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Temephos Residues in Stagnant Ponds After Mosquito Larvicide Applications by Helicopter

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Temephos, (0,0,0',0'- tetramethyl,0,0'-thiodi-p-phenylene phosphorothioate,) is an organophosphate insecticide commonly used for control of mosquito larvae. In Florida during 1983, 10,416 L of Abate 4E®, an emulsifiable concentrate formulation of temephos, were applied (FAMA 1983). Applications of temephos as a mosquito larvicide are often made in remote areas where the size and depth of the ponds are estimated by aerial reconnaissance. Also, in many cases, the ponds are covered by a tree canopy that prevents some of the spray from reaching the water. The intended application rate, commonly one fluid ounce/acre (73 mL/ha), is therefore rarely verified in the target areas.

The purpose of this study was to measure the concentrations of temephos in the water so that the application rates could be confirmed. This paper reports the results obtained from residue analysis of water from two stagnant ponds following actual field applications of larvicide by helicopter at treetop level in South Florida. Site 1 was less than 0.05 ha in surface area, less than 10 cm deep, and completely covered by a tree canopy. Site 2 was greater than 0.5 ha in surface area, less than 10 cm deep and the canopy was open in the middle.

A number of investigators have measured temephos residues in water after sprays and the rate of disappearance of temephos from these waters. Sanders et al. (1981) reported concentrations ranging from 0.15 µg/L to 10 µg/L in small freshwater ponds 24 h after application. Henry et al. (1971) measured temephos concentrations after simulating salt marsh application rates (26 to 131 µg/L) and reported disappearance rates as a function of temperature. Our results are similar to the results of Henry et al. (1971) and can be used to approximate the actual application rates.
Several methods have been employed for analysis of temephos residues residues in water samples: Henry et al. (1971) and Otsuki and Takaku (1969) used high-pressure liquid chromatography (HPLC); Wright et al. (1967), Dale and Miles (1969), Miles et al.(1976), Bowman et al. (1968), and Shafik (1968) used gas-liquid chroma-

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tography (GLC). These methods generally used organic solvent extraction prior to analysis of temephos residues.
Our method is a combination of several of these methods, and includes capillary gas chromatography with electron-capture detection with a new silica-gel cleanup method of Lores et al. (1984). Selected samples were confirmed with HPLC.

MATERIALS AND METHODS

A Hewlett-Packard 5730 gas chromatograph, equipped with an electron-capture detector and a split/splitless capillary inlet system operated in the splitless mode, was used for analysis of temephos residues. A 15 m x 0.32 mm i.d. fused silica capillary column with a 1 micron Durobond DB-1® film thickness was used for separations. The oven was programmed from 275 to 320° C at 4 °/min with an 8 min hold at 320° C. The temephos residues were quantitated by a Hewlett Packard 3357 computer using an internal standard method with fenvalerate as the internal standard. The carrier gas was helium and the head pressure was 10 psi. The analysis was confirmed with a Waters high-pressure liquid chromatograph equipped with a Model 4000A pump and a WISP® injection system, a radial compression module, and a Model 440 UV absorbance detector operated at 254 nm and 0.01 absorbance units full scale. The column was a Radial-Pak® 5 micron C-8 cartridge; the mobile phase, 80% methanol and 20% water at a flow rate of 4 mL/min. The injection volume was 100 µL (1.0 ng/µL standard).

Water samples were collected in 1.0-L borosilicate glass bottles (Wheaton), 2 mL each of mineral oil and 30% formalin were added, the samples shaken, stored in styrofoam containers packed with ice, then transported to the laboratory where extraction was completed within 24 h. Water samples were extracted twice with 100 mL aliquots of petroleum ether by shaking for 1 minute in a 2-L separatory funnel. The solvent was dried by passing it through a funnel containing heat-treated glass wool (600° C) and collected in a 500 mL Kuderna-Danish evaporator fitted with a three-ball Snyder column and a 25-mL concentrator tube. The solvent was evaporated on a steam cabinet to 10 mL then concentrated to 1.0 mL with a gentle stream of nitrogen at 35° C.

Silica gel was stored at 130° C, deactivated by addition of 20% (w/w) distilled water, and tumbled on a roto rack for 2 h prior to use. Cleanup columns were prepared by adding 3.5 g of deactivated silica gel, followed by 2.0 g of anhydrous sodium sulfate (granular), to a 200 mm x 9 mm i.d. Chromaflex column (Kontes Glass Co., Vineland, NJ) plugged with glass wool.
Columns were washed with 10 mL of 1% (v/v) acetic acid in hexane. Since the mineral oil extracted from the samples tended

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to inhibit flow through the column, each sample concentrate was transferred to the column by using 10 mL of 1% acetic acid in hexane followed by two 1 mL washes. Columns were then eluted with 20 mL of 5% ethyl ether in hexane followed by 20 mL of 10% ethyl ether in hexane. Temephos eluted in the 10% ethyl ether fraction and was concentrated to 1.0 mL with a gentle stream of nitrogen. Samples to be confirmed by HPLC were evaporated to dryness and redissolved in 1 mL of methanol prior to injection.

RESULTS AND DISCUSSION

One of the major difficulties encountered in this study, as in many field studies, was stabilization of temephos residues in samples until analysis could be performed. Since laboratory facilities were unavailable for immediate extraction of residues at the field site, samples were shipped to the laboratory by air immediately following collection. While developing our method, we found that without addition of an organic solvent to preserve the samples, recovery of temephos residues in raw seawater decreased from 60-70% after 24 h to less than 30% after 3 days. Henry et al. (1971) concluded that temephos was adsorbed rapidly to organic particulate matter, which was confirmed by our laboratory test since the recovery decreased rapidly in the presence of any organic particulate material in the water. Since volatile solvents are unsafe for transport by air, 2 mL of mineral oil were added as the organic matrix to absorb temephos residues until the samples could be returned to the laboratory. To prevent microbial degradation, 2 mL of 30% formalin were added to each sample. Generally samples were returned to the laboratory within 24 h. The results of the residue analyses are shown in Figure 1. At Site 1, which was completely covered by a tree canopy, initial concentrations of temephos were much lower than at Site 2. A rain approximatley 9 h after the first spray, apparently washed the temephos residues from the treetops, significantly increasing residues in the water. Just after the 48-h sample at Site 1 and just prior to the 48-h sample at Site 2, a second application was made at each site and only the 15-min post spray samples were taken. Slightly higher concentrations were attained than in the first spray, Site 1 reaching 49 µg/L and Site 2 reaching 62 µg/L.

The recommended application rate on the label of the 4-E formulation (45% active ingredient) for control of mosquito larvae is 0.5 to 1.5 fl oz/acre (37 to 110 mL/ha). Calculated residue concentrations for the intended 1 oz/acre (73 mL/ha) application rate used in this study range from 250 µg/L for water that is 1 inch deep to 42 µg/L for waters that are 6 inches deep. These calculations predict concentrations of temephos that are very close to the measured concentrations found immediately after the sprays (Figure 1).

Figure 2 shows gas chromatograms from selected samples to indicate the effectiveness of the cleanup procedure. Background interference in the blank was too great for quantitative measurement; however, after cleanup the baseline in the chromatogram was much improved, allowing accurate quantitation.

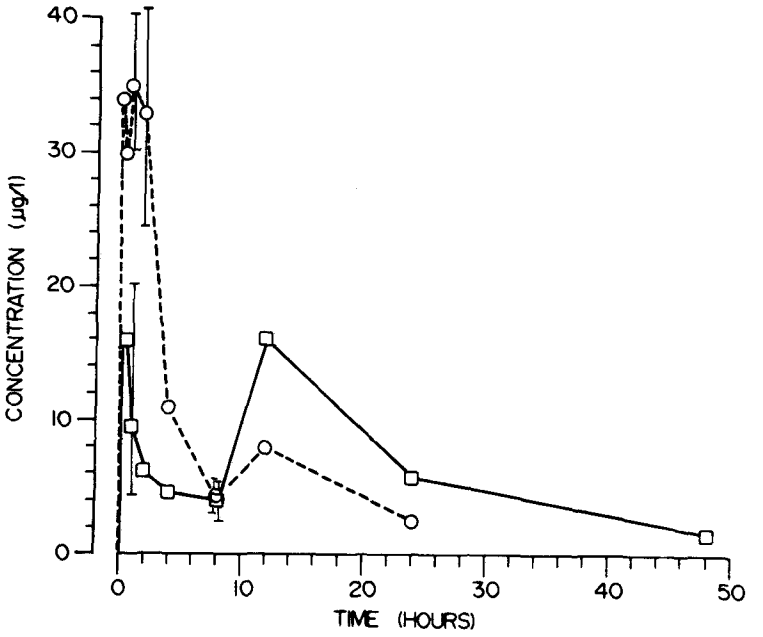

Figure 1. Concentrations of temephos found in water samples after larvicide applications —□— represents Site 1 and -- O -- represents site 2. The bars represent the ranges for duplicate and triplicate samples.

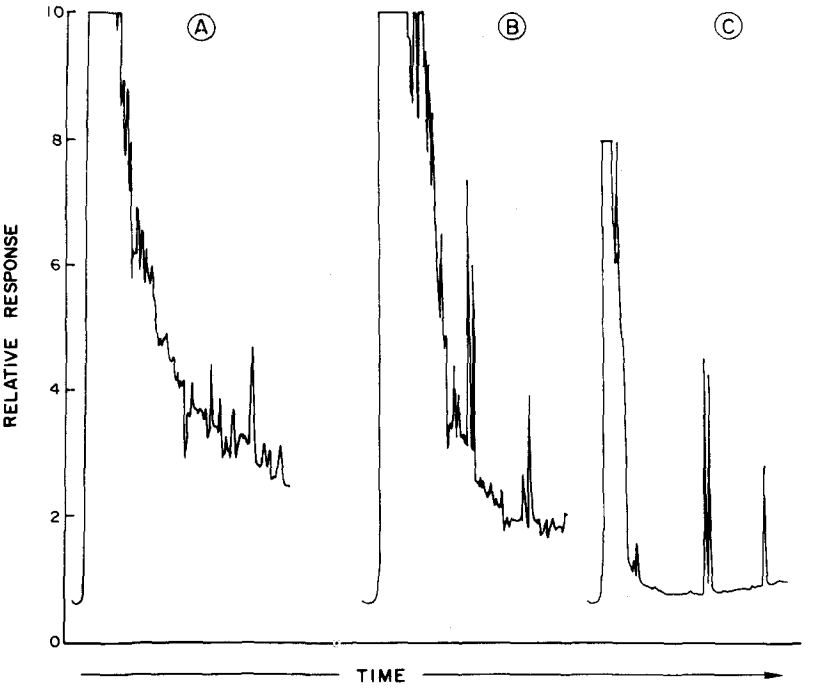

Figure 2. Capillary gas chromatograms. A) Chromatogram of blank water sample without cleanup. B) Chromatogram of spiked water sample without cleanup. C) Chromatogram of spiked water sample after cleanup.

During the course of our field study, nine blank samples of pond water were fortified with a known quantity of temephos at the field site and returned with the field samples to measure stability of residues in the field samples. The average recovery from samples spiked in the field and returned to the laboratory for analysis was 83% $\pm$ 12. Duplicate and triplicate samples were taken several times to determine the variability in sampling and analysis. As demonstrated by the range bars in Figure 1, the variability is much higher in the early samples probably due to uneven distribution of the temephos. The response on the GC and HPLC was checked and found to be linear over the range of 0 to 2 ng/µL.

This paper has shown that temephos, in actual field applications as a mosquito larvicide, behaves in much the same way as was predicted by Henry et al. (1971) and Sanders et al. (1981). The results indicate that the pesticide remains intact in water up to 48 hours, and residues as high as 62 µg/L were found. A problem of stabilization in this study was overcome by using mineral oil as an extracting solvent until the samples could be returned to the laboratory. The two new methods presented for analysis, although similar to methods already published, incorporate some newer technology.

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