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Comprehensive two-dimensional gas chromatography of the 209 polychlorinated biphenyls

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Abstract

Comprehensive two-dimensional gas chromatography (GC × GC) of the 209 polychlorinated biphenyls (CBs) was carried out using a longitudinally modulated cryogenic system (LMCS) and liquid carbon dioxide as cryogen. The effluent from a non-polar column was modulated and further separated on either a polar or a shape-selective second-dimension column. Five GC × GC column combinations were evaluated, with DB-XLB as the first column in each case. DB-XLB separates more congeners than any other GC column currently available. When combined with a biscyanopropyl siloxane (SP-2340 or BPX70) or smectic liquid crystal (LC-50) second-dimension column in a GC × GC system many additional CBs can be separated. In total, 176 and 181 of the 209 congeners were separated ($R_s = 0.5$) on the column combinations DB-XLB/SP-2340 and DB-XLB/LC-50, respectively. Of the 136 CBs present in any Aroclor mixture at concentrations greater than 0.05% (w/w), 126 were resolved using either of these two column combinations. The seven frequently measured CBs 28, 52, 101, 118, 138, 153, 180, and the WHO-PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189 were all separated from Aroclor CBs on the DB-XLB/LC-50 column set, whereas CBs 118 and 131 coeluted on the DB-XLB/SP-2340 column set. In addition, three technical CB formulations (Aroclors 1232, 1248 and 1260) and a seal blubber sample (*Halichoerus grypus*) from the Baltic Sea were analyzed. Similar peak patterns were found for Aroclor 1260 and the CBs in the seal blubber, facilitating use of this technical CB formulation to identify the CBs in the blubber by GC × GC. Individual CBs in environmental samples, such as seal blubber, may be identified semi-automatically by matching the samples GC × GC profiles to a template generated using a standard containing all 209 CBs. Using such a template, 64 CBs were identified in the grey seal blubber sample.

Keywords: Gas chromatography, comprehensive two-dimensional; Baltic grey seal; Polychlorinated biphenyls; Aroclors

1. Introduction

There are 209 polychlorinated biphenyl congeners (CBs), 136 of which have been found in technical CB mixtures [1] and hence may be found in the environment as a result of either intentional or unintentional release. Much attention has been focused on the CB congeners that are non- or mono-*ortho*-chlorine-substituted. These compounds may adopt a coplanar conformation and become isosteric with the toxic

Abbreviations: CB, polychlorinated biphenyl; 1D-GC, single-dimension gas chromatography; μECD, micro-electron capture detection; EPC, electronic pressure control; FID, flame ionization detection; GC × GC, two-dimensional gas chromatography; LMCS, longitudinally modulated cryogenic system; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TEF, toxic equivalency factor; TEQ, dioxin-like toxic equivalence

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2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs). Consequently, twelve of them (CBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189) have been assigned dioxin toxic equivalency factors (TEF) by the World Health Organization (WHO) [2,3]. The TEFs of the WHO-PCBs are much lower than those of the most potent PCDD/Fs, but since they are often present at higher concentrations they contribute significantly to the total dioxin-like toxicity (TEQ). The non-*ortho* CBs 77, 126 and 169 and the mono-*ortho* CBs 105, 118 and 156 are major TEQ contributors in most biological samples [4–6].

However, in many environmental monitoring programs a smaller selection of CBs commonly found in aquatic as well as terrestrial environments are monitored as representatives of the CB family, namely CBs 28, 52, 101, 118, 138, 153 and 180.

Some regulatory bodies and researchers have suggested that broader sets of congeners that reflect both environmental abundance and dioxin-like toxicity should be used [7,8]. A balanced set of congeners, such as those suggested by Hansen, which includes 79 CBs with varying degrees of chlorination and *ortho*-chlorine substitution, would probably be most useful in environmental risk assessment for CBs.

However, the gas chromatographic (GC) determination of individual CBs is not straightforward, because of the many potentially coeluting congeners. The more congeners that have to be determined, the higher the risk of positive bias due to coelution with non-target CBs. So far no GC column has been developed that allows unambiguous determination of even the seven regulatory CBs. Many of these compounds coelute with one or more of the other CB congeners in single-dimension GC (1D-GC), and not even the use of high performance GC with mass spectrometric (MS) detection solves the problem. Unambiguous peak assignment and quantification of the WHO-PCBs is still more demanding since these compounds are generally present at much lower concentrations than most of the CBs. Usually pre-fractionation on a carbon or pyrenyl-silica column is performed prior to analysis to separate the planar non- and mono-*ortho* CBs from the non-planar di- through tetra-*ortho* CBs [9,10] and thus decrease the risk of biased results. While this is a common and well-validated procedure, it is labor intensive and makes the analyses costly. Naturally, a method allowing simultaneous determination of a broad set of CB congeners, such as those proposed by Hansen would be very attractive and useful.

Comprehensive two-dimensional (2D) gas chromatography (GC × GC) is a novel technique that provides unprecedented peak capacity [11] and thus improves the separation not only between analytes, but also between the analytes and the matrix background, thereby decreasing background-associated bias. This allows individual CBs in complex matrices to be analyzed without the extensive sample fractionation that is usually required. One such complex matrix, seal blubber, contains a wide range of persistent halogenated hydrocarbons, e.g. CBs, PCDD/Fs, polybrominated diphenyl ethers (PBDEs), hexachlorobenzene (HCBs), toxaphenes, DDTs, chlordanes, hexachlorocyclohexanes (HCHs), and their metabolites. An additional advantage of the high peak capacity provided by GC × GC is that a lot of chromatographic space is empty, making it easy to determine the baseline level [12]. In 1D-GC, the true baseline is difficult to define due to the almost continuous elution of sample constituents from the column, which inevitably affects the precision of the peak area determination.

In GC×GC, two columns with different kinds of selectivity are serially connected. The separation in the first column, usually a non-polar column, is mainly related to differences in the compounds' vapor pressure, while the separation in the second column is based on specific analyte–stationary phase interactions. The key element of a GC × GC system is the modulator, which is positioned between the two columns and prevents components separated in the first column from remixing in the second column. It also refocuses (compresses in time) the material eluting from the first column before re-injecting it into the second column, thereby increasing the detector signal intensity and decreasing the limits of detection (LODs) [13,14].

Temperature programming is normally employed to obtain an orthogonal separation. Under such conditions the second-dimension separation takes place under (essentially) isothermal conditions. Since the components eluting from the first column have roughly the same volatility the dispersion along the second column becomes dependent on solute–stationary phase interactions. This may result in an ordered peak

distribution on the 2D chromatographic plane [15] based on the structural similarity of the compounds; i.e. chemical classes are separated. These peak distribution patterns facilitate analyte identification.

Phillips, who originally proposed the idea of modulating the first-dimension column effluent, pioneered the development of GC × GC. The first interface consisted of a metal-clad, thick-film capillary column. The material eluting from the first column was retained by phase-ratio focusing and then remobilized by resistive heating [16]. This system was difficult to manufacture and was replaced by a thermal sweeper system [17], which was not robust enough to allow routine use and was too limited in the range of analyte volatility it could handle. It was therefore phased out and replaced by a number of cryogenically cooled modulator systems [18–21] in which the analytes are cryogenically trapped and later thermally desorbed by means of convection currents of oven air, thermal jets, or heating wires. An alternative GC × GC instrument is the differential flow modulation in which the majority of the effluent from the first column is sampled by a valve onto the second-dimension column, this system have proven to be effective for high volatility compounds [22].

For this project, we used the first of the cryogenically cooled systems—the longitudinally modulated cryogenic system (LMCS) of Marriott and Kinghorn [18], which is robust enough for routine analysis. The effluent from the primary column is focused in the cryogenically cooled (mechanically actuated) moving trap and rapidly revolatilized when the trap moves away upstream. The revolatilized fraction is then subjected to fast chromatography over a few seconds in the secondary column. The revolatilization rate is dependent on the temperature difference between the trap and the oven, the carrier gas flow rate and the column dimensions, as well as the column's physico-chemical properties, requiring appropriate tuning of the trap temperature [23]. The column type is of major importance, since a column with a narrow internal diameter, a thin capillary wall and a thin stationary phase improves focusing and revolatilization.

Although a wide range of columns with different kinds of selectivity is available, none of them can separate all the 209 CBs, even in conjunction with mass spectrometry. However, a joint inter-laboratory study coordinated by Frame [24] resulted in a comprehensive database with relative retention times for all 209 CBs on 20 types of capillary columns.

A few research groups have tested GC × GC in attempts to resolve many of the CBs that coelute in 1D-GC. In these studies a non-polar first-dimension column has been combined with liquid crystal (LC-50) [25,26] or (semi-)polar stationary phases (BPX-50, HT-8 and SupelcoWax-10) [27].

The objective of this study was to tune the column set, temperature programming and GC modulator parameters of the GC × GC system for optimum separation of CBs. The column combinations tested included tailor-made (non-polar) narrow-bore columns for CB analysis for the first-dimension separation and polar or selective phases for the second-dimension separation.

2. Material and methods

2.1. Chemicals

Nine CB mixtures (C-CS-01 through C-CS-09) were purchased as *iso*-octane solutions from Larodan Fine Chemicals Inc. (Malmö, Sweden). Collectively, these mixtures contain all 209 CBs. An aliquot of each mixture was transferred to separate GC vials for use during the column and system evaluation. Vials were also prepared containing all 209 CBs and the 136 congeners (C-CS-01 through C-CS-05) present in the Aroclor formulations 1242, 1254, or 1260 in excess of 0.05% (w/w) [24], further, three vials with the Aroclor 1232, 1248 and 1260, respectively, was prepared (Larodan Fine Chemicals Inc.). Each vial was spiked with 1,2,3,4-tetrachloronaphthalene (TCN) for use as a retention time reference standard.

2.2. Samples and sample preparation

A blubber sample of a female grey seal (*Halichoerus grypus*) from the Baltic Sea was supplied by the Swedish Museum of Natural History and stored at −20 °C until analysis. A 4 g fraction (wet weight) was taken, macerated, dried with anhydrous Na_2SO_4 and an internal standard, CB 142 (supplied by Dr. Ehrenstorfer, Augsburg, Germany), was added. Lipids and lipophilic compounds were extracted with acetone/*n*-hexane (5:2, v/v) followed by a mixture of *n*-hexane/diethyl ether (9:1, v/v) and the lipid content

was determined gravimetrically. The lipids were removed using a multi-layer column containing, from the bottom, basic silica (KOH treated), activated silica, 40% H_2SO_4 silica (w/w), 20% H_2SO_4 acidic silica (w/w) and a thin layer of anhydrous Na_2SO_4. The CBs were eluted with 200 ml *n*-hexane, 2 ml of *iso*-octane was added and the extract was concentrated to 1 ml, which was transferred to a vial for GC × GC analysis.

2.3. Gas chromatographic analysis

GC × GC was performed using a LMCS retrofitted on an Agilent HP6890 (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with a split/splitless injector and two detectors: a micro-electron capture detector (μECD) and a flame ionization detector (FID). The GC oven temperature program and flow rate used for each GC × GC column combination is described in detail in Table 1. Hydrogen was used as carrier gas in the constant flow mode, controlled by the electronic pressure control (EPC). Two detection techniques were used, with either a μECD (280 °C; 150 ml/min of N_2 makeup gas) or a FID (280 °C; 450 ml/min air with 40 ml He/min makeup gas and 40 ml H_2/min) at a data collection rate of 50 or 100 Hz.

Sample portions of 1 μl were injected (at 250 °C) in either split (20:1) or splitless mode (split opening at 1 min) depending on the type of sample, detector and mode of chromatography (1D-GC or GC × GC). The compounds eluting from the first GC column were cryogenically modulated using the LMCS. The flow of cryogen was regulated to maintain a trap temperature 130–150 °C below oven temperature and the LMCS modulation period was set in the interval 2.5–5 s. The true second-dimension retention times were determined according to De Geus et al. [28]. Two sets of experiments were performed, one with a 4 s modulation period and one with a 5 s modulation period. The second-dimension retention times were calculated using the two sets or retention data.

The GC × GC column combinations were selected by consulting an extensive relative retention time (RRT) database including RRTs for all 209 CBs. The database included figures obtained from an extensive inter-laboratory effort coordinated by Frame [24] and from an in-house GC/MS evaluation of 10 additional columns with a wide spread of stationary phase properties (polar, non-polar and shape-selective) (in preparation). The DB-XLB column was selected as the first-dimension column for its low-bleed and high CB separation capacity [29,30]. As second-dimension column, a liquid crystal or cyanopropyl column was used due to their dissimilarity in selectivity compared to the non-polar columns.

Five combinations of columns were evaluated. In each case the first column was a 60 m × 0.18 mm, 0.18 μm DB-XLB (Agilent Technologies Inc.), except in combination 5, when a 30 m × 0.25 mm, 0.25 μm DB-XLB column was used (also from Agilent Technologies Inc.). In combinations 1 and 2, the second column was a 2.25 m × 0.10 mm, 0.1 μm 70% cyanopropyl polysilphenylene-siloxane (BPX70; SGE Inc., Victoria, Australia) and a 3 m × 0.10 mm, 0.1 μm 100% biscyanopropyl polysiloxane (SP-2340; Sigma–Aldrich, St. Louis, MO, USA), respectively. In combinations 3 and 4, the second column was a 2 m × 0.15 mm and a 0.9 m × 0.15 mm, 0.1 μm poly(50% liquid crystalline/50% dimethyl) siloxane (LC-50; J&K Environmental Ltd., Sydney, NS, Canada), respectively. In combination 5, the second column was a 1.4 m × 0.15 mm, 0.1 μm LC-50. A thin wall narrow bore capillary (0.15 m × 0.10 mm i.d. (0.18 mm o.d.), 0.10 μm 007-1 100% dimethyl polysiloxane column (Quadrex Corp., Woodbridge, CT, USA)) was inserted between the two columns in all but the first column set. The low thermal mass of this capillary increases the desorption rate when the trap moves away from the focusing region. Standard pressfits were used to couple the columns and polyimide resin was used to further secure the columns and ensure a leak-free connection.

Nine mixtures (C-CS-01 through C-CS-09) containing all the 209 CBs were run on these column sets. Each mixture contains congeners that are easily resolved using 5% phenyl methylpolysiloxane types of stationary phase [24]. Since only non-polar phases were used in the first-dimension columns it was possible to determine first and second-dimension retention times for all 209 CBs using only nine injections per column set.

CBs 77 and 188 were used as second-dimension retention window markers. CB 188 is a tetra-*ortho* CB with no *para*-chlorines and CB 77 is a non-*ortho* CB with two *para* (4,4′)-chlorines. For this reason, CB 188 elutes early and CB 77 elutes late from polar and/or shape-selective columns. By comparing the

Table 1
Capillary GC×GC system characteristics

Column combinations			Temperature program								Carrier gas data		
Column set	First-dimension DB-XLB	Second dimension	Initial temperature (°C)	Initial hold (min)	First ramp (°C/min)	First level (°C)	Second ramp (°C/min)	Second level (°C)	Third ramp (°C/min)	Final Temp (°C)	Head pressure (KPa[a])	Flow rate, 1D[b] (cm/s)	Flow rate 2D[c] (cm/s)
	60 m × 0.18 mm, 0.18 μm film	None	80	2	30	140	1.5	n/a	n/a	260	228	35	n/a
1	–	BPX-70; 2.25 m × 0.1 mm, 0.1 μm film	80	2	30	140	0.5	n/a	n/a	260	407	36	115
2	–	SP-2340; 3 m × 0.1 mm, 0.1 μm film	80	2	30	140	1.5	n/a	n/a	260	438	35	115
3	–	LC-50; 2 m × 0.15 mm, 0.1 μm film	80	2	30	140	1.5	n/a	n/a	270	438	53	76
4	–	LC-50; 0.9 m × 0.15 mm, 0.1 μm film	80	2	30	140	1.5	n/a	n/a	270	386	52	76
5	30 m × 0.25 mm, 0.25 μm film	LC-50; 1.4 m × 0.15 mm, 0.1 μm film	80	2	20	160	2	220	3	270	212	31	86

[a] Measured at 80 °C.
[b] Average flow rate in the columns, determined by measuring the holdup time for a headspace injection of dichloromethane.
[c] Calculated flow rate in the second column.

time differences between runs performed at 4 and 5 s GC × GC modulation periods the actual retention times in the second-dimension were calculated. On the SP-2340 and LC-50 column combinations the second-dimension CB retention times ranged from 15.8 to 18.2 s and from 3.2 to 6.2 s, respectively. Thus, the second-dimension retention time span was 3–4 s, which is sufficient to allow at least three modulations over a first-dimension peak.

To evaluate chromatographic resolution in the first-dimension the peak widths at half height for CBs 52 and 180 were measured and the peak widths at half height for all the other CBs were estimated by linear interpolation. Likewise, the resolution in the second-dimension was determined by linear interpolation of the peak widths at half height for CBs 188 and 77: the first and last eluting congeners, respectively, in the second-dimension.

The GC × GC resolution was calculated by combining the resolutions gained from each consecutive dimension, as suggested by Giddings [31] and Schure [32]. Giddings claimed that the total resolution is the Euclidean norm of the resolution in each coordinate axis, so, when the peak-to-peak resolution in the first- and second-dimensions ($R_{s,x}$ and $R_{s,y}$, respectively) has been calculated, the total resolution can be calculated:

$$R_s{}^2 = R_{s,x}^2 + R_{s,y}^2$$

Thus, due to the dimensionless nature of R_s, the two-dimensional peak-to-peak separation can easily be compared to the individual resolutions, $R_{s,x}$ and $R_{s,y}$, gained from each dimension.

2.4. Data collection and evaluation

HPCHEM software (v6.3, Agilent Technologies Inc.) running on a standard PC was used to control the GC × GC and 1D-GC instruments and to collect raw data, which where transformed to csv-files and exported to GC-Image v1.1: a program for GC × GC image processing and analysis developed by the Computer Science and Engineering Department of the University of Nebraska and Zoex Corp. (Lincoln, NE, USA) for qualitative and quantitative analysis and graphical presentation. Some csv-files were also converted to an ASCII text matrix using software developed by Marriott and Kinghorn (RMIT University, Melbourne, Australia) which was then read in Spyglass Transform (Fortner Research, Savoy, IL, USA) and presented as black-and-white contour plots.

GC × GC peak identifications, baseline corrections, area and volume determinations were all done automatically within GC-Image [33]. To identify CBs in the technical CB and seal blubber samples a template was generated using a standard with all 209 CBs. TCN was used as an internal retention time marker for the technical CBs, while CB 142 was used as a marker, as well as an internal standard, for the seal blubber extract. The template was applied to the sample profiles and the search algorithm was set to find peaks within two modulations (2× 4 s) in the first-dimension and 200 ms (10 data points at 50 Hz) in the second-dimension. Only a few mis-assignments were made by this search algorithm, for some poorly resolved and close-eluting CBs in the 209 CBs standard. In these cases, a manual correction was applied.

3. Results and discussion

3.1. Effect of oven temperature ramping on resolution

In GC × GC analysis, each trap and release cycle typically takes 3–6 s, with first column peak widths ranging between 8 and 20 s giving, on average, three to four modulations per peak, which is barely enough to trace the first-dimension peak shape. To accurately reproduce the peak shapes of the first-dimension peaks at least eight modulations per peak are required [34–36]. However, modulating peaks into more than three or four fractions is not practical, since it would require excessive retention times, or very short modulation periods and short second-dimension columns; with a consequent loss in second-dimension resolution. The use of very thin columns (50 μm or less) would circumvent the latter restriction, but such columns have low loadability and are only offered as “tailor-made” columns outside the normal quality control system. Another way to increase the number of modulations per peak is to increase the peak width in the first-dimension by using first columns with thicker stationary phases or larger internal diameters. However, this would inevitably lead to loss in peak capacity from the first-dimension.

To evaluate the gain in (first-dimension) "peak shape integrity" due to the use of slow oven temperature gradients the 1D-GC and reconstructed 1D-GC chromatograms of runs performed at 3, 1.5 and 0.5 °C/min and 5, 4 (and 2.5 s) and 4 s modulation periods, respectively, were compared. The peak width at baseline (6σ) for the first column increased from 13 s at 1.5 °C/min to 35 s at 0.5 °C/min, corresponding to three modulations per peak at 1.5 °C/min with a modulation period of 4 s and eight modulations per peak at 0.5 °C/min. CBs 169 and 196 were not significantly resolved in any of the runs. CB 203 was partially resolved from the CBs 169 and 196. As expected, the resolution increased as the gradient decreased, from ≈0.6 at 3 °C/min to ≈0.8 and 0.9 at 1.5 and 0.5 °C/min, respectively (Fig. 1, top). Accordingly, the reconstructed 1D-GC chromatogram from the modulated run at 0.5 °C/min showed a clear separation of CB 203 from CBs 169 and 196, very similar to that obtained in 1D-GC (Fig. 1a), while the reconstructed 1D-GC chromatogram from the modulated run at 1.5 °C/min showed much lower resolution compared to the corresponding 1D-GC separation (Fig. 1b). To improve the GC × GC resolution at 1.5 °C/min the second-dimension column was cut to 0.9 m and a run was performed using 2.5 s modulations. This modification decreased the peak capacity of the second column while increasing the number of modulations per peak from three to five. The resulting reconstructed 1D-GC chromatogram exhibited a resolution that was only slightly poorer than the original 1D-GC separation (Fig. 1b). However, there is clearly a trade-off between the conservation of 1D resolution and reductions in peak capacity of the second-dimension column.

3.2. Effect of detector type on peak width and shape

FID is a type of detector that has a large linear response range and causes virtually no post-column peak broadening [37,38]. However, the μECD devices

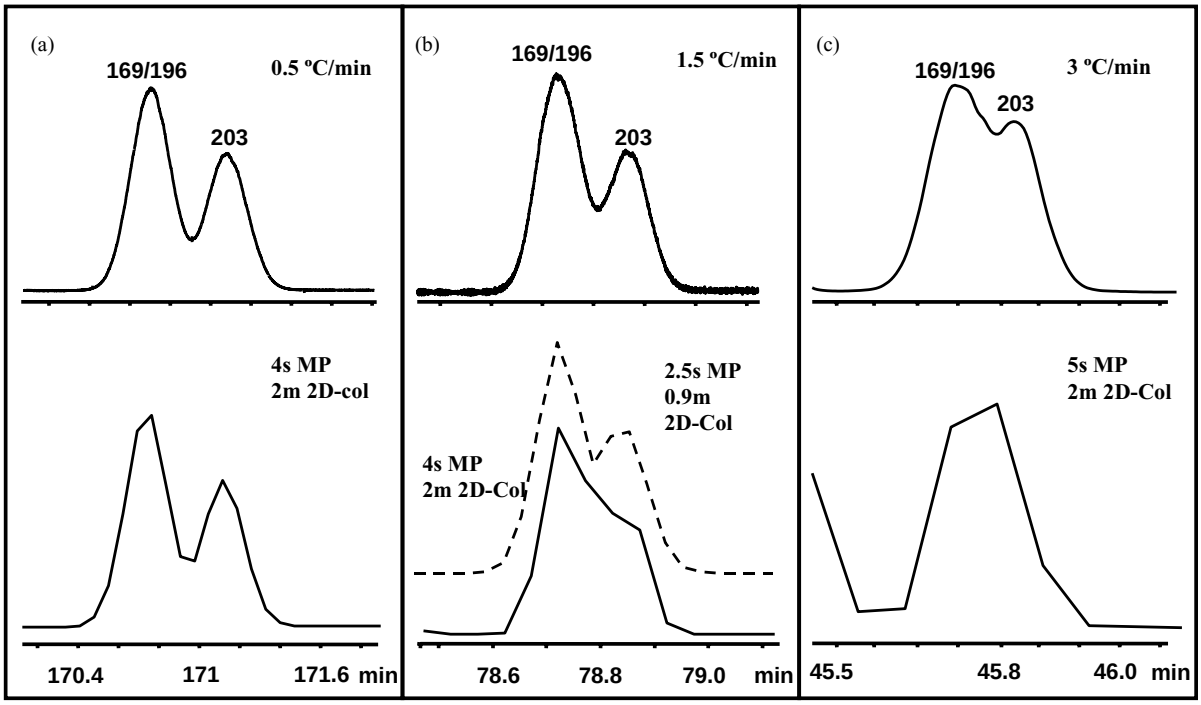

Fig. 1. Peak shape comparisons: (top) 1D-GC chromatograms; (bottom) reconstructed first-dimension chromatograms. The peak envelopes of CBs 169, 196 and 203 are shown for different GC oven gradients, modulation periods (MP) and second column length (2D-col): (a) 0.5 °C/min; (b) 1.5 °C/min; (c) 3 °C/min.

of Agilent [28] and the TOF-MS systems [39] are also rapid response detectors that are suitable for GC × GC. We have tested and evaluated the FID and the μECD.

The cyanopropyl and liquid crystal columns were tested with the FID and the results showed that the SP-2340 column gave slightly better peak symmetry (0.9–1.0) than the LC-50 column (0.8–0.9). The peak widths at baseline (4σ) were slightly wider with the liquid crystal column (200–300 ms) than with the cyanopropyl column (200–230 ms). Early eluting peaks are narrower than later eluting peaks since the second-dimension separation takes place under essentially isothermal conditions.

Notably, the liquid crystal columns show poor performance in 1D-GC of CBs in terms of both resolution and symmetry. Nevertheless, the second-dimension performance is fairly good.

The peak widths at baseline (4σ) were larger with the μECD. For instance, with the SP-2340 peak widths were between 270 and 340 ms: 30–70 ms more than with the FID. The main factor contributing to the wider peaks of the μECD is the more pronounced peak tailing, which is probably due to turbulence in the detector causing some of the sample to be retained. This drawback is reflected in the peak symmetry, which was much poorer for the μECD (0.5–0.6) than for the FID (0.8–1.0). The peak tailing is worse at low detector temperatures and makeup gas flow rates. It might be possible to reduce the tailing effect by operating the system with auxiliary purge gas (and then extending beyond the 150 ml/min maximum of the HP6890). However, that would naturally reduce the sensitivity of the detector.

3.3. Separation of the 209 CBs

The liquid crystal column (LC-50) is shape-selective and selectively retains non- and mono-*ortho* CBs with chlorine substituents in the 4,4′-position. When the DB-XLB was combined with the LC-50 column (column combination 3), 181 of the 209 CBs were separated ($R_s = 0.5$) within a time frame of 90 min (Fig. 2). The congeners that coelute are listed in Table 2 . The data in this table also show that the DB-XLB column alone separates 117 congeners. Thus, a significant improvement in the number of separated congeners was achieved using GC × GC as compared with 1D-GC.

Table 2
Coeluting CB congeners ($R_s < 0.5$) in order of elution

1D-GC	GC × GC		
DB-XLB	Column set 1 (BPX-70)	Column set 2 (SP-2340)	Column set 3 (LC-50)
4/**10**	–	–	**4**/**10**
11/**18**[a]	–	–	–
13/**27**[a]	–	–	–
34/54[a]	–	–	–
26/50[a]	–	–	–
31/**53**[a]	**31**/**53**[a]	–	–
20/21/**33**	–	21/**33**	20/**33**
36/**43**[a]	–	–	**43**/69
38/39/**47**/62/65/**75**/104[a]	**47**/62/65	**47**/62/65	62/65
–	**42**/**59**	**42**/**59**	–
37/**40**	–	**37**/**40**[a]	–
57/94[a]	57/94[a]	57/94[a]	–
58/**67**	–	58/**67**	58/**67**
63/76/93[a]	–	**63**/76	**63**/76
88/**95**	–	–	88/**95**
70/121[a]	–	–	–
66/**91**[a]	–	–	–
56[a]/84/**89**/**92**	–	–	**84**/**89**
60[a]/90/**101**	–	90/**101**	–
99/150[a]	–	–	–
83/**119**	–	–	**83**/**119**
86/125	86/112	86/125	86/125
111/154[a]	–	–	–
82/**151**[a]	–	–	–
139/140	–	–	–
106/**123**	106/**109**	–	–
109/**134**[a]		**109**/**134**[a]	–
118/**131**[a]	–	**118**/**131**[a]	–
142/188[a]	–	–	–
122/**184**[a]	–	–	–
114/**146**[a]	–	–	–
132/**153**/168	–	**153**/168	–
127/186[a]	–	–	–
160/**163**	–	160/**163**	160/**163**
129/**178**[a]	–	–	–
175/182	**175**/182	**175**/182	**175**/182[a]
166/**183**	–	–	–
171/**201**/204	–	**201**/204	**201**/204
193/**200**[a]	–	–	–
169/**196**	–	**196**/**203**	**196**/**203**
195/207[a]	–	–	–
∑92	∑15	∑33	∑28 CBs[b]
∑42	∑4	∑10	∑10 Aroclors[c]
∑46	∑11	∑25	∑26 CBs[a,b]
∑19	∑2	∑4	∑10 Aroclors[a,c]

CBs present in any of the Aroclors [1] at concentrations higher than 0.05 or 1.0% (w/w) are displayed in bold and bold + underlined typefaces, respectively.

[a] Resolved by MS.

[b] Total number of coeluting CBs (maximum 209).

[c] Number of coeluting Aroclor CBs (maximum 136).

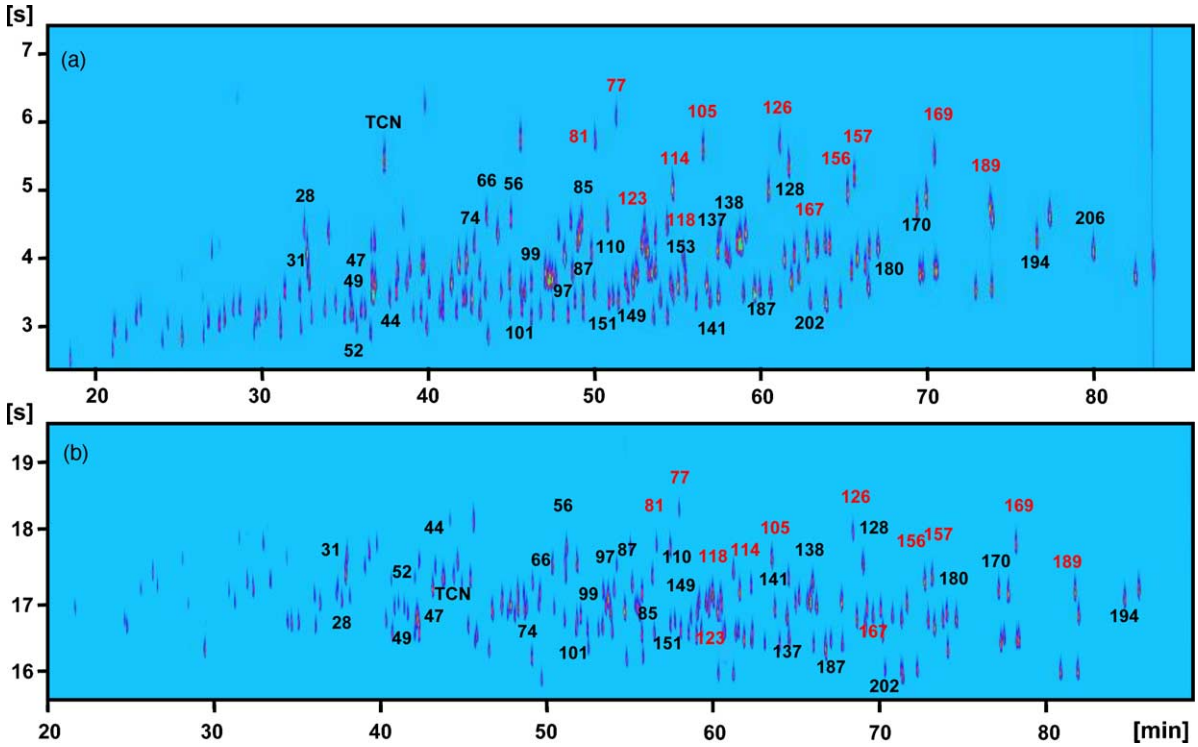

Fig. 2. Contour plots of the 209 CBs. Column set: 60 m × 0.18 mm, 0.18 μm DB-XLB as first-dimension column; and 2 m × 0.15 mm, 0.1 μm LC-50 (a), or 3 m × 0.1 mm, 0.1 μm SP-2340 (b), as second-dimension column. The oven temperature gradient was 1.5 °C/min and the detector a μECD.

Column combination 2 (DB-XLB and SP-2340) is equally successful and separates 176 congeners. The peaks are more evenly spread using the SP-2340 rather than the LC-50, as clearly seen in Fig. 2. This is not unexpected since the polar interactions of the cyanopropyl columns are not as selective as the shape-discriminating effect of the liquid crystal phase LC-50. Planar CBs with chlorines in *para* position are more easily polarisable giving a higher interaction with a polar stationary phase as SP-2340 and LC-50 but the added interaction with planar CBs gained from the surface of the liquid crystal phase is more similar to the CB interaction found for a carbon column.

Generally, the LC-50 has stronger retention, when used as second-dimension column, as compared to the SP-2340. It therefore produces slightly narrower peaks. Consequently, a 2 m column was sufficient while a 3 m column was needed for SP-2340.

To take full advantage of the analytical power of GC × GC, the GC oven ramping rate should be reduced. Using column combination 1 (a 60 m DB-XLB column connected to a 2.25 m BPX70) and a gradient of 0.5 °C/min, a total analysis time of 4 h was obtained. The increased analysis time mainly improved the congener resolution in the first-dimension. This led to an increase in the number of separated CBs, i.e. 194 of the 209 CBs. This is a significant improvement, but it is still questionable whether the increase in resolution is sufficient to justify the use of such excessive retention times.

Alternatively, increasing the oven temperature gradient to 3–5 °C/min, the number of coeluting peaks in GC × GC is expected to increase and the analysis time to decrease. Therefore, a 30 m DB-XLB column was connected with a 1.4 m LC-50 narrow bore (0.15 mm i.d.) second column (column set 5 in Table 1). At a programming rate of 2–3 °C/min the total analysis time was cut to 53 min. The shorter and wider bore capillary column in combination with the increase in temperature programming speed gives similar peak

widths as for column combination 3, with an average of three modulations per peak at 5 s each. Consequently, 18 more CBs coeluted, due to the loss of resolution in the first-dimension. While some of the first-dimension peak resolution was lost, the resolution in the second-dimension was generally maintained.

3.4. Separation of CBs present in technical formulations

The congener distributions in environmental samples resemble those of the parent commercial CB mixtures (e.g. Aroclors and Clophens), and heavy CB mixtures, such as the Aroclors 1254 and 1260 or the Clophens A50 and A60, have frequently been used as standards for comprehensive, quantitative, congener-specific (CQCS) analysis of CBs. However, there have been problems determining the fractional contribution of each congener to the total amount of CBs in technical samples. A few researchers, e.g. Schulz et al. [40] and Frame [1] have worked extensively on Aroclor congener assignments and percentage determination (w/w) of each individual CB in technical formulations. Consequently, some technical CBs have been fully characterized. The large batch-to-batch variations amongst these products causes problems since it prevents direct transfer of these data to other laboratories using other batches of the characterized products. However, all 209 CBs are now available as individual congeners and CQCS analysis of CBs present in technical CBs may be performed without the use of these technical mixtures.

According to the data shown in Table 2, both column combinations 2 (DB-XLB and SP-2340) and 3 (DB-XLB and LC-50) separate 126 of the 136 CBs that are present in the Aroclors 1242, 1254 and 1260 at levels greater than 0.05% (w/w) [1]. This is significantly more than the 94 congeners that may be separated on DB-XLB alone. The congeners that coelute are listed in Table 2 and congeners present in any Aroclor above 0.05% or 1% are marked in bold and bold + underlined typefaces, respectively.

Six additional congeners were separated using column combination 1 (60 m DB-XLB and 2.25 m BPX70) and a gradient of 0.5 °C/min compared to the equivalent column combination 2 at 1.5 °C/min. The increased analysis time mainly improved the congener resolution in the first-dimension. Thus, only 4 of the 136 Aroclor CBs were not separated with these modifications. Although this is an extraordinary result, the long run time (4 h) will prevent this from being adopted as a routine method. However, in the future, narrow bore thin film columns (50 μm with 0.05 μm film) may be available and allow the same separation to be achieved within a reasonable time.

The use of TOF-MS would not increase the number of CBs resolved by column combination 3 (LC-50), but six additional congeners would be separated using column combination 2 (SP-2340). Thus, more congeners would be resolved using slow oven temperature gradients and μECD detection (see earlier) than with a faster gradient and TOF-MS detection. The gain in total analysis time given by the latter system would easily make up for the difference, but the cost of such a system would be rather high.

In our opinion, the use of an oven temperature ramp rate of 1.5 °C/min and a μECD detector is the preferred combination. Many components are separated using such a system and the analysis time is reasonable: less than 90 min. Fig. 3 shows the separation of Aroclors 1232, 1248, and 1260 on such a system. As expected, there are great similarities in the peak patterns of the three Aroclors. Roughly the same congeners are present in all the products, but their relative abundance differs in the three mixtures. Aroclor 1232, which is a 50/50 blend of Aroclors 1221 and 1242, contains mainly di- through penta- CBs while Aroclor 1248 contains mainly tri- through hexa-CBs and Aroclor 1260 contains mainly penta- through octa-chloro congeners. Of the congeners that can be found in Aroclor 1260, some coelute on the DB-XLB column (for instance, CBs 153 and 132) both of which are found at high levels in technical products. All of these congeners that coelute in 1D-GC (CBs 84/92, 82/151, 132/153, 129/178, 171/201 and 193/200) were resolved on column combination 2 (SP-2340) while 84/92 coeluted on column combination 3 (LC-50). In Aroclor 1242, the CBs 20/33 and 31/53 were not resolved by 1D-GC and were barely separated in GC × GC ($R_s = 0.5$–0.6). CB 56 can be found at high levels in Aroclor 1248, but on the DB-XLB these congeners coelute with the CBs 84, 89 and 92 while they are partially separated ($R_s > 0.5$) in GC × GC. Further, Aroclor 1242 contains high levels of the congeners 101 and 118, which are frequently detected in environmental samples. While CB 101 can be

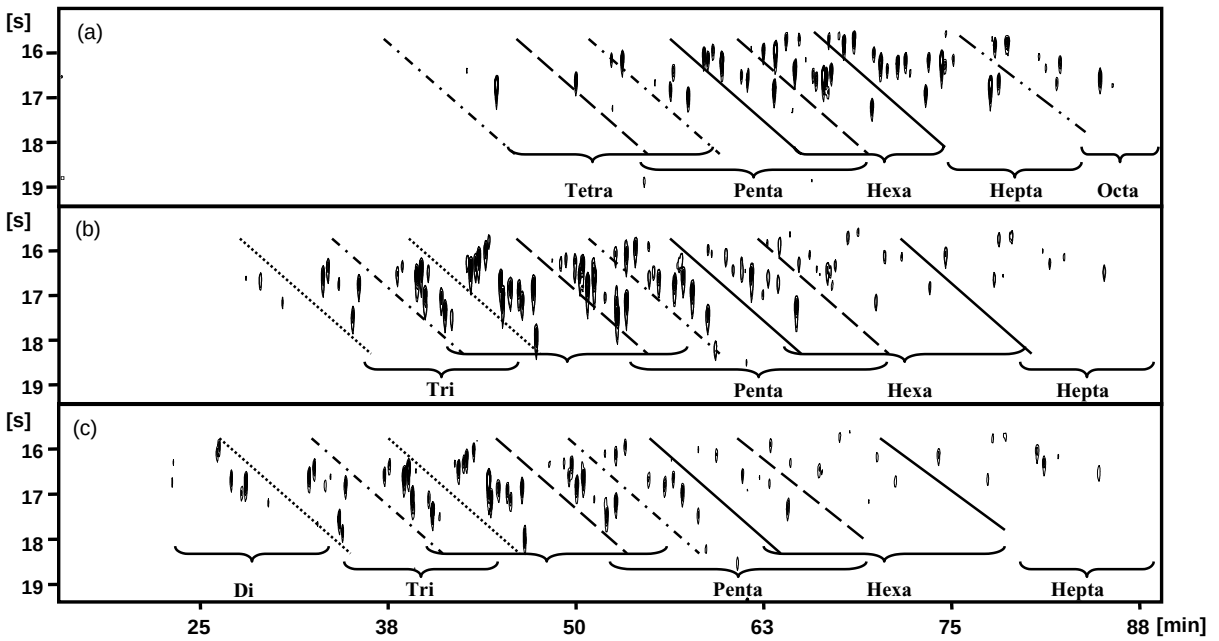

Fig. 3. Contour plots of the technical CB mixtures: (a) Aroclor 1260; (b) Aroclor 1248; (c) Aroclor 1232. Column set: DB-XLB (60 m × 0.18 mm, 0.18 μm film) and SP-2340 (3 m × 0.1 mm, 0.1 μm film). The oven temperature gradient was 1.5 °C/min and the detector a μECD.

resolved in GC × GC by either column set specified in Table 1, CB 118 is only resolvable in GC × GC by a column combination with shape-selective characteristics (LC-50). In Aroclor 1232 we found coelutions in 1D-GC between 4/10, 31/53, and 66/91, amongst others. CB 4 can be resolved in the second-dimension by the SP-2340 column, but not on LC-50 while CB 66 is resolved on the LC-50 but not SP-2340.

3.5. Separation of WHO-PCBs

Eight mono-*ortho* and four non-*ortho* CBs have been assigned TEF values by WHO, i.e., CBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189. Four WHO-CBs can be separated ($R_s \geq 1$) with 1D-GC (CBs 105, 126, 156 and 189), but the remaining eight congeners are not resolved (Table 3). CBs 123 and 157 coelute with non-Aroclor congeners in 1D-GC, and since these are not present in the Aroclors in excess of 0.05% (w/w), one could assume that quantification would be fairly accurate, even if complete separation is not achieved. The remaining six WHO-PCBs (CBs 77, 81, 114, 118, 167 and 169) coelute with CBs that are abundant in Aroclors and have to be separated in the second-dimension of a GC × GC analysis. This is especially important for the non-*ortho* CBs 77, 81 and 169 since they are found at trace levels in the environment, which increases the probability of biased analytical results. In GC × GC, CBs 77, 81, 114, 167 and 169 are resolved from Aroclor congeners in GC × GC using either column combination. CBs 118 and 131 are not resolved using column set 2 (SP-2340), but this pair does separate on column combination 3 (2 m LC-50) (Table 3). Therefore, we conclude that the best performance was achieved by column combination 3, which separates all the WHO-CBs from all the Aroclor CBs and allows their complete resolution, or at least partial separation, from all non-Aroclor CBs.

Using column combination 5 and a steeper temperature gradient, 3 °C/min, as compared to the gradient used for column combination 3, many congeners coelute in the first-dimension, while the second-dimension separation is acceptable (Fig. 2a). The smectic liquid crystal column used as the second-dimension in column combinations 3–5 offers a separation based

Table 3
Coeluting environmentally significant CBs[a]

Substitution order	Significant CBs	1D-GC		GC × GC	
		$R_s < 1$	$R_s < 0.5$	SP-2340[b]	LC-50[c]
Non-*ortho*	**77**[a]	**144**	–	–	–
	81[a]	**110**/120	–	–	–
	126[a]	–	–	–	–
	169[a]	**196**	**196**	–	–
Mono-*ortho*	**28**[d]	–	–	–	–
	31[d]	**53**	**53**	**53** (0.6)	–
	56[d]	**84**/**89**/**92**	**84**/**89**/**92**	**89** (0.9)	–
	66[d]	**91**/155	**91**	**91** (0.6)	–
	74[d]	88/**95**	–	–	–
	105[a,d]	–	–	–	–
	114[a]	**146**	**146**	–	–
	118[a,d]	**131**	**131**	**131**	–
	123[a]	106	106	106 (0.6)	106/107 (0.7)
	156[a,d]	–	–	–	–
	157[a]	192	–	–	–
	167[a]	181/**202**	–	–	181 (0.9)
	189[a]	–	–	–	–
Di-*ortho*	**44**[d]	–	–	–	–
	47[d]	38/39/62/65/**75**/104	65	62/65	62/65 (0.5)
	49[d]	–	–	–	–
	52[d]	–	–	–	–
	85[d]	111/154	111/154	–	–
	87[d]	**136**	–	–	–
	97[d]	–	–	–	–
	99[d]	150	150	150 (0.7)	–
	101[d]	**60**/90	**60**/90	90 (0.5)	90 (0.7)
	110[b]	–	–	–	–
	128[b]	162/**185**	–	–	–
	137[d]	127/186	–	186 (0.9)	127 (0.8)
	138[d]	–	–	–	–
	141[d]	–	–	–	–
	153[d]	**132**/168	**132**/168	168	168 (0.5)
	170[d]	**198**	–	–	–
	180[d]	–	–	–	–
	194[d]	–	–	–	–
Tri-*ortho*	**149**[d]	–	–	–	–
	151[d]	**82**	**82**	–	–
	187[d]	–	–	–	–
	206[d]	–	–	n/a	–
Tetra-*ortho*	**202**[d]	**167**/181	–	–	–

CBs present in any of the Aroclors [1] at concentrations higher than 0.05 or 1.0% (w/w) are displayed in bold and bold + underlined typefaces, respectively. Estimated congener resolution (R_s) in parenthesis.

[a] WHO-PCBs.

[b] Column set 2, $R_s < 1.0$.

[c] Column set 3, $R_s < 1.0$.

[d] QUASIMEME CBs.

on the number of *ortho*-chlorines. This makes the separation of non- and mono-*ortho* CBs virtually independent of the oven temperature gradient, provided the length of the second-dimension column is appropriate. Consequently, all WHO congeners, except CB 167 (which coelutes with the non-Aroclor CB 181) were successfully separated. At higher temperature gradients, e.g. 5–10 °C/min, there might be a risk of coelution of the mono-*ortho* CBs 118 and 123.

3.6. Separation of CBs in grey seal (H. grypus) blubber

In environmental programs for monitoring CBs in aquatic organisms a selection of CBs is generally included. For example, a number of European laboratories have agreed to measure 31 congeners and the quality of the data are checked within the QUASIMEME network (a holistic quality assurance programme for marine environmental monitoring information in Europe) [41].

The DB-XLB column has been reported to separate many of these QUASIMEME CBs [24] and this was verified by our 1D-GC data (Table 3). On our 1D-GC system, 15 of the 31 congeners were separated ($R_s > 1$; Table 3) and will not be further discussed. Of the remaining 16 congeners, CBs 85, 99 and 137 coelute with non-Aroclor congeners and are not of major concern. However, these three CBs are fairly well separated from coelutants ($R_s > 0.7$) on either GC × GC column combination. The remaining 13 congeners coelute with CBs that can be found at moderate levels in the environment as well as in technical CB mixtures. The best GC × GC column combination for the separation of these CBs was column set 3 (LC-50), on which all 31 congeners were fully resolved or partially separated from all other CBs. However, CB 153 partially coelutes with CB 168 ($R_s \approx 0.5$) while CB 168 is only found at trace levels and CB 153 is normally the most abundant CB in aquatic species. Thus, the bias is expected to be negligible. Column set 2, with a polar cyanopropyl phase as the second-dimension column, separates 27 of 31 CBs while coelutions occur for CBs 31, 56, 66 and 118. The CBs 31, 56 and 66 are partially separated ($R_s > 0.5$) and could probably be quantified, but with greater uncertainty (Table 3).

Table 4
Concentration levels in μg/g lipid weight of CBs found in an adult female Baltic grey seal blubber sample by GC × GC

No.	Level	No.	Level	No.	Level
52	0.04	135	0.04	175	0.02
67	0.58	137	0.07	177	0.24
74	0.02	138	4.14	178	0.26
77	0.07	139	0.01	180	2.84
80[a]	0.05	141	0.03	183	0.66
85	0.04	144	0.01	185	0.01
87	0.02	146	0.67	187	1.07
91	0.02	147	0.01	189	0.09
92	0.04	149	0.26	190	0.17
95	0.02	151	0.04	191	0.04
97	0.01	153	3.07	193	0.24
99	0.30	154[a]	0.02	194	0.55
101	0.15	156	0.11	195	0.18
105	0.03	157	0.02	197	0.02
110	0.02	158	0.11	198	0.02
114	0.01	163	0.21	199	0.45
118	0.16	166	0.01	202	0.14
128	0.18	170	0.91	205	0.05
130	0.13	171	0.13	207	0.06
132	0.11	172	0.21	208	0.10
134	0.01	174	0.08	196/203	0.30

[a] Identified as non-Aroclor congeners.

A seal blubber sample was analyzed to evaluate the suitability of this system for routine analysis of CBs in environmental samples (the main compounds of interest being the QUASIMEME CBs, followed by the WHO-PCBs). Seal blubber contains large amounts of CBs and split injections (20:1) were used to avoid column overload. Correct retention times in both dimensions and a signal to noise ratio (S/N) of 3 were required for positive identification of any CB. Small retention time drifts were noticed in the chromatogram of the seal blubber sample, as compared to a standard run of all 209 CBs, with a 4 s increase in first-dimension retention times, equivalent to one modulation period, and a 70–90 ms shift in the second-dimension. This drift in retention time was compensated for by using an internal standard (CB 142) in both the quantification standard mixture and the seal blubber sample. By applying the peak template, based on the quantification standard, a small retention time drift in the sample could easily be compensated for. With this peak template 64 CBs could be identified in the blubber sample (Table 4). Clearly, highly chlorinated CBs, i.e. penta- through

nona-chloro CBs dominate in the blubber. The most abundant were CBs 138, 153 and 180 and *p,p′*-DDE (a metabolite of *p,p′*-DDT), as usually found in grey seal samples. Many CBs are not visible in the chromatogram since they are present in low concentrations (Fig. 4). In total, 23 of the 31 QUASIMEME CBs were detected while many of the lightly chlorinated tri- and tetra-chloro CBs (28, 31, 44, 47, 49, 56 and 66) were close to or below determination levels, while CB 206 elutes after the set modulation period.

Many of the 64 congeners detected by GC × GC in the seal sample could be accurately quantified in 1D-GC, especially those present at high levels, but for many other CBs this would be problematic. For instance, CB 101 coelutes with the mono-*ortho* CB 60, CB 144 is only partially resolved from the non-*ortho* CB 77, and CB 153 is not separated from CB 132 in 1D-GC. In the last of these cases, the large concentration difference between CB 153 compared to CBs 132 and 168 (CB 153 is present at five times higher concentration than CBs 132 and 168) would result in a 5% overestimation of the CB 153 concentration, and make quantification of CBs 132 and 168 impossible.

Due to the low levels of planar CBs in technical mixtures, only trace levels are likely to be found in seal blubber. However, since all of the non- and mono-*ortho* CBs are separated from other CBs it is easy to positively identify them and limit the risk of bias due to coelution. Seven of the 12 WHO-PCBs were detected. Among the non-*ortho* CBs, i.e. CBs 77, 81, 126 and 169, only 1 (CB 77) was found. The remaining six CBs found were the mono-*ortho* CBs 105, 114, 118, 156, 157 and 189. Weak signals were also detected at the retention times of CBs 123 and 167. It is possible that additional WHO-PCBs could have been detected if splitless injection was utilized, but the high levels of CBs 138, 153, 180 and *p,p′*-DDE might then have overloaded the second-dimension column, which can only handle limited loads due to its narrow bore and thin film. It would also be difficult to keep all congeners within the working range of the μECD detector. A separate injection for the detection of WHO-PCBs would partially overcome those problems.

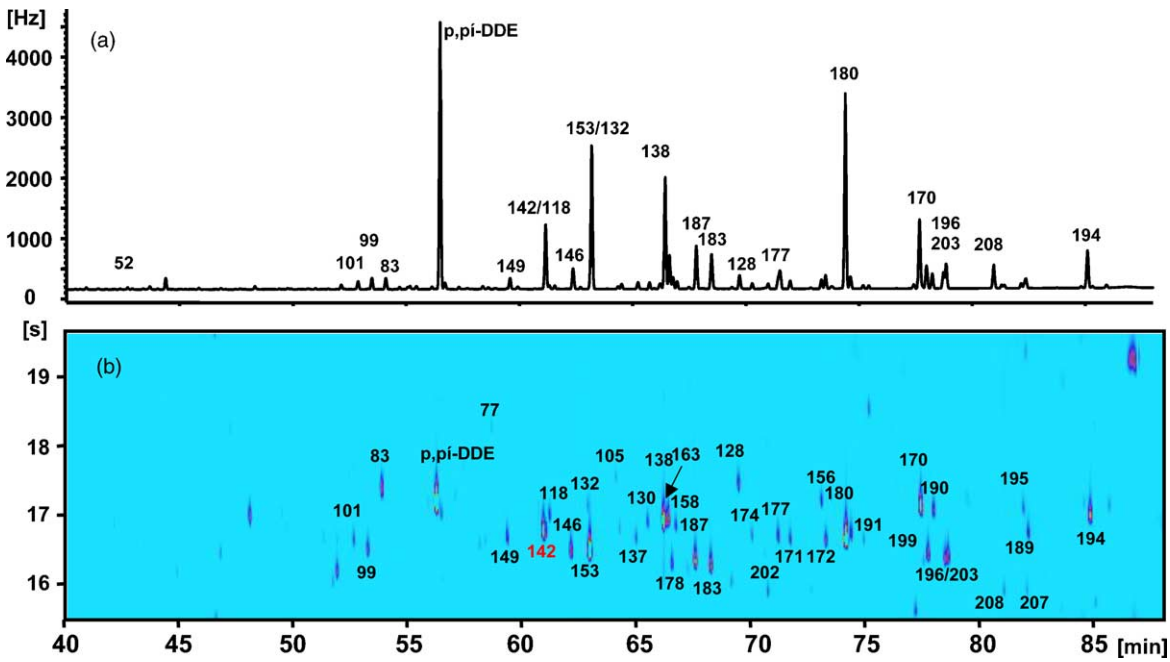

Fig. 4. Seal blubber (*H. grypus*) extract analyzed by: (a) 1D-GC; (b) GC × GC. Column set: DB-XLB (60 m × 0.18 mm, 0.18 μm film) and SP-2340 (3 m × 0.1 mm, 0.1 μm film). The oven temperature gradient was 1.5 °C/min and the detector a μECD.

4. Conclusions

GC × GC are superior to 1D-GC in separating CB. Almost all CBs present in technical CB formulations were separated by the use of GC × GC/μECD and the CB 2D-patterns facilitate the identification of CBs in the technical mixtures, as well as in environmental samples. The twelve WHO-PCBs and the seven indicator congeners (CBs 28, 52, 101, 118, 138, 153, and 180) that are frequently analyzed in environmental monitoring programmes are chromatographically separated by GC × GC system used, although further optimization is needed, depending on the analytical task at hand, to obtain sufficient separation in shortest possible analysis time.

Decreasing the temperature programming rate give a general improvement of the CB resolution in 1D-GC. An oven programming rate of several hours increases the resolution of many partially coeluting congeners. In GC × GC, it is important to maintain a reasonable number of modulations over a first-dimension peak to maintain 1D resolution. This is especially important in CB separations as many congeners have a similar retention in both dimensions. As a next step, we would recommend that the second-dimension column is changed to an ultra narrow bore column with very thin coating, e.g. a 50 μm column with a 0.05 μm film. Columns of this type are highly efficient and allow faster second-dimension separations. Thus, more data points could be collected over a 1D-GC peak profile, thereby better conserving the 1D resolution. However, the load they can handle is low, and such columns are not readily available.

Although the μECD give raise to peak broadening, as compared to the FID, it is still to prefer as it is much more sensitive to organochlorine compounds. A TOF-MS system gives low post column band broadening similar to the FID and provide the added benefit of MS separation, which increases the number of resolved CBs (by a few congeners) on the studied column combinations. The sensitivity is however lower than for the μECD unless electron-capture negative ion chemical ionization (ECNI) is used. The GC × GC modulation process also results in peak compression and lower detection limits. Consequently, the GC × GC with μECD or TOF-MS detection is useful alternatives to the HRGC/MS systems for environmental analysis.

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