering with rainwater. These results show that the amount of temephos added from rain washings could represent a significant influx to shallow tidal pools, prolonging temephos exposure to aquatic organisms.

Although the simulated tidal pools did not contain as much temephos as was recovered from the tidal water, the concentrations were within the lower range reported to cause mortality from extended exposure (American Cyanamid 1980). These data raise concern for temephos impact in saltmarsh pools not experiencing daily tidal flushing, especially regarding invertebrate larvae released into that environment.

Interpretation of oyster temephos data requires careful attention to the tidal cycle and collection techniques. Oysters from episode 2 and the September 29 application of episode 3 exhibited similar temephos concentrations for the first few hours after application. No temephos was recovered from oysters 24 h after episode 2, whereas the episode 3 oysters exhibited an increase in temephos at 24 h and retained the larvicide through 48 h at one of the sites. The 24-h increase is consistent with additional temephos influx with rain between the 7 h and 24 h sampling.

Oysters collected following the October 2 application of episode 3 retained much higher concentrations of temephos than either of the previous studies, which is consistent with the greater influx to the mangrove floor observed on the ground-level filters and in the simulated tidal pool tubs. These results show that oysters did concentrate temephos at least 1,000 times over that in the water, on a weight/weight basis. Oysters continued to accumulate the larvicide for 4 to 7 h following applications with gradual depuration indicated by the detection of elevated concentrations for 24 to 48 h. These data show the need to investigate the sublethal effects of temephos on oysters and oyster larvae.

Although temephos concentrations did exceed toxic levels in the water around the caged organisms, the exposure time was sufficiently short to avoid appreciable mortalities. The only significant mortality observed was for mysids during the September 29 application of episode 3. Based on this mortality, it would appear that site 2 received the highest concentration, followed by site 3, then site 1 (Table 5). These mortalities could not be compared with temephos concentration in ambient water, however, because none was detected in water following the September 29 application. Ground-level filters at sites 2 and 3 did receive more than twice as much temephos as site 1, indicating greater larvicide influx at these sites. Interpretation is further complicated by the fact that no mortalities were observed following the October 2 application when temephos residues were observed in ambient water and 2 to 9 times as much temephos was collected on the ground-level filters. Therefore, no direct correlation was found between temephos concentration and organism mortality.

Natural functions and behavior of organisms were also observed, including ovigerous mysids that continued to molt and release their larvae. Although no quantitative data were kept for the larvae, they appeared to be healthy.

Water quality parameters for each episode showed no appreciable differences between control and test sites and no conditions that would be detrimental to the caged organisms, except for the *C. undecimalis* stranded at low tide at the 96-h monitoring of episode 3 (Pierce et al. 1988).

CONCLUSIONS

These results demonstrate that under normal larviciding conditions, temephos applied to an intertidal mangrove system did not persist in the water in sufficient quantities to affect appreciable mortality to 5 representative species of estuarine animals. Temephos did persist in mangrove leaves and in simulated tidal pools for more than 72 h, and in mangrove oysters up to 48 h after application. Additional temephos en-

tered the water and mangrove floor with rainwater dripping from the mangrove leaves. These data indicate the need to consider chronic impact to mangrove herbivores and their predators, as well as to marine organisms inhabiting salt-marsh pools not flushed by diurnal tides, and to the growth and reproduction of oysters repeatedly exposed to larviciding applications.

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