Cleanup Procedure for Water, Soil, Animal and Plant Extracts for the Use of Electron-Capture Detector in the Gas Chromatographic Analysis of Organophosphorus Insecticide Residues[1]

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Numerous cleanup procedures for the analysis of organophosphorus insecticide residues in plant and animal tissues have been developed.

Conventional partitioning methods have been demonstrated as efficient and useful (1, 3, 4, 5, 6, 8, 9, 10). Their disadvantage is the interference they cause in operating the electron capture-detector in gas chromatography. Partitioning methods must be preceded or followed by an adsorption step prior to gas chromatography. In the methods polar solvents

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used such as dimethyl sulphoxide and dimethyl formamide are expensive and unpleasant to use and nitromethane is explosive and immiscible with water.

Egan et al. and Laws and Webley (4, 8) separately reported elution and recovery difficulties with alumina column that preceded their partitioning methods. Therefore, a simple, effective method to isolate insecticides from fats, waxes, and other biological substances should be widely applied.

Primary purpose of work reported here was to develop a cleanup partition method to separate some common organophosphorus insecticides from several types of interfering biological materials. Four solvent partitioning systems with reproducible high recovery of insecticides were established.

Materials and Methods

Reagents and Equipment. Insecticide standards were prepared in hexane from analytical grade diazinon (Geigy Agricultural Chemicals, Ardsley, N. Y.), parathion, methyl parathion, malathion and thimet (American Cyanamid Co., Princeton, N. J.).

Solvent systems were hexane with 50%, 60%, 70% or 80% (v/v) solutions of acetonitrile in distilled water. All solvents were nanograde (Mallinckrodt Chemical, St. Louis, Mo.).

Gas chromatographs included: (1) RSCO model #600 series; and (2) Barber Coleman #5000 series, both equipped with electron-capture detectors.

Experimental

The partition characteristics of the insecticides used between hexane and four separate aqueous acetonitrile solutions (Table I) were first examined separately on the microgram scale. Fifty ug of each individual insecticide were dissolved in 10 ml hexane saturated with the aqueous solution of acetonitrile and extracted with four successive 20-ml portions of the aqueous acetonitrile saturated with hexane. Each of the aqueous acetonitrile phases was transferred into an evaporator to reduce volume to the amount of distilled water in the 20 ml of aqueous acetonitrile solution. Using small portions of hexane totaling 20 ml, the aqueous extract was then transferred to a 250-ml separatory funnel. The mixture was shaken thoroughly with an additional 100 ml of water. After the two phases separated, the lower (aqueous) layer was discarded and the upper phase (hexane) was collected and reduced in volume to 1 ml of which a 4-ul portion was used for gas chromatographic analysis.

Recovery Experiments. Extracts of ground water, soil, animal and plant tissues were prepared by standard techniques (2). The extracts were fortified with 0.1 ppm of the indicated insecticides and carried through according to the aforementioned cleanup procedures except that the four successive 20-ml portions of aqueous acetonitrile were combined. If the extracts were prepared in a solvent other than hexane, the solvent should be

evaporated and replaced with 10 ml hexane. It is preferable to centrifuge the hexane insoluble contaminants. Recoveries were determined by gas liquid chromatography with an electron capture-detector.

Results and Discussion

Partitioning residue extracts between immiscible solvents is a convenient process to separate insecticides from interfering biological substances. The partition characteristics of tested insecticides between hexane and four separate aqueous acetonitrile solvent systems (Table I) were determined and calculated as previously published (7). With the 80% aqueous acetonitrile system (Table I), the average efficiency of extraction with four successive partitions for all insecticides examined was 99% with a mean deviation below 0.1. However, the lowest recoveries were obtained with 50% aqueous acetonitrile system.

Recovery experiments were conducted with hexane extracts of grain, soil, ground water, foliage, and animal tissues to which microgram amounts of insecticides examined had been added. In the application of the four separate solvent systems for cleanup, complete recovery was obtained of all tested insecticides except thimet and diazinon; their complete recovery was obtained using 80% aqueous acetonitrile. Although use of 50% and 60%

TABLE I

Partition of insecticides between hexane and successive aqueous acetonitrile extracts

Solvent system (with hexane)	Insecticide (50 ug)	Percentage recovered in aqueous acetonitrile extracts: 1st extract	2nd extract	3rd extract	4th extract	Total recovery	% in final hexane extract
50% acetonitrile in distilled water	Diazinon	35.0[a]	26.4	18.2	7.7	87.3 ± 0.8[b]	13.3
	Malathion	64.3	24.2	7.9	2.3	98.7 ± 0.3	1.3
	Thimet	27.2	22.6	16.2	9.2	75.2 ± 1.5	24.8
	Parathion	49.7	27.1	14.0	4.6	95.4 ± 0.2	4.6
	Methyl parathion	68.2	23.5	5.7	1.7	99.1 ± .03	0.8
60% acetonitrile in distilled water	Diazinon	45.3	28.0	15.4	5.1	93.8 ± 0.7	6.2
	Malathion	78.2	18.2	2.9	0.5	99.8 ± 0.1	0
	Thimet	35.8	26.5	17.4	7.3	87.0 ± 0.9	13.0
	Parathion	59.3	26.3	9.8	2.7	98.1 ± 0.2	1.9
	Methyl parathion	82.9	15.1	1.7	0.3	100.0 ± 0.03	0
70% acetonitrile in distilled water	Diazinon	53.2	25.8	14.3	3.5	96.8 ± 0.5	3.1
	Malathion	84.1	14.6	1.2	0.1	100.0 ± 0.0	0
	Thimet	47.8	27.1	13.7	5.4	94.0 ± 0.2	6.0
	Parathion	71.5	22.3	5.4	0.6	99.8 ± 0.0	0.3
	Methyl parathion	89.6	9.5	0.7	0.2	100.0 ± 0.03	0
80% acetonitrile in distilled water	Diazinon	61.0	24.9	9.6	2.8	98.3 ± 0.0	1.8
	Malathion	92.6	7.1	0.3	0	100.0 ± 0.5	0
	Thimet	61.3	24.7	9.4	2.8	98.2 ± 0.03	1.8
	Parathion	79.6	17.9	2.2	0.2	99.9 ± 0.0	0.0
	Methyl parathion	94.2	5.5	0.3	0.0	100.0 ± 0.0	0

(a) Recovery is an average of 4 replicates.
(b) ± indicates mean deviation.

aqueous acetonitrile solvent systems resulted in low recovery of diazinon and thimet, those systems were the most efficient in removing the contaminants. The two separate solvent systems with hexane should be used for extracts that have large amounts of fats, waxes and/or pigments. Choice of the aqueous acetonitrile solvent system depends on the nature of the substrate, the extent of the cleanup necessary, and recovery percentage required.

Cleanup of extracts with the described procedure was satisfactory for electron capture-gas chromatographic analysis with 0.01 ppm detected using 10 gm soil, grain, plant and animal tissues and 0.01 ppb using 10 liters of ground water. This cleanup method should apply equally well for paper and thin-layer chromatography.

Although there were indications that very small amounts of plant pigments were not completely eliminated by this procedure, a minimum background was encountered in the chromatogram.

The aqueous acetonitrile hexane solvent system could be used for larger amounts of substrate by adjusting the volumes of the various solvents accordingly. It is simple, reproducible and convenient to use and should be adaptable to recovery of organophosphorus insecticides from both plant and animal products.

Summary

A simple, aqueous acetonitrile partition cleanup method for analyses of some common organophosphorus insecticide residues

is described. The procedure described is for cleanup and quantitative recovery of parathion, methyl parathion, diazinon, malathion and thimet from different extracts. Those insecticides in the purified extracts of ground water, grain, soil, plant and animal tissues can be detected quantitatively by gas chromatography with an electron capture-detector at 0.01 ppm. Cleanup is satisfactory for paper and thin-layer chromatography for further identification of individual insecticides in the extracts.

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