Modifications of the Micromethod of Sample Cleanup for Thin-Layer and Gas Chromatographic Separation and Determination of Common Organic Pesticide Residues[1]

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Success in the cleanup of microquantities of pesticides in ground water, soil, plant and animal tissue extracts for gas-liquid chromatography has been achieved using a rapid microcolumn method (1,2). However, the microcolumn cleanup method has limited the size of samples that can be processed for analysis. The micromethod using high purity grade 950 silica gel has been extended to include high purity grade 923 silica gel (100-200 mesh) and has been modified for the cleanup of 5 to 100 gm samples for thin-layer and gas chromatographic analysis.

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[1] Contribution No. 961, Department of Entomology, Kansas Agricultural Experiment Station, and Contribution No. 21, Kansas Water Resources Research Institute, Manhattan, Kansas, U. S. A. This project was supported in part by Regional Research Projects MCM-37 and NC-85, Kansas projects 687 and 481; and by the U. S. Department of the Interior, Office of Water Resources No. B-007-KAN. The assistance of Mrs. Marie Finocchio is greatly appreciated.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 3, No. 6, 1968, published by Springer-Verlag New York Inc.

Materials and Methods

Reagents and Equipment. Materials and equipment were identical with those used in research previously published (1, 2) with the following additions:

Activated silica gel, high purity grade 923 (100-200 mesh) (Fisher Scientific Company, St. Louis, Mo.) was used as adsorbent. Chromatographic columns included: (1) chromaflex chromatographic column size A (8 mm O.D.), B (9 mm O.D.) and C (11 mm O.D.) with a 50-ml capacity reservoir; and (2) macrochromatographic column, with 2 mm bore stopcock at bottom with a 2A teflon plug; 25 mm O.D., 22 mm I.D., 350 mm length, and reservoir capacity 300 ml (Kontes, Franklin Park, Ill.).

Chromogenic agents and nonaqueous solvent systems of Mitchell (3) have been used with the exception that 1% (v/v) hydrogen peroxide in redistilled acetone was used prior to the chromogenic agent. Hydrogen peroxide treatment provides a nearly white background which aids in visualization of pesticide residues. Silica gel G. and cellulose thin-layer plates were purchased from Brinkman Instruments, Westbury, N. Y.

Procedure. The cleanup method was identical with that previously described (1, 2) except different volumes of eluting solvents directly proportional to the amount of adsorbent were collected as indicated in Table I and II. Glass wool was used as a plug at the narrow tip of the columns. Thin-layer chromatography technique of Walker and Beroza (4) was used for detection of pesticide residues with the following minor variations. Silica gel G (or cellulose) plates were washed with 1% (v/v) hydrogen peroxide in redistilled acetone, then reactivated in a vertical position for 60 minutes at 120°C prior to use. Five minutes after development the plate was sprayed with 1% hydrogen peroxide in redistilled acetone, dried and then sprayed with fluorescein solution, exposed to ultraviolet (U.V.) light for 5 minutes, sprayed again with fluorescein solution, re-exposed to U.V. light for 5 minutes, sprayed with silver nitrate solution, and finally exposed to U. V. light for 5 minutes. R_f values were recorded throughout the procedure.

Results and Discussion

Results in Table I indicate that cleanup of pesticide residue extracts using high purity grade 923 (100-200 mesh) silica gel was satisfactory. However, total recovery of pesticide residues was obtained using grade 923 silica gel in the 12 ml elution instead of 8 ml which is used with 950 grade silica gel (1). Because of the lack of a reservoir for the microcolumns and the limited amount of sample used, further study was undertaken using three sizes of Chromaflex columns with 50 ml reservoir capacity and a macrocolumn with 350 ml reservoir capacity. Recoveries of different pesticides using three different sizes of column, containing variable amounts of silica gel 950 and 923, were shown in Table II. Complete recovery of all pesticides listed was obtained.

Cleanup of soil, water, animal and plant tissue extracts was satisfactory for thin-layer and gas chromatographic detection using different

TABLE I

Elution of pesticides from microcolumns[(a)] packed with 1 gm grade 923 silica gel

Pesticide	Distribution in eluate, % recovery estimated from GLC		Pesticide	Distribution in eluate, % recovery estimated from GLC	
	8 ml	12 ml		8 ml	12 ml
	(b) (c)				
Aldrin	90 ± 1	100 ± 1	Thiodan (low m.p. isomer)	98 ± 1	101 ± 1
Dieldrin	86 ± 2	100 ± 2	Thiodan (high m.p. isomer)	84 ± 2	98 ± 2
Endrin	96 ± 1	101 ± 1	Methoxychlor	45 ± 2	98 ± 3
Heptachlor	84 ± 3	101 ± 3	BHC Alpha	80 ± 1	99 ± 1
Heptachlor epoxide	95 ± 1	100 ± 1	BHC Beta	88 ± 2	97 ± 1
o,p' DDT	95 ± 1	99 ± 1	BHC Delta	60 ± 1	99 ± 2
p,p' DDT	92 ± 1	101 ± 1	Lindane	69 ± 1	98 ± 2
DDD	90 ± 1	98 ± 2	Ronnel	89 ± 2	99 ± 2
DDE	97 ± 1	100 ± 1	Parathion	82 ± 3	97 ± 3
Ovex	85 ± 2	99 ± 3	Methyl parathion	30 ± 2	93 ± 3
Perthane	96 ± 1	99 ± 1	Trithion	70 ± 4	97 ± 3
Telodrin	95 ± 2	101 ± 2	Thimet	90 ± 2	99 ± 1
Kelthane	90 ± 2	95 ± 2	Diazinon	70 ± 4	101 ± 2
			Malathion	80 ± 1	99 ± 2

(a) Scientific Products, Evanston, Ill.

(b) Recovery is the average of 4 replicates to nearest percent.

(c) Standard deviation.

TABLE II

Elution of pesticides from three types of columns and
a macrocolumn packed with silica gel 923 (100-200 mesh) and 950 (60-200 mesh), separately.

Chromatograph column:	A (6 mm I.D.)		B (7 mm I.D.)		C (9 mm I.D.)		Macrocolumn (22 mm I.D.)	
Amount of silica gel:	1 gm		1.5 gm		3 gm		20 gm	
Sample size:	5 gm		25 gm		15 gm		100 gm	
Grade of silica gel:	950	923	950	923	950	923	950	923
Volume of eluting solvent:	8	12	12	18	24	36	160	240
	% recovery determined by GLC							
	(a) (b)							
Aldrin	100 ± 0	98 ± 1	99 ± 0	98 ± 1	98 ± 2	99 ± 1	98 ± 2	97 ± 1
Dieldrin	99 ± 1	95 ± 1	99 ± 1	93 ± 1	100 ± 1	93 ± 2	99 ± 1	98 ± 1
Heptachlor	99 ± 1	98 ± 2	98 ± 1	95 ± 3	99 ± 1	96 ± 3	96 ± 2	96 ± 1
Heptachlor epoxide	100 ± 0	100 ± 1	99 ± 1	98 ± 1	100 ± 0	99 ± 1	100 ± 0	99 ± 0
o,p DDT	98 ± 2	99 ± 1	96 ± 1	92 ± 2	98 ± 2	97 ± 3	96 ± 1	99 ± 1
p,p DDT	99 ± 1	98 ± 1	98 ± 1	96 ± 1	98 ± 1	97 ± 1	98 ± 1	98 ± 1
Endrin	99 ± 0	99 ± 1	100 ± 1	99 ± 1	100 ± 0	99 ± 1	99 ± 1	100 ± 1
DDE	99 ± 2	98 ± 1	98 ± 1	98 ± 2	100 ± 2	99 ± 1	100 ± 0	100 ± 1
DDD	99 ± 2	98 ± 2	98 ± 1	97 ± 1	97 ± 2	97 ± 1	99 ± 1	96 ± 2

(a) Recovery is the average of 4 replicates to nearest percent.
(b) Standard deviation.

amount of samples as indicated in Table II. Application of this method will enable investigators to use larger amounts of samples. Chromaflex columns are simple designs which are quickly set up and easily cleaned, and are capable of producing rapid separation whenever cleanup is needed for a small number of samples. Since larger amounts of samples are needed for thin-layer chromatography, high purity grade silica gel macrocolumns should be used prior to chromatography. A complete recovery of a mixture of all pesticides was obtained using benzene instead of an eluting solvent for each pesticide except malathion and diazinon where an 8% Ethyl acetate in benzene was needed to achieve 100% recovery.

The modified thin-layer chromatography reported herein provides an effective method for detection and visualization of the pesticides. Organophosphorus and chlorinated hydrocarbon compounds generally gave brilliant greenish-yellow and pink spots, respectively, on a white background which was obtained by washing the plates, using 1% (v/v) hydrogen peroxide in redistilled acetone and 1% (v/v) hydrogen peroxide spray prior to use of fluorescein and silver nitrate spray. The manner in which the plates were sprayed enhanced the appearance of all spots.

References

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