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Liquid–gel partitioning using Lipidex in the determination of polychlorinated biphenyls, naphthalenes, dibenzo-*p*-dioxins and dibenzofurans in blood plasma

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Abstract

A method was developed for the transfer of fat, polychlorinated biphenyls (PCBs), naphthalenes (PCNs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) from blood plasma into the lipophilic gel Lipidex 5000. Subsequent elution of the gel separated about 70% of the fat from the analytes. Different adsorbents and activated charcoal were applied for further purification of the sample and separation of analytes. Identification and determination of the chlorinated compounds were made by gas chromatography with electron-capture detection (GC–ECD) or gas chromatography–mass spectrometry (GC–MS). Recoveries were studied by addition of Halowax 1014 and different congeners of PCBs, PCNs, PCDDs and PCDFs to 50 ml of plasma. The mean recoveries of the individual compounds studied were 72–99%. By using the liquid–gel partitioning technique emulsions were avoided. Concentrations of lipids in plasma obtained by the present method agreed well with the concentrations obtained using liquid–liquid partitioning with chloroform–methanol.

1. Introduction

Polychlorinated biphenyls (PCBs), naphthalenes (PCNs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are four classes of anthropogenic chlorinated aromatic hydrocarbons that are structurally and toxicologically related. They are highly lipophilic and resistant to chemical and enzymatic degradation. This results in persistence and bioaccumulation in the environment, particularly in fatty tissues of animals, including man. The toxicity of PCDDs and PCDFs is well documented [1] and more recently, attention has been focused on the toxicity of compounds that act by the same mechanism as TCDD, particularly on the non-*ortho*- and mono-*ortho*-chlorinated congeners of PCBs [1,2] and certain PCNs [3]. In order to investigate the presence and the concentrations of such toxic compounds, it is necessary to have reliable techniques for their determination. In humans, measurements can be performed in, e.g., mother's milk, adipose tissue or blood. Since it is less complicated to collect blood, this is the preferred choice of material for exposure assessment. With the increasing number of pollutants

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and the possibility of interaction between compounds in their toxic activity, there is a need for multicomponent methods for simultaneous analysis of a large number of halogenated compounds. To our knowledge, cogener specific analysis of PCBs, PCNs, PCDDs and PCDFs in blood, milk or adipose tissue in one sample has not been reported. However, PCBs, PCDDs and PCDFs have been determined simultaneously in serum [4] and PCNs and PCBs in plasma [5].

The determination of low levels of organochlorine compounds in biological samples is complicated because of the complexity of the matrices. Generally, extractions of blood samples (whole blood, blood plasma or serum) are made by liquid–liquid partitioning [6–9]. This technique has drawbacks since emulsions are often formed and repeated extractions and centrifugations are needed. In recent years, successful attempts have been made to sorb organochlorine compounds from blood to a solid phase. Päpke et al. [10] used a modified silica gel (Chem-Elut) and Chang et al. [11] used an octadecylsilane-bonded silica cartridge for the extraction and enrichment of lipophilic compounds in whole blood and blood plasma, respectively. However, the lipids were not completely extracted by these methods and other techniques had to be used for the determination of the lipid content.

The aim of the present study was to develop a simple and non-destructive method for the determination of PCBs, PCNs, PCDDs and PCDFs in human blood plasma. The present paper describes a technique to transfer lipids and lipid-soluble compounds from blood plasma into the lipophilic gel Lipidex. This gel has been shown to have high capacity for lipids and lipid-soluble compounds [12,13] and it has previously been used for the extraction of organochlorine compounds from cod liver oil [14] and aqueous samples, e.g., water [15], urine [15] and human milk [16]. Elution of the gel with solvents of different polarity enables the isolation and partial purification of the organochlorine compounds. Using different adsorbents for subsequent separation, the method can be applied to multicomponent determination of PCBs, PCNs, PCDDs and PCDFs in blood plasma.

2. Experimental

2.1. Samples

Three different batches of human blood plasma were studied. These were obtained from the Blood Center, Karolinska Hospital, Stockholm, Sweden. In the production of the plasma, citrate phosphate dextran (CPD) was used as an anticoagulant. The plasma was stored at −20°C.

2.2. Solvents

Hexane, dichloromethane (DCM), acetonitrile, methanol, 2-propanol and chloroform were of HPLC-grade (Rathburn, Walkeburn, Scotland) and were redistilled prior to use. Toluene was of Pestanal quality (Riedel de Häen, Seelze, Germany) or of Distol quality (Fison, Loughborough, UK) and was used as supplied. Formic acid was of pro analysi quality (Merck, Darmstadt, Germany). Water was deionized and purified with a Milli-Q cartridge system (Millipore, Bedford, MA, USA).

2.3. Standards

Unlabelled PCB congeners were obtained from Ehrenstorfer (Augsburg, Germany) or Cambridge Isotope Laboratories (CIL, Woburn, MA, USA). The following $^{13}C_{12}$-labelled compounds, purchased from CIL, were used as internal standards: 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta- and 3,3′,4,4′,5,5′-hexaCB; 2,3,7,8-tetra-, 1,2,3,7,8-penta-, 1,2,3,6,7,8-hexa-, 1,2,3,4,6,7,8-hepta- and octaCDD; 2,3,7,8-tetra-, 1,2,3,7,8-penta, 1,2,3,4,7,8-hexa-, 1,2,3,4,6,7,8-hepta- and octaCDF. Unlabelled 1,2,3,4,7,8-hexa and 1,2,3,7,8,9-hexaCDD, 2,3,4,7,8-penta-, 1,2,3,6,7,8-hexa-, 2,3,4,6,7,8-hexa-, 1,2,3,7,8,9-hexa- and 1,2,3,4,7,8,9-heptaCDF, used for identification, were obtained from the World Health Organization (WHO, Copenhagen, Denmark) interlaboratory quality control study (1991). The PCN product Halowax 1014 was obtained from Koppers (Pittsburgh, PA, USA). 1,2,3,4,6,7-, 1,2,3,5,6,7-, 1,2,4,5,6,8-Hexa-, 1,2,3,4,5,6,7-hepta- and octaCN were synthesized as described [17–19]. 1,2,3,4,6,7-HexaCN

and 1,2,3,5,6,7-hexaCN were obtained as a mixture. All standards above were dissolved in hexane or 2,2,4-trimethylpentane. [4-^{14}C] Cholesterol and 1,2-di[1-^{14}C]palmitoyl-L-3-phosphatidylcholine (^{14}C-phosphatidylcholine) were purchased from Amersham International (Bucks, UK) and were dissolved in toluene.

2.4. Open column chromatography

Column chromatography was performed in glass columns. The glassware was washed in an ultrasonic bath with detergents, thoroughly rinsed with water and then heated overnight to 280°C. Prior to use, the glassware was rinsed with hexane. Lipidex 5000 (Packard Instruments, Downers Grove, IL, USA) was washed and stored in methanol at 4°C [20]. Lipidex which had been used for extraction could be reused (at least once) after washing with different solvents [20,21]. Immediately before addition to plasma samples, the gel was washed with 2-propanol as described elsewhere [14]. Sodium sulphate (pro analysi)(Merck), aluminium oxide 90 (activity grade II–III) (Merck), basic aluminium oxide (Woelm, Eschwege, Germany) and silica gel 60 (70–230 mesh) (Merck) were prepared as described elsewhere [16]. Activated charcoal (SP-1) (Serva, Heidelberg, Germany), 100 mg, and 2 g of Chromosorb W HP (Chrompack, Middelburg, Netherlands) were thoroughly mixed prior to use.

2.5. Gas chromatography

GC analyses were performed using a Varian 3500 gas chromatograph equipped with an on-column injector, 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. Helium was used as carrier gas and nitrogen as make-up gas. The oven temperature program was as follows: 60°C for 1 min, to 190°C at 20°/min, hold for 0.5 min, to 235°C at 2°C/min, hold for 5 min, then to 280°C at 20°C/min, hold for 10 min. The injector temperature was programmed from 60°C to 240°C at 120°C/min, held for 20 min, then to 280°C at 120°C/min, held for 25 min. The detector temperature was 320°C.

2.6. Gas chromatography–mass spectrometry

GC–MS analyses were performed using a VG 70-250 mass spectrometer equipped with a VG 11-250 data system (Fisons Instruments, VG Analytical, Manchester, UK) and a Hewlett-Packard gas chromatograph HP 5890A (Hewlett-Packard, Geneva, Switzerland). The GC column was of the same type as above. Two different oven temperature programs were used: program A; 180°C for 0.5 min, to 240°C at 4°C/min, held for 4 min, to 270°C at 12°/C min and held at this temperature for 10 min; program B: 160°C for 0.5 min, programmed to 230°C at 4°C/min, held for 1 min, programmed to 270°C at 12°C/min and held at this temperature for 10 min. An all-glass falling-needle injector was used with an injector heater set at 260°C. The carrier gas was helium. Electron ionization (EI) was performed in an EI-only ion source with an electron energy of 30–40 eV. The temperature of the ion source was 260°C. A very low flow of perfluorokerosene (PFK) was allowed to leak into the ion source from the heated inlet. The acceleration voltage was 6 kV and the resolution at m/z 293 was 7000–9000. A sensitivity corresponding to a S/N ratio of 2.5 was attained for 0.1 pg TCDD at a resolution of 7000. Compounds were monitored using selected ion monitoring in groups determined by the number of chlorine atoms in the molecule. Two ions in each molecular ion cluster were monitored, Table 1. A deviation of the isotope ratio of less than ±15% from the theoretical value was accepted, usually the deviation was within ±10%. For each group, one ion from PFK 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 10 ms.

2.7. Radioactivity measurements

Radioactivity was determined in an LKB 1211 Minibeta liquid scintillation counter (Wallac, Turku, Finland). OptiPhase "Hi-Safe" 2 (Wallac) was used as scintillation liquid. Efficiency of

Table 1
Ions (m/z) selected in the GC–MS determinations of PCBs, PCNs, PCDDs and PCDFs

Compound	m/z
TriCB	255.961 (M^+), 257.958
TetraCB	289.922 (M^+), 291.919
[$^{13}C_{12}$]TetraCB	301.963 (M^+), 303.960
PentaCB	323.883 (M^+), 325.880
[$^{13}C_{12}$]PentaCB	335.924 (M^+), 337.921
HexaCB	359.841 ($[M+2]^+$), 361.838
[$^{13}C_{12}$]HexaCB	371.882 ($[M+2]^+$), 373.879
HeptaCB	393.802 ($[M+2]^+$), 395.799
DecaCB	425.748 ($[M-2Cl+2]^+$), 427.745
TetraCN	263.907 (M^+), 265.904
PentaCN	297.868 (M^+), 299.865
HexaCN	333.826 ($[M+2]^+$), 335.823
HeptaCN	367.787 ($[M+2]^+$), 369.784
OctaCN	401.748 ($[M+2]^+$), 403.745
TetraCDD	319.897 (M^+), 321.894
[$^{13}C_{12}$]TetraCDD	331.937 (M^+), 333.934
PentaCDD	353.858 (M^+), 355.855
[$^{13}C_{12}$]PentaCDD	365.898 (M^+), 367.895
HexaCDD	389.816 ($[M+2]^+$), 391.813
[$^{13}C_{12}$]HexaCDD	401.856 ($[M+2]^+$), 403.853
HeptaCDD	423.777 ($[M+2]^+$), 425.774
[$^{13}C_{12}$]HeptaCDD	435.817 ($[M+2]^+$), 437.814
OctaCDD	459.735 ($[M+4]^+$), 461.732
[$^{13}C_{12}$]OctaCDD	471.775 ($[M+4]^+$), 473.772
TetraCDF	303.902 (M^+), 305.899
[$^{13}C_{12}$]TetraCDF	315.942 (M^+), 317.939
PentaCDF	337.863 (M^+), 339.860
[$^{13}C_{12}$]PentaCDF	349.903 (M^+), 351.900
HexaCDF	373.821 ($[M+2]^+$), 375.818
[$^{13}C_{12}$]HexaCDF	385.861 ($[M+2]^+$), 387.858
HeptaCDF	407.782 ($[M+2]^+$), 409.779
[$^{13}C_{12}$]HeptaCDF	419.822 ($[M+2]^+$), 421.819
OctaCDF	441.743 ($[M+2]^+$), 443.740
[$^{13}C_{12}$]OctaCDF	451.786 ($[M^+]$, 453.783

counting was assessed using a quench-correction curve.

2.8. Method

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

Sample preparation, liquid-gel extraction and preliminary purification

Plasma (50 ml) was weighted into a 250-ml Erlenmeyer flask with PTFE-lined screw cap.

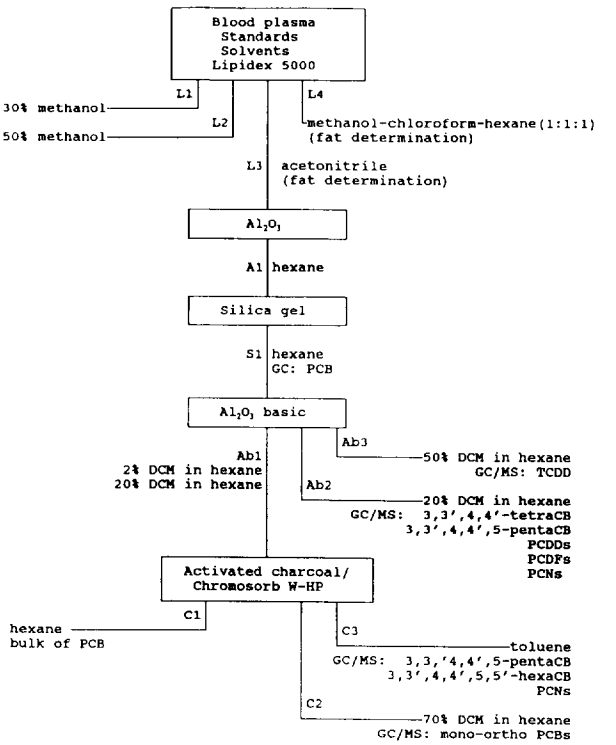

Fig. 1. Flow scheme of the analytical method.

One blank sample (50 ml of water) was run in parallel with each set of sample. Formic acid (50 ml) was mixed with the sample and the mixture was left for 15 min. 2-Propanol (20 ml) and 20 ml of water were mixed with the sample solution and 12 g of washed Lipidex were added. The mixture was shaken at 35°C in a water-bath for 3 h and then transferred into a glass column (2 cm I.D.). The gel was drained and then washed with 40 ml of methanol–water (30:70, v/v) (fraction L1) and 40 ml methanol–water (50:50, v/v) (fraction L2). The chlorinated compounds and part of the lipids were eluted with 175 ml of acetonitrile (fraction L3), and remaining lipids with 60 ml of methanol–chloroform–hexane, (1:1:1, v/v/v) (fraction L4).

Fat determination

Fractions L3 and L4 were taken to near dryness under reduced pressure at 35°C and the residues were dried to constant mass in a desiccator with silica gel at room temperature. The sum of the weights of the material in fractions L3

and L4 defined the total amount of fat in the samples.

Purification and group separation

For further purification and separation, previously described methods [14,16,22,23] were modified. Partly deactivated aluminium oxide (5.0 g) was transferred to a glass column (1 cm I.D.) containing 5 ml of hexane and washed with a total of 10 ml of hexane. The stopcock was closed and the residue in fraction L3 was quantitatively transferred to the column with small volumes of hexane. Most of the hexane on the top of the column was then evaporated with a gentle stream of nitrogen. The organochlorine compounds were eluted with 20 ml of hexane (fraction A1). This fraction was concentrated and applied to silica gel (0.6 g) as described [16]. The PCDDs, PCDFs, PCBs and the PCNs were eluted in 4 ml of hexane (fraction S1) and were separated from most of the pesticides. Fraction S1 was concentrated to 0.5 ml and 0.5 ml of 4,4′-diCB (90 ng/ml) was added for volume correction in the GC–ECD analyses. Compounds occurring at high concentrations were determined after dilution of the sample and addition of 0.5 ml of 4,4′-diCB (90 ng/ml) to an appropriate aliquot of the diluted sample.

Activated basic aluminium oxide (1 g) was dry-packed in a column (0.4 cm I.D.) followed by a 0.3 cm layer of anhydrous sodium sulphate. Fraction S1 was concentrated to ca. 50 μl and quantitatively transferred to the column with small volumes of hexane. Most of the PCBs and the PCNs were eluted with 16 ml of DCM–hexane (2:98, v/v) and 1 ml of DCM–hexane (20:80, v/v) (fraction Ab1). PCDDs (except TCDD), PCDFs, 3,3′,4,4′-tetraCB, 3,3′,4,4′,5-pentaCB (partly) and certain PCNs were eluted in 5 ml of DCM–hexane (20:80, v/v) (fraction Ab2) and TCDD in 5 ml of DCM–hexane (50:50, v/v) (fraction Ab3). Fraction Ab2 and Ab3 were concentrated to near dryness and 50 μl of decaCB (1 pg/μl hexane) were added. After concentration to ca. 10 μl, half of the sample was injected and analysed by GC–MS (program A). Fraction Ab1 was further subfractionated as follows. A column (0.4 cm I.D.) was packed with a mixture of activated charcoal and Chromosorb W HP and washed with 25 ml of DCM–hexane (70:30, v/v), 30 ml of toluene and 20 ml of hexane. Fraction Ab1 was concentrated to ca. 100 μl and quantitatively transferred to the column with hexane. The column was eluted with 50 ml of hexane (fraction C1). This fraction contained the PCBs, except mono-*ortho*- and non-*ortho*-PCBs. Mono-*ortho*-PCBs were eluted with 50 ml of DCM–hexane (70:30, v/v). 3,3′,4,4′,5-PentaCB (partly), 3,3′,4,4′,5,5′-hexaCB and the PCNs were eluted with 30 ml of toluene (fraction C3). Fraction C2 and C3 were concentrated to near dryness. A 0.5-ml volume of decaCB (9 pg/μl hexane) and 100 μl of decaCB (1 pg/μl hexane) were added to fractions C2 and C3, respectively. After dilution of fraction C2 with 0.5 ml hexane and after concentration of fraction C3, the fractions were analysed by GC–MS (program B).

2.9. Preliminary study of transfer of lipids into Lipidex

Three samples from different batches of plasma were fortified with ^{14}C-cholesterol (2 μg, 33 nCi) and three samples from different batches of plasma with ^{14}C-phosphatidylcholine (0.2 μg, 25 nCi). The samples were extracted and separated from the Lipidex gel as described in method. The volumes of fractions L1 to L4 were measured and 0.5 ml aliquots from each fraction were taken for determination of the radioactivity.

2.10. Fat determination according to Folch

Five samples of plasma, three from one batch of plasma and two from two other batches, were used in the determinations according to Folch et al. [24]. Plasma (6 ml) was weighed into a test tube and transferred into a separatory funnel with 1 ml of saline (0.9%, w/w). The sample was shaken with 125 ml of chloroform–methanol (2:1, v/v) for 5 min and then 25 ml of saline (0.9% w/w) were added. The mixture was shaken for another 5 min and the phases were then left to separate. The lower phase was transferred to a preweighed round-bottomed flask. The layer between the two phases was

transferred to a test tube and centrifuged at 1000 *g* for 10 min. The lower phase was added to the phase in the round-bottomed flask. The solvents were evaporated under reduced pressure at 35°C and the residue was dried to constant mass in a desiccator with silica gel at room temperature. The weight of the residue defined the total amount of fat in the sample.

2.11. Recovery and reproducibility studies of PCBs, PCNs, PCDDs and PCDFs

Five samples from the same batch of plasma were fortified with ^{13}C-labelled non-*ortho*-PCBs, PCDDs and PCDFs. Two of these samples were also fortified with unlabelled compounds of di- and mono-*ortho*-PCBs and different congeners of PCNs. Two samples from a second batch of plasma were fortified with unlabelled compounds of di- and mono-*ortho*-PCBs and Halowax 1014. One of these samples was also fortified with ^{13}C-labelled non-*ortho*-PCBs, PCDDs and PCDFs. One sample from a third batch of plasma was fortified with ^{13}C-labelled non-*ortho*-PCBs, PCDDs and PCDFs, unlabelled compounds of di- and mono-*ortho*-PCBs and different congeners of PCNs. Samples fortified with the unlabelled compounds were compared with at least one unfortified sample from the same batch of plasma. The blank samples ($n = 3$) were fortified with the ^{13}C-labelled compounds. The total volume of standard compound solutions added to each sample of plasma was below 0.5 ml. After fortification of the samples, they were treated as described in method.

3. Results and discussion

Fat and fat-soluble compounds in blood plasma were effectively sorbed into the Lipidex gel using the procedure described. The additions of formic acid and 2-propanol were essential for a complete transfer into the gel. It has been shown by others that denaturation of proteins in serum and plasma before extraction enhances the recoveries of organochlorine compounds. For this purpose, methanol [25], saturated ammonium sulphate in combination with ethanol [8] or formic acid [11,26–28] have been added to the sample. Formic acid has also been used in the extraction of human milk with Lipidex gel [16]. The need for 2-propanol for quantitative extraction of lipids in plasma with Lipidex was first noted in studies of sterols and sterol esters in plasma [29]. In our investigation, the minimum volumes of formic acid and 2-propanol for 50 ml of plasma were 50 ml and 20 ml, respectively.

3.1. Determination of fat content

In previous studies using Lipidex for extraction, e.g., milk [16] and fish oil [14], the lipids mainly consisted of triacylglyceroles. In order to validate the method for cholesterol and more polar phospholipids occuring in blood, ^{14}C-cholesterol and ^{14}C-phosphatidylcholine were added to the plasma. The recoveries, calculated from the sum of radioactivity in fractions L3 and L4 were 95–98% ($n = 3$) of ^{14}C-cholesterol and 92–101% ($n = 3$) of ^{14}C-phosphatidylcholine. Thus, both of these compounds were efficiently transferred into Lipidex and released by the elution solvents.

The average level of fat in five samples from the same batch of plasma, calculated from fractions L3 and L4, was 0.54% (range 0.51–0.56%). The distribution of the fat in fractions L3 and L4 in these samples was 26–31% (mean 28, R.S.D. 8) and 69–74% (mean 72, R.S.D. 3), respectively. Thus, about 70% of the fat were removed from the analytes by the elution system used for Lipidex. Many of the methods used for extraction of organochlorine contaminants in blood do not give quantitative recoveries of the lipids. Thus, the variability of the fat levels obtained in the quality control study, arranged by the WHO, was considered unsatisfactory [30] and laboratories, in general, were encouraged to improve the reliability of their fat determinations. We evaluated the present procedure by comparing it with the well established liquid–liquid partitioning method of Folch et al. [24] for quantitative extraction of lipids from biological tissues. The fat concentration obtained in three

samples when using Lipidex and 50 ml of plasma (from one batch of plasma) was 0.62% (range 0.61–0.63%). The value obtained when using the method by Folch et al. and 6 ml of plasma from the same batch as above was 0.61% (range 0.60–0.62%). Corresponding comparisons were made with plasma from two other batches of plasma. The concentrations obtained in the two batches when using Lipidex were 0.49% ($n = 1$) and 0.67% ($n = 1$) and when using the method by Folch et al. 0.53% ($n = 1$) and 0.72% ($n = 1$). Thus, the agreement between the two methods was good.

3.2. Determination of PCBs

The concentrations of di-*ortho*-PCBs were calculated from GC-ECD analysis of fraction S1, Fig. 2. From the mixture of PCBs and PCNs in fraction Ab1, the mono-*ortho*-PCBs were separated on activated charcoal and recovered in fraction C2, leaving the bulk of PCBs in fraction C1. Non-*ortho*-PCBs were recovered in fraction C3. The recoveries of di- and mono-*ortho*-PCBs added to plasma ranged from 74 to 107% (Table 2). The total recoveries of the three ^{13}C-labelled non-*ortho*-PCBs were 66–111% (Table 2). 3,3',4,4',5-PentaCB was recovered in fraction Ab2 and fraction C3. Since the corresponding ^{13}C-labelled compound was used as an internal standard and the total recovery was high, and the endogenous level of this compound is well above the detection limit in human plasma, it could be determined in either of these two fractions. The reproducibility of the method is demonstrated by three analyses of the same batch of plasma (Table 3).

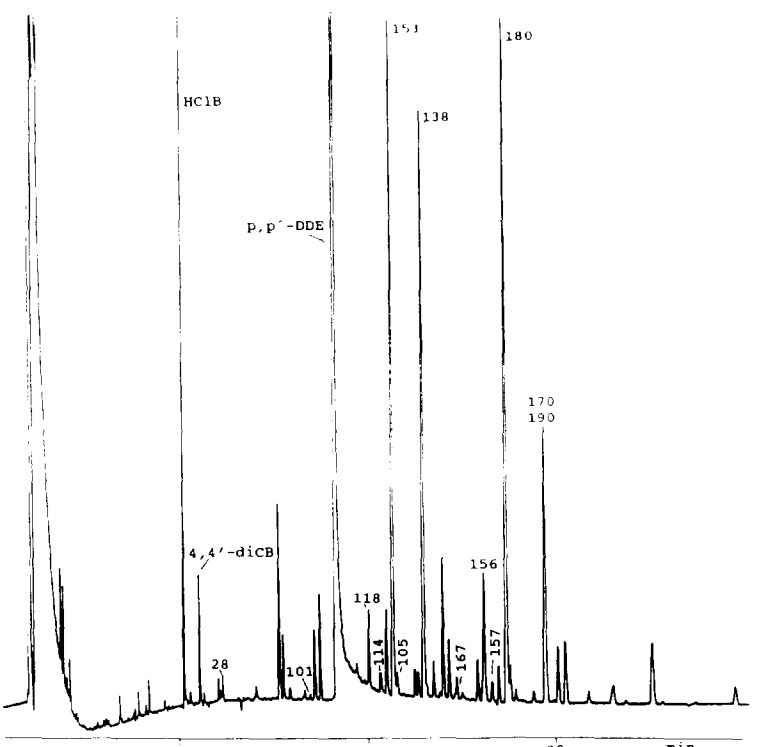

Fig. 2. Gas chromatogram (GC–ECD) of a plasma sample after separation on silica gel (fraction S1). For structures of the PCB congeners, see Table 2. HClB = hexachlorobenzene.

Table 2
Recoveries of different congeners of PCBs, PCNs, PCDDs and PCDFs added to plasma

Structure	IUPAC No.	Amount added	Recovery (%)[a]		
			Mean	Range	*n*
PCB					
Di-ortho-		(ng)			
2,2',4,4'	47	4–9	99	93–106	5
2,2',5,5'	52	10–20	92	87–97	5
2,2',4,5,5'	101	10–20	93	80–101	5
2,2',3,4,4',5'	138	10–20	91	78–101	5
2,2',4,4',5,5'	153	10–20	89	74–107	5
2,2',3,3',4,4',5	170	4–9	92	84–106	5
2,2',3,4,4',5,5'	180	4–9	86	77–93	5
Mono-ortho-					
2,4,4'	28	10–20	81	74–91	5
2,3,3',4,4'	105	5–10	96	82–105	5
2,3,4,4',5	114	4–9	91	89–92	5
2,3',4,4',5	118	5–10	89	81–92	5
2',3,3',4,5	122	4–9	95	90–101	5
2,3,3',4,4',5	156	5–10	94	81–104	5
2,3,3',4,4',5'	157	5–11	91	82–101	5
2,3',4,4',5,5'	167	4–9	84	82–90	5
Non-ortho-		(pg)			
3,3',4,4'	77	50	84	78–90	7
3,3',4,4',5	126[b]	50	86	74–111	7
3,3',4,4',5,5'	169	50	83	66–99	7
PCN					
1,2,3,4,6,7[c]		100	82	72–97	3
1,2,3,5,6,7[c]					
1,2,4,5,6,8		139	88	73–107	3
1,2,3,4,5,6,7[b]		132	78	72–84	3
1,2,3,4,5,6,7,8		100	86	76–97	3
PCDD					
2,3,7,8		10	72	63–83	7
1,2,3,7,8		10	84	69–97	7
1,2,3,6,7,8		10	89	80–104	7
1,2,3,4,6,7,8		10	92	79–104	7
OctaCDD		110	84	71–92	7
PCDF					
2,3,7,8		10	72	63–90	7
1,2,3,7,8		10	86	76–95	7
1,2,3,4,7,8		10	80	76–86	7
1,2,3,4,6,7,8		10	79	60–90	7
OctaCDF		10	79	61–94	7

[a] Recoveries of non-*ortho*-PCBs, PCDDs and PCDFs correspond to ^{13}C-labelled congeners.
[b] Eluted in two fractions. The recoveries correspond to the sum of the recoveries of the compound in fraction Ab2 and C3.
[c] 1,2,3,4,6,7- and 1,2,3,5,6,7-hexachloronaphthalene coelute from the GC column and were determined as one compound.

Table 3
Levels of PCBs, PCNs, PCDDs and PCDFs in plasma from one individual.

Structure	IUPAC No.	Level[a]			
		Mean	Range	R.S.D. (%)	*n*
PCB					
Di-ortho-					
2,2',4,4'	47	<1.5	ND	ND	3
2,2',5,5'	52	<1.5	ND	ND	3
2,2',4,5',5	101	2.7	2.5–2.8	6	3
2,2',3,4,4',5'	138	221	230–280	5	3
2,2',4,4',5,5'	153	142	138–146	3	3
2,2',3,3',4,4',5[b]	170	59	57–60	3	3
2,2',3,4,4',5,5'	180	155	152–158	2	3
Mono-ortho-					
2,4,4'	28	3.7	3.6–3.9	4	3
2,3,3',4,4'	105	2.5	2.3–2.6	6	3
2,3,4,4',5	114	1.0	0.8–1.2	20	3
2,3',4,4',5	118	15	14–16	8	3
2',3,3',4,5	122	<0.3	ND	ND	3
2,3,3',4,4',5	156	17	17–18	2	3
2,3,3',4,4',5'	157	3.9	3.5–4.1	9	3
2,3',4,4',5,5'	167	5.1	4.4–5.8	12	3
Non-ortho-		(pg/g fat)			
3,3',4,4'	77	18	15–22	28	2[d]
3,3',4,4',5	126	59	56–66	10	3
3,3',4,4',5,5'	169	104	102–105	2	3
PCN					
1,2,3,4,6,7[c]		260	243–284	8	3
1,2,3,5,6,7[c]					
1,2,4,5,6,8[f]		<20	ND	ND	3
1,2,3,4,5,6,7		<3	ND	ND	3
1,2,3,4,5,6,7,8		<3	ND	ND	3
PCDD					
2,3,7,8		3	2–3	21	5
1,2,3,7,8		8	7–9	12	5
1,2,3,4,7,8[g]					
1,2,3,6,7,8		37	34–42	9	5
1,2,3,7,8,9		7	6–9	18	5
1,2,3,4,6,7,8		60	55–63	5	5
OctaCDD		601	544–651	7	5
PCDF					
2,3,7,8		<1	ND	ND	5
1,2,3,7,8		<1	ND	ND	5
2,3,4,7,8		22	19–24	10	5
1,2,3,4,7,8		4	3–5	18	5
1,2,3,6,7,8		7	6–8	12	5
2,3,4,6,7,8		<1	ND	ND	5
1,2,3,7,8,9		<1	ND	ND	5
1,2,3,4,6,7,8		11	10–13	12	5
1,2,3,4,7,8,9		<1	ND	ND	5
OctaCDF		<1	ND	ND	5

Levels of non-*ortho*-PCBs, PCDDs and PCDFs were determined using ^{13}C-labelled standards.
ND = not determined.
[a] The fat levels in the samples were 0.51–0.53%.
[b] Separation from 2,2',3,4',5-pentaCB (IUPAC No. 90) not confirmed.
[c] Separation from 2,3,3',4,4',5,6-heptaCB (IUPAC No. 190) not confirmed.
[d] Only two values are given due to interference in one of the samples.
[e] 1,2,3,4,6,7- and 1,2,3,5,6,7-hexachloronaphthalene coelute from the GC column and were determined as one compound.
[f] Separation from 1,2,4,5,7,8-hexaCN not confirmed.
[g] Determination of 1,2,3,4,7,8-hexaCDD were made together with 1,2,3,6,7,8-hexaCDD since these compounds were poorly separated on the GC column. 1,2,3,4,7,8-HexaCDD contributes to about 10% of the levels of 1,2,3,6,7,8-hexaCDD given above.

3.3. Determination of PCNs

The recoveries of 1,2,3,4,6,7-, 1,2,3,5,6,7-, 1,2,4,5,6,8-hexaCN and octaCN (fraction C3) added to plasma ranged from 72 to 107% (Table 2). In some samples 1,2,3,4,5,6,7-heptaCN was recovered both in fraction Ab2 and C3 (Table 2). Thus, the endogenous 1,2,3,4,5,6,7-heptaCN occurring at low levels, could not be determined. The reproducibility of the method is demonstrated by analyses of three samples from the same batch of plasma (Table 3). In the blank samples, run in parallel with each set of samples, traces of 1,2,3,4,6,7-, 1,2,3,5,6,7-, and 1,2,4,5,6,8-hexaCN and 1,2,3,4,5,6,7-heptaCN were found. The amounts of these in the blank samples were 1–6 pg. Two plasma samples were fortified with Halowax 1014 (4 ng) and compounds labelled on the ion current chromatograms in Fig. 3 were studied. Seventeen PCN congeners present in Halowax 1014 have so far been tentatively identified as follows: 1,3,5,7-tetraCN (4a); 1,2,4,6-tetraCN (4b); 1,4,6,7-tetraCN (4c); 1,2,3,5,7-pentaCN (5a); 1,2,3,4,6,7- and/or 1,2,3,5,6,7-hexaCN (6a); 1,2,4,5,6,8- and/or 1,2,4,5,7,8-hexaCN (6d); 1,2,3,4,5,6-hexaCN (6e); 1,2,3,4,5,6,7-heptaCN (7a); 1,2,3,4,5,6,8-heptaCN (7b); octaCN [14]. The total recoveries were as follows: tetraCNs 68–103%, pentaCNs 91–113%, hexaCNs 71–111%, heptaCNs 68–113% and octaCN 77–88%. However, compounds 4f, 4g, 6e and 7a were recovered in two fractions (fraction Ab2 and C3).

3.4. Determination of PCDDs and PCDFs

TCDD was recovered in fraction Ab3 and the other PCDDs and PCDFs in fraction Ab2. The recoveries of the ^{13}C-labelled PCDDs and PCDFs added to plasma were 60–104% (Table 2). The reproducibility of five analyses of PCDDs and PCDFs is shown in Table 3. In blank samples traces (0–10 pg) of octaCDD were found.

4. Conclusions

The mean recoveries of individual congeners of PCBs, PCNs, PCDDs and PCDFs added to plasma were 72–99%. Similar results were reported by others using liquid–liquid extraction [6–9] but not all the compounds were determined in the same sample. The liquid–gel partitioning technique described here has several advantages compared to methods based on liquid–liquid partitioning. The lipids are efficiently extracted and no emulsions are formed. The gel with sorbed compounds constitutes a column bed that is easily eluted with solvents of different

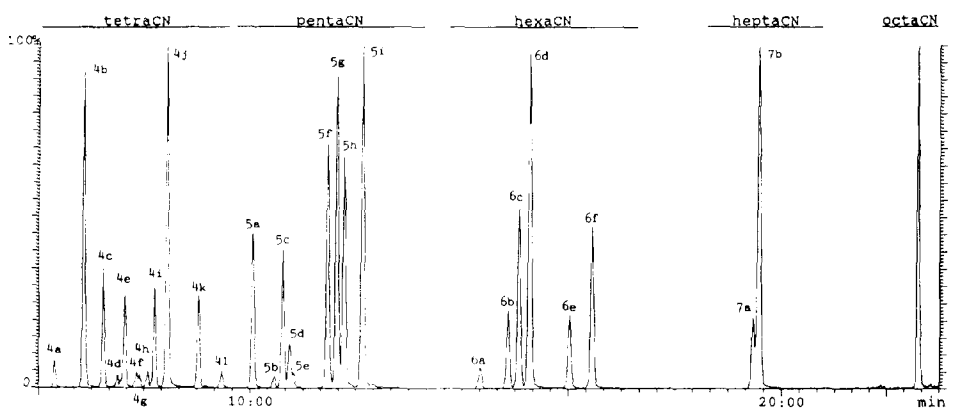

Fig. 3. Selected chromatograms of tetraCN to octaCN in Halowax 1014 (program B). The compounds are numbered according to their number of chlorines and elution order from the GC column. The peaks are normalized to the highest intensity for each m/z value.

polarity and thus, a preliminary purification is attained. The concentration of fat in the sample is also obtained in the same analysis. Thus, additional procedures for fat determination are not needed. The method requires relatively small sample sizes and solvent volumes. Consequently, the contamination risk from glassware, solvents and adsorbents is reduced.

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