Partitioning Method for Sample Cleanup for Gas Chromatographic Analysis of Common Organic Pesticide Residues in Biological Materials[1]

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A cleanup procedure for gas chromatographic analysis of common organic phosphorus pesticide residues has been reported (3). The cleanup method advocating the use of four aqueous acetonitrile-hexane solvent systems has been modified and extended to include several additional pesticides, especially the chlorinated hydrocarbon pesticides.

Materials and Methods

Reagents and Equipment. Materials and equipment were the same as previously published (3). The pesticides used were analytical grades of DDT isomers (o,p'; p,p'), DDD, DDE,

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trithion, methoxychlor, ronnel, BHC isomers (Alpha, Beta, Delta), lindane, thiodan, telodrin, endrin, ovex, perthane, aldrin, dieldrin, heptachlor, heptachlor epoxide, Kelthane, furnished by various pesticide manufacturers.

Procedure. The method used was identical with that previously published (3) except that 85% and 95% aqueous acetonitrile in distilled water were used separately for partitioning with hexane to determine percent recovery of pesticides in plant and animal samples. The pesticides were added to the extracts of plant and animal tissues at the 0.1 ppm level. Recoveries of pesticide residues were determined by comparing retention time and peak area or peak height of each pesticide with that of a known quantity of pesticide in hexane added to the pre-cleaned extract.

Results and Discussion

An attempt was made to utilize the partitioning cleanup procedure of organophosphorus pesticides (3) for isolation of chlorinated hydrocarbon pesticides from biological substances. During the experimental testing of this procedure, low recovery ($<$70%) of pesticides was obtained. These preliminary results indicated the necessity for using different solvent systems such as 85% and 95% aqueous acetonitrile-hexane

TABLE I

Partition of pesticides between hexane and successive 85% aqueous acetonitrile extracts

Pesticide (20 ug)	Percentage recovered in aqueous acetonitrile extract 1st	2nd	3rd	4th	Total recovery	% in final hexane extract
Ronnel	52.8[a]	26.8	10.6	5.1	95.3 ± 4[b]	4.3
Trithion	55.0	26.4	11.7	4.7	97.8 ± 3	2.7
Ovex	84.0	14.5	1.5	0.0	100.0 ± 0.0	0.0
Kelthane	72.6	21.3	4.6	0.8	99.3 ± 2	0.8
Perthane	36.4	21.3	13.3	9.3	80.3 ± 1.8	19.5
Telodrin	23.6	16.9	13.3	10.5	64.3 ± 1.4	35.6
Thiodan	31.9	24.8	14.9	11.0	82.6 ± 0.5	16.6
BHC Alpha	67.6	24.4	6.0	1.5	99.5 ± 0.2	0.4
BHC Beta	93.8	6.0	0.3	0.0	100.1 ± 0.0	0.0
Lindane	81.7	15.9	2.5	0.0	100 ± 0.0	0.0
BHC Delta	88.7	10.0	1.1	0.0	99.8 ± 0.0	0.0
DDD	56.3	25.5	10.3	4.2	96.3 ± 0.1	3.4
DDE	11.4	10.0	9.1	8.1	38.6 ± 0.8	61.3
o,p' DDT	14.7	13.2	11.5	10.0	49.4 ± 1.0	50.7
p,p' DDT	21.6	18.2	15.5	13.3	68.6 ± 1.2	31.5
Aldrin	9.1	8.3	8.2	7.3	32.9 ± 1.5	67.1
Dieldrin	42.3	24.6	14.4	7.8	89.1 ± 0.1	10.9
Heptachlor	17.6	14.9	12.5	8.8	53.8 ± 0.4	46.2
Heptachlor epoxide	49.4	24.8	13.0	5.9	93.1 ± 0.1	6.9
Endrin	39.4	24.2	14.7	8.4	86.7 ± 0.3	13.4
Methoxychlor	76.5	18.9	3.7	0.8	99.9 ± 0.0	0.2

(a) Recovery is an average of 5 replicates.

(b) ± indicates mean deviation.

TABLE II

Partition of pesticides between hexane and successive 95% aqueous acetonitrile extracts

Pesticide (20 ug)	Percentage recovered in aqueous acetonitrile extract 1st	2nd	3rd	4th	Total recovery	% in final hexane extract
Ronnel	72.8[a]	22.7	3.2	0.9	99.6 ± 0.4[b]	0.5
Trithion	76.5	20.1	2.7	0.6	99.9 ± 0.0	0.0
Ovex	93.9	5.9	0.4	0.0	100.2 ± 0.0	0.0
Kelthane	86.2	12.3	1.3	0.0	99.8 ± 0.1	0.1
Perthane	56.9	24.2	10.2	4.6	95.9 ± 0.8	4.0
Telodrin	43.2	25.8	14.1	7.2	90.3 ± 1.3	9.7
Thiodan	55.4	26.5	11.0	5.2	98.1 ± 0.5	2.5
BHC Alpha	82.2	15.5	2.0	0.3	100.0 ± 0.2	0.1
BHC Beta	97.4	2.7	0.0	0.0	100.1 ± 0.0	0.0
Lindane	90.3	8.6	0.9	0.1	99.9 ± 0.0	0.0
BHC Delta	93.7	5.7	0.5	0.0	99.9 ± 0.0	0.0
DDD	76.0	18.3	4.2	1.0	99.5 ± 0.0	0.5
DDE	25.7	21.0	14.0	11.3	72.0 ± 1.0	28.1
o,p' DDT	33.4	22.5	16.5	9.0	81.4 ± 0.6	18.6
p,p' DDT	48.2	26.3	13.2	6.0	93.7 ± 0.3	6.4
Aldrin	16.6	14.3	12.6	9.4	52.9 ± 0.5	47.2
Dieldrin	62.3	22.7	9.1	3.5	97.6 ± 0.2	2.0
Heptachlor	33.8	24.5	14.0	10.1	82.4 ± 0.8	17.6
Heptachlor epoxide	66.8	23.3	6.5	2.3	98.9 ± 0.1	1.2
Endrin	57.6	26.1	9.2	4.2	97.1 ± 0.3	2.9
Methoxychlor	86.8	12.0	1.2	0.1	100.1 ± 0.0	0.0

(a) Recovery is an average of 5 replicates.

(b) ± indicates mean deviation.

systems. The characteristic distribution of the pesticides between aqueous acetonitrile and hexane is summarized in table I and II. Four successive partitions with 85% aqueous acetonitrile systems resulted in lower recoveries of pesticides compared to that obtained with 95% aqueous acetonitrile solvent system. However, 95% aqueous acetonitrile solvent system resulted in low recovery (<95%) of telodrin, DDT, o,p'DDT, p,p'DDT, aldrin and heptachlor. Recovery experiments were conducted by adding known amounts (0.1 ppm) of pesticides to extracts of animal and plant tissues. The pesticides were isolated from all fats and waxes and most pigments using 85% and 95% aqueous acetonitrile solvent systems. The average results of six replicates for each pesticide are given in table III. The average recoveries of all tested pesticides were usually lower and more variable than expected, as indicated in table III which shows that mean deviation ranges from $\pm$ 0.5 to $\pm$ 3.1. Low recovery could be attributed to the amounts of fats and waxes in the extract. Although use of 85% aqueous acetonitrile solvent system resulted in low recovery of tested pesticides, it was more efficient in removing the contaminants, as indicated by the minimum background encountered in the chromatogram. Because

TABLE III

Recoveries of pesticides from extracts of 10 grams of animal and plant tissues after cleanup[a]

Pesticide	Percent recovery determined by gas chromatography	
	85% aqueous acetonitrile-hexane system	95% aqueous acetonitrile-hexane system
	[b] [c]	
o,p' DDT	42.1 ± 2.5	75.2 ± 1.8
p,p' DDT	60.5 ± 2.0	87.4 ± 1.0
p,p' DDE	32.4 ± 3.0	67.1 ± 3.1
p,p' DDD	89.1 ± 2.1	92.0 ± 1.5
Lindane	97.1 ± 1.2	97.5 ± 0.8
BHC Alpha	95.5 ± 0.8	96.7 ± 0.5
BHC Beta	97.1 ± 1.0	98.1 ± 0.6
BHC Delta	97.2 ± 1.0	98.5 ± 0.7
Methoxychlor	96.7 ± 1.1	98.3 ± 0.9
Aldrin	30.8 ± 1.1	51.4 ± 1.5
Dieldrin	86.2 ± 0.9	95.2 ± 0.5
Heptachlor	51.3 ± 2.4	80.3 ± 2.5
Heptachlor epoxide	90.2 ± 0.7	96.5 ± 0.5
Endrin	84.1 ± 1.3	95.1 ± 0.8

(a) Samples were fortified with the indicated pesticides at 0.1 ppm level.

(b) Recovery is an average of six replicates.

(c) ± indicates mean deviation.

of low recoveries of DDT (o,p'; p,p'), DDE, heptachlor and aldrin, the cleanup procedure is not recommended for quantitative analysis of these pesticides. However, the use of n-hexane and 95% aqueous acetonitrile for the isolation of chlorinated hydrocarbon pesticides from fats and waxes was more efficient than the original partition technique of Maunder et al. (4) and Jones and Riddick (1) which gave high background in the chromatogram. Detection level using electron capture gas chromatography ranges from 0.01 to 0.1 ppm depending on the sensitivity of the gas chromatographic detector for the pesticide.

Since the nature of the sample and the quantity of fats and waxes affect the efficiency of the cleanup procedure as well as the recovery, the partitioning procedure could be used alone or followed by the microcleanup method (2). This cleanup procedure could be used for paper and thin layer chromatographic analysis of the tested pesticides as long as the various solvents in the partitioning method are adjusted according to the sample size.

Although intended primarily for the determination of tested chlorinated hydrocarbon pesticides, this procedure will apply equally well for the analysis of ronnel, trithion, ovex, Kelthane and perthane.

References

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