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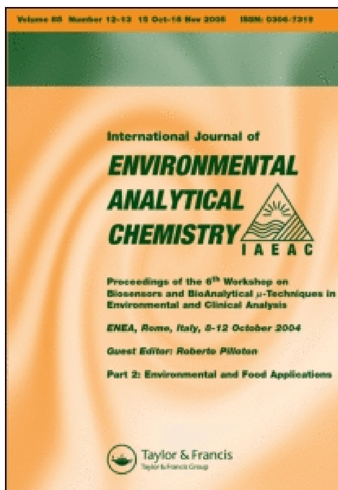

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Mes, Jos , Davies, David and Bryce, Fred(1983) 'The Determination of Polychlorinated Biphenyl in Small Samples of Monkey Milk and Tissues. II. Extraction Efficiency', International Journal of Environmental Analytical Chemistry, 15: 1, 25 — 37

To link to this Article: DOI: 10.1080/03067318308071910

URL: http://dx.doi.org/10.1080/03067318308071910

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Intern. J. Environ. Anal. Chem., 1983, Vol. 15, pp. 25–37
0306-7319/83/1501-0025 $18.50/0
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Printed in Great Britain

The Determination of Polychlorinated Biphenyl in Small Samples of Monkey Milk and Tissues. II. Extraction Efficiency

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(*Received January 6, 1983*)

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The extraction efficiency of benzene, toluene, dichloromethane, acetone:hexane and chloroform:methanol with respect to lipids and polychlorinated biphenyls was investigated using small samples of monkey adipose tissue, liver, kidney, brain, skin, feces and milk. The most efficient solvents were: acetone:hexane and chloroform:methanol for brain, feces, kidney, liver and milk; acetone:hexane and dichloromethane for adipose tissue; acetone:hexane and toluene for blood and dichloromethane for skin tissue. Within these solvent pairs acetone:hexane was the most outstanding with respect to an average of 90% PCB recoveries from fortified samples in the range of 0.02–2 ppm.

In addition, a comparison was made between the lipid determination before and after Florisil column chromatography. Only adipose and blood lipids were sufficiently recovered from Florisil to make a lipid determination after chromatography feasible.

KEY WORDS: Monkey, extraction, PCBs, lipid, recovery.

INTRODUCTION

In a previous study, the importance of method development for polychlorinated biphenyls (PCBs) in small samples of monkey tissues and fluids was outlined.[1] However, this study was based on the use of benzene as extraction solvent, the use of which has been restricted in many laboratories to minimize a potential health hazard. In the

present study, the efficiency of several extraction solvents for the determination of PCBs in monkey tissues and fluids was investigated in anticipation of an alternative to benzene. At the same time, methodology was developed to accommodate small samples of monkey tissues and fluids, especially monkey milk.

EXPERIMENTAL

Solvents and chemicals

All solvents were glass distilled and free from interfering residues as tested by gas liquid chromatography (GC) (concentration 300:1). Florisil, anhydrous Na_2SO_4 and glass wool were decontaminated as previously described.[2]

Fortification and blanks

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Samples were fortified at levels shown in Table I. Dilute solutions were made by serial dilution of the most concentrated fortification solution, except in the case of monkey milk. A 100 μl aliquot of a solution, containing 50 ng of Aroclor 1254/μl acetone, was added to 5 gm of a pooled monkey milk sample. The milk was then thoroughly mixed and serial dilutions for fortification experiments obtained by mixing equal quantities of fortified and non-fortified pooled monkey milk.

Fortification of tissues was carried out by addition of the appropriate volume of fortification solution and leaving the latter to penetrate for several hours before extraction. Solvent blanks were run through the entire analytical procedure for each extraction solvent and the results were used to correct for background, where applicable.

Sampling

Monkey tissue samples except for blood, were obtained at autopsy, and stored at −20°C in previously cleaned glass jars (washed, heated at 300°C (1 hr) and rinsed with acetone and hexane). The jars were covered with screw caps, lined with hexane rinsed aluminium foil.

TABLE I

Scheme for the fortification of monkey tissues and fluids with Aroclor 1254

Substrate	Average fortification level (ppm)	Fortification solution[a] (ng Aroclor 1254/ μl acetone)	Average sample weight (mg)	Volume used (μl)
Adipose	0.45	1	110	50
	1.82	4		
Blood	0.02	2	5000	50
	0.2	20		
	2	200		
Brain	0.0153	0.08	262	50
	0.153	0.8		
	1.53	8		
Feces	0.0192	0.5	26000	1000
	0.192	5		
	1.92	50		
Kidney	0.191	0.5	131	50
	1.91	5		
Liver	0.192	0.5	130	50
	1.92	5		
Milk	0.0625	0.125	2000[b]	1000[c]
	0.125	0.25		
	0.25	0.5		
	0.5	1		
Skin	0.158	0.5	158	50
	1.58	5		

[a]In the case of milk: ng Aroclor 1254/μl of milk.
[b]Total weight of fortified sample, from which subsamples of ~0.25 ml were withdrawn for triplicate extractions.
[c]Fortified milk.

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Blood was obtained by venipuncture and collected in a jar containing 0.01 ml of 10% EDTA in water/1 ml blood. Monkey milk was obtained by restraining the animal and manually expressing the milk directly into a large vial, cleaned as above. Monkey feces were collected over a period of 24 hrs and stored as above.

Extraction

All extractions were carried out in triplicate and according to the following procedures:

1. *Blood.* Five gram samples were extracted with one of the following solvents or solvent mixtures:

a) Toluene, benzene or dichloromethane. The sample was weighed in a centrifuge tube (Pyrex no. 8240) and extracted with 15 ml of one of the three solvents for 2 min, using a Silverson homogenizer. The extraction was started by slowly lowering the homogenizer shaft into the solvent layer in order to create a vortex of blood in the solvent. This procedure prevented emulsions, which can otherwise be broken at $< -70°C$ in an ethanol-dry ice bath. The shaft was rinsed with solvent and the extract plus rinsings centrifuged at 2000 rpm for 15 min in a Beckman TJ-6 centrifuge. Next the solvent layer was decanted, filtered through anhydrous Na_2SO_4 and the filtrate collected in a 250 ml round bottom flask (RBF). The filtrate was evaporated on an all glass rotatory evaporator at 60°C for toluene and at $<30°C$ for benzene and dichloromethane. The residue was redissolved in hexane.

b) Acetone:hexane (2:1, v/v). The sample was extracted as under 1(a), except that after centrifugation the entire liquid phase was decanted into a 250 ml RBF and evaporated at $<30°C$ until water and hexane separated. At that point more hexane was added and the procedure continued as above by filtering the extract through anhydrous Na_2SO_4.

c) Chloroform:methanol (2:1, v/v).[3] Extraction and subsequent centrifugation of the badly emulsified extract were carried out as under 1(a), but with 19 ml $CHCl_3$:CH_3OH as extraction solvent and 13 ml $CHCl_3$ as rinsing solvent. Centrifugation separated the emulsified extract into a $CHCl_3$ bottom—and H_2O:CH_3OH top layer with the blood pellet sandwiched in between. The liquid was carefully poured into a 50 ml centrifuge tube (Pyrex no. 8424), 13 ml H_2O added and the contents shaken. The emulsion which formed was centrifuged as above, the top layer removed by aspiration and the $CHCl_3$ extract filtered and evaporated as under 1(a).

2. *Adipose.* Approximately 150 mg were extracted with one of the following solvent or solvent mixtures:

a) Toluene, benzene, dichloromethane or acetone:hexane (2:1, v/v). After sample extraction as under 1(a), but using 20 ml of solvent, the extract was not centrifuged, but subsequently filtered through anhydrous Na_2SO_4. The redissolved residue in hexane was

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transferred to a pre-weighed aluminum weighing dish and the solvent evaporated in a fumehood to determine solvent soluble lipids. The lipid residue was redissolved in hexane and transferred back to the same 250 ml RBF.

b) Chloroform:methanol (2:1, v/v). Extraction was carried out as under 1(c), but with 20 ml $CHCl_3$:CH_3OH as extraction solvent and 10 ml $CHCl_3$ for rinsing. After extraction 8 ml H_2O was added to the extract plus rinsings and the latter mixed by bubbling N_2 through for 2 min, which resulted in the formation of an emulsion. The emulsion was centrifuged, the top layer removed and the $CHCl_3$ dried and evaporated as under 1(c), followed by lipid determination as under 2(a).

3. *Liver.* Samples of approximate 150 mg were extracted with the various solvents according to the procedures for adipose tissue (2(a) and (b)). However, upon concentration of the extract prior to the lipid determination a very fine particulate matter was observed. This was removed by centrifugation at 2000 rpm for 30 min in a Beckman model TJ-6 centrifuge.

4. *Kidney.* Extractions were carried out as for liver, using approximate 130 mg. The particulate matter observed in the final liver extracts also appeared in toluene and dichloromethane extracts of kidney tissue and was removed as described above.

5. *Brain.* Samples of approximate 220 mg were extracted with toluene, benzene and dichloromethane as for adipose tissue. Extraction with solvent mixtures was carried out as follows:

a) Acetone:hexane (2:1, v/v). The sample was extracted as under 1(a), but using 20 ml of solvent. The Silverson shaft was rinsed with 10 ml hexane and to the extract plus rinsings 15 ml H_2O was added. The solution was mixed by bubbling N_2 through and the hexane separated by centrifugation at 2000 rpm for 20 min as above. The hexane layer was filtered and its lipid content determined as under 2(a).

b) Chloroform:methanol (2:1, v/v). Extraction was carried out as under 2(b), except the Silverson shaft was rinsed with 13 ml $CHCl_3$ and 17 ml H_2O was added to the extract plus rinsings.

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6. *Skin.* At necropsy an area of approximately 10 × 20 cm was shaven and removed. Fatty tissue from the back of the skin was removed by scalpel. The skin was placed on a solvent cleaned teflon sheet and disks of approximately 12 mm diameter were punched out by means of a corkbore. The average weight of the disks was 160 mg ± 10%. Individual disks were weighed and saponified by refluxing with 10 ml of 1N KOH until completely disintegrated (~1 hour). After cooling, the condenser was rinsed with 5 ml of one of the solvents (toluene, dichloromethane, acetone:hexane or chloroform:methanol) and the sample transferred to a 50 ml stoppered centrifuge tube. The Erlenmeyer flask was rinsed several times with small portions of the appropriate solvent (total ~5 ml) and the rinsings added to the centrifuge tube. Each sample was mixed, except the one containing acetone:hexane, and the organic solvent phase transferred to another 50 ml centrifuge tube. To the sample containing the acetone:hexane, water was added until the hexane phase separated. The sample was then vigorously shaken and transferred as above. Each collected organic solvent phase was washed with 3 × 10 ml hexane-extracted H_2O. The washed extracts were filtered and their lipid content determined as under 2(a). The remaining KOH layer of each sample was acidified with 6N HCl (pH ~5) and subsequently extracted with 15 ml hexane. The hexane was removed, dried and its lipid content determined as under 2(a).

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7. *Milk.* Samples weighing ~700 and 240 mg were used for benzene and all other solvent extractions respectively. Extractions were carried out with the following solvents or solvent mixtures:

a) Toluene, benzene and dichloromethane. The procedure was carried out as under 2(a), except for the lipid determination. Lipids were determined by transfer of the concentrated extract to 2.5 ml centrifuge tubes, followed by careful evaporation of the solvent. The residue was redissolved in 100 μl hexane and transferred with appropriate rinsings to a preweighed cut-off disposable test tube (8 × 10 mm). The solvent was left to evaporate and the lipids weighed.

b) Acetone:hexane (2:1, v/v). Samples were extracted as above 7(a), except after extraction, the sample was first filtered through glass wool into a 100 ml RBF and concentrated on an all glass

rotary evaporator (<30°C) to remove acetone. The acetone free residue was diluted with some hexane, filtered and its lipid content determined as in other milk extractions.

c) Chloroform:methanol. Extraction was carried out as under 2(b), but using 15 ml extraction solvent, 9 ml rinsing solvent and 7 ml H_2O.

8. *Feces.* Twenty-five gram samples were extracted with the following solvents or solvent mixtures:

a) Toluene, benzene and dichloromethane. Samples were weighed in 500 ml stoppered Erlenmeyer flasks and 300 ml solvent added. The samples were extracted with a Silverson homogenizer for 1.5 min at high speed. After extraction, no rinsings were added to the extract, but the particles allowed to settle before a 100 ml aliquot was removed by volumetric pipette. This aliquot was filtered, evaporated and its lipid content determined as above 2(a).

b) Acetone:hexane (2:1, v/v). After extraction as in 8(a), ~200 ml H_2O was added and the extract stirred for 5 min at high speed by magnetic stirrer. A 50 ml aliquot was removed from the hexane phase by volumetric pipette, filtered, evaporated and its lipid content determined as above 2(a).

c) Chloroform:methanol (2:1, v/v). Samples were extracted as above 8(b). However, particles were between the water and $CHCl_3$ phases, as well as at the bottom of the $CHCl_3$ layer. The $CHCl_3$ aliquot was carefully removed.

Extractions, centrifugations, filtrations and evaporations involving the use of benzene were carried out in the fumehood or specially vented areas. All monkey tissues were handled with gloves.

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Clean-up

Florisil clean-up was carried out as previously described,[1] except for monkey milk extracts. The latter were applied in 100 μl hexane to 1 g of Florisil in glass columns (6 mm O.D. and 260 mm long) with 50 ml reservoir and 1–2 mm teflon® stopcock. The Florisil was topped with

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5 mm anhydrous Na_2SO_4 and prewetted with hexane. PCBs were eluted in 8 ml hexane. Lipids were eluted with 10 ml of 20% ethylacetate in dichloromethane in preweighed aluminum weighing dishes as an alternative to the precolumn chromatography lipid determination (2(a)). All blood lipids were determined by this alternative method.

Identification and quantification

The PCB fractions were chromatographed on a glass column (0.6 × 183 cm) packed with 4% SE-30 + 6% OV-210 on Chromosorb W(AW) 100/120 in a Varian Series 3700 gas chromatograph with ^{63}Ni detector. Injector, column and detector temperatures were 220, 208 and 299°C respectively. PCBs were quantitated by summation of peak heights, using peaks no. 4–10 as earlier reported.[1]

Confirmation

GC-MS was carried out using a Varian Series 3700 gas chromatograph with a DB-5 capillary column (0.2 mm × 28 m), temperature programmed from 80–250°C in 2 stages: from 80–180°C at 50°C/min and from 180–250°C at 5°C/min and held at the final temperature. The GC was coupled to a Zab-2F (VG Analytical Co.) MS with electron energy of 70 eV and a resolution of $M/\Delta M = 4000$ (10% valley definition). Extracts were analysed for the presence of Aroclor 1254 by multiple ion monitoring of $m/z = 324$, 326 and 328.

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RESULTS AND DISCUSSION

Table II shows the extraction efficiency of various solvents with respect to the fat extracted from small samples of monkey tissues and fluids. Toluene was included as an alternative to the more toxic benzene and $CHCl_3$:CH_3OH as one of the most widely used lipid solvents.[4] The importance of the lipid content lies in the lipophilic nature of the PCBs. The data in Table II suggest that there is no universal solvent for the extraction of lipids from all substrates under the conditions used, although $CHCl_3$:CH_3OH gave the highest percentage fat in four out of eight substrates.

TABLE II
The effect of extraction solvent on the fat determination in monkey tissues and fluids

Substrate	% Fat[a]									
	Toluene	(CV)	Benzene	(CV)	CH_2Cl_2	(CV)	Ac:H[b]	(CV)	C:M[c]	(CV)
Adipose	87.7	(2)	101.3	(1)	103.6	(5)	92.3	(7)	92.6	(4)
Blood	0.1	(6)	N.D.[d]		0.1	(7)	0.2	(3)	0.2	(3)
Brain	11.7	(5)	8.8	(2)	10.4	(4)	9.7	(2)	10.4	(3)
Feces	0.8	(11)	0.9	(0)	1.8	(0)	1.7	(0)	2.4	(17)
Kidney	1.3	(3)	1.6	(2)	1.5	(5)	2.7	(4)	3.3	(2)
Liver	3.8	(3)	4.3	(4)	5.1	(5)	5.4	(1)	6.5	(1)
Milk	0.9	(7)	4.9	(4)	0.8	(10)	1.9	(4)	1.0	(6)
Skin	8.9	(27)	N.D		7.7	(22)	7.5	(24)	10.1	(33)

[a]The coefficient of variation (CV) is given in brackets.
[b]Acetone:hexane (2:1, v/v).
[c]Chloroform:methanol (2:1, v/v).
[d]Not determined.

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During part of this investigation there was a temporary restriction on the use of benzene in our laboratory. Therefore at that particular time only four out of five extraction solvents were evaluated for blood, adipose, liver, kidney and milk. A comparison between the best of these four solvents (after further fortification experiments) and benzene was made at a later date using the same substrate, except for blood. Because initially an insufficient volume of monkey blood was collected, a new supply of blood was obtained and extracted with benzene and acetone:hexane. The two solvents gave 0.2(CV = 2) and 0.1(CV = 14)% fat respectively. Benzene extracted an unusually large amount of lipids from milk compared to other solvents. Unfortunately no milk was left to verify this unexpected result.

During the manipulations of the lipid determination step in small tissue samples, not only small amounts of lipids, but also some of the PCBs dissolved in these lipids may be lost. To minimize this loss, the possibility of determining lipids after separation from PCBs was investigated. This separation is normally accomplished by Florisil column chromatography. The results in Table III indicate that only blood and adipose tissue lipids can be determined after column chromatography. Approximately 60–70% of the liver and kidney lipids were retained on the Florisil column. The extent of the lipid

TABLE III
The effect of column chromatography on the lipid determination of some monkey tissues

Substrate	Average sample weight (mg)	% Fat[a] Florisil column chromatography Before	(CV)	After	(CV)
Blood	2270	0.12	(12)	0.11	(13)
Adipose	130	92.3	(7)	91.4	(7)
Liver	158	5.38	(1)	1.85	(2)
Kidney	121	2.73	(4)	0.71	(11)

[a]Average of triplicate determinations.

retention may depend on the type and polarity of the lipids present in particular tissues.

In addition to the effect of column chromatography on the lipid determination in monkey tissues, the extent of lipid variation in small samples was investigated, using monkey milk. The data in Table IV show less variation in lipid content with sample size after than before column chromatography. Approximately 20% of the milk lipids are retained on the column.

TABLE IV
The effect of sample size and column chromatography on the fat determination in monkey milk

Average sample size(mg) ± S.D.	% Fat[a] Florisil column chromatography Before	(CV)	After	(CV)
238 ± 26	4.1	(15)	3.1	(4)
515 ± 2	3.5	(3)	3.0	(0)
1018 ± 6	3.1	(13)	2.7	(11)
Average	3.6	(11)	2.9	(6)

[a]Average of triplicate determinations.

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Table V shows the effect of extraction solvent on the determination of PCBs in monkey tissues and fluids. In four out of eight substrates $CHCl_3$:CH_3OH extracted more PCBs than other solvents, correlating in all, but one, with higher fat content (Table II). In a study of extraction efficiency of polybrominated biphenyls from rat liver and adipose tissue, Fawkes *et al.*[5] reported $CHCl_3$:CH_3OH to be superior to hexane. In this study the combination of hexane and acetone resulted in equally good PCB extractions and overall slightly better coefficients of variation.

The new blood supply obtained for reasons mentioned earlier, and extracted with benzene and acetone:hexane, contained 0.9(CV = 10) and 1.0(CV = 28) ppb PCBs.

TABLE V

The effect of extraction solvent on the determination of PCBs in monkey tissues and fluids

	ng PCB/g substrate									
Substrate	Toluene	(CV)	Benzene	(CV)	CH_2Cl_2	(CV)	Ac:H	(CV)	C:M	(CV)
Adipose	832	(2)	884	(5)	924	(7)	870	(14)	846	(7)
Blood	2.3	(28)	N.D.		1.1	(11)	2.2	(13)	1.7	(26)
Brain	6	(37)	12	(20)	2	(82)	8	(6)	6	(79)
Feces	2.3	(22)	2.7	(33)	3.3	(15)	3.0	(0)	4.3	(12)
Kidney	33	(15)	27	(5)	42	(7)	40	(22)	44	(15)
Liver	95	(3)	102	(16)	101	(9)	103	(12)	114	(4)
Milk	20	(31)	25	(10)	22	(21)	16	(11)	30	(27)
Skin	92	(23)	N.D.		95	(12)	56	(8)	70	(25)

Table VI shows the PCB recoveries of fortified monkey tissues and fluids when extracted with selected solvents or solvent mixtures. This solvent selection was based on data reported in Tables II and V, taking into consideration lipid content, PCB level and coefficient of variation. For blood, adipose, kidney and liver tissues two solvents were selected, because of their similar extraction efficiencies. Fortified samples extracted with acetone:hexane gave recoveries of PCBs higher than or equal to those extracted with other solvents. In particular this was true for PCB recoveries from kidney and liver tissues fortified at the 0.19 ppm level. In addition, in many instances, the coefficient of variation decreased as expected with an increase in fortification level. Recoveriés of PCBs from fortified blood. brain and

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TABLE VI

Recoveries of PCBs from monkey tissues and fluids in selected solvent extracts

Substrate[a]	Average level of fortification (ppm)	% Recovery[b]							
		Extraction solvent							
		Toluene	(CV)	CH_2Cl_2	(CV)	Ac:H	(CV)	C:M	(CV)
Adipose	0.45			81	(16)	89	(10)		
	1.82			90	(1)	91	(3)		
Blood	0.02	92	(3)			92	(17)		
	0.2	84	(9)			87	(1)		
	2	79	(6)			96	(4)		
Brain	0.0153					93	(11)		
	0.153					83	(1)		
	1.53					94	(1)		
Feces	0.0192					90	(8)		
	0.192					78	(5)		
	1.92					85	(6)		
Kidney	0.191					96	(17)	75	(5)
	1.91					91	(3)	92	(1)
Liver	0.192					86	(8)	75	(13)
	1.92					96	(2)	95	(4)
Milk	0.0625					88	(5)		
	0.125					88	(8)		
	0.25					92	(6)		
	0.5					91	(3)		
Skin	0.158			92	(16)				
	1.58			99	(3)				

[a]For average sample weight see Table I.
[b]Average of triplicate determinations.

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feces at the 0.2, 0.15 and 0.19 ppm levels respectively, were lower than those at the corresponding lowest level of fortification for no apparent reason.

No recovery studies were carried out for those tissues and fluids, which are best extracted with benzene, such as blood, milk and adipose, because recovery data were earlier reported for human blood, milk and monkey adipose tissue.[2] The presence of PCBs in adipose, kidney, liver and milk extracts were confirmed as pentachlorobiphenyl by GC-MS.

Acknowledgements

The authors wish to thank Dr. P.-Y. Lau for the GC-MS analysis and Dr. N. Sen and Mr. R. Graham for reviewing this manuscript.

References

1. J. Mes, D. Davies and J. Truelove, *Intern. J. Environ. Anal. Chem.* **8,** 89 (1980).
2. J. Mes, D. J. Davies and P.-Y. Lau, *Chemosphere* **9,** 763 (1980).
3. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37,** 911 (1959).
4. P. W. Albro, *Ann. N.Y. Acad. Sci.* **320,** 19 (1979).
5. J. Fawkes, P. W. Albro, D. B. Walters and J. D. Mckinney, *Anal. Chem.* **54,** 1866 (1982).

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