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Simplified Cleanup Procedures for Adipose Tissue Containing Polychlorinated Biphenyls, DDT, and DDT Metabolites

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The usual procedure for the analyses of chlorinated pesticides in adipose tissue requires that the fat be separated from the connective tissue and then partitioned by liquid-liquid extraction to remove many endogenous interferants. The solvent phase containing the pesticides is further cleaned up by column chromatography (MILLS et al. 1963). The partitioning step is tedious and time-consuming and can be a source of incomplete recovery of some chlorinated pesticides. An effective but potentially hazardous method, in which concentrated sulfuric acid is used, has also been reported for the cleanup of animal tissues (MURPHY 1972).

Anticipating the need for an analytical micro procedure for determining polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and DDT metabolites in several hundred adipose samples taken by needle biopsy, surgery, or autopsy, we developed an analytical micro procedure that eliminates the need for liquid-liquid partitioning in the cleanup of chlorinated pesticide residues in fat samples. In our procedure, the column chromatography step fulfills the function of both the partitioning and column chromatography steps. For the analysis of DDT and DDT metabolites, a 44% sulfuric acid on silica gel chromatography column is used to remove the bulk of lipids and other oxidizable components from the hexane extract. For the analysis of PCBs, a 10% silver nitrate on silica gel chromatography column is used to retain the lipids, DDT and DDT metabolites. We have reported the use of this column for analyzing serum samples for PCBs (NEEDHAM et al. 1980).

MATERIALS AND METHODS

Apparatus. The gas chromatography/data system used was a Perkin-Elmer Sigma I equipped with a constant current ^{63}Ni detector and an AS-100 autosampler. A 1.83 m by 2 mm (i.d.) glass gas chromatography column packed with 1.5% SP2250/1.95% SP2401 on 100/120 mesh Supelcoport was used for DDT analysis. A 1.83 m by 2 mm (i.d.) glass chromatography column packed with 3% SE-30 on 80/100 Supelcoport was used for PCB analysis.

Chromatographic Columns. Size 22, i.d. 7 mm, length 200 mm, Kontes No. 420100.

0007-4861/82/0028-0718 $1.00

Tissue Grinder. 15 mL, size 23, Kontes No. 885350.

Standards. Analytical standards of DDT and DDT metabolites were obtained from the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711. Aroclor 1260, Lot AK 3, was supplied by Monsanto Chemical Company.

Reagents. Hexane, iso-octane, benzene and petroleum ether were Burdick and Jackson, distilled-in-glass quality. Sulfuric acid, anhydrous sodium sulfate and silver nitrate were ACS grade from Fisher Scientific. The 44% sulfuric acid on 100-200 mesh Woelm Activity I silica gel and the 10% silver nitrate on 100-200 mesh Woelm Activity I silica gel were prepared as described in the literature (LAMPARSKI et al. 1979).

Micro Extraction Procedure. Macerate 100-mg adipose tissue samples with approximately 100 to 200 mg of anhydrous sodium sulfate by using a tissue grinder. Isolate the fat from the nonlipid tissue by extracting three times with successive 10-mL portions of petroleum ether. After each extraction, cap and then centrifuge the homogenizer tube for 10 min. at 2000 rpm to isolate the fat-containing petroleum ether from the connecting tissue and the sodium sulfate. Transfer the petroleum ether extracts to a tared 30-mL beaker. Exercise caution when handling the beakers so that no extraneous weight is added to the tared beaker. Evaporate the combined extracts to dryness under a hood, then desiccate over phosphorus pentoxide overnight. Weigh the beaker and the fat and subtract the weight of the beaker tare to obtain the weight of the extracted fat. Quantitatively transfer the fat, using hexane as the solvent, to a 15-mL conical screw-cap tube that has been calibrated to 10 mL. Fill to the mark with hexane and mix thoroughly.

Cleanup Procedures for DDT and Metabolites. Prepare the chromatographic column by layering a silanized glass wool plug, 6 cm (1 g) of Woelm Activity I silica gel, and 3 cm (0.52 g) of 44% sulfuric acid on silica gel. After a prewash of the column contents with 10 mL of 90:10 hexane:benzene, pipette 2 mL of the fat extract (1/5 of total fat weight) onto the head of the column. After the bulk of the extract has passed into the column, add an additional 13 mL of 90:10 hexane:benzene to the column and collect the first 15 mL of eluate in a 15-mL conical screw-cap tube that has been calibrated at a volume of 2mL. Place the tube containing the eluate in a 40°C water bath, apply a gentle stream of nitrogen and evaporate the eluate nearly to dryness. Adjust volume to exactly 2 mL with iso-octane, mix by vortex, and transfer a sample to the autosampler vials. Make 2-µl injections into the gas chromatograph, which is operated under the following conditions: temperature of injector, column and detector are 230, 197 and 340°C, respectively; 95% argon/5% methane column flow and detector makeup flow are 15 and 20 mL/min, respectively.

Cleanup-Procedure for PCB. Prepare the 10% silver nitrate on silica column as described in our previous paper (NEEDHAM et al. 1980). Prewash the column contents with 10 mL of hexane. Pipet 2 mL of the fat extract (1/5 of total fat weight) onto the head of the column, and then add 28 mL of hexane to the column. Collect 30 mL of eluate in a 50-mL conical screw-cap tube that has been calibrated to 2 mL. Place the tube containing the eluate in a 40°C water bath, apply a gentle stream of nitrogen and evaporate the eluate nearly to dryness. Adjust the volume to exactly 2 mL with iso-octane mix by vortex, and transfer a sample to autosampler vials. Make 5-µl injections into the gas chromatograph, which is operated at the following conditions: temperature of injector, column and detector are 230, 202 and 340°C, respectively; 95% argon/5% methane column flow and detector makeup flow are 15 and 20 mL/min, respectively.

RESULTS AND DISCUSSION

To evaluate the efficiency of the 44% sulfuric acid on silica gel column for cleanup of fat extracts, we used the reported procedure to analyze 10 samples of a hexane pool (Blank pool A) containing 10 mg of lamb's fat/mL and 10 samples of a hexane pool B, fortified with o,p'-DDE (29.2 ng/mL), p,p'-DDE (390.2 ng/mL), o,p'-DDD (9.7 ng/mL), o,p'-DDT (9.7 ng/mL), p,p'-DDD (9.8 ng/mL), p,p'-DDT (19.6 ng/mL), and 10 mg of lamb's fat/mL. No peaks were observed at the retention times of DDT or DDT metabolites on the chromatograms of the blank pool, which indicates that none of the fat and oxidizable components were eluted through the column. The recovery data for DDT and DDT metabolites of the fortified pool are given in Table 1. Both the mean recovery and the precision for all analytes were excellent.

TABLE 1. Recoveries[a] of DDT and DDT Metabolites

	Spiked Level (ng)[b]	Mean Recovery (%)	Std. Dev. of % Recovery
o,p'-DDE	58.4	99.6	1.9
p,p'-DDE	780.4	106.8	1.9
o,p'-DDD	19.4	100.7	2.8
o,p'-DDT	19.4	97.3	2.2
p,p'-DDD	19.6	100.3	4.2
p,p'-DDT	39.2	94.9	2.2
Total as DDT	1033.0	105.3	1.7

[a] Number of analyses = 10

[b] Number of ng per 2 mL of sample, each of which also contained 20 mg of lamb's fat.

Using the 10% silver nitrate on silica gel column (KREISS et al. 1981), we have analyzed more than 450 human serum samples for PCB (Aroclor 1260). To evaluate the cleanup

efficiency of the silver nitrate-based adsorbant for fat extracts, we analyzed samples of each of the following: blank pool A; hexane pool C, containing Aroclor 1260 (30 ng/mL) and 10 mg of lamb's fat/mL; and hexane pool D, containing Aroclor 1260 (30 ng/mL), o,p'-DDE (29.2 ng/mL), p,p'-DDE (390.2 ng/mL), o,p'-DDD (9.7 ng/mL), o,p'-DDT (9.7 ng/mL), p,p'-DDD (9.8 ng/mL), p,p'-DDT (19.6 ng/mL) and 10 mg of lamb's fat/mL. The method of WEBB & MCCALL (1973) was used to quantitate the PCBs.

The recovery data for total PCBs and each PCB peak are given in Table 2. In general, the recoveries are excellent, particularly for those PCBs with relative retention times of 117 or greater. These latter PCBs are very important in analyzing biological samples for PCBs, since they are more avidly accumulated in animal fat. This is true whether the Aroclor involved is Aroclor 1254 or Aroclor 1260.

TABLE 2. Recoveries of Aroclor 1260 in Fat (by Peak and Total)

		Pool C[a]		Pool D[b]	
RRT[c]	Peak[d] Weight (ng)	Mean[e] Recovery(%)	Std.Dev. of Mean Recovery	Mean[e] Recovery(%)	Std.Dev. of Mean Recovery
70	1.62	85.5	1.5	82.1	6.9
84	2.82	90.7	3.6	87.5	5.3
89+104	2.28	60.8	5.5	68.1	6.2
117	1.98	100.1	3.7	97.5	3.7
125	7.20	95.3	3.2	92.6	4.7
146	8.46	97.6	2.1	95.0	3.1
160	2.94	100.1	3.5	96.3	5.2
174	7.44	81.0	3.1	80.3	2.9
203	5.58	100.0	3.0	97.6	4.9
232+244	5.88	100.1	2.3	98.1	3.8
280	6.60	99.8	3.3	98.8	3.3
332	2.52	102.9	2.8	103.4	3.9
372	2.40	102.1	3.9	101.5	3.9
448	0.36	102.2	6.6	99.1	4.7
528	0.90	101.3	6.0	103.7	8.0
Total PCB	60	93.1	2.1	91.1	2.6

[a] Contains 60 ng Aroclor 1260 and 20 mg lamb's fat per 2-mL sample.

[b] Contains 60 ng Aroclor 1260, 58.4 ng o,p'-DDE, 780.4 ng p,p'-DDE, 19.4 ng o,p'-DDD, 19.4 ng o,p'-DDE, 19.6 ng p,p'-DDD, 39.2 ng p,p'-DDT and 20 mg lamb's fat per 2-mL sample.

[c] Retention time relative to p,p'-DDE = 100 according to WEBB & MCCALL (1973).

[d] 60 ng of Aroclor 1260 in each sample. Weights in this column were determined by multiplying 60 ng by the weight percent (WEBB & MCCALL 1973).

[e] Number of analyses = 10.

The two methods described for the analysis of adipose tissue samples show high recoveries of the pesticides, and, since no liquid-liquid partitioning is required, they are time- and cost-effective. The disadvantage of the two methods is that if they are used as a general method for detecting pesticides, some pesticides may chemically react with the column material.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

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