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IMPROVED RECOVERY OF HEXACHLOROBENZENE IN ADIPOSE TISSUE WITH A MODIFIED MICRO MULTIRESIDUE PROCEDURE

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SUMMARY

Using the described methodology the recovery of hexachlorobenzene from adipose tissue was significantly increased over that normally obtained with other multiresidue procedures. The recovery of other commonly encountered chlorinated hydrocarbon pesticides was not affected nor was the "background" from adipose tissue intolerable. Although extensive work has not been done, it is likely that improved recovery of hexachlorobenzene could be expected from other tissues.

INTRODUCTION

Recovery of low concentrations of hexachlorobenzene (HCB) from fatty substances with conventional multiresidue methods has been reported to be only 60%[1]. Other investigators[2] have pointed out that the poor recovery of this fungicide with the procedure of Mills *et al.*[3] is due to its non-polar nature. In a petroleum ether (b.p. 30–60°)–acetonitrile solvent system, HCB is preferentially soluble in the hydrocarbon phase. The poor recovery is to be expected since the *p*-value for HCB was determined to be about 0.3.

Stijve[4], who also reported on the slight solubility of HCB in acetonitrile, resorted to direct elution through a Florisil® column for cleanup prior to determination of HCB in fatty substances. A direct elution cleanup and quick confirmation method for HCB by derivatization and electron capture gas chromatography was recently reported[5].

Our experiences in determining HCB using a modification of the procedure of Mills *et al.* yielded recoveries in the 60–65% range. We reasoned that recoveries with this methodology could not be improved because of the petroleum ether–acetonitrile partitioning step. Therefore, a micro multiresidue procedure developed by Enos *et al.*[6] was tried using acetonitrile to extract the pesticides from the fat. The acetonitrile was then diluted with a 2% sodium sulfate solution and the pesticides were partitioned into hexane.

Results again were disappointing, with recoveries still in the 60–65% range. However, the use of 20% acetone in acetonitrile (v/v) as the extracting solvent pro-

duced a marked improvement in the recovery of HCB without sacrificing recovery for other common chlorinated pesticides.

EXPERIMENTAL

Apparatus[*]

A Tracor MT 220 gas chromatograph, equipped with a tritium electron capture detector operated in the d.c. mode, was used. The gas chromatographic column was 1.8 m × 4 mm I.D. borosilicate glass, packed with 1.5% OV-17/1.95% QF-1 on 80–100 mesh Supelcoport. The column was operated at 200° with a nitrogen flow-rate of 60 ml/min. Other temperatures were: detector, 210°; inlet, 230°; transfer line, 230°.

The following glassware were used: Chromaflex column, 200 × 7 mm I.D., reservoir volume 50 ml, size 22 (Kontes[**] Cat. No. 420100); Kuderna-Danish concentrator assembly (K-570000) and 25-ml graduated tubes size 2525 (K-570050); Chromaflex column, 200 mm × 10.5 mm I.D., size 212 (Kontes Cat. No. 420530); tissue grinder, 150 mm × 15 mm O.D., size 22 (Kontes Cat. No. K-885450); 50-ml round-bottom tube with glass stopper; modified micro Snyder distilling column.

Reagents and materials

Hexachlorobenzene, β-hexachlorocyclohexane, aldrin, heptachlor epoxide, dieldrin, *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT were 99% analytical reference standards.

Sodium sulfate was used either in the form of a 2% aqueous solution or anhydrous, granular, which was Soxhlet-extracted with benzene and oven-dried at 130°.

Florisil® (60–100 mesh) was used in a form activated by the manufacturer at 1200°F.

MgO powder, reagent grade, was Soxhlet-extracted with benzene, and oven-dried at 130°.

Celite 545 must be cleaned up by adding 6 *N* HCl and heating on a steam-bath for 1 h; HCl should be decanted and Celite washed with distilled water until neutral, and then washed successively with several portions of methanol, acetone, ethyl acetate, and petroleum ether. Traces of solvent should be removed by suction and air drying. Drying is to take place in a 130° oven for a minimum of 7 h. The end product should be stored in a glass container.

Acetone, acetonitrile, hexane, petroleum ether (b.p. 30–60°), and methanol were pesticide quality or equivalent.

Preparation of adsorbent columns

Florisil® column. Place a small plug of hexane-extracted glass wool in the tip of a size 22, 200 × 7 mm I.D. Chromaflex column. Slowly add 1.6 g Florisil with gentle tapping of the column to ensure even packing. Add 1.6 g anhydrous sodium sulfate on top of the Florisil. Wash the column with 50 ml Nanograde® hexane (Mallinckrodt, St. Louis, Mo., U.S.A.) followed by 50 ml Nanograde® methanol. Dry and store columns at 130° for a minimum of 15 h before using.

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[*] Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Environmental Protection Agency.

[**] Kontes Glass Company, Vineland, N.J. 08360, U.S.A.

MgO–Celite 545 column. Weigh out 2.5 g each of MgO and cleaned-up Celite 545. Mix the two powders thoroughly. Transfer to a small beaker and add enough petroleum ether to form a slurry. Carefully pour the slurry into a size 212, 200-mm × 10.5-mm-I.D. Chromaflex column. Allow the solvent to drain through the column until the solvent level is at the top of the MgO–Celite.

Method

Approximately 500 mg of rendered chicken fat was extracted in a tissue grinder with 2.5 ml of 20% acetone in acetonitrile. The sample was then centrifuged and the supernatant was transferred by disposable pipet to a 50-ml round-bottom tube containing 25 ml of 2% sodium sulfate solution. The sample was extracted twice more, centrifuged, and transferred as described to the 50-ml tube. Partitioning was accomplished by adding 4 ml hexane and shaking vigorously for 2 min. After the phases separated the top layer (hexane) was transferred to a 25-ml concentrator tube with a disposable pipet. The extract was partitioned two additional times with 3 ml hexane each time and transferred to the tube. The solvent was evaporated to 0.3 ml under a gentle stream of nitrogen and on a water-bath at 50° or on a steam-bath using a micro Snyder column. The sample was quantitatively transferred with hexane to a 200-mm Chromaflex column containing 1.6 g Florisil® topped with 1.6 g sodium sulfate. The column was eluted with 12 ml hexane followed by 12 ml 1% methanol in hexane (first fraction) and finally with 12 ml 1% methanol in hexane (second fraction). The eluates were collected in 25-ml concentrator tubes and evaporated with either a nitrogen stream or a micro Snyder column to 1.0 ml.

The second fraction from the Florisil® column was reduced in volume to 0.5 ml and eluted through a 200-mm column containing 5 g MgO–Celite (1:1) with 70 ml of petroleum ether. This step is recommended to remove excessive amounts of interfering material.

RESULTS AND DISCUSSION

Table I shows the recovery of HCB using 20% acetone in acetonitrile as the extraction solvent. A slight improvement in recovery could be obtained by using nitrogen to reduce the volume of the extract to approximately 0.5 ml prior to hexane

TABLE I

RECOVERY OF HCB FROM FORTIFIED CHICKEN FAT WITH 20% ACETONE IN ACETONITRILE AS EXTRACTION SOLVENT*,**

Fat (mg)	*Fortification level (μg/g)*	*Recovery (%)*
407	0.01	80.7
397	0.03	81.5
420	0.10	82.4
558	0.30	82.5
411	1.0	87.6

* Each value is the average of four determinations.

** The volume of sample extract was not reduced prior to hexane partitioning.

TABLE II

HCB RECOVERY USING A MODIFIED MICRO SNYDER COLUMN OR NITROGEN STREAM TO EVAPORATE HEXANE*

Fat (mg)	*Fortification level (μg/g)*	*Means of evaporation of hexane*	*Recovery (%)*
243	0.16	micro Snyder column	80.4
346	0.12	micro Snyder column	84.2
285	0.14	nitrogen stream	86.1
304	0.13	nitrogen stream	88.0

* The volume of sample extract was reduced to 0.5 ml prior to hexane partitioning.

partitioning. This is indicated by the data in Table II, as the sample extract was concentrated in the above manner. However, the evaporation of the extract requires additional analysis time so that the improved recovery must be considered in relation to the increased time involved. The data in Table II also show that the evaporation of the hexane using a steam-bath and a micro Snyder column results in a slight loss of hexachlorobenzene. Consequently, for maximum recovery of HCB with this modified multiresidue procedure, the use of a nitrogen stream and a warm water-bath to evaporate the hexane is recommended.

Several trials were made using different concentrations of acetone in acetonitrile as the extracting solvent. Lesser amounts of acetone gave lower recoveries of HCB while higher amounts produced a somewhat better extraction efficiency but also more interference. A good compromise seemed to be reached with 20% acetone.

Recovery of other selected chlorinated pesticides was quantitative using the modified methodology (Table III). Moreover, aldrin recovery was several per cent greater than that normally obtained with the unmodified procedure.

The chromatograms in Fig. 1 show the separation and sensitivity achieved with a chicken fat sample (fraction 1) fortified with 10 ng/g HCB and various concen-

TABLE III

RECOVERY OF SELECTED PESTICIDES FROM CHICKEN FAT USING THE MODIFIED MICRO MULTIRESIDUE PROCEDURE

Pesticide	*Fortification level (μg/g)*	*Recovery (%)**
β-HCH	0.2	89.6
Aldrin	0.3	88.2
p,p'-DDD	0.3	104.2
Heptachlor epoxide	0.5	93.5
Dieldrin	0.6	93.6
p,p'-DDT	1.0	97.8
p,p'-DDE	4.0	93.3

* Average of three sample determinations.

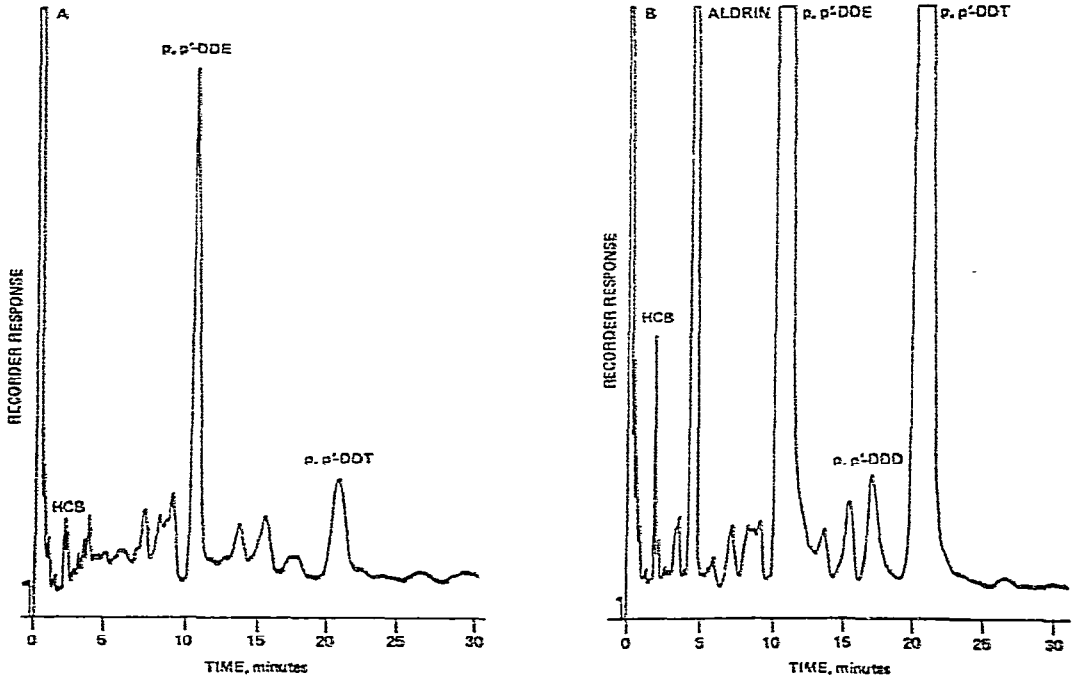

Fig. 1. Chromatograms of (A) 489 mg unfortified chicken fat sample and (B) the first fraction of chicken fat sample fortified with 10 ng/g HCB and other selected pesticides. Injections, 5 μl from 1.0 ml hexane; column, 1.8 m × 4 mm I.D., Pyrex glass, packed with 1.5% OV-17/1.95% QF-1 on 80–100 mesh Supelcoport; oven temperature, 200°; nitrogen flow-rate, 60 ml/min.

trations of certain other chlorinated insecticides. Chromatogram A is an unfortified sample blank and indicates the presence of a small background level of HCB that is, however, well below 10 ng/g. A large response for *p,p'*-DDE and a somewhat smaller response for *p,p'*-DDT in the blank fat is evident.

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