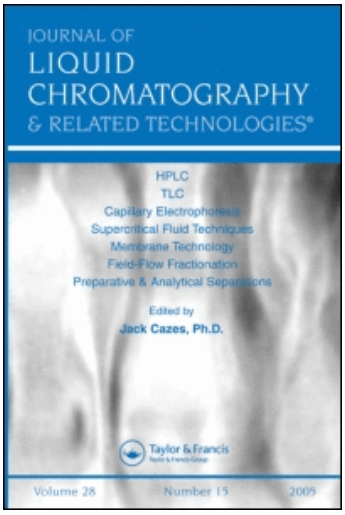

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A Liquid Chromatographic Study of the Effect of Adsorption on the Concentration of Aqueous Temephos Solutions

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A LIQUID CHROMATOGRAPHIC STUDY OF THE EFFECT OF ADSORPTION ON THE CONCENTRATION OF AQUEOUS TEMEPHOS SOLUTIONS

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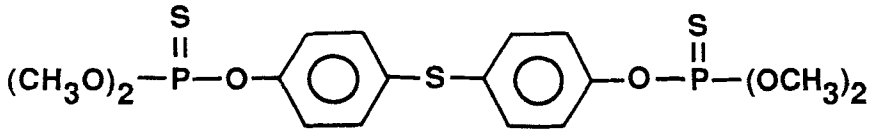
ABSTRACT

A new HPLC method to determine temephos in water was developed and tested. The method is reliable for measuring concentrations of temephos in aqueous solutions from 0.005 to 5.0 ppm. The detection limit at 254 nm is 1 ng. The standard curve is not linear over the entire range; separate curves are required for concentrations above and below 0.1 ppm. The method was used to study adsorption of temephos on plastic and glass surfaces. The size of the container, is important to minimize adsorption effects if solutions of 0.01 ppm or less are used. For these solutions, glass containers at least as large as a standard 50 mL beaker should be used for experiments in which a relatively constant concentration of temephos must be maintained for a period of hours.

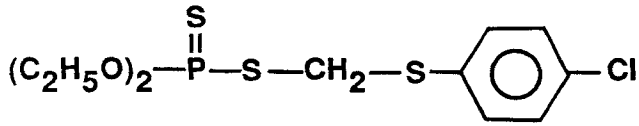
INTRODUCTION

Temephos is an organophosphorus insecticide (0,0,0,0,-tetramethyl 0, 0-thiodi-p-phenylene phosphorothioate -Fig. 1) widely used in public health programs to control arthropod vectors of disease. An efficient mosquito larvicide, temephos is also effective against black flies in onchocerciasis control and against freshwater cyclopoid copepods in dracunculiasis (guinea worm disease) control. Temephos is approved by the World Health Organization for use in drinking water at concentrations up to 1 ppm; using temephos in drinking water is one of the primary strategies for control of dracunculiasis, a disease acquired by ingesting freshwater cyclopoid copepods infected with the third stage larva of the parasite Dracunculus medinensis. Determination of the LC50 and LC90 for these freshwater microcrustaceans requires a simple, reliable method for quantification of this organophosphate in water samples both before and after exposure of the organisms.

Since copepods are very small organisms ($\leq$ 3 mm in length), their exposure to the pesticide in small containers should



Temephos



Carbophenothion

Figure 1. The structural formulae of temephos and the internal standard, carbophenothion.

expedite assessment of the effects of temephos on the organism. However, during the initial 24-hour exposures of single organisms in 2-mL volumes of temephos solution in each of 24 wells in a plastic tissue culture plate, the concentration of temephos dropped substantially. Adsorption is a documented property (5,6) of temephos, but a careful study of this problem has not been made, and our initial findings indicated the necessity to examine this phenomenon on both plastic and glass surfaces. The effects of such adsorption on temephos concentration required quantification before any accurate measurements of LC50 or LC90 could be determined for any organism.

A colorimetric method (1) as well as both gas-liquid (2, 3, 4) and high performance liquid (5, 6, 7) chromatographic methods for the quantitation of temephos are available. Although both the colorimetric and the gas-liquid chromatographic methods require a large volume of sample (300-1500 mL), one of the high performance liquid chromatographic (HPLC) methods (6) achieved parts per billion sensitivity using 1 mL samples. This method was not used in the current study since the column is not readily available, but it did prove that reverse-phase HPLC for sensitive temephos assay is feasible.

This paper describes an analytical HPLC method for extraction and quantitation of temephos in 5-mL water samples, which uses a reverse phase column (C-18) and ultraviolet detection at 254 nm. This method was applied to studies of the adsorption of temephos on plastic and glass surfaces and the relation of adsorption to the surface area/volume ratio of test containers.

EXPERIMENTAL*

Chemicals

Organic solvents were HPLC grade. Purified water was obtained from a laboratory purification system equipped with

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

both ion-exchange and carbon filters (Millipore, Bedford, MA, USA). The analytical standard temephos insecticide (99.1% purity) was supplied by American Cyanamid Company, Agricultural Division (Princeton, NJ). Carbophenothion, (Fig. 1) technical grade, 95% pure, was obtained from Pesticides Research Laboratory (Perrine, FL, USA).

Preparation of Standard Solutions

Temephos standard stock solution (103.55 mg analytical standard temephos diluted to 100 mL in a volumetric flask with acetonitrile as the solvent) contained 1.0355 mg/mL, and was serially diluted to produce working standard solutions containing 103.55, 10.355 and 1.0355 µg/mL in acetonitrile.

Individual, 5-mL samples of temephos in water at 1.0355, 0.103555, and 0.010355 ppm were prepared by adding 50 µL of each working standard solution to 5 mL of deionized water in a 15-mL conical centrifuge tube. A sample containing 0.0010355 ppm was prepared by adding 5 µL of the 1.0355 µg/mL working standard solution to 5 mL of deionized water in a centrifuge tube.

One milliliter of a standard solution of carbophenothion (67.00 mg in a 25-mL volumetric flask and diluted to volume with methanol) containing 2.68 mg/mL was diluted to 100 mL with methanol in a volumetric flask to produce a solution with a concentration of 26.8 µg/mL, which was used as the internal standard solution in the temephos analysis.

Equipment

The samples were analyzed with a Varian Model 5060 liquid chromatograph equipped with a Rheodyne Model 7125 injector, an LDC/Milton Roy Spectromonitor 3000 variable wavelength detector set at 254 nm and Hewlett Packard 5880A terminal as integrator. The column used with this instrument was an Altex Ultrasphere ODS, 5 µm, 2 mm x 15 cm, using a flow rate of 0.3 mL per minute.

Analysis of Water Samples

All glassware used in the analyses was washed first with detergent and water, rinsed twice with tap water, twice with deionized water, and twice with ACS grade acetone. The 2 acetone rinses are necessary to eliminate all traces of temephos from glassware previously exposed to it. Intermediate transfers of solutions containing temephos should be avoided since temephos will be lost from the sample by adsorption onto the surface of the intermediate container. Pipets or plastic tips for automatic pipets should be rinsed twice with the solution to be measured before the actual measurement is made.

Five mL of water sample was measured, using a P-5000 Pipetman (Rainin, Woburn, MA), directly into a 15-mL conical, glass centrifuge tube. Internal standard solution (50 µL, 1.340 µg carbophenothion) was added to each sample. Standards, prepared in glass centrifuge tubes as described above, were handled in exactly the same manner as the samples. Ethyl acetate (1.5 mL) was added to each tube and the tubes were vortexed for 1 min. After the layers were separated, the ethyl acetate layer (1 mL) was transferred to a clean centrifuge tube. The ethyl acetate was evaporated by heating in a water bath at 45°C under N_2. The residue was dissolved in 100 µL of mobile phase (82% methanol/water) by vortex mixing.

The mobile phase was prepared by premixing and filtering 410 mL of methanol and 90 mL water. The flow rate was 0.3 mL per minute. A 20 µL sample loop was used on the injector and was overfilled by adding 30 µL from a syringe. After each injection the syringe was cleaned twice with acetone and then twice with methanol to avoid contaminating subsequent samples. Mobile phase was injected frequently to check for contamination from the syringe.

The standards, extracted in the same tubes in which they were prepared to prevent loss by adsorption, were handled in the same way as the samples. A typical chromatogram is shown

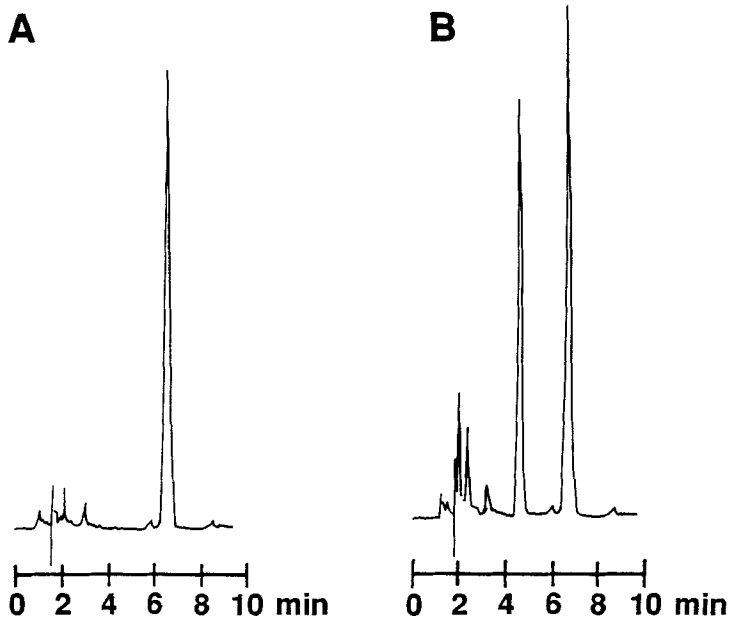


Figure 2. Representative chromatographic traces. A. Extract of control water sample showing internal standard, carbophenothion. B. Extract of water sample spiked with 1 ppm temephos.

in Figure 2. The area count ratio of temephos/internal standard was calculated for each standard; this ratio and the concentration of the standard were used in a linear least-squares program to calculate the concentration of the unknown samples.

Adsorption Studies

Plastic tissue culture multi-well plates from two sources were tested: Linbro from Flow Laboratories, McLean, VA and Costar from Data Packaging Corporation, Cambridge, MA. The wells in both plates were 16mm in diameter and had a maximum volume of 3.5 ml. Glass vials (1.5 dram) with a diameter of 15mm were used for comparison. Sixteen vials were silanized by

filling them with a 5% (V/V) solution of dimethyldichlorasilane in toluene. After 15 minutes this solution was discarded and the vials rinsed with methanol and acetone and then dried. Sixteen identical vials were rinsed with water and acetone and dried. A 2-ml sample of freshly prepared solution containing either 0.1, 1 or 5 ppm temephos was placed in each well of the plastic plates and in the glass vials. A separate plastic plate was used for each of the three concentrations to avoid contamination. Four silanized and 4 non-silanized glass vials were used for each concentration. Two sets of samples of each of the solutions were placed in glass centrifuge tubes, one set analyzed immediately and the other set at 24 hours along with samples taken from the plastic plates and glass vials. After 24 hours, samples were removed from 3 wells or vials and combined to obtain 1 5-ml sample for each concentration. These samples were analyzed and the percentage of temephos remaining in solution after 24 hours was calculated.

Five sizes of beakers (250, 50, 30, 20, 10 ml) were filled with 200, 40, 25, 15, and 10 ml volumes, respectively, then covered with aluminum foil. Two beakers of each size were used for each of three different concentrations, 0.01, 0.1, and 1.0 ppm (nominal concentrations). Five-ml samples of each concentration were placed in glass centrifuge tubes at the same time the beakers were filled in order to provide initial concentrations. After 24 hours, duplicate 5-ml samples were taken from each beaker and analyzed.

To test the variation of adsorption among beakers, triplicate sets of 50 mL beakers were filled with 45 ml of 0.005, 0.01, 0.1, and 1 ppm. The beakers with their solutions sat overnight, covered with aluminum foil, before a 5-ml sample was removed from each beaker. To check precision, 4 5-ml samples were removed from one beaker containing 0.01 and one containing 0.1 ppm solutions.

RESULTS AND DISCUSSION

Analytical Method

The stock solutions used an organic solvent, acetonitrile, in which temephos is both soluble and stable over a period of several months. It was necessary to extract standard aqueous solutions in the same tubes in which they were prepared to avoid loss of analyte by adsorption on the surface of the tube. Concentrations of temephos in dilute aqueous solutions, decreased rapidly as indicated by analysis of aliquots at various time intervals. These dilute aqueous solutions show no changes in concentration if measured into several glass centrifuge tubes at the same time and analyzed at various time intervals (Table 1) in these same tubes. This indicates that the temephos has not decomposed and can be extracted by an organic solvent added to the tube, which suggests that the temephos is on the interior surface of the glass tube.

When each standard is prepared and extracted in the same glass tube, a reliable standard curve can be obtained by using the simple extraction method described. The chromatographic conditions provide good separation of temephos, internal standard, and the minor peaks found near the solvent front and before and after the internal standard (Fig. 2). Typical standard curve data are given in Table 2. Although there is a minor change in the slope of the line at approximately 0.1 ppm,

TABLE 1

Stability of Temephos Solutions

Nominal concentration (ppm)	0.1	1.0	5.0
Concentration at t=0	0.1156	0.9468	4.213
at t=24 hr.	0.1041	0.9228	4.291
at t=48 hr.	0.1134	0.9521	4.644
Average concentration	0.1110	0.9406	4.383
Standard deviation	.0050	.0127	.188
Coefficient of variation (%)	4.5	1.3	4.3

TABLE 2

Standard-Curve Data for Temephos

All points on 1 standard curve

Temephos added (X) µg/mL (ppm)	Area Ratio (Y) Temephos/ Carbophenothion	Temephos Calc. (X') µg/mL
0	0	-0.002578
0.001036	0.006140	-0.001667
0.01036	0.06172	0.006577
0.1036	0.7316	0.1059
1.036	7.094	1.050
2.072	13.94	2.065

Calculated from the least-squares straight line, y= mx+b (m= 6.742, b= .01738), r^2 = .99993, n=6.

Points calculated for 2 separate curves

Temephos added (X) µg/mL (ppm)	Area Ratio (Y) Temephos/ Carbophenothion	Temephos Calc. (X') µg/mL
0	0	0.0005753
0.001036	0.006140	0.001441
0.01036	0.06172	0.009276
0.1036	0.7316	0.1037

Calculated from the least-squares straight line, y= mx+b (m= 7.094, b= .00408), r^2 = .99978, n = 4.

0	0	-0.005691
0.1036	0.7316	0.1030
1.036	7.094	1.048
2.072	13.94	2.066

Calculated from the least-squares straight line, y= mx+b (m= 6.729, b= .03830), r^2 = .99992, n = 4.

the calculated values of points above 0.1 ppm are negligibly affected by the inclusion of the lower concentrations. However, if very low levels of temephos are to be analyzed (.001-.01 ppm), a standard curve using only points up to and including 0.1 ppm should be used.

Adsorption Studies

Dilute solutions of temephos are stable as shown by data in Table 1. All of these solutions were extracted in the same

glass tube in which they had been stored, thereby including any temephos adsorbed on the glass tube. When the solutions are stored in other containers and samples removed for analysis, the concentration appears to decrease because adsorption increases. The extent of adsorption depends upon the size of the container (surface area/volume ratio) and the concentration of the solution.

The results of the comparison of the plastic, silanized glass and non-silanized glass containers are summarized in Table 3.

These data show non-silanized glass to be the best material of those tested for containers of temephos solutions but there remains a serious problem with solutions of 0.1 ppm or less. It appears that very small containers should not be used with dilute solutions of temephos.

Table 4 summarizes the data collected using 5 sizes of glass beakers and 3 temephos concentrations. All sizes of beakers were acceptable for solutions of 0.1 ppm or higher but beakers of 50 ml volume or less were unacceptable for solutions of 0.01 ppm.

In another experiment, the size of the beaker was standardized at 50 mL so that reproducibility and precision of the analytical method could be checked at 5 different concentrations. Reproducibility in triplicate beakers was poor

TABLE 3

Percentage of Temephos Remaining in Solution After 24 Hours in Very Small Containers Made of Various Materials

Concentration (ppm)	Plastic	Silanized Glass	Non-Silanized Glass
0.1	16.9%	13.0	38.7
1	46.1	40.4	67.9
5	75.6	83.0	89.0

All containers held a volume of 2 ml and had a surface area/volume ratio of approximately 350 mm^2/mL.

TABLE 4

Changes in Temephos Concentration of Aqueous Solutions Stored in Glass Beakers for 24 Hours

Beaker Size (mL)	Surface Area/Volume (mm^2/mL)	% Temephos remaining in solution after 24 hours		
		0.01	0.10	1.0 ppm
250	78	66.6	90.3	84.7
50	132	33.5	69.3	85.6
30	163	42.1	87.8	87.2
20	188	16.0	66.5	90.6
10	213	18.3	89.9	88.5

Two beakers of each size were used for each concentration and the average value reported.

TABLE 5

Reliability of Concentration Data Obtained from Different 50-mL Beakers

0.005 ppm	nominal conc.	0.01 ppm	nominal conc.
Beaker A	.003014	Beaker A	.007473 ± 0.00071(n=4)
B	.003296	B	.006883
C	.004689	C	.007194
mean	.003666	mean	.007183
Std. Dev.	.000897 (24.5%)	Std. Dev.	.000295 (4.1%)

0.1 ppm		1 ppm	
Beaker A	.08422 ± .006355(n=4)	Beaker A	1.1430
B	.07040	B	1.1932
C	.09006	C	1.0890
mean	.08157	mean	1.1417
Std. Dev.	.01010 (12.4%)	Std. Dev.	.0521 (4.6%)

at 0.005 ppm and good at 0.01, and 0.1 and 1 ppm as shown in Table 5. When 4 replicates were taken from the same beaker (0.01 and 0.1 ppm) the precision was good. It was critical that the pipet tips used to measure these samples were rinsed twice with the solution before samples were taken; otherwise, great variation occurred.

Conclusion

The data from these studies indicate that the analytical method for the determination of temephos in aqueous samples is reliable and precise. The adsorption studies proved non-silanized glass containers to have less adsorption than either plastic or silanized glass containers. If solutions of 0.1 ppm or less of aqueous temephos are required to remain constant over a period of several hours, the containers should be non-silanized glass with a surface area/volume ratio of approximately 130 mm^2/mL, or the size of a 50-ml beaker.

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