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SEPARATION OF POLYCHLOROBIPHENYLS FROM CHLORINATED PESTICIDES IN SEDIMENT AND OYSTER SAMPLES FOR ANALYSIS BY GAS CHROMATOGRAPHY

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SUMMARY

Polychlorobiphenyls (PCBs) have been separated from DDT and its analogs and from the other common chlorinated pesticides by adsorption chromatography on columns of alumina and charcoal. Elution from alumina columns with increasing fractional amounts of hexane first isolates dieldrin and heptachlor epoxide from a mixture of chlorinated pesticides and PCBs. The remaining fraction, when added to a charcoal column, can be separated into two fractions, one containing the chlorinated pesticides, the other containing the PCBs, by eluting with acetone–diethyl ether (25:75) and benzene, respectively. The PCBs and the pesticides are then determined by gas chromatography on the separate column eluates without cross interference. The method is applicable to samples prepared by multi-pesticide residue methodology and should be applicable to sample extracts prepared for gas chromatography. Recoveries of the PCBs (Aroclors) and the chlorinated pesticides are good and the method is applicable to sediment and marine biota samples.

INTRODUCTION

The ubiquitous nature of the polychlorobiphenyls (PCBs) in the environment adds to the problems always present in the analysis of biota and soil samples for chlorinated pesticides. Attempts, more or less successful, have been made by Reynolds[1,2], Holden and Marsden[3], Armour and Burke[4], Feltz[5] and Berg *et al.*[6] to solve this problem by various partitioning procedures utilizing alumina, silica gel, Celite and/or charcoal as adsorbents. Other problems which hamper these procedures include the difficulty in obtaining subsequent supplies of alumina with repetitive adsorbent characteristics and maintaining the required technical expertise of the analyst in preparing the adsorbents for analytical use.

A method of analysis is presented which isolates the PCBs from pesticides, thereby permitting the qualitative and semi-quantitative analyses of each of these chemical groupings separately. The procedure incorporates features of the previous studies with strict modifications necessary to successfully complete the analyses.

Biological samples and soil samples are used to illustrate the applicability of the analytical procedure.

EXPERIMENTAL

Reagents

Woelm Neutral Alumina (or an equivalent) for column chromatography. Fisher No. 5-690 activated charcoal (50–200 mesh) (no substitute) from Fisher Scientific, Fair Lawn, N.J., U.S.A. 6 *N* Nitric acid (reagent grade). Sodium sulfate, anhydrous granular, heated and stored at 200°. QUSO-G30, unreductionized precipitated silica (Philadelphia Quartz Co., Philadelphia, Pa., U.S.A.). Desiccant mix (10% QUSO, 90% anhydrous sodium sulfate). Ottawa sand (acid washed), Cat. No. 3382 (J. T. Baker, Phillipsburg, N.J., U.S.A.). Isooctane (redistilled). Diethyl ether, Mallinckrodt No. 0844. Acetone, benzene, hexane and light petroleum (nanograde; Mallinckrodt St. Louis, Mo., U.S.A.).

Copper powder, as received from the supplier (J. T. Baker, Phillipsburg, N.J., U.S.A.), was treated for *ca.* 30 sec with 6 *N* nitric acid to remove surface oxides. The acid was decanted, the copper was rinsed several times with distilled water, followed by a rinse with acetone, and then air dried under a stream of nitrogen.

The alumina was treated as follows. 95 g of alumina (heated at 120° for 5 h) were weighed into a dry 500 ml ground-glass-stoppered erlenmeyer flask. 3 ml of water were slowly added and the flask was shaken until the heat dissipated. The procedure was repeated with the addition of 2 ml of water, and the treated alumina was mixed for 2 h and then stored in a desiccator. It was necessary to check the relative activity of each new batch (or manufacture's lot number) of alumina.

The charcoal was treated as follows. The charcoal was refluxed with acetone on a steam-bath, cooled and the solvent was removed by suction: the procedure was repeated and the charcoal was filtered. The filter-cake in the funnel was washed with cold acetone, air dried and stored at 135° until needed.

Standard pesticide and PCB solutions

Stock solutions were prepared of technical grade chlordane (1 mg/ml), of a mixture containing 1 μg/ml each of lindane, heptachlor, heptachlor epoxide, aldrin, *p,p'*-DDD, *p,p'*-DDT, *p,p'*-DDE and dieldrin and of 1 mg/ml each of the PCBs (Aroclors) 1242, 1248, 1254 and 1260 (Monsanto, St. Louis, Mo., U.S.A.). Solutions used for fortifying sample materials were as follows: chlordane (0.1 μg/μl); PCBs (1 μg/10 μl); lindane (2.5 ng/5 μl); heptachlor, heptachlor epoxide and aldrin (5 ng/5 μl); DDT, DDE, DDD and dieldrin (each 10 ng/5 μl). Standard solutions for gas chromatography (GC) were as follows: α- and γ-chlordane (each 0.5 ng/5 μl); technical grade chlordane (1 ng/5 μl); lindane (0.2 ng/5 μl); aldrin, heptachlor, heptachlor epoxide, DDD, DDE, DDT and dieldrin (each 0.4 ng/5 μl); PCBs (each 10 ng/5 μl). All of the pesticide and PCB standard solutions were prepared with nanograde hexane.

Apparatus

Aerograph 1200 and 204 gas chromatographs were used. The Aerograph 1200 contained a glass column (6 ft. × 0.125 in.) packed with 4% SE-30–6% SP-4201 on

Chromosorb W (100–120 mesh). The Aerograph 204 contained a glass column (6 ft. × 0.125 in.) with 4% SE-30–6% QF-1 on Chromosorb W (80–100 mesh). The operating conditions of the gas chromatographs were:

	1200	204
Column temperature	180°	185°
Injector temperature	215°	200°
Detector temperature	200°	200°
Nitrogen gas flow-rate	25 ml/min	30 ml/min

Both instruments contained an electron capture detector with a tritium-foil source.

For GC–mass spectrometry (MS), a Varian 1400 gas chromatograph coupled to a Finnegan 3000 mass spectrometer. The 1400 was equipped with a glass column (6 ft. × 2 mm I.D.) packed with 4% SE-30–6% SP-4201 on Supelcoport (100–120 mesh). The operating conditions were: column temperature, 210°; transfer-line temperature, 250°; gas-jet separator temperature, 225°; flow-rate of helium gas, 12 ml/min; sensitivity, 10^{-7} A/V; electron-multiplier voltage, 2.25 kV; electron-ionization current, 6.95 eV.

Biota samples were homogenized in either a Sorvall Omni-Mixer or a Waring Blendor. Sample extracts were concentrated on a rotary evaporator. Prior to use, all of the glassware was soaked in acidic dichromate solution, followed by a thorough rinse with water and acetone. The dried glassware was stored in an oven at 200° until used. Glass wool was rinsed with hexane and acetone and heated overnight at 200°.

Sample preparation

Oyster shells were opened by cracking the hinge; water was removed by shaking the shell after it had opened but before the adductor muscle was cut. The entire animal was removed and mixed in a Waring Blendor. A 30-g amount of the homogenate was weighed into a pint Mason jar and the jars were chilled (but not frozen) in a freezer for 30 min. Desiccant mix (120 g) was added to the chilled sample and thoroughly incorporated with the oyster tissue by means of a spoon after which the mixture was frozen. The frozen mixture was ground in the Sorvall Omni-Mixer until the material was free-flowing. The sample was maintained at freezing temperature during the mixing stage, and stored frozen until needed for analysis.

Screened soil samples were air dried at room temperature for at least 72 h, then blended in a Waring Blendor for 1 min. The soil was transferred to Mason jars and stored in a freezer until needed for analysis.

The oyster and soil samples were soxhlet extracted for 8 h with light petroleum for the oysters and acetone–light petroleum (1:9) for the soils.

Sample clean-up

Alumina column. To a chromatographic column (10 × 30 mm, Kontes Chromaflex, Cat. No. K-420320 or an equivalent) containing a glass-wool plug were added 1.0 cm of anhydrous sodium sulfate, 8 cm of alumina adsorbent and finally 1.0 cm of anhydrous sodium sulfate. The column was tapped to ensure proper settling of the granules. Each layer must be flat and not tilted otherwise poor chromatography will result.

The hexane solution of the concentrated extract (less than 1.0 ml) was carefully added to the top of the dry adsorbent in the column. Hexane eluates were collected in 50 ml graduated cylinders and transferred to round bottom flasks for concentration. Eluate volumes will vary from batch to batch of alumina. The following elution scheme details the pesticide separation:

Sample (hexane extract)	Alumina column	First fraction
	↓	(0–30 ml hexane)
	Second fraction	↓
	(31–60 ml hexane)	aldrin, chlordane, *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, lindane, heptachlor, and PCBs
	↓	
	dieldrin, heptachlor epoxide	

Charcoal column. A Pyrex micro chromatographic tube (140 × 6 mm I.D.) with a 50 ml reservoir was plugged by a small wad of solvent-washed glass wool. *ca.* 25 mm of acid-washed Ottawa sand was added to the column to retain the fines from the charcoal. An acetone slurry of the adsorbent was add until the height of the charcoal was 90 mm. [The acetone drains almost instantaneously through the micro tube, and the analyst should be ready to immediately add the concentrated residue of the first fraction from the alumina column (concentrated in acetone to 1 ml) to the charcoal column.] The flask was rinsed with several 1.0 ml portions of acetone and the rinsings were added to the column.

The DDT group of pesticides was eluted with 90 ml of 25% acetone in diethyl ether. A subsequent elution of the same column with 60 ml of benzene removed the PCBs from the column as noted in the following elution scheme:

Sample (First fraction from alumina in acetone)	Charcoal column	First fraction
	↓	[90 ml acetone–diethyl
	Second fraction	ether (25:75)]
	(60 ml benzene)	↓
	↓	aldrin, heptachlor, *p,p'*-DDE, *p,p'*-DDT, *p,p'*-DDD, lindane, chlordane
	PCBs	

Analysis

Analyses were made by GC, and the identification of the PCBs was confirmed by MS.

Elemental sulfur may be present in soils and perhaps in some biological materials; if present it will pass through the described analytical schemes and will be evident in the gas chromatograms[7] thus confusing the interpretation of the chromatograms. In order to avoid this problem, the extract or any fraction of the extract from the clean-up procedures may be treated as follows. The sample extract is evaporated to dryness; 1 ml each of hexane and isooctane, plus 100 mg of treated copper powder,

are added to the residue. The mixture is shaken vigorously and allowed to stand for 1 h; it is then evaporated to dryness and appropriate dilutions are made for GC analysis. Additional amounts of copper may be necessary depending the amount of sulfur present in the sample.

RESULTS AND DISCUSSION

A summation of the elution of the chlorinated organic pesticides and the PCBs from the alumina column is given in Table I. Heptachlor epoxide and dieldrin were removed from the column by extending the elution solvent beyond the 30 ml volume with an additional, but separate, elution volume of 30 ml (total 60 ml). The PCBs remained an integral part of the mixture containing the pesticides in the first 30 ml of eluate. The elution pattern of alumina column Fraction I on the charcoal column, Table II, shows that the pesticides were separated from the PCBs by means of the acetone–diethyl ether eluent. The PCBs were subsequently removed from the charcoal

TABLE I

PERCENTAGE RECOVERY OF PESTICIDES ELUTED FROM NEUTRAL ALUMINA

Compound		*First fraction*				*Second fraction*
		0–15 ml	*15–20 ml*	*20–25 ml*	*25–30 ml*	*30–60 ml*
Lindane				10	90	
Heptachlor		100				
Aldrin		100				
Heptachlor epoxide						100
p,p'-DDE		100				
Dieldrin						100
p,p'-DDD				50	50	
p,p'-DDT		100				
PCBs		100				
Chlordane	γ	10	80	10		
	α	80	20			

TABLE II

PERCENTAGE RECOVERY OF PESTICIDES ELUTED FROM CHARCOAL

Compound	*First fraction 90 ml acetone–diethyl ether (25:75)*			*Second fraction 60 ml benzene*	
	0–30 ml	*30–60 ml*	*60–90 ml*	*0–30 ml*	*30–60 ml*
Lindane	30	40	30		
Heptachlor	100				
Aldrin	100				
p,p'-DDE	50	50			
p,p'-DDT	80	20			
PCBs				80	20
Chlordane	100				

column with benzene. Known amounts of pesticides and PCBs (Aroclor 1254) were added to soils and oyster samples; the samples were analyzed as described herein to check the efficiency of the analytical procedure. Recoveries of the added chemicals to the soils and the oysters were consistent and acceptable (Tables III and IV). The limits of detectability of the chemicals examined (Table V) refer to those obtained from pure solutions and they are also applicable to sample extracts; at times, however,

TABLE III

RECOVERY OF PESTICIDES AND PCBs FROM FORTIFIED SOIL SAMPLES

Pesticide	*Fortified (ppm)*	*Recovered (ppm)*	*Recovered (%)*
Lindane	0.0013	0.0011	84.6
Heptachlor	0.0027	0.0022	81.5
Aldrin	0.0027	0.0023	85.2
Heptachlor epoxide	0.0027	0.0025	92.6
p,p'-DDE	0.0027	0.0025	92.6
Dieldrin	0.0027	0.0026	96.3
p,p'-DDD	0.0027	0.0027	100
p,p'-DDT	0.0027	0.0027	100
γ-Chlordane	0.0033	0.0035	106
α-Chlordane	0.0033	0.0037	112
PCB (Aroclor 1254)	0.066	0.066	100

TABLE IV

RECOVERY OF PESTICIDES AND PCBs FROM FORTIFIED OYSTERS

Pesticide	*Fortified (ppm)*	*Recovered (ppm)*	*Recovery (%)*
Heptachlor	0.0027	0.0019	70.4
Aldrin	0.0027	0.0021	77.8
Heptachlor epoxide	0.0027	0.0028	102
α-Chlordane	0.0033	0.0033	100
PCB (Aroclor 1254)	0.066	0.045	68.2

TABLE V

LIMITS OF DETECTABILITY OF PESTICIDES AND PCBs USING THE DESCRIBED PROCEDURE UNDER IDEAL CONDITIONS

Pesticide	*Detectability (ppb*)*
Lindane	0.04
Heptachlor	0.05
Aldrin	0.06
Heptachlor epoxide	0.10
p,p'-DDE	0.14
Dieldrin	0.14
p,p'-DDD	0.25
p,p'-DDT	0.33
γ-Chlordane	0.10
α-Chlordane	0.11
Aroclors 1254, 1260 (PCBs)	6.5

* The American billion (10^9) is meant.

it may be difficult, if not impossible, to minimize background contaminant levels sufficiently to observe this low level of detectability. Many PCBs are present in the environment and their composition is variable, resulting usually in multicomponent peaks on the gas chromatogram. However, when using the recommended analytical procedure, there is always a sufficient number of identifiing peaks to illustrate that they are indeed PCBs and this fact can be confirmed by mass spectrometry. It may be possible to estimate the amount of PCBs in a sample using the procedure described but, in many instances, it is more important to acertain their presence or absence under a given set of circumstances.

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