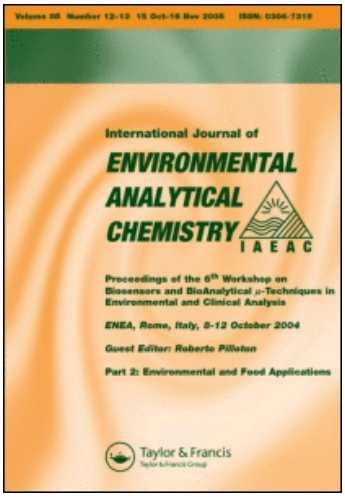

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Problems in the Determination of Polychlorinated Biphenyls

V. Zitko[a]
[a] Fisheries Research Board of Canada Biological Station, St. Andrews, New Brunswick, Canada

To cite this Article Zitko, V.(1972) 'Problems in the Determination of Polychlorinated Biphenyls', International Journal of Environmental Analytical Chemistry, 1: 3, 221 — 231
To link to this Article: DOI: 10.1080/03067317208076373
URL: http://dx.doi.org/10.1080/03067317208076373

Intern. J. Environ. Anal. Chem., 1972, Vol.. 1 pp. 221–231

Printed in Great Britain

Problems in the Determination of Polychlorinated Biphenyls†

V. ZITKO

Fisheries Research Board of Canada
Biological Station, St. Andrews
New Brunswick, Canada

(*Received June 2, 1971*)

The presence of aromatic hydrocarbons in pesticide-grade hexanes affects the elution patterns of polychlorinated biphenyls (PCB) and chlorinated hydrocarbon pesticides from silicic acid columns. Several batches of silicic acid Silicar® (Mallinckrodt) were contaminated with 0.031–0.163 mcg/g of PCB expressed as Aroclor® 1254. Chlorinated naphthalenes accompany PCB on silicic acid chromatography and their presence can be detected from an absorption maximum at 306 nm, $A^{1\%}_{1cm}$ = 329. PCB of the Aroclor® 1254 type were detected in American eel (*Anguilla rostrata*), herring (*Clupea harengus*), and Atlantic salmon (*Salmo salar*) in concentrations of 0.63–0.75, 0.32–0.54, and 0.45 mcg/g wet weight, respectively. Commercial fish oil contained 3.55 mcg/g of PCB.

INTRODUCTION

Since about 1930, polychlorinated biphenyls have been used as plasticizers, paint additives, hydraulic fluids, heat transfer media, transformer oils, etc. In 1966, PCB residues were found in wildlife, and it was soon realized that the fate of PCB in the environment is similar to that of chlorinated hydrocarbon pesticides, *i.e.*, PCB are readily accumulated by living organisms, and the occurrence of PCB in the environment is ubiquitous.[1,2,3] PCB residues not only interfere with the determination of several chlorinated hydrocarbon pesticides but are also toxic, and hence their levels in the environment need to

† First presented at the Symposium on Recent Advances in the Analytical Chemistry of Organic Pollutants; 54th Canadian Chemical Conference, Halifax, May 31–June 2, 1971.

be measured. PCB are multicomponent mixtures of chlorinated biphenyls, and both the separation from chlorinated hydrocarbon pesticides and the measurement of PCB present problems. Some of the problems are discussed in this paper.

RESULTS AND DISCUSSION

Separation of PCB from Chlorinated Hydrocarbon Pesticides

Chromatography on Florisil was used to separate PCB from some of the chlorinated pesticides, including DDT.[4] PCB were eluted from the column by hexane; DDT and other pesticides were eluted by a mixture of diethylether and hexane. It was reported[5] that DDT was partly eluted from Florisil columns with hexane and it was recommended that the pesticide elution characteristics of Florisil were examined prior to its use for the separation of PCB and chlorinated pesticides. A simultaneous clean-up of the sample extract and a separation of PCB from all common pesticides except p,p'-DDE was accomplished by successive chromatography on alumina and silica.[6] The separation of PCB from all common chlorinated pesticides was obtained by chromatography on silicic acid-Celite.[7] PCB were eluted with petroleum ether and pesticides were eluted with a mixture of acetonitrile, hexane, and methylene chloride. The chromatographic conditions were carefully controlled, since there was only a small margin of separation between PCB and p,p'-DDE. Rather large volumes of solvents (250 ml of petroleum ether and 200 ml of the acetonitrile-hexane-methylene chloride mixture per sample) were required. Alumina-silica chromatography is more suitable for routine analyses and was used in this work. The interference of PCB with the determination of p,p'-DDE is usually small.

In order to achieve reproducible chromatographic conditions, the activity of the adsorbents must be carefully controlled, and the activation procedure must be described in detail. The quality of solvents used for elution may also affect the chromatographic separation. It is usually specified only that pesticide-grade solvents were used, which implies that electron-absorbing impurities are absent. Commercial pesticide-grade hexanes contain varying amounts of aromatic hydrocarbons, as demonstrated by u.v. spectra of hexanes from three suppliers (Figure 1). The differences in the aromatic hydrocarbon content have a pronounced effect on the elution patterns of PCB and chlorinated pesticides from silica columns. In the routine procedure, using the Fisher Scientific Company hexane, PCB (Aroclor® 1254) are eluted in fractions I and II (Figure 2). Fraction I contains 56%, fraction II 44% of PCB and 100% of p,p'-DDE. On silica of the same activity, only 18% of

PCB and 56% of p,p'-DDE appear in fractions I and II, respectively, when the Matheson Coleman & Bell hexane is used. The addition of benzene to this hexane (5 ml/l) results in separation patterns similar to those obtained with the Fisher Scientific Company hexane (69% PCB in fraction I, 100% p,p'-DDE in fraction II). When the concentration of benzene is increased further

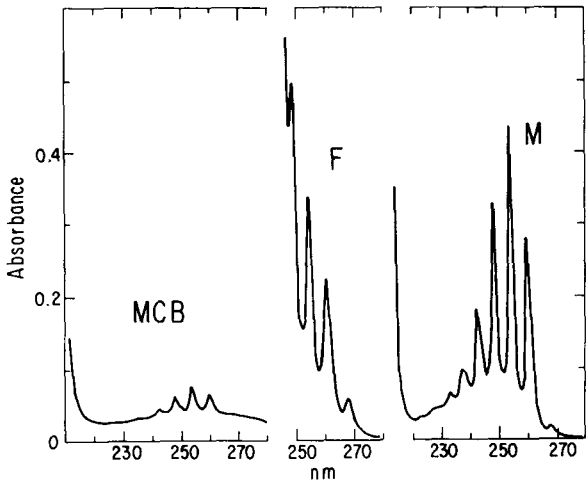

FIGURE 1 U.v. spectra of commercial pesticide-grade hexanes, concentration 10% in Fisher spectrograde hexane. M = Mallinckrodt, F = Fisher, MCB = Matheson Coleman & Bell.

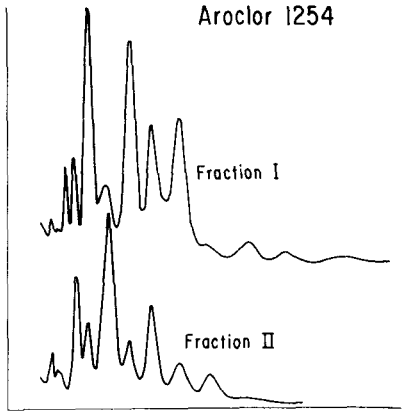

FIGURE 2 PCB (Aroclor® 1254) eluted from the silica column in fractions I and II, respectively.

(20 ml/l), 86% of PCB and 31% of p,p'-DDE are eluted in fraction I, and 68% of p,p'-DDT appears in fraction II (in both previous cases p,p'-DDT is eluted in fraction III). Regardless of the solvent, hexachlorobenzene is always eluted in fraction I; lindane, heptachlor epoxide, dieldrin, and p,p'-DDD are eluted in fraction III. To achieve reproducible separation patterns, the hexanes used should be characterized for example by their u.v. spectra. It is possible that the discrepancies in the elution patterns of PCB and p,p'-DDT from Florisil columns[4,5] were caused by different hexanes.

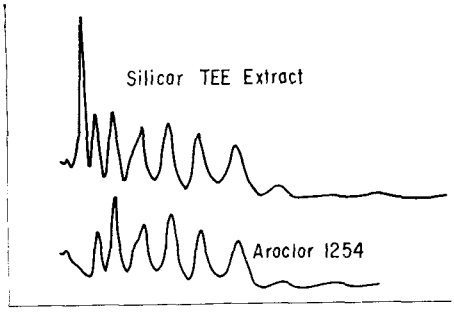

FIGURE 3 PCB eluted from silicic acid SILICAR® (Mallinckrodt), Lot TEE, and Aroclor® 1254.

PCB Contamination of Samples during Analysis

Since PCB are used in a great variety of commercial products, care must be taken to avoid the contamination of analyzed samples from such sources. For example, PCB of the 40% chlorine variety were detected in various cardboards.[8] It was found that several batches of silica (Silicar® Mallinckrodt) were contaminated by PCB of the Aroclor® 1254 type (Figure 3). The amounts of PCB present (Table I) would seriously affect the results of PCB deter-

TABLE I

Contamination of Silicar® with PCB.

Lot	Container	PCB, mcg/g			Av.
TEE	1	0.034	0.027	0.033	0.031
	2	0.037	0.029	0.027	0.031
	3	0.033	0.027	0.033	0.031
PXR		0.26	0.15	0.08	0.163
TSB		PCB not detectable			

minations. PCB can be removed from the contaminated silica by washing with acetone, but the treatment changes irreversibly the chromatographic properties of the adsorbent, and the required activity cannot be restored.

Chlorinated Naphthalenes and Polychlorinated Terphenyls

Both groups of compounds have uses similar to those of PCB and may possibly be present in environmental samples. It has been shown[9] that chlorinated naphthalenes can be separated from chlorinated hydrocarbon

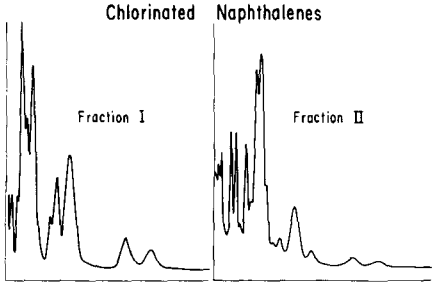

FIGURE 4 Chlorinated naphthalenes eluted from the silica column in fractions I and II, respectively.

pesticides by the silicic acid-Celite chromatography, previously used for the separation of PCB, and occur in the same fraction as PCB (petroleum ether eluate).Chlorinated naphthalenes are also eluted in the same fractions as PCB on the alumina-silica chromatography (Figure 4). If present alone, or in concentrations comparable to the concentration of PCB, their presence could be recognized from the characteristic peak patterns on gas chromatography. The u.v. spectrum of chlorinated naphthalenes has a maximum at 306 nm (Figure 5) with $A_{1\,cm}^{1\%} = 329$. PCB[10] and chlorinated terphenyls (Figure 5) have only negligible absorbance at this wavelength, so that chlorinated naphthalenes could be determined in the presence of PCB and chlorinated terphenyls by u.v. spectrophotometry. The limitation for this approach is the background generated by u.v.-absorbing compounds eluted from the alumina and silica columns, and u.v.-absorbing compounds present in hexane extracts of biological samples and eluted in fractions I and II. Chlorinated naphthalenes in concentrations below 3 mcg/ml could easily escape detection.

Chlorinated terphenyls are not eluted from the SE-30 column at 200°C, and thus their presence would not be detected. Halogenated biphenyls containing chlorine and fluorine atoms, such as trifluoropentachlorobiphenyl, or trifluorotrichlorobiphenyl may be used in hydraulic fluids.[11] Diaminopolyhalobiphenyls,[12] and dihydroxypolyhalobiphenyls[13] may be used for the preparation of flame-resistant polymers and reinforced plastics. All of these compounds could be encountered in environmental samples and appropriate detection methods should be developed.

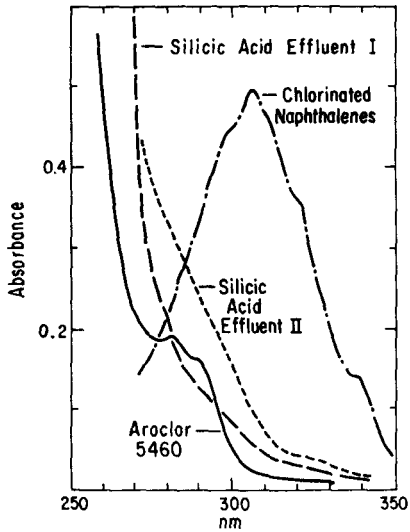

FIGURE 5 U.v. spectra of chlorinated naphthalenes, polychlorinated terphenyls, and u.v.-absorbing compounds eluted from the silica column.

PCB Quantification

The presence of many unidentified compounds in PCB preparations makes the quantification of PCB difficult. Some authors use total peak area[7] or area under one,[14] two,[15] or more[16,17] peaks, others use peak heights.[17-20] The values thus obtained are compared with values obtained in the same way on commercial PCB preparations. A method based on the average electron-capture detector response to biphenyls containing from one to seven chlorine atoms using commercial Aroclor® preparations as standards, was recently described.[21] This method is no more accurate than any of the other quantification procedures, since it has been shown that the detector response to chlorinated biphenyls with the same degree of chlorine substitution may vary

significantly according to the substitution patterns.[22] It has been estimated[23] that due to the outlined uncertainties the quantification of PCB may be accurate only within a factor of 2, whereas according to others[24] it is impossible at the moment to quantify PCB with any degree of confidence.

The presence of PCB in the environment has been well established, and the levels of PCB have to be measured to determine the degree of contamination of different areas, of different trophic levels, etc. Since the PCB components are not fully identified as yet, the measurement of PCB cannot be accurate,

TABLE II

Recovery of Aroclor® 1254

Added (mcg)	Quantification peak numbers — Found (mcg)			
	1	2	3	Av.
0.689	0.775(103)[a]	0.745(99)	0.725(97)	0.748
0.689	0.728(103)	0.670(95)	0.710(101)	0.703
0.689	0.850(114)	0.687(92)	0.705(94)	0.747
0.689	0.760(107)	0.693(98)	0.668(94)	0.707
				0.726
				105.5%

[a] ( ) per cent of average.

but it can be reasonably precise. To achieve maximum precision, the isolation and separation of PCB from organochlorine pesticides and, particularly, the quantification of PCB should be standardized.

PCB concentrations given in this paper were determined from areas under three of the six major peaks in Aroclor® 1254 preparations (quantification peak numbers 1, 2, and 3 corresponding to the second, fourth, and sixth major peak in Aroclor® 1254). For comparison, PCB in some samples were quantified using the total height of five of the six major Aroclor® 1254 peaks (the third peak was not used since it overlaps with the peak of p,p'-DDE).

The average recovery of Aroclor® 1254 on alumina-silica chromatography was 105.5% (Table II). The quantification peak No. 1 yielded somewhat higher results than the quantification peak No. 3. The opposite case was observed when analysing solutions of Aroclor® 1254, solubilized in water with Corexit 7664[10] (Table III). These solutions were used in bioassay experiments with trout. The decrease of PCB concentration with time is due to the PCB uptake by fish and possibly to the adsorption of PCB on the walls of

the bioassay tanks. In PCB extracted from the exposed trout tissues, PCB levels calculated from the quantification peak No. 1 are again higher than those calculated on the basis of the quantification peak No. 3. It is thus possible that PCB with shorter retention times are taken up faster than those with longer retention times. PCB levels calculated by the peak-height method are in good agreement with those obtained from the quantification peaks.

TABLE III

Relative distribution of PCB peaks in different substrates

	Quantification peak numbers				
	1	2	3	Av.	PCB peak height
Water, mcg/l					
44 hr	140(76)[a]	193(105)	218(119)	184	184
72 hr	124(77)	161(100)	196(122)	160	153
96 hr	102(81)	119(95)	157(125)	126	115
Trout, mcg/g wet weight					
Muscle	7.40(118)	6.0(95)	5.45(87)	6.3	5.50
Liver	68(119)	53(93)	50(88)	57.0	52.5
Caecae	276(121)	207(91)	202(89)	228.3	208.0

[a] ( ) per cent of average.

TABLE IV

Precision of PCB determination. Whole herring, two batches consisting of five specimens each, analysed in duplicate. Concentrations are in mcg/g, wet weight.

Quantification peak numbers							
1	2	3	Av.	PCB peak height	p,p′-DDE	p,p′-DDD	p,p′-DDT
0.37(127)[a]	0.26(90)	0.23(79)	0.29	0.41	0.07	0.03	0.10
0.39(118)	0.30(91)	0.29(88)	0.33	0.46	0.09	0.03	0.09
0.48(141)	0.32(94)	0.21(63)	0.34	0.45	0.10	0.02	0.08
0.43(139)	0.29(94)	0.31(64)	0.31	0.41	0.10	0.01	0.06
		Av.	0.32	0.43			
		SD	0.022	0.026			

[a] ( ) per cent of average.

Comparison of these two quantification methods and precision of the PCB measurement is presented in Table IV. The peak-height method yielded higher results than the peak-area method; the precision, however, expressed as standard deviation was practically the same. Levels of PCB (peak-area method) and chlorinated hydrocarbon pesticides in selected samples of fish are given in Table V. The levels of PCB in herring, Atlantic salmon, and commercial fish oil are comparable to those reported from Sweden.[2,3]

TABLE V

PCB and chlorinated hydrocarbon pesticides in fish. Concentrations are in mcg/g, wet weight (average of two duplicate determinations).

Species	Station	PCB	p,p′-DDE	p,p′-DDD	p,p′-DDT
American eel					
(*Anguilla rostrata*)	A	0.63	0.55	not detectable	0.21
Muscle	B	0.75	0.57	0.27	0.32
Herring[a]		0.54	0.24	0.04	0.15
(*Clupea harengus*)	C				
Whole fish		0.32	0.09	0.02	0.08
		0.27[b]			0.40[b]
Commercial fish oil		3.55 ± 0.21[c]	2.27 ± 0.24	—	0.37 ± 0.03
		3.5[b]			7.3[b]
Atlantic salmon					
(*Salmo salar*)	D	0.45	0.22	0.07	0.08
Muscle		0.30[b]			1.5[b]

[a] Average weight 222 and 59 g, resp.
[b] Specimens from the Baltic Sea[2,3]
[c] Standard deviation

A = Chamcook Lake, N.B.
B = St. John River System, N.B.
C = Chedabucto Bay, N. S.
D = Miramichi River, N.B.

CONCLUSIONS

In order to achieve reproducible separation of PCB from most chlorinated hydrocarbon pesticides, not only the activity of the adsorbents, but also the composition of the chromatographic solvents should be carefully controlled. PCB from different sources may contaminate the analysed samples. All materials used in the analysis must be checked for possible presence of PCB. The lack of identification of individual chlorobiphenyls precludes accurate quantification of PCB. The quantification is, however, reasonably precise.

The quantification method should be standardized to obtain results comparable between laboratories. PCB have been detected in fresh-water and marine fish from Canadian Atlantic provinces, and a more detailed monitoring program should be established. The fate of PCB in commercial fish oil on its further processing should be investigated.

EXPERIMENTAL

Conditions of the gas chromatographic procedure have been described.[22] Clean-up chromatography was carried out and alumina was activated according to Holden and Marsden;[6] silicic acid was activated according to Armour and Burke.[7] The procedure is summarised in Table VI.

TABLE VI

Procedure

Sample (5 g) ground with anhydrous Na_2SO_4.
Ground sample extracted with pesticide-grade hexane, Soxhlet, 1 hr, final volume of extract 100 ml.
Chromatography on alumina (Fisher # A-540), aliquot of extract (1–50 ml) in 1.5 ml hexane, alumina activated at 800°C (4 hr), 5% water added. Column 45 × 0.7 cm, 2 g of alumina, 20 ml of effluent collected.
Chromatography on SILICAR®. Effluent from alumina in 1.5 ml hexane, SILICAR activated at 130°C (overnight), 3% water added, column 45 × 0.7 cm, 2 g of SILICAR®. Effluent: hexane, 10 ml fraction I 20 ml fraction II 10% ether in hexane, 10 ml fraction III

References

1. R. Risebrough, P. Rieche, D. B. Peakall, S. G. Herman, and M. N. Kirven, *Nature* **220**, 1098 (1968).
2. G. D. Veith and G. F. Lee, *Water Res.* **4**, 265 (1970).
3. D. B. Peakall and J. L. Lincer, *BioScience* **20**, 958 (1970).
4. L. M. Reynolds, *Bull. Environ. Contam. Toxicol.* **4**, 128 (1969).
5. A. Bevenue and J. N. Ogata, *J. Chromatog.* **50**, 142 (1970).
6. A. V. Holden and K. Marsden, *J. Chromatog.* **44**, 481 (1969).

7. J. A. Armour and J. A. Burke, *J. Ass. Offic. Anal. Chem.* **53**, 761 (1970).
8. S. Bailey, P. J. Bunyan, and F. B. Fishwick, *Chem. Ind. (London)* 705 (1970).
9. J. A. Armour and J. A. Burke, *J. Ass. Offic. Anal. Chem.* **54**, 175 (1971).
10. V. Zitko, *Bull. Environ. Contam. Toxicol.* **5**, 279 (1970).
11. R. H. Boschan, D. H. Nail, and J. P. Holder, U.S. 3,514,406, 26 May 1970; CA **73**, 27321 (1970).
12. L. Sobel, L. Parvi, A. Isard, Ger. Offen. 1,945,681, 30 April 1970; CA **73**, 66244 (1970).
13. Anon., *Chem. Eng. News* **49**(7), 85 (1971).
14. J. H. Koeman, M. C. Ten Noever De Brauw, and R. H. De Vos, *Nature* **221**, 1126 (1969).
15. K. Vermeer and L. M. Reynolds, *The Canadian Field-Naturalist* **84**, 117 (1970).
16. J. E. Keil, L. E. Priester, and S. H. Sandifer, *Bull. Environ. Contam. Toxicol.* **6**, 156 (1971).
17. I. Prestt, D. J. Jeffries, and N. W. Moore, *Environ. Pollut.* **1**, 3 (1970).
18. D. L. Grant, W. E. J. Phillips, and D. C. Villeneuve, *Bull. Environ. Contam. Toxicol.* **6**, 102 (1971).
19. D. J. Hansen, P. R. Parrish, J. T. Lowe, A. J. Wilson, Jr., and P. D. Wilson, *Bull. Environ. Contam. Toxicol.* **6**, 113 (1971).
20. R. W. Risebrough, *Chemical Fallout*, M. W. Miller and G. G. Berg (Eds.) (Charles C. Thomas Publisher, Springfield, Ill., 1969).
21. J. W. Rote and P. G. Murphy, *Bull. Environ. Contam. Toxicol.* **6**, 377 (1971).
22. V. Zitko, O. Hutzinger, and S. Safe, *Bull. Environ. Contam. Toxicol.* **6**, 160 (1971).
23. J. Jensen, A. G. Johnels, M. Olson, and G. Otterlind, *Nature* **224**, 247 (1969).
24. A. Richardson, J. Robinson, A. N. Crabtree, and M. K. Baldwin, *Pesticides Monitoring J.* **4**, 169 (1971).