Journal of Chromatography, 630 (1993) 179-186
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 696

Liquid-gel partitioning using Lipidex in the determination of polychlorinated biphenyls in cod liver oil

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(First received July 14th, 1992; revised manuscript received October 19th, 1992)

ABSTRACT

A technique was developed for transfer of fat and polychlorinated biphenyls from cod liver oil into the lipophilic gel Lipidex 5000. Subsequent elution of the gel separated about 60% of the fat from the sample. Following further purification on aluminium oxide and silica gel, toxic non-ortho- and mono-ortho-PCB congeners were isolated in two separate fractions on charcoal. Recoveries were studied by addition of twelve different PCB congeners to 0.2 g of fat. The non-ortho-PCBs were labelled with ^{13}C. The recoveries of 5–50 ng of the unlabelled compounds were 80–100% and those of 50–100 pg of the labelled compounds were 76106%.

INTRODUCTION

In recent years, attention has been focused on the toxicity of polychlorinated biphenyls (PCBs), particularly on the congeners that elicit toxic responses similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These compounds induce aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-0-deethylase (EROD) enzyme activities. The potency to induce such activities ***in vitro*** has been used to evaluate the toxicological significance of PCB congeners and their activities in relation to TCDD [1,2]. According to these investigations, the non-ortho-congeners, IUPAC Nos. 77, 126 and 169 (for structures, see Table I), are the most toxic. Among the mono-*ortho*-congeners, IUPAC Nos. 105, 118 and 156 are considered to be the most potent.

The determination of PCBs in biological samples is complicated by their presence at trace levels in highly complex matrices. The first step in the analysis of biological samples is to extract the organochlorine compounds from the material. This is usually done by liquid-liquid partitioning, Soxhlet extraction or the sample is mixed with sodium sulphate and eluted with an organic solvent [3]. The co-extracted lipids can be removed by treatment with sulphuric acid or alkali (saponification). However, strong alkali decomposes certain polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-p-dioxins (PCDDs) [4,5] and DDT [6] and concentrated sulphuric acid destroys dieldrin [7]. Complementary purification and separation from lipids and other interfering compounds have been achieved by column chromatography using aluminium oxide, silica gel and Florisil [3]. Gel permeation chromatography (GPC) with Bio-Beads is more efficient in removing lipids than these adsorbents [3]. However, this method requires special equipment for a forced liquid flow.

In studies of toxic ***non-ortho-*** and mono-ortho-PCBs, chromatography on activated charcoal has frequently been used for the separation of the non-*ortho*-congeners from the bulk of PCBs [8–10]. Only a few methods have been reported concerning the isolation of the toxic non-ortho- and ***mono-ortho-*** PCBs in separate fractions. Athanasiadou et al. [11] used multiple charcoal columns to isolate non- and

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0021-9673/93/$06.00

TABLE I

IUPAC NUMBERS AND CHLORINE ATOM POSITIONS OF PCBs

IUPAC No.	Structure	IUPAC No.	Structure
	Dichlorobiphenyl		***Hexachlorobiphenyls***
15	4,4′-	132	2,2′,3,3′,4,6′-
		138	2,2′,3,4,4′,5′-
	Trichlorobiphenyl	***149***	2,2′,3,4′,5′,6-
28	2,4,4′-	***153***	2,2′,4,4′,5,5′-
	Tetrachlorobiphenyls	***156***	2,3,3′,4,4′,5-
52	2,2′,5,5′-	169	3,3′,4,4′,5,5′-
77	3,3′,4,4′-		
			Heptachlorobiphenyl
	Pentachlorobiphenyls	171	2,2′,3,3′,4,4′,6-
101	2,2′,4,5,5′-	***180***	2,2′,3,4,4′,5,5′-
105	2,3,3′,4,4′-		
118	2,3′,4,4′,5-		*Docedachlorobiphenyl*
126	3,3′4,4′,5-	209	2,2′,3,3′,4,4′,5,5′,6,6′-

mono-ortho-PCBs in a technical PCB product, and Wilson-Yang *et al.* [12] employed charcoal for isolation of non- and mono-ortho-congeners in a biological sample. Recently, Haglund *et al.* [13] separated PCBs into similar groups by high-performance liquid chromatography (HPLC) on 2-(1-pyrenyl)ethyldimethylsilylated silica. Depending on the concentrations, final determination of PCBs has been performed by gas chromatography with electron-capture detection (GC-ECD) or gas chromatography-mass spectrometry (GC-MS).

The aim of this study was to develop a simple and non-destructive method for the extraction and purification of organochlorine compounds from materials rich in lipids (e.g., solution of lipids). Extraction with Lipidex was chosen as this gel has been shown to have a high capacity for lipids and lipid-soluble compounds [14] and it has been used successfully for the enrichment of organochlorine compounds from aqueous samples, e.g., water [15], urine [15] and human milk [16]. In attempts to adapt the method for fatty samples, cod liver oil was chosen as a model matrix. This product is used as a vitamin supplement, and as organochlorine contaminants, such as PCBs, accumulate in cod liver, the investigation of this oil was of interest. A technique was developed to transfer lipids and lipid-soluble compounds from an organic solvent into the gel. The subsequent elution of the gel with solvents of different polarity permits the isolation of the compounds and partial purification of the sample. No special equipment is required for the sample preparation. The separation of toxic *non-ortho-* and mono-*ortho*-PCBs from the bulk of PCBs was achieved by chromatography on a charcoal column, which was eluted with a modified solvent system.

EXPERIMENTAL

Sample

The cod liver oil (Apoteksbolaget, Solna, Sweden) was a common pharmacy product used as a vitamin A and D supplement. A 10-g amount of oil was weighed into a volumetric flask (100 ml), dissolved in hexane and diluted to the mark with hexane.

Solvents

All solvents were of analytical-reagent grade. Methanol was treated with sodium hydroxide and redistilled twice [15]. Acetonitrile, toluene and 2-propanol were redistilled once. Hexane, chloroform and methylene chloride were redistilled twice. Hexane used for the elution of silica gel was dried with sodium sulphate. Water was deionized and purified with a Milli-Q cartridge system (Millipore, Bedford, MA, USA).

Standards

Clophen A50 (Bayer, Leverkusen, Germany) was

used as a standard for the determination of total PCBs. ^{13}C-labelled PCBs Nos. 77, 126 and 169 (Cambridge Isotope Laboratories, Woburn, MA, USA) were used for the determination of non-*ortho*-PCBs. Standards for determination of PCBs Nos. 28, 52, 101, 105, 118, 138, 153, 156 and 180 were obtained from Ehrenstorfer (Augsburg, Germany), or were received as gifts from Dr. Åke Bergman, Wallenberg Laboratory, Stockholm University. Standards used for volume correction, PCBs Nos. 15 and No. 209, were obtained from Ehrenstorfer.

Column chromatography

Glass chromatographic columns were of 0.4, 1 and 2 cm I.D. [9,15]. Lipidex 5000 (Packard Instruments, Downers Grove, IL, USA) was washed and stored in methanol at 4°C [17]. Immediately before use, it was rinsed with 2-propanol(2 × 25 ml per 20 g of Lipidex) in a separating funnel, equipped with a sintered-glass disc and a PTFE stopcock at the end. A gentle stream of purified nitrogen was used to remove most of the remaining 2-propanol. Aluminium oxide 90 (activity grade II-III) (Merck, Darmstadt, Germany), silica gel 60 (70–230 mesh) (Merck) and activated charcoal (SP-1) (Serva, Heidelberg, Germany) were prepared as described elsewhere [9,10].

Gas chromatography

The GC analyses of di-ortho- and mono-*ortho*-substituted PCBs were performed using a Pye Unicam gas chromatograph equipped with an all-glass falling-needle injector with the heater at 220°C, a fused-silica capillary column coated with SE-54 (25 m × 0.32 mm I.D., 0.25 μm film thickness) (Quadrex, New Haven, CT, USA) and a ^{63}Ni electron-capture detector. Nitrogen was used as the carrier gas. The column temperature was kept at 190°C for 15 min, programmed to 260°C at 5°C/min and then kept isothermal at 260°C for 30 min. An on-column injector maintained at 220°C was used for the GC determination of the total amount of PCBs. The glass column (2 m × 2 mm I.D.) was packed with a mixture of 3% SF-96 and 6% QF-1 (32:68, w/w) on Chromosorb W HP (100–120 mesh) [18]. The column temperature was 185°C.

Gas chromatography-mass spectrometry

GC-MS analyses were performed with a VG 7070 E mass spectrometer equipped with a DAN1 gas chromatograph and a VG 1 l-250 data system (VG Analytical, Manchester, UK). A fused-silica capillary column coated with SE-30 (25 m × 0.32 mm I.D., 0.25 μm film thickness) (Quadrex) was directly connected to the ion source. The oven temperature was 190°C for 15 min, programmed to 270°C at 8°C/min and kept at this temperature for 8 min. An all-glass falling-needle injector was used with an injector heater at 270°C. The carrier gas was helium. Electron impact (EI) ionization was performed in an EI-only ion source at 48 eV. The acceleration voltage was 6 kV and the resolution was 8000–9000. Compounds were monitored in groups determined by the number of chlorine atoms in the molecule. Two ions of each molecular ion cluster were monitored. For each group, one ion from the column bleeding was selected as the reference mass for correction of mass spectrometer drift (lock mass). For each ***m/z*** value the dwell time was 80 ms and the delay time 20 ms.

Method

A flow scheme of the analytical procedure is shown in Fig. 1.

Extraction and preliminary *purification.* An aliquot of 2 ml of the oil solution (0.1 g/ml) was transferred into a 100 ml erlenmeyer flask. All samples were fortified with ^{13}C-labelled PCBs Nos. 77, 126, and 169. A number of samples were also fortified with unlabelled PCBs Nos. 28, 52, 101, 105, 118, 138, 153, 156 and 180 for recovery studies. One blank sample (2 ml of hexane) was run in parallel with each set of samples. A 15-ml volume of 2-propanol was mixed with the sample solution and 5.0 g of washed Lipidex were added. A dropping funnel equipped with a pressure equalizer was attached to the top of the flask. A 40-ml volume of water was added to the funnel, which was closed with a glass stopper at the top. The subsequent procedure was performed while shaking the flask at 35°C in a water-bath for a total of 2.5 h. After 5 min, water was added from the funnel (Teflon stopcock) with continuous shaking at a rate of about 0.5 ml/min. When all the water had been added, shaking was continued with the stopcock closed. The mixture was then transferred into a glass column (2 cm I.D.)

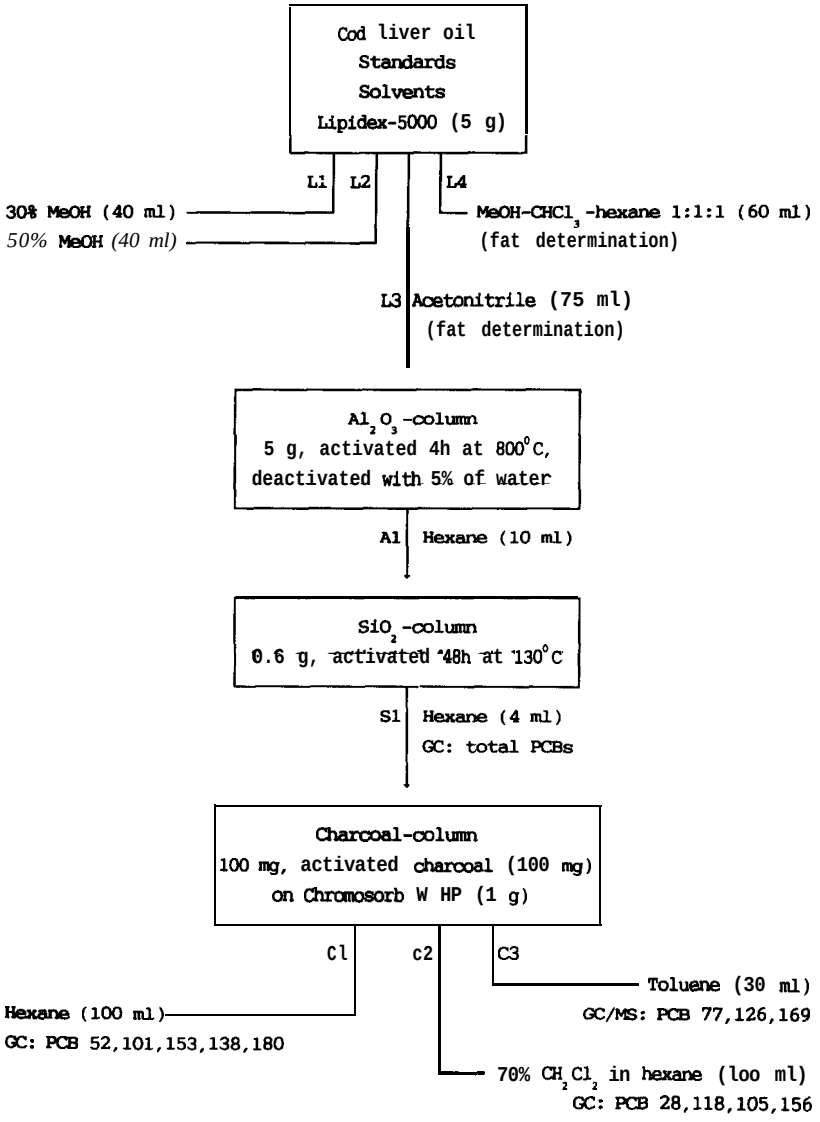

Fig. 1. Flow scheme of the method

and the solvent was drained. The gel was washed with 40 ml of methanol-water (30:70, v/v) (fraction L1) and 40 ml of methanol-water (50:50, v/v) (fraction L2). The chlorinated compounds and some lipids were eluted with 75 ml of acetonitrile (fraction L3). Remaining lipids were eluted with 60 ml of methanokhloroforn-hexane, (1: 1: 1, v/v/v) (fraction L4).

Fat determination. Fractions L3 and L4 were taken nearly to dryness in a rotary evaporator at 35°C and the residues were dried to constant mass in a desiccator at room temperature. The sum of the masses of fractions L3 and L4 defined the total amount of fat in the sample.

Purification and group separation. Partly deactivated aluminium oxide and activated silica gel were used for further purification and separation of the sample, according to ref. 16 and Fig. 1. The silica

gel fraction (S1) contained the PCBs. A standard (40 ng of PCB No. 15) was added for volume correction and the total amount of PCBs was determined by GC-ECD.

A column (0.4 cm I.D.) was packed with 100 mg of a mixture of activated charcoal and Chromosorb W HP [9,10] and washed with 30 ml of toluene, 20 ml of methylene chloride-hexane (10:90, v/v) and 20 ml of hexane. Fraction S1 from the silica gel was evaporated with a gentle stream of nitrogen to cu. 100 μl and quantitatively transferred into the column with hexane. The column was then eluted with 100 ml of hexane (fraction Cl). This fraction contained the PCBs, except for certain mono-*ortho*-PCBs and the non-ortho-PCBs. Mono-*ortho*-PCBs (IUPAC Nos. 28, 105, 118, 156) were eluted with 100 ml of methylene chloride-hexane (70:30, v/v) (fraction C2) and the non-ortho-PCBs with the following 30 ml of toluene (fraction C3). Fractions Cl and C2 were concentrated to l-2 ml. After addition of internal standard (40 or 80 ng of PCB No. 15), the fractions were analysed by GC-ECD. Fraction C3, was evaporated to cu. 50 μl. Internal standard (100 pg of PCB No. 209) was added and after further concentration the fraction was analysed by GC-MS.

RESULTS AND DISCUSSION

Lipidex proved to be an effective sorbent for fat and fat-soluble compounds in cod liver oil. The transfer of lipids from a hexane-Zpropanol extract of testicular tissue into Lipidex has been demonstrated by Anderson and Sjövall [193 in the determination of steroids. They mixed the extract with Lipidex 1000 and evaporated the solvent with a rotary evaporator. The steroids were eluted from the dried gel with aqueous methanol leaving the lipids in the gel. We utilized the same approach for the determination of organochlorine compounds in cod liver oil. However, the method of evaporation was not suitable, as the material spread on the surface of the glassware and the resulting recoveries were low. Other methods were therefore tested for the transfer of fat and PCBs into the gel. Water was added in 10-ml portions to the hexane-2-propanol extract during shaking. This provided better results than the evaporation method, but the recoveries were still not acceptable. The continuous addition of water during shaking proved to be essential for complete transfer into the gel.

About 60% of the lipids were removed from the sample by the elution system used for Lipidex (Table II). The average amount of fat in nine samples was 0.206 g (range 0.203-0.211 g). Further removal of lipids and separation from most of the pesticides were achieved with column chromatography on aluminium oxide and silica gel. The total amount of PCBs was determined in two samples (fraction Sl). These determinations were made by GC-ECD using a packed column and Clophen A50 as a standard as described previously [18]. Congener-specific analyses were made for twelve compounds (IUPAC Nos. 28, 52, 77, 101, 105, 118, 126, 138, 153, 156, 169 and 180). Fig. 2 shows high-resolution gas chromatograms of an unspiked oil sample. From the complex mixture of PCBs in fraction S1 (Fig. 2a) the mono-ortho-PCBs were separated on charcoal and recovered in fraction C2 (Fig. 2c), leaving the bulk of PCBs in fraction Cl (Fig. 2b). By this procedure the mono-ortho-PCBs Nos. 105, 118 and 156 could be separated from the interfering compounds Nos. 132, 149 and 171, respectively. The selected-ion chromatograms of non-ortho-PCBs (fraction C3) obtained in the GC-MS analyses are shown in Fig. 3. The recoveries of di- and mono-ortho-PCBs added to 0.2 g of oil ranged from 80 to 100%, and the recoveries of added ^{13}C-labelled non-*ortho*-PCBs ranged from 76 to 106% (Table III).

The cod liver oil was intended as a vitamin A and D supplement and was purified from PCDDs according to the supplier. The total PCB concentration in this oil was 1 μg/g fat and it contained toxic non- and mono-ortho-congeners of PCBs (Table III). Safe [1] and Ahlborg *et al.* [20] have proposed

TABLE II

DISTRIBUTION OF FAT IN THE FRACTIONS ELUTED FROM LIPIDEX (n = 9)

Fraction	Distribution (%)		
	Mean	Range	R.S.D.
L1	0	–	–
L2	0	–	–
L3	38	32-44	11
L4	62	56–67	7

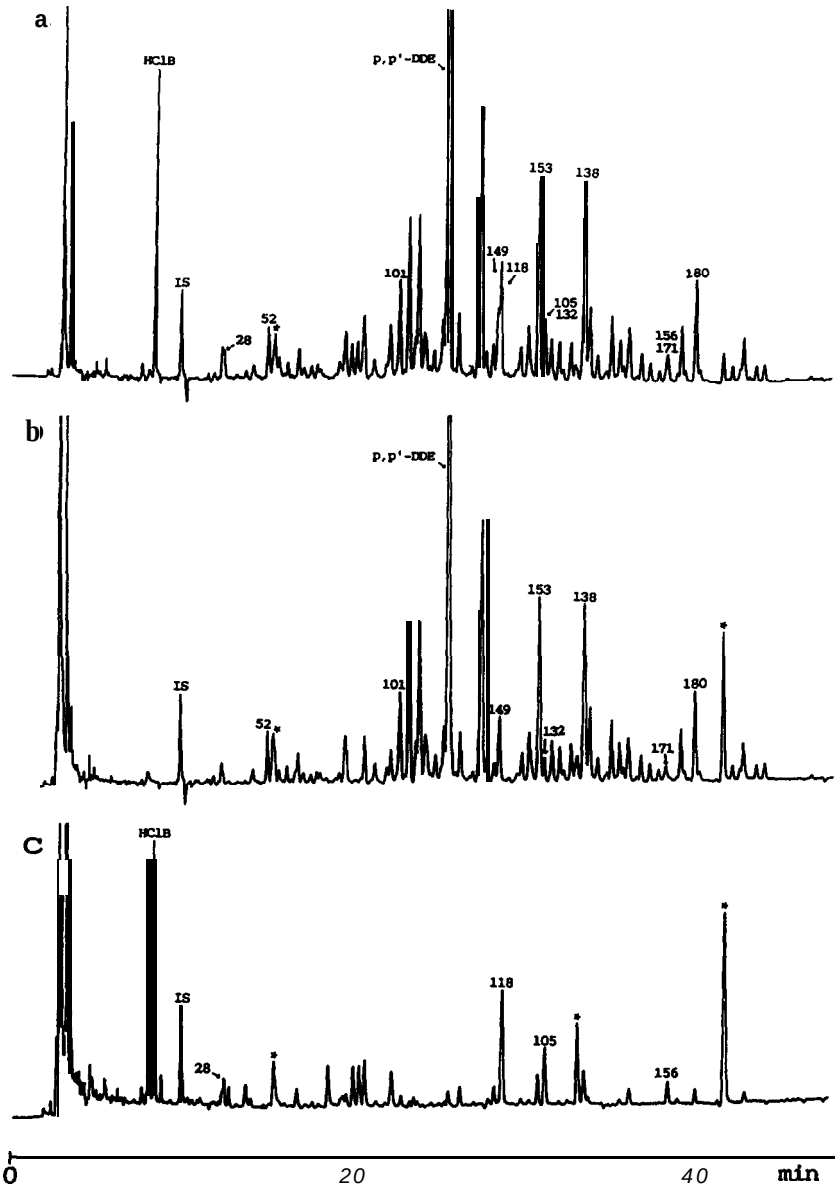

Fig. 2. High-resolution gas chromatograms of (a) cod liver oil before separation on charcoal (fraction S1); (b) the PCB pattern after group separation on charcoal with certain mono-*ortho*-PCBs and non-*ortho*-PCBs removed (fraction Cl); and (c) mono-*ortho*-PCBs (fraction C2). The peaks marked with asterisks represent impurities, HClB = Hexachlorobenzene.

toxic equivalency factors (TEFs) for different PCB congeners. These factors express the toxicity of a compound relative to that of TCDD. Using these' factors for non- and mono-ortho-PCBs in the cod liver oil, the toxic equivalents (TEQs) were calculated as 69 and 20 pg/g, respectively (Table IV). According to the Nordic risk assessment [21], a tolerable intake would be O-35 pg TCCD per kg body-mass per week.

The proposed method for liquid-gel partitioning

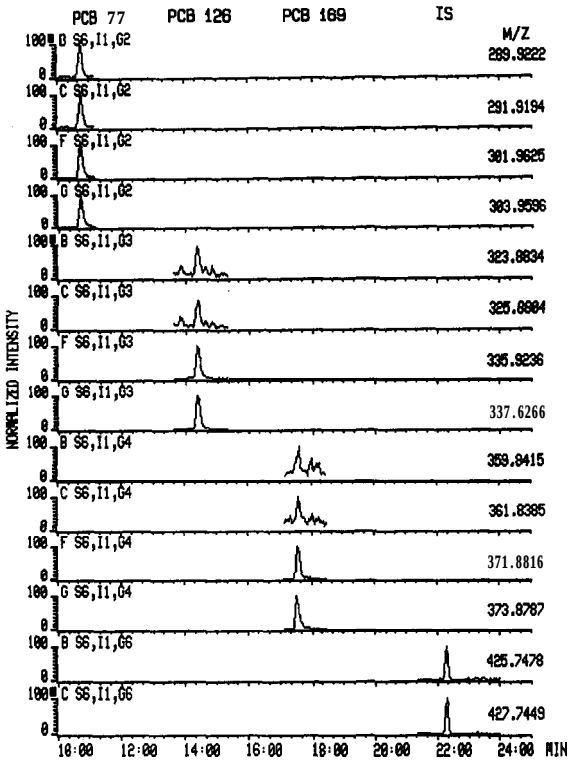

Fig. 3. Selected ion chromatograms of non-ortho-PCB congeners (fraction C3) obtained in the GC-MS analyses. The peaks with the two highest m/z values for each non-ortho-PCB represent ^{13}C-labelled compounds.

of lipids and lipid-soluble organochlorine compounds is advantageous compared with liquid–liquid partitioning as no emulsions are formed and repeated extractions and centrifugations are avoided. In recent years, GPC has been applied successfully for the purification of fatty extracts. However, although the lipids are efficiently removed, further purification is required for determinations of low levels of organochlorine compounds. In contrast to the present method, GPC requires special equipment and the throughput is limited unless multiple HPLC systems and columns are available. Lipidex has a high capacity to incorporate lipids and lipid-soluble compounds. In the proposed method 0.2 g of oil was used for analysis. However, the same distribution of fat in fractions L3 and L4 was obtained using up to 0.5 g of fat. The subsequent elution of the gel with solvents of different polarity facilitates partial purification of the sample. Only simple laboratory equipment is needed for the extraction procedure and the elution of the gel. The relatively long extraction time is compensated for by the fact that many extractions can be performed simultaneously. The technique adopted is partly based on a non-destructive method for the determination of organochlorine pesticides, PCDDs, PCDFs and PCBs in human milk [9,16]. Such compounds can also be included and analysed by the proposed method.

TABLE III

LEVELS OF CERTAIN PCB CONGENERS IN COD LIVER OIL AND RECOVERIES OF ADDED PCBs

PCB IUPAC No.	Level (ng/g fat)		Amount added (ng)	Recovery (%)		
	Mean[a]	Range		Mean	Range	R.S.D.
Di-ortho-						
52	*28 (2)*	*27-28*	10–50 (7)	91	83-89	5
101	40 (2)	40–41	10–50 (7)	93	85-99	5
138	73 (2)	70-76	10–50 (7)	88	81-94	6
153	84 (2)	81-88	10–50 (7)	94	86-99	6
180	30 (2)	28-3 1	10–50 (7)	91	84–98	5
Mono-ortho-						
28	10 *(2)*	*9-10*	*lo-50 (7)*	*90*	*81-98*	7
105	*14 (2)*	14–15	*5-25 (7)*	*91*	*88-98*	*4*
118	*36 (2)*	34–39	5–25 *(7)*	*88*	80–100	*8*
156	*5 (2)*	*5-5*	*5-25 (7)*	*90*	80–97	7
Non-ortho-						
77	*0.26 (5)*	0.23–0.27	0.05–0.10 *(5)*	*89*	*70-106*	*12*
126	*0.10* (5)	0.09-O. 11	0.05-O. 10 (5)	90	79-102	11
169	0.02 (5)	0.02-0.03	0.05-O. 10 (5)	88	83-97	11

[a] Number of samples analysed is given in parentheses.

TABLE IV

TEF VALUES PROPOSED BY SAFE [1] AND AHLBORG ET AL. [20] AND TEQ VALUES FOR PCBs IN COD LIVER OIL

PCB IUPAC No.	TEF		TEQ (pg/g)	
	Ref. 1	Ref. 20	Ref. 1	Ref. 20
Non-ortho-				
77	0.01	0.0005	2.6	0.1
126	0.1	0.1	10.0	10.0
169	0.05	0.01	1.0	0.2
Mono-ortho-				
105	0.001	0.000 1	***14.0***	1.4
118	0.001	0.0001	36.0	3.6
156	0.001	0.001	5.0	5.0
Total			68.6	20.3

The method can also be applied to the analysis of organic solvent extracts of other biological samples. The method was developed for small amount of samples, using small column systems and solvent volumes. Consequently, the risks of contamination from solvents and adsorbents are reduced, and the costs of the analyses are decreased.

ACKNOWLEDGEMENTS

This study was financially supported by the Swedish Environmental Protection Agency, contract No. 5323 19 1-6, the Swedish Medical Research Council (03X-219), Stiftelsen Lars Hiertas Minne and Karolinska Institutet.

REFERENCES

1 S. Safe, ***CRC Crit. Rev.*** Toxicol., 21 (1990) 51.
2 A. Hanberg, F. Wearn, L. Asplund, E. Haglund and S. Safe, ***Chemosphere, 20 (1990)*** 1161.
3 M. D. Erickson, ***Analytical Chemistry of PCBs***, Butterworths, Stoneham, MA, 1986.
4 P. W. Albro, J. S. Schroeder, D. J. Harvan and B. J. Corbett, ***J. Chromatogr., 312 (1984) 165.***
5 J. J. Ryan, R. Lizotte, L. G. Panopio and B. P.-Y. Lau. ***Chemosphere,*** 18 (1989) 149.
6 R. Benecke, J. Brotka, J. Wijsbeek and R. A. Zeeuw, ***J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 30.***
7 D. E. Wells, ***Pure Appl. Chem., 9 (1988) 1437.***
8 S. Tanabe, N. Kannan, T. Wakimoto and R. Tatsukawa, ***J. Environ. Anal. Chem., 29 (1987) 199.***
9 K. Norén, A. Lundén, J. Sjovall and A. Bergman, ***Chemosphere, 20 (1990) 935.***
10 B. Jansson, R. Andersson, L. Asplund, A. Bergman, K. Litzen, K. Nylund, L. Reutergård, U. Sellström, U-B. Uvemo. C. Wahlberg and U. Wideqvist, ***Fresenius' Z. Anal. Chem., 340 (1991) 439.***
11 M. Athanasiadou, S. Jensen and E. Wehler, ***Chemosphere, 23 (1991) 957.***
12 K. M. Wilson-Yang, J. P. Power, E. A. Chrisholm and D. J. Hallett, ***Chemosphere, 23 (1991)*** 1139.
13 P. Haglund, L. Asplund, U. Järnberg and B. Jansson, ***Chemosphere, 20*** (1990) ***887.***
14 B. Egestad, T. Curstedt and J. Sjövall, ***Anal. Lett., 15 (1982) 293.***
15 K. Norén and J. Sjovall, ***J. Chromatogr., 414 (1987) 55.***
16 K. Norén and J. Sjovall, ***J. Chromatogr., 422 (1987) 103.***
17 M. Axelsson and J. Sjovall, ***J. Chromatogr., 126 (1976) 705.***
18 K. Norén, ***Arch. Environ. Contam. Toxicol., 12 (1983) 277.***
19 S. H. G. Andersson and J. Sjövall, ***Anal. Biochem.. 134 (1983) 309.***
20 U. G. Ahlborg, A. Hanberg and K. Kenne, ***Risk Assessment of Polychlorinated Biphenyls (PCBs)***, Nordic Council of Ministers, Copenhagen, 1992.
21 U. G. Ahlborg, H. Håkansson, F. Waern and A. Hanberg, *Nordisk Dioxinriskbedömning*, Nordic Council of Ministers, Copenhagen, 1988.