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ORIGINAL PAPER

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An improved clean-up strategy for simultaneous analysis of polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated biphenyls (PCB) in fatty food samples

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Abstract The study and extension of a simple automated clean-up method for polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) to a broad range of polychlorinated biphenyls (PCB) is described. The isolation of seven PCDD, ten PCDF, and three coplanar PCB (cPCB) is extended to eight mono-*ortho* substituted PCB and seven so-called "marker PCB" (Aroclor 1260) for fatty food samples. This enables quantification of 35 compounds – including all congeners with a WHO toxic equivalent factor (TEF) – in a single extraction and single purification step. The chromatographic behaviour of mono-*ortho* PCB and marker PCB on a variety of adsorbents, including basic alumina, has been studied. Partitioning of analytes through multi-column sequences is described and correlated with their structural and electronic properties, by use of molecular modelling calculations. The fractionation process available with the Power-Prep automated clean-up system enables rapid independent analysis of the different groups of compounds. Gas chromatography with high resolution mass spectrometry (GC–HRMS) is used for the PCDD/F and cPCB fraction and quadrupole ion-storage tandem in time mass spectrometry (GC–QISTMS) for analysis of the remaining PCB. A comparison study was performed on quality-control samples and real fatty food samples to evaluate the robustness of the new strategy compared with a reference method. On the basis of this simultaneous clean-up, a rapid simplified strategy for PCDD/F and selected PCB analysis determination is proposed for fatty food samples.

Keywords PCB · Dioxins · Clean-up · Partitioning · Alumina

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Introduction

Polycholorinated biphenyls (PCB) are a family of man-made chemicals that contain 209 individual compounds that differ in the number and position of the chlorine atoms. All are toxic, but the most hazardous are those called "dioxin-like". Because of their structural similarity with dioxins, their mechanism of toxicity is the same and have been assigned a toxic equivalence factor (TEF) that refers to the toxicity of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [1]. The specific compounds are the four non-*ortho* substituted (coplanar) PCB (cPCB) and the eight mono-*ortho* substituted PCB. The toxicity of the other PCB is not 2,3,7,8-TCDD related; they have different toxicity, particularly neurobehavioural effects [2].

Because of the persistence and accumulation of these compounds in the environment, their analysis has become part of monitoring programmes. Recent events that occurred in Belgium have demonstrated the possible economic impact of underestimating the potential danger the chemicals represent [3, 4]. Many countries have instituted norms for their foodstuffs to keep a check on the background level to enable objective reaction to any contamination problem. In Belgium, for example, after the so-called "dioxin crisis", the government imposed a norm for PCB (200 ng g^{-1} fat) and dioxins (5 pg TEQ g^{-1} fat) levels in foodstuffs containing more than 2% animal fat. The dioxin analysis includes seven polychlorinated dibenzo-*p*-dioxins (PCDD) and ten polychlorinated dibenzofurans (PCDF) (substituted in the 2,3,7,8 position), whereas only the seven marker PCB (Aroclor 1260; PCB 28, 52, 101, 118, 138, 153, and 180) are taken into account in the estimation of the PCB concentration present in samples [3].

Currently, for a given sample, PCB and dioxins are usually analysed by use of two distinct procedures each with their own extraction and sample preparation steps, and different analytical tools. In both procedures the lipid fraction containing the compounds of interest must be isolated. Pressurised liquid extraction (PLE, also called accelerated solvent extraction or ASE) is one of the most widely used techniques used to replace Soxhlet or

liquid–liquid extraction for extraction of persistent organic pollutants (POPs) in biological samples [5, 6]. After gravimetric determination of lipid content the fat must be processed by a clean-up step to enable physicochemical analysis of analytes. In that step the efficiency of the automated Power-Prep system (FMS, Waltham, MA, USA) for the purification of sample extracts for dioxin analysis has already been demonstrated in recent years for different types of matrix, for example environmental [7], biological [8], and food [9]. The multi-step procedure is based on the use of well-established sets of adsorbents such as acidic, basic, and neutral silica, basic alumina, Florisil, and PX-21 carbon [10], which can be combined depending on the target analytes. This enables the isolation of dioxins with good recovery rates for up to ten samples in parallel, even for high-fat-content samples [11]. Although many laboratories performing dioxin analysis of fatty food samples must also produce PCB data, few use a single sample-preparation procedure yielding both dioxins and PCB with a TEF and/or concerned by the norms.

The aim of this study was to apply the same automated clean-up for both dioxins and PCB. The isolation of some PCB and persistent pesticides has already been reported for human serum samples [12, 13, 14] during PCDD/F determination but, because of the reactive nature of some of the pesticides towards acidic silica and basic alumina, only neutral silica and carbon columns were used in these studies. Because in this study quantities of fat were far greater than for serum samples, acidic silica and basic alumina were required as additional adsorbents. To understand the mechanisms of action of the complex set of adsorbents used and, therefore, to enable prediction of the elution of compounds during analysis, the fractionation of dioxins and PCB was investigated. This single preparation step was evaluated by comparison with a well established manual clean-up procedure for PCB to demonstrate the feasibility of isolation of seven PCDD, ten PCDF, twelve non- and mono-*ortho* PCB, and the seven marker PCB by processing a single PLE fat extract on the Power-Prep system.

Experimental

Chemicals and reagents

Hexane, toluene, ethyl acetate, cyclohexane, benzene and dichloromethane were Pestanal reagents (Riedel–de-Haën, Seelze, Germany). Nonane puriss p.a. standard for GC was purchased from Fluka (Steinheim, Germany). Anhydrous sodium sulfate was Baker analysed (J.T. Baker, Deventer, Netherlands), silica gel 60 (0.063–0.200 mm) was for column chromatography (Merck, Darmstadt, Germany). Liquid nitrogen was purchased from Air Liquide (Liege, Belgium). The $^{13}C_{12}$-labelled internal standard solution containing dioxins, furans, and coplanar PCB was from Cambridge Isotope Laboratories (Andover, MS, USA). This EDF-4144 internal standard solution, used for isotopic dilution, contains 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, OCDF, 3,3′,4,4′-TCB (PCB-77) (PCB's numbering following Ballschmiter and Zell rules [15]), 3,4,4′,5-TCB (PCB-81), 3,3′,4,4′,5-PeCB (PCB-126) and 3,3′,4,4′,5,5′-HxCB (PCB-169) at concentrations ranging from 24 to 125 pg μL^{-1} in nonane [11]. The recovery standard solution EDF-4145 (Cambridge Isotope Laboratories) contains [$^{13}C_{12}$]3,3′,5,5′-TCB (PCB-80) at 48 pg μL^{-1}, [$^{13}C_{12}$]1,2,3,4,7,8,9-HpCDF at 62.5 pg μL^{-1} and [$^{13}C_6$]1,2,3,4-TCDD at 25 pg μL^{-1} in nonane. Multi-analyte calibration solutions (EDF-4143, Cambridge Isotope Laboratories) were used to calculate the relative response factors (RRF) for each congener [11].

Table 1 Concentration levels of PCB in "in-house" quality-control (QC) samples of fortified beef fat

PCDD/F	pg g^{-1} fat	pg TEQ g^{-1}fat	PCB # [18]	ng g^{-1} fat	pg TEQ g^{-1} fat
Dioxins			Aroclor f260		
2,3,7,8-TCDD	0.4	0.4	28	2.2	–
1,2,3,7,8-PeCDD	2	2	52	2.2	–
1,2,3,4,7,8-HxCDD	2	0.2	101	2.2	–
1,2,3,6,7,8-HxCDD	2	0.2	118	2.2	0.22
1,2,3,7,8,9-HxCDD	2	0.2	153	2.2	–
1,2,3,4,6,7,8-HpCDD	2	0.02	138	2.2	–
1,2,3,4.6,7.9-HpCDD	2		180	2.67	–
OCDD	4	0.0004	209	2.2	–
Furans			Mono-*ortho* PCB		
2,3,7,8-TCDF	4	0.4	105	0.44	0.044
1,2,3,7,8-PeCDF	2	0.02	114	0.44	0.22
2,3,4,7,8-PeCDF	2	1	123	0.44	0.044
1,2,3,4,7,8-HxCDF	2	0.2	156	0.44	0.22
1,2,3,6,7,8-HxCDF	2	0.2	157	0.44	0.22
1,2,3,7,8,9-HxCDF	2	0.2	167	0.44	0.0044
2,3,4,6,7,8-HxCDF	2	0.2	189	0.089	0.044
1,2,3,4,6,7,8-HpCDF	2	0.02	cPCB		
1,2,3,4,7,8,9-HpCDF	2	0.02	77	0.01	0.001
OCDF	4	0.0004	126	0.055	5
			169	0.1	1

The $^{13}C_{12}$-labelled internal standard solution containing non- and mono-*ortho* PCB was from Wellington Laboratories (Ontario, Canada). This WP-LCS internal standard solution contains 3,3′,4,4′-TCB (PCB-77), 3,4,4′,5-TCB (PCB-81), 2,3,3′,4,4′-PeCB (PCB-105), 2,3,4,4′,5-PeCB (PCB-114), 2,3′,4,4′,5-PeCB (PCB-118), 2′,3,4,4′,5-PeCB (PCB-123), 3,3′,4,4′,5-PeCB (PCB-126), 2,3,3′,4,4′,5-HxCB (PCB-156), 2,3,3′,4,4′,5′-HxCB (PCB-157), 2,3′,4,4′,5,5′-HxCB (PCB-167), 3,3′,4,4′,5,5′-HxCB (PCB-169) and 2,3,3′,4,4′,5,5′-HpCB (PCB-189), each at a concentration of 1 ng μL^{-1}. The MBP-MXE (Aroclor 1260 congeners) internal standard solution (Wellington Laboratories) contains 2,4,4′-TriCB (PCB-28), 2,2′,5,5′-TCB (PCB-52), 2,2′,4,5,5′-PeCB (PCB-101), 2,2′,3,4,4′,5-HxCB (PCB-138), 2,2′,4,4′,5,5′ HxCB (PCB-153), 2,2′,3,4,4′,5,5′-HpCB (PCB-180) and decachlorobiphenyl (PCB-209) each at concentration of 5 ng μL^{-1}.

Samples

To evaluate the accuracy of the multi-analyte method a batch of "in-house" quality-control (QC) samples was prepared by fortifying beef fat with 40.4 pg g^{-1} fat (5.3 pg TEQ g^{-1} fat) for the 17 PCDD/F, 160 pg g^{-1} fat (6 pg TEQ g^{-1} fat) for the 4 coplanar PCB, 4.9 ng g^{-1} fat (1 pg TEQ g^{-1} fat) for the 8 mono-*ortho* PCB and 18 ng g^{-1} for Aroclor 1260 PCB (Table 1). Pork, beef, poultry, and horse meat samples were from Belgian supermarkets.

Sample-preparation procedure

A general procedure for high-fat-content sample preparation has been described elsewhere [11]. Briefly, samples were homogenised by use of dissecting and/or mortar equipment and frozen under liquid nitrogen before freeze-drying. The freeze-dried products were ground to obtain a fine powder. Pressurised-liquid extraction (PLE) was performed on dried powder using an ASE 200 extractor (Dionex, Sunnyvale, CA, USA) with hexane as solvent. Fat extracts were dried over sodium sulfate before determination of their lipid content by gravimetric analysis. Subsequent clean-up steps were performed on these samples of fat.

Clean-up procedures

Reference manual method for PCB

This clean-up is described in detail in official documents [16]. Briefly, the procedure is based on the use of an open chromatographic multi-layer glass column freshly packed with 6 g acid silica, 1 g deactivated alumina and 0.5 g sodium sulfate. The fat extract (0.5 g) diluted with 2 mL hexane was applied on the top of the column and eluted with 20 mL hexane. After completion of the collection step, the solution containing the PCB was evaporated by rotary evaporation. Dodecane was added as keeper and the remaining solution was analysed by GC–MS.

Multi-analyte automated method

Because PCDD/F levels are much lower than PCB levels, greater quantities of lipids were processed through the clean-up step to ensure proper identification of compounds during the final analysis. PLE-extracted fats were processed by gel permeation chromatography (GPC) to enable lipid fraction reduction (typically, 4 g fat were processed through the GPC) [11]. This was achieved by use of a Latek LC-12-3 glass column (Latek, Eppelhein, Germany) connected to a Latek P100 piston pump equipped with a Superfrac fraction collector (Amersham Pharmacia Biotech, Uppsala, Sweden). The column was packed with 70 g S-X3 Bio-Beads (Bio-Rad, Nazareth, Belgium) using ethyl acetate–cyclohexane (1:1) as solvent. The flow rate was set at 5 mL min^{-1} and fractions were collected between 25 min and 60 min.

The ethyl acetate–cyclohexane was evaporated from the GPC fractions, by use of a Turbovap II concentration workstation (Zymark, Hopkinton, MA, USA), and replaced with hexane. Hexane solutions were loaded on the first column of the Power-Prep (Fluid Management Systems, Waltham, MA, USA). This system has been described elsewhere [7, 8, 9, 11]. Briefly, the automated clean-up system comprises a valve-drive module connected to a pump module responsible for the solvent flow in the valve module. The programming of solvent volumes, types, flow-rates and directions is performed by FMS patented software operating under Windows. This system uses disposable multi-layer silica columns (4 g acid, 2 g base and 1.5 g neutral), basic alumina (8 g) and PX-21 (2 g) carbon columns to separate analytes of interest from matrix interferences, by use of a strategy reported elsewhere [17]. The configuration of the system enables the operator to collect different fractions at different stages of the purification. Collected fractions can therefore be concentrated and analysed by GC–MS.

Instrumentation

Gas chromatography–high-resolution mass spectrometry (GC–HRMS)

PCDD/F and cPCB analysis was performed by GC–HRMS using a Hewlett–Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph and a MAT95XL high-resolution mass spectrometer (Finnigan, Bremen, Germany). GC and mass spectrometer conditions and quality insurance procedures have been described in detail elsewhere [11]. Briefly, the GC column was a RTX-5SIL-MS (30 m×0.25 mm i.d., 0.25 μm; Restek, Evry, France); splitless injection of the extract (2 μL) at 275 °C, initial oven temperature 140 °C; temperature programming: 140 °C, held for 2 min, then increased at 15 ° min^{-1} to 220 °C, then increased to 240 °C at 1.2 °C min^{-1}, then increased to 270 °C at 4 ° min^{-1}, then increased at 10 ° min^{-1} to 300 °C and held at this temperature for 1 min. Pure GC grade He, 99.9999% (Air Products, Vilvoorde, Belgium) was used as carrier gas. The mass spectrometer was operated in electron-impact-ionization mode, using selected-ion monitoring (SIM). The electron energy was set to 60 eV. Source temperature was 270 °C. The MS was tuned to a minimum resolution of 10,000 (10% valley) and masses obtained from FC-5311 (perfluorophenanthrene; tuning compound) were used as lock mass. The linear response zone and relative response factor (RRF) for each congener were determined periodically using calibration solutions. Daily checks, blanks, and QC samples were performed to ensure the system was under control [11]. Quantification was performed using internal standards and the isotopic dilution technique.

Gas chromatography–tandem mass spectrometry (GC–MS–MS)

GC–MS–MS analysis was performed with a Saturn 2000 GC–MS–MS mass spectrometer coupled with a Star 3400CX gas chromatograph and a 8200CX autosampler (Varian, Walnut Creek, KS, USA). The Saturn 5.1 software version of the workstation was used. Mixtures were chromatographed on an RTX-5SIL-MS (30 m× 0.25 mm i.d., 0.25 μm; Restek) capillary column. GC conditions were optimised to enable the separation of the eight mono-*ortho* PCB and the seven marker PCB as follows: on-column injection of 1 μL at 140 °C, initial oven temperature of 140 °C for 1 min, then increased at 25 ° min^{-1} to 180 °C, held for 1 min, then increased at 2 ° min^{-1} to 210 °C, held for 8 min, finally increased at 3 ° min^{-1} to 280 °C, held for 2 min. He (N60, Air Liquide, France) was used as carrier gas. Optimised conditions for the mass spectrometer are reported on in Table 2. Trap temperature was set at 220 °C, the transfer line at 280 °C and a maximum number of 5000 ions in the trap.

Molecular modelling

During this study the electronic properties of some PCB were investigated. Dipole moments were calculated by use of Sybyl 6.2

Table 2 Optimised conditions for MS–MS analysis of PCB

Congeners		Molecular ions (m/z)	CID Amplitude (V)	CID rf (m/z)	Daughter ions
TriCB	$^{12}C_{12}$	258 (M+2)	1.80	113.7	186/188
	$^{13}C_{12}$	270 (M+2)	1.80	119.0	198/200
TCB	$^{12}C_{12}$	292 (M+2)	1.20	128.8	220/222
	$^{13}C_{12}$	304 (M+2)	1.20	134.0	232/234
PeCB	$^{12}C_{12}$	326 (M+2)	1.60	143.8	254/256
	$^{13}C_{12}$	338 (M+2)	1.60	149.0	266/268
HxCB	$^{12}C_{12}$	360 (M+2)	1.80	164.0	288/290
	$^{13}C_{12}$	372 (M+2)	1.80	158.8	300/302
HpCB	$^{12}C_{12}$	396 (M+4)	1.90	174.8	324/326
	$^{13}C_{12}$	408 (M+4)	1.90	180.1	336/338
DeCB	$^{12}C_{12}$	500 (M+6)	2.10	220.8	428/430
	$^{13}C_{12}$	512 (M+6)	2.10	226.0	440/442

molecular modelling software (Tripos, St Louis, MO, USA). For computations, the geometry of each molecule was optimised minimise the potential energy. When the stable geometry had been selected dipole moments were obtained using the Gasteiger–Hückel method available in the software. Dipole moments were expressed in Debye.

Results and discussion

Fractionation

The classical sequence of events constituting the program for a run on the automated system depends on solvent types, flow rates, and pass or by-pass on different columns. During this study, the goal was to use the system under conditions as close as possible to those routinely used for PCDD/F and cPCB analysis and to extend the range of analytes to additional PCB. The same program as that used for PCDD/F and cPCB isolation was used. Because our clean-up scheme on the Power-Prep employs a succession of three different types of column (multi-layer silica, basic alumina, and PX-21 carbon), fractions resulting from each event were sequentially collected to establish the fractionation pattern for the marker and mono-*ortho* PCB (Fig. 1).

After PLE and GPC the extract in hexane (15 mL) was loaded on the multi-layer silica column, which had previously been conditioned for fat removal. Hexane (90 mL) was used to elute compounds from the silica through the alumina column; this was the first fraction collected (F1). A mixture (60 mL) of hexane–dichloromethane (98:2) was then applied to the alumina column and collected as F2. After valve switching, compounds remaining on alumina column were eluted with 120 mL hexane–dichloromethane (50:50) through the carbon column (F3). An ethyl acetate–benzene (1:1) mixture (5 mL) was then applied to the carbon column in the forward direction (F4) for additional clean-up of the PCDD/F and cPCB fraction that was adsorbed on this column. The carbon column was then back-flushed with 65 mL toluene; this was collected as F5 and called the “dioxin fraction”.

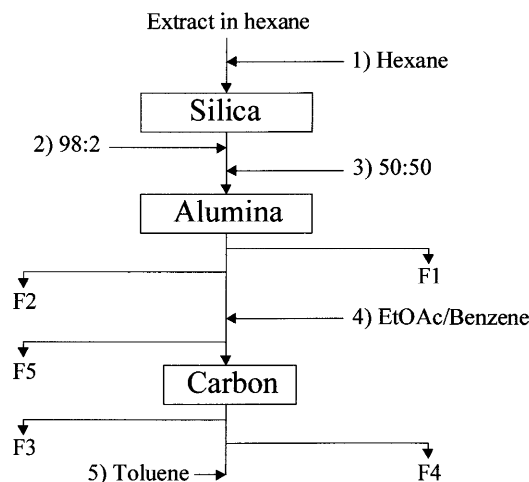

Fig. 1 Schematic diagram of events and fraction collection in automated clean-up using multi-layer silica, basic alumina and PX-21 carbon disposable columns

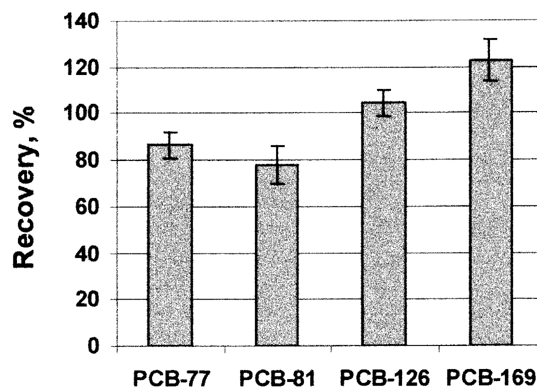

Fig. 2 Recovery rates for cPCB congeners collected in the PCDD/F fraction (F5)

Whereas non-*ortho* PCB (cPCB) with planar geometry were isolated with good recovery rates (Fig. 2) in the PCDD/F fraction (F5) collected after toluene back-flush of the carbon column, other PCB were dispersed in different fractions according their degree of substitution and position of the chlorine atoms. (Fig. 3). Both figures show, however, that all the targeted congeners were nearly quantitatively recovered in collected fractions. Recoveries ranged between 70 and 120%, which is acceptable [18, 19].

Correlation of the retention of PCB with their electronic and structural properties

The distribution of the PCB in the different fractions was investigated. The use of a carbon column has previously been demonstrated to be suitable for the separation of *ortho*-substituted PCB, non-*ortho* substituted PCB, and PCDD/F according to their structure–affinity relationship [20]. In this study, however, only the cPCB were actually isolated via the carbon column, because their coplanar

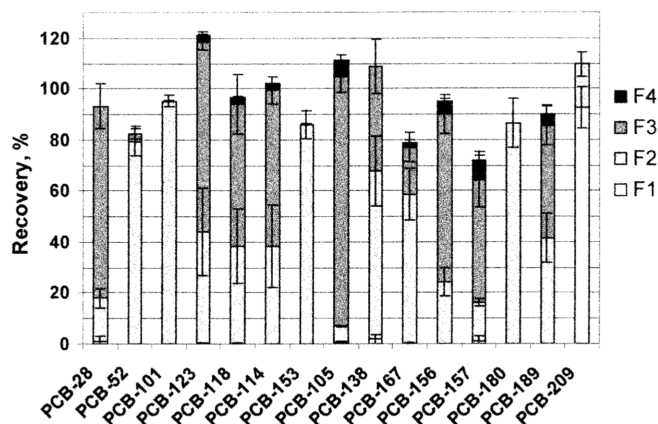

Fig. 3 Distribution of eight mono-*ortho* and seven marker PCB in different fractions collected during automated clean-up (details of the fractionation are given in Fig. 1)

properties enable them to be trapped between planar carbon layers until their displacement by use of the structurally related toluene solvent [21]. Other congeners with chlorine atoms in *ortho* positions cannot can not assume this planar geometry and pass through the carbon. Their separation is performed upstream, on the basic alumina column, where they are selectively desorbed as a function of their polarity and that of the selected solvent.

To correlate the fractionation pattern with analyte polarity dipole moments were calculated by use of molecular modelling software. The geometry of each molecule was optimised to locate the minimum of potential energy. Dipole moments were obtained by the Gasteiger–Hückel method and were expressed in Debye. Fig. 4 illustrates the correlation between PCB dipole moment (μ) and solvent polarities (fractionation pattern) for each mono-*ortho* and marker PCB. In this table PCB are listed in order of increasing dipole moment and their distribution in different fractions collected during the clean-up (details of the fractionation are given in Fig. 1). It is apparent from the range of very low to higher dipole moments that elution of PCB congeners correlates with solvent polarities. The non-polar PCB-209 congener was eluted with non-polar hexane but as dipole moments increased more polar solvents were necessary to elute the PCB from the basic alumina column. This correlation was not, however, sufficient to explain the order of elution of PCB congeners with similar dipole moment values. Because their distribution was not the same in fractions F2 and F3, other effects must play an important role and must thus be taken into account to ex-

Fig. 4 Chemical structure, dipole moment (μ) and distribution of the mono-*ortho* and marker PCB in the fractions collected (details of the fractionation are given in Fig. 1)

PCB	μ	Fractions
209	0.00	
101	0.01	
153	0.02	
52	0.03	
180	1.00	
189	1.01	
167	1.01	
114	1.01	
118	1.01	
28	1.01	
156	1.49	
138	1.74	
123	1.75	
157	2.02	
105	2.34	

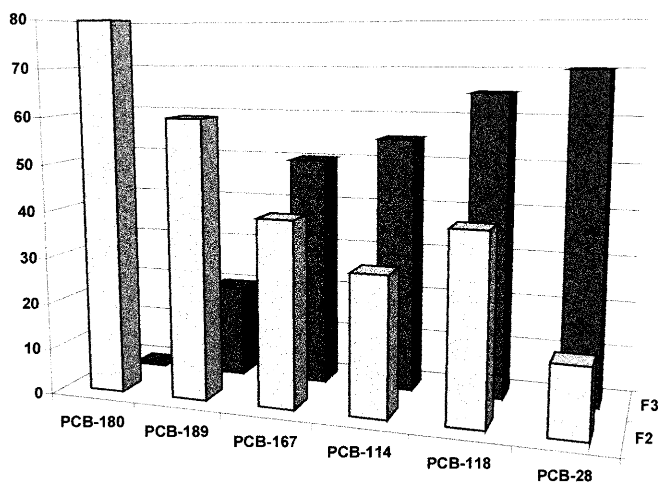

Fig. 5 Distribution of PCB congeners with same dipole moment values. The degree of chlorination decreases from left to right (details of the fractionation are given in Fig. 1)

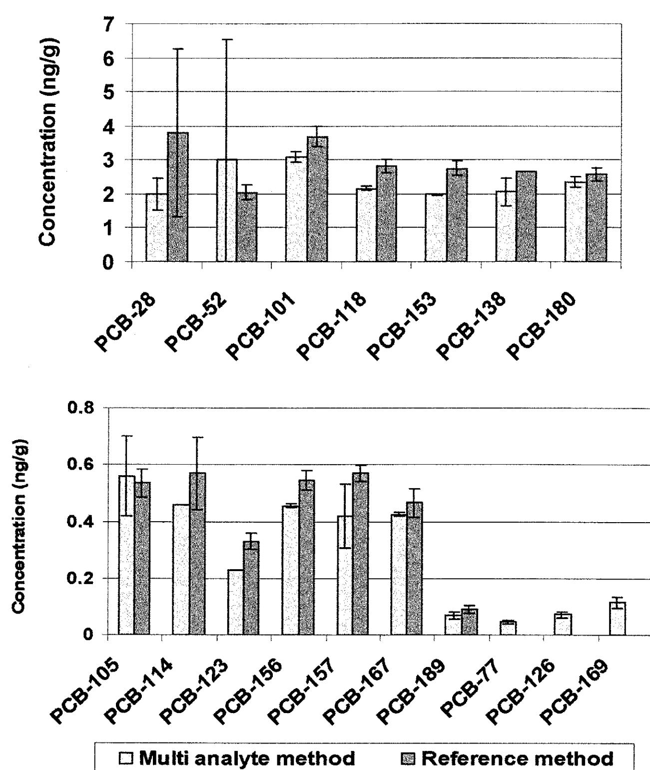

Fig. 6 Results obtained from the automated multi-analyte method and from the manual reference method for seven marker PCB, non and mono-*ortho* PCB in QC samples

Table 3 Concentrations of some PCB in a blend of solvents representative of the types and quantities used during the automated clean-up. Results are expressed in pg mL^{-1} of the solvent mixture used

Species	PCB # [18]	Level (pg mL^{-1})
Aroclor 260	28	8.1
	52	26.53
	101	11.56
	118	1.31
	153	0.79
	138	1.26
	180	nd
Mono-*ortho* PCB	105	0.61
	114	nd
	123	nd
	156	0.02
	157	nd
	167	nd
	189	nd

Table 4 Recovery (%) for PCB present in QC samples

Congeners PCB # [18]	Reference method		Multi-analyte method	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
28	53	6.9	66	4.4
52	48	1.3	71	6.6
101	55	4.2	71	1.8
118	52	8.7	66	14.6
153	61	4.3	78	1.7
138	60	1.6	55	6.0
180	64	3.0	70	7.2
105	72	8.9	85	9.7
114	73	5.0	94	6.3
123	74	8.7	95	4.7
156	59	3.0	80	8.1
157	65	16.2	83	7.5
167	72	2.1	78	18.2
189	59	0.6	67	8.1
77	–	–	65	4.2
126	–	–	80	4.6
169	–	–	74	9.3

plain the experimentally observed order of elution. Assuming that some of the interactions of PCB with basic alumina arise as a result of hydrogen bonding, the less chlorinated congeners should have more tendency to become involved in hydrogen bonding. They should, therefore, interact more strongly with basic alumina and be eluted later than more chlorinated congeners.

PCB-180, 189, 167, 114,118, and 28 had the same dipole moment – 1.01 Debye – and PCB-180 and 189, which were the most chlorinated of these – with seven substituting chlorine atoms – were the most abundant in the fraction F2 (80% and 60%, respectively) (Fig. 5). The hexachlorinated CB-167 was equally distributed between F2 and F3, whereas the two pentachlorinated CBs, 114 and 118, were mainly present in F3 (60% and 70% respectively). The less chlorinated PCB-28, with even more hydrogen atoms available, ended up almost exclusively (80%) in fraction F3. Finally, the combined effect of dipole moment and degree of chlorination accounted for the very weak retention of PCB-209 (no available hydrogen

Table 5 Results from analysis of poultry, horse, pork, and beef samples by use of the Power Prep (n=3 for each matrix)

Congeners	Poultry (n=3)			Horse (n=3)			Pork (n=3)			Beef (n=3)		
	pg g^{-1}	pg g^{-1} I-TEQ g^{-1}	%Rec	pg g^{-1}	pg g^{-1} I-TEQ g^{-1}	%Rec	pg g^{-1}	pg I-TEQ g^{-1}	%Rec	pg g^{-1}	pg I-TEQ g^{-1}	%Rec
Dioxins												
2,3,7,8-TCDD	0.0	0.00	95	0.4	0.45	90	0.0	0.00	77	0.0	0.04	92
1,2,3,7,8-PeCDD	<LOQ	0.01	99	1.9	1.93	103	0.0	0.00	75	0.3	0.28	108
1,2,3,4,7,8-HxCDD	0.0	0.00	107	1.4	0.15	96	0.0	0.00	82	0.0	0.00	98
1,2,3,6,8,9-HxCDD	<LOQ	0.01	87	3.3	0.33	89	0.0	0.00	69	0.8	0.05	93
1,2,3,7,8,9-HxCDD	0.0	0.00	94	0.9	0.09	93	0.0	0.00	76	0.0	0.00	100
1,2,3,4,6,7,8-HpCDD	<LOQ	0.02	91	12.6	0.13	88	<LOQ	0.02	78	<LOQ	0.02	85
OCCD	0.0	0.00	64	23.2	0.00	69	0.0	0.00	69	0.0	0.00	66
Furans												
2,3,7,8-TCDF	<LOQ	0.01	94	0.8	0.08	88	0.0	0.00	76	0.0	0.00	83
1,2,3,7,8-PeCDF	0.0	0.00	92	0.9	0.05	94	0.0	0.00	74	0.0	0.00	101
2,3,4,7,8-PeCDF	0.2	0.07	98	4.9	2.46	97	0.1	0.03	75	1.2	0.08	98
1,2,3,4,7,8-HxCDD	<LOQ	0.01	99	1.4	0.14	90	<LOQ	0.01	79	0.4	0.04	93
1,2,3,6,7,8-HxCDD	0.0	0.00	86	2.6	0.26	85	0.0	0.00	71	0.4	0.04	88
2,3,4,6,7,8-HxCDD	0.0	0.00	91	1.4	0.14	79	0.0	0.00	74	0.4	0.04	90
1,2,3,7,8,9-HxCDD	0.0	0.00	99	0.5	0.05	96	0.0	0.00	84	0.0	0.00	95
1,2,3,4,6,7,8-HpCDF	<LOQ	0.02	83	3.8	0.04	76	<LOQ	0.02	91	1.5	0,02	76
1,2,3,4,7,8,9-HpCDF	0.0	0.00	100	0.1	0.00	100	0.0	0.00	100	0.0	0.00	100
OCDF	0.0	0.00	65	0.0	0.00	67	0.0	0.00	72	0.0	0.00	60
Total PCDD/F pg I-TEQ (% TEQ)		0.15 (26%)			6.29 (42%)			0.07 (15%)			0.69 (25%)	
cPCB												
PCB-77	<LOQ	0.01	90	<LOQ	0.01	79	<LOQ	0.01	73	<LOQ	0.01	74
PCB-81	0.2	0.00	79	19.3	0.00	77	0.0	0.00	65	2.8	0.00	65
PCB-126	3.1	0.42	59		6.76	62	3.1	0.31	76	15.3	1.53	100
PCB-169	0.5	0.01	94	11.1	0.11	91	0.5	0.00	80	3.4	0.03	84
Total cPCB pg I-TEQ (% TEQ)		0.43 (50%)			6.88 (46%)			0.33 (68%)			1.58 (57%)	
Mono-*ortho* PCB												
PCB-105	130.7	0.02	92	827.4	0.15	100	112.8	0.01	83	124.3	0.03	80
PCB-114	9.8	0.00	79	4154.1	0.70	92	293.7	0.00	88	4.2	0.02	80
PCB-118	815.6	0.08	105	614.3	0.09	88	225.6	0.02	87	2228.6	0.22	89
PCB-123	9.0	0.01	107	67.5	0.01	84	11.4	0.00	62	10.9	0.00	70
PCB-156	138.1	0.08	56	734.4	0.66	63	78.1	0.04	60	177.8	0.19	73
PCB-157	27.9	0.02	96	198.3	0.13	81	11.7	0.01	82	29.0	0.03	86
PCB-167	27.2	0.00	91	340.2	0.01	96	39.8	0.00	84	70.5	0.00	87
PCB-189	38.9	0.00	93	101.7	0.01	79	12.4	0.00	74	21.7	0.00	80
Total mono-*ortho* PCB pg I-TEQ (% TEQ)		0.21 (24%)			1.76 (12%)			0.08 (17%)			0.49 (18%)	
Marker PCB												
PCB-28	543.4	–	64	1001.2	–	69	2194.5	–	64	640.8	–	68
PCB-52	420.6	–	60	499.4	–	71	2792.8	–	58	600.3	–	79
PCB-101	258.3	–	77	343.8	–	74	1044.4	–	76	318.4	–	73
PCB-138	1408.5	–	73	5487.9	–	101	1386.2	–	84	1657.1	–	69
PCB-153	1541.9	–	80	10702.0	–	82	1142.9	–	90	1951.3	–	68
PCB-180	597.1	–	82	3553.2	–	72	266.7	–	71	741.7	–	85

For levels below the LOQ values, concentrations in pg I-TEQ g^{-1} are set as LOQ/2

atoms) on basic alumina compared with PCB-101 (five hydrogen atoms available for hydrogen bonding) with almost the same dipole moment but which ended up entirely in fraction F2.

Method evaluation

The applicability of the multi-analyte method was evaluated by comparing it with a well-established manual

clean-up method for PCB [16] for various types of sample. QC samples were cleaned by use of both methods. For multi-analyte clean-up, QC samples (4 g) were treated by GPC before Power-Prep clean-up. Fractions F2 and F3 collected by different sequences were pooled to include all PCB of interest. Results presented in Fig. 6 enable comparison of the procedures. It seems that results were similar for both methods. Relative standard deviations were generally acceptable, except for PCB 28 and 52, for which results were not reproducible. Troubles were encountered in the determination of the more volatile congeners (PCB 28, 52, 114, and 105). The use of gentle heating and a stream of nitrogen during the evaporation step was found to be potentially responsible for cross-contamination, and analysis of residual levels present in solvents used for sample preparation revealed the presence of significant levels of less chlorinated PCB, especially tri-, tetra- and penta-CBs. Table 3 shows residual PCB levels per millilitre of solvent used. Because quite large quantities of solvent are used (cyclohexane–ethyl acetate for GPC; hexane, hexane–dichloromethane for Power-Prep), high levels of PCB 28, 52, 101, 118 and 138 are introduced with the solvent during clean-up. Because part of these are evaporated to concentrate the extracts during the process, high standard deviations and differences from expected values can be partly explained. This solvent effect was reduced by insertion of blanks in the sample series but, because it was also apparent that solvent contamination was not constant with time, interferences still persisted, affecting accurate estimation of some congeners. Recovery percentages for QC samples are illustrated in Table 4. These are on average higher for the multi-analyte procedure (74% average compared with 62% average for the manual method), for which rates were in the range 55–94%.

To evaluate the robustness of the method, several types of fatty food matrix were processed. Meat samples were representative of most of the samples analysed in our laboratory last year. Analysis of poultry, horse, pork, and beef samples was performed using Power-Prep multi-analyte method. PCDD, PCDF, cPCB, mono-*ortho* PCB and marker PCB were isolated with good recovery from all the matrices investigated (Table 5). PCDD/F levels were below the limit of 5 pg TEQ g^{-1} fat (poultry 0.23 pg TEQ g^{-1} fat, pork 0.07 pg TEQ g^{-1} fat, beef 0.69 pg TEQ g^{-1} fat), except for horse samples, for which the average value was 6.3 pg TEQ g^{-1} fat. This higher background level for horses has previously been reported [22]. Marker PCB levels were far below the critical value of 200 ng g^{-1} fat (poultry 4.8 ng g^{-1} fat, horse 21.6 ng g^{-1} fat, pork 8.8 ng g^{-1} fat, beef 5.9 ng g^{-1} fat) for all matrices. Because this method also gives access to other TEQ values, it is interesting to see that, if cPCB and mono-*ortho* PCB TEQ values are added in the evaluation of the total 2,3,7,8-TCDD toxicity, TEQ response increases significantly without, however, pushing the values over the limit imposed by the norm. The relative contribution of PCDD/F to the TEQ is lower than 50% for all samples.

Conclusions

This simple and automated multi-analyte clean-up procedure enables the separation and the analysis of PCDD, PCDF, cPCB, mono-*ortho* PCB and marker PCB. The method furnishes clean extracts with good recovery rates. Robustness during quality control and for a variety of types of meat sample was satisfactory for all the 34 selected analytes. This enables estimation of the TEQ including not only PCDD/F, but also cPCB and mono-*ortho* PCB by use of a single sample clean-up step. This simplification is a time- and cost-effective solution for laboratories that must produce results for both dioxins and PCB for evaluation of the total toxicity of the sample including all congeners that have been attributed a TEF by the WHO. In addition to these analytes, other PCB congeners (no TEF) are also present in fractions and can be further analysed by GC–MS if necessary. Finally, it is also possible to modify the Power-prep system so that it can directly accept larger quantities of fat; this simplifies the GPC step and increases the sample throughput of the laboratory. The analyte list could also be extended to other compounds, e.g. persistent pesticides and other halogenated compounds without complex modification; this makes the method a useful tool in the analysis of persistent organic pollutants (POPs).

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