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Gas-chromatographic Analysis of Residues of Pirimiphos-methyl in Water, Fish, and Snails

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Pirimiphos-methyl, O-{2-diethylamino)-6-methyl-4-pyrimidinyl} O,O-dimethyl phosphorothioate, is an organophosphorus pesticide which was introduced in 1970 (WORTHING 1979). It has been shown to be effective against various pests of stored agricultural products (VARCA et al. 1975, REDLINGER & SIMONAITIS 1977, and MENSAH et al. 1979), certain mosquito vectors of malaria (RISHIKESH et al. 1977 and SHAW et al. 1979), and the blackfly vector of onchocerciasis (river blindness) (LEBERRE et al. 1972). There has been an increased interest in pirimiphos-methyl as an alternate insecticide for use in vector-control programs where resistance to temephos and other organophosphorus compounds has developed. Before using pirimiphos-methyl in large-scale field operations, studies are needed to determine its effect on certain nontarget organisms found in mosquito and blackfly habitats. Improved methods of residue analysis are needed for this work.

A general review of analytical methodology for the analysis of residues of pirimiphos-methyl has been published by BULLOCK (1976) which includes a lengthy procedure for extraction and cleanup of animal tissues. Specific methods for residue analysis have been reported including a TLC-UV method for residues in water, soil, and plants (KRASNYKH 1978), an HPLC method for determining pirimiphos-methyl and five metabolites in fortified samples of plasma and urine (BREALEY & LAWRENCE 1979), and gas-chromatographic methods for residues in stored grain (VARCA et al. 1975), farmer's stock peanuts (REDLINGER & SIMONAITIS 1977), and in milled fractions of dry or tough wheat (MENSAH et al. 1979). In addition, a gas-chromatographic method has been proposed for the simultaneous determination of residues of pirimiphos-methyl and malathion found in peanuts (SIMONAITIS et al. 1981), and GC-MS was used to identify residues of pirimiphos-methyl found in imported foods (BARRY et al. 1981). Since none of these methods met all of the desired criteria for specificity, simplicity of sample preparation, and recovery after cleanup, the new methods presented herein were developed for extraction and analysis of residue levels of pirimiphos-methyl in water, bluegill fish, and ramshorn snails.

EXPERIMENTAL

Materials.

1. Actellic (R), 86.5% pirimiphos-methyl stabilized with epoxidized soybean oil (standard).

2. Actellic (R)7E, 74.4% pirimiphos-methyl emulsifiable concentrate (EC).

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3. Actellic(R), experimental formulation JF 6914A, 20% microencapsulated pirimiphos-methyl.

(1 and 2 furnished by ICI Americas, Inc., Biological Research Center, Goldsboro, NC; 3 by Imperial Chemical Industries, Ltd., Plant Protection Division, Jealott's Hill Research Station, Bracknell Berks, England.)*

Equipment.

Gas Chromatograph equipped with dual-flame photometric detector operated at 280°C with 530 nm P filter; 1.8 m x 6.4 mm o.d. x 2 mm i.d. glass column packed with 7.5% OV-210 on Gas Chrom Q, 100/120 mesh; injector block at 220°C; column oven for water samples isothermal at 190°C, for fish and snail samples at 190°C for 3 min and then increasing rapidly to 270°C in order to clear column of extraneous material prior to next injection; flow rate for N_2 carrier gas was 40 mL/min.

Standard Solutions.

The solutions used as external standards were prepared by serial dilution in n-hexane from a stock standard of pirimiphos-methyl of known purity dissolved in acetone (ca. 1.0 mg/mL). Gas-chromatographic response was linear over the range of 0.2 to 61.5 ng injected. Quantitation by peak height and by automated peak-area integration gave equally satisfactory results. The minimum detection limit (2 x noise) was 0.04 ng.

Solutions used for fortification of water samples were prepared so that addition of 1 mL of pirimiphos-methyl (formulated either as EC or microencapsulate) in deionized water would yield the desired concentration.

Solutions used for fortification of fish and snail samples were prepared so that addition of <50 µL of pirimiphos-methyl in acetone would yield the desired concentration.

Extraction Procedures.

Water. The method used for extraction of water samples was essentially the same as that reported by MILES et al. (1976). A 300-mL aliquot was transferred to a 500-mL volumetric flask, 10 mL n-hexane added, and the sample stirred on a magnetic stirrer for 30 min; sufficient deionized water was added to bring the hexane layer into the neck of the flask, and, after separation of the phases, a 5-mL aliquot of the hexane was removed for GC analysis.

Fish and Snails. Fish and snails were extracted by a method similar to that reported for extraction of chlorphoxim from bluegill fish (ZAKITIS 1979). Individual bluegill fish weighing 1 to 3 g and sufficient ramshorn snails to make up a 1- to 3-g sample (4-7 snails) were thoroughly ground using a mortar and pestle and enough anhydrous Na_2SO_4 to dry. Each sample was then quantitatively transferred with acetone to a 250-mL ground-glass stoppered Erlenmeyer flask, and the final volume of acetone increased to about 100 mL. After shaking on a wrist-action shaker for 30 min, the sample was filtered through

* Mention of commercial sources does not constitute endorsement by the Public Health Service or the U. S. Department of HHS.

Whatman #1 filter paper into another 250-mL Erlenmeyer flask. The original flask and solid residue were rinsed 3 times with small amounts of acetone. The combined filtrate was then evaporated over a steambath to about 2 to 3 mL and quantitatively transferred with a minimum amount, but no more than 20 mL, of acetone to a 500-mL volumetric flask containing 300 mL deionized water. After 10 mL of n-hexane had been added, each sample was extracted and analyzed as described for water.

Efficiency of Recovery.

Water. Dechlorinated water which had been fortified with pirimiphos-methyl (EC) at levels ranging from 0.0056 to 9.17 ppm was extracted as described above. As shown in Table 1, average recovery was 101 ± 5%.

Fish and Snails. Solutions of pirimiphos-methyl dissolved in acetone were used to fortify bluegill fish with 0.051 to 48.8 ppm and ramshorn snails with 0.509 to 50.8 ppm of the pesticide. As shown in Table 1, recoveries were 93 ± 8% and 95 ± 2%, respectively. No data are reported for recovery of pirimiphos-methyl from snails at levels less than 0.5 ppm due to the fact that control snail samples were found which contained substances that interfered with analysis for pirimiphos-methyl at levels <0.5 ppm.

TABLE 1
Recovery of Pirimiphos-methyl from Fortified Substrates

Substrate	Pirimiphos-methyl (ppm) Added	Recovered	Recovery (%)	$\bar{X} \pm s$ * (%)
Dechlorinated water	0.0056	0.0057	102	
	0.0092	0.0089	97	
	0.054	0.051	94	
	0.092	0.10	109	
	0.527	0.517	98	
	0.917	0.947	103	
	5.24	5.32	102	
	9.17	9.73	106	101±5
Bluegill fish	0.051	0.052	102	
	0.501	0.409	82	
	5.45	5.18	95	
	48.8	45.9	94	93±8
Ramshorn snails	0.509	0.484	95	
	5.07	4.75	94	
	50.8	49.5	97	95±2

* Mean recovery based on duplicate injections of duplicate samples at each concentration.

RESULTS AND DISCUSSION

These methods have been and are currently being used to analyze a number of samples resulting from environmental studies of the effects of pirimiphos-methyl on nontarget organisms. The following data represent a small portion of the samples analyzed

for these studies and are given only to demonstrate application of the methodology. The results of all studies together with discussions of environmental significance will be published later (MC CRAY et al. in preparation).

Before actual exposure studies were begun, it was necessary to determine the number of organisms which could be tested in a 30-L aquarium without causing stress due to insufficient oxygen. Constant aeration of the water in the aquaria would provide more available oxygen during static testing and allow more organisms to be placed in each aquarium for exposure studies; however, previous work in our lab indicated that some pesticides are sensitive to aeration. Therefore, to determine the effect of aeration on concentration of pirimiphos-methyl in water, two 30-L aquaria, one aerated and one not, were fortified at each of two concentrations of pirimiphos-methyl, 5.2 ppm and 0.52 ppm. Water samples taken from each aquarium at the time of dosing and at 24-h intervals thereafter for 96 h were extracted and analyzed using the methodology described above. As shown in Figure 1, the rate of decrease in concentration of pirimiphos-methyl was greater in the two aerated aquaria.

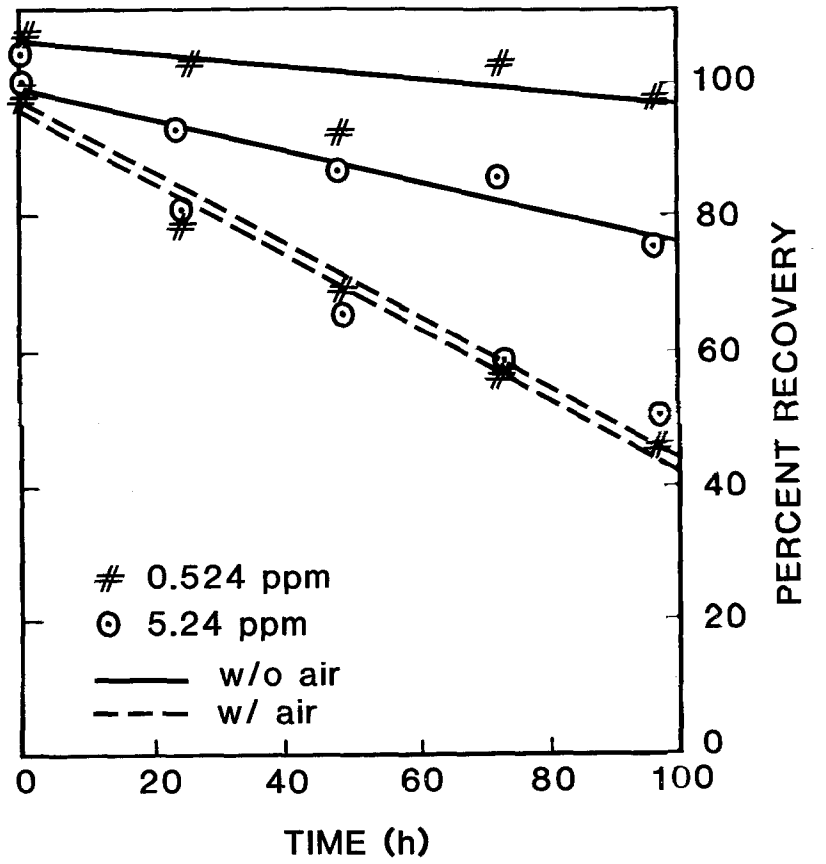

Figure 1. Effect of aeration on concentration of pirimiphos-methyl over 96-hour period.

Another study was designed to compare the uptake of pirimiphos-methyl by two nontarget species exposed to the same concentrations of pirimiphos-methyl by way of two different formulations, an emulsifiable concentrate and an experimental microencapsulated formulation furnished by the manufacturer.

Bluegill fish and ramshorn snails which had been exposed to water containing 0.5 ppm pirimiphos-methyl over a 96-h period

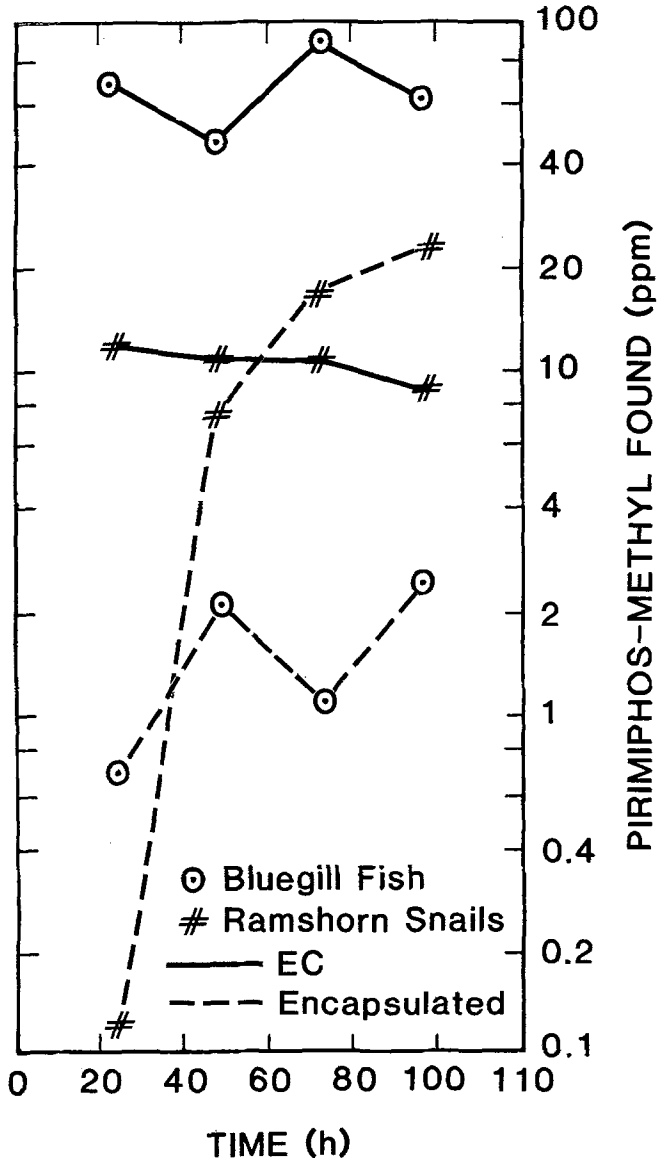

Figure 2. Uptake of pirimiphos-methyl by fish and snails exposed to water containing 0.5 ppm of the pesticide as (a) an EC and (b) a microencapsulate.

were extracted and analyzed as described above. As shown in Figure 2, the concentration of pirimiphos-methyl found in bluegill fish exposed to the encapsulated formulation was substantially less than that found in those exposed to the EC, whereas there was little difference in final uptake of the two formulations by ramshorn snails.

Within the concentration limits discussed above for each substrate, i.e., water, fish, and snails, the recovery of pirimiphos-methyl is essentially quantitative. Direct analysis of the hexane extract without additional cleanup of the fish and snail samples reduces the number of steps from that usually required for extraction of pesticides from animal tissues. This decreased handling increases recovery of the pesticide and reduces the time required for each analysis. The simplicity of these methods combined with the sensitivity and selectivity of GC-FPD analysis provides a useful means of determining residue concentrations of pirimiphos-methyl.

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