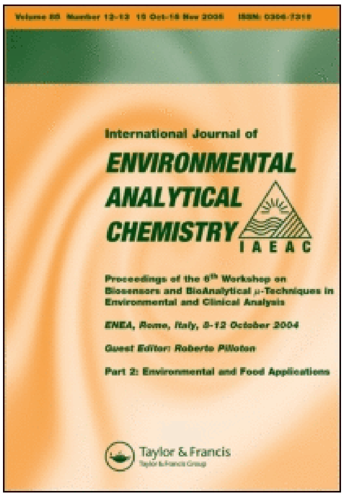

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HRGC SEPARATIONS OF 160 CHLOROBIPHENYLS IN TECHNICAL MIXTURES ON FOUR POLAR NARROW-BORE COLUMNS

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The separation of 160 chlorobiphenyl (CB) congeners from technical polychlorinated biphenyl (PCB) mixtures was investigated on four narrow-bore, fused silica gas chromatography (GC) columns with high temperature resistant polar stationary phases: 15% diphenyldimethylsiloxane (0.20 µm SIL-13, 50 m × 0.25 mm), 50% diphenyl dimethylsiloxane (0.25; gmm DB-17, 60 m × 0.25 mm), a series combination of 5% diphenyldimethylsiloxane (0.25 µm SIL-8, 25 m × 0.25 mm) with 1,10-dicarba-*closo*-dodecarborane dimethylsiloxane (0.1 µm HT-5, 25 m × 0.2 mm) and a 70% cyanopropyl silphenylene dimethylsiloxane copolymer (0.25 µm BPX-70, 50 m × 0.22mm). Technical PCB mixtures (Aroclors) and authentic standards (totally 149 CBs) were injected on the narrow-bore columns and analyzed by GC-ECD and GC-MS.

Structural principles for retention of CBs and the retention mechanism of diphenyl and cyanopropyl in the stationary phase are discussed.

SIL-13 proved to be an interesting alternative to the widely used SE-54 type columns. DB-17 showed a significantly changed elution order and seems the best choice of column together with SIL-8/HT-5 for parallel analysis of CB congeners. A total of 144 CBs in technical mixtures can be analyzed free of interference from other CBs imploying these two columns. Too many CB congeners co-eluted on BPX70 for this column to be useful in routine analysis of CBs.

KEY WORDS: PCB congeners, HRGC-ECD/MS, polar GC-stationary phases, Aroclors.

INTRODUCTION

The analysis of polychlorinated biphenyls (PCBs) as separated individual compounds on capillary column gas chromatography (HRGC) has become routine in most laboratories. A total of 209 possible chlorobiphenyl (CB) configurations (congeners) exists and around 150 have been reported at significant concentrations in the environment and technical PCB mixtures [1] etc. Each individual CB congener has been given a IUPAC number. For three congeners, CB-199, CB-200 and CB-201, their original numbers [1] have been found to be in disagreement with the IUPAC numbering system [2]. In the present paper the new IUPAC numbers [3] will be used for these three CBs.

Certain CB congeners have been given special priority owing to their toxicity and occurrence in the environment[4] (18, 37, 44, 49, 52, 70, 74, 77, 81, 87, 99, 101, 105, 114, 118, 119, 123, 126, 128, 138, 151, 153, 156, 157, 158, 167, 168, 169, 170, 177, 180, 183, 187, 189, 194 and 201). The separation of these congeners from the bulk of PCBs is complicated by their relative low particular concentrations compared with the bulk of PCBs and by the high number of possible co-elutants.

In the legislation of some European countries and international bodies, the monitoring of PCBs is prescribed as the quantification of a set of seven indicator CBs (28, 52, 101, 118, 138, 153 and 180). These congeners have been selected on the basis of their presence at high concentrations in technical PCB mixtures and in the environment. The interference-free analysis of these congeners on various GC phases is of great commercial interest and has been studied recently in detail[5-7].

To date, only a few stationary GC phases have been adequately investigated with respect to CB congener separations. In 1984 Mullin *et al.*[8] published the retention time of all 209 CB congeners on a 50 m × 0.2 mm fused silica capillary column coated with chemically bounded 5% diphenyl, 1% vinyl dimethyl polysiloxane (SE-54) and so far, this is still the most popular choice of stationary phase for PCB analysis. Unfortunately, only 18 congeners out of the 36 priority CBs and five out of the seven indicator CBs can be separated from co-eluting congeners on this phase[9]. In 1988 Fisher and Ballschmiter[10] characterized the elution profile of technical and environmental PCB mixtures on a 50 m × 0.25 mm fused silica capillary column coated with 50% dioctyldimethylpolysiloxane. Although this column facilitated the separation of 33 out of the 36 priority CBs and six out of the seven indicator CBs from any CB interference[9], the chemical stability of this stationary phase requires that the analysis is performed at a mild temperature programme which causes undesirably prolonged run periods.

Recently, the elution profiles of technical PCB mixtures (Aroclors) have been characterized in our laboratory on six narrow-bore GC columns with stationary phases representing a broad range of polarities: SIL-5, 50 m × 0.25 mm dimethylsiloxane; SIL-8, 50 m × 0.25 mm 5% diphenyldimethyl siloxane; SIL-19, 50 m × 0.25 mm 14% cyanopropylphenyl + 1% vinyldimethylsiloxane; SIL-88, 50 m × 0.25 mm bis-cyanopropylphenylsiloxane; HT-5, 25 m × 0.22 mm 1,10-dicarba-*closo*-dodecarborane dimethylsiloxane and SIL-8/HT-5, a series combination of the above mentioned columns SIL-8 with HT-5. The results of these studies[11-14] have received great interest and have assisted in the optimum choice of GC column for CB congener separations. In the present paper we present the separation of 160 chlorobiphenyl (CB) congeners from technical polychlorinated biphenyl (PCB) mixtures on three polar stationary phases with chemical stabilities resisting an upper temperature of 270–285°C, necessary for total run periods around 60 min used in routine PCB analysis. Furthermore, we present retention data on a shorter version of the previously published[12,14] SIL-8/HT-5 series combination (25 m SIL-8 as opposed to 50 m).

EXPERIMENTAL

Materials

A total of 149 CBs were available for this study. Of these 105 were available as either authentic reference standards or certified standard mixtures. Individual CBs were obtained either as neat crystals from the Community Bureau of Reference (BCR), Brussels, Belgium (IUPAC numbers: 18, 20, 28, 35, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170 and 180), from Promochem, Germany (8, 37, 42, 44, 48, 49, 53, 55, 60, 69, 70, 74, 75, 81, 84, 87, 92, 100, 110, 114, 115, 119, 123, 124, 136, 141, 151, 158, 167, 171, 172, 183, 187, 189, 194, 202), from Ultra Scientific, U.S.A. (CBs 1, 2, 3, 4, 6, 10, 11, 13, 16, 26, 66, 80, 95, 133, 185, 190), or were synthesized and purified as described previously (77, 122, 126, 163, 169)[6,15]. Certified CB standard solutions were obtained from the National Research Council, Canada (CLB-1A: 18, 31, 40, 44, 49, 54, 77, 86, 87, 121, 153, 156, 159, 209; CLB-1B: 15, 52, 60, 103, 105, 128, 143, 154, 173, 182, 202, 205, 207, 208, 209; CLB-1C: 15, 114, 129, 137, 153, 171, 183, 185, 189, 191, 199, 201, 203, 206, 209; CLB-1D: 15, 101, 118, 138, 141, 153, 170, 180, 187, 194, 195, 196, 199, 209) and from Supelco, Italy (DCMA: 1, 11, 29, 47, 121, 136, 185, 194, 206 and 209). A further 39 CB congeners were synthesized in well defined mixtures according to Mullin *et al.*[8] (17, 19, 23, 24, 25, 27, 29, 34, 36, 43, 45, 46, 48, 57, 59, 63, 64, 67, 71, 74, 83, 84, 89, 90, 91, 97, 99, 107, 110, 118, 125, 130, 132, 134, 138, 147, 152, 155, 160, 165, 177, 178, 179, 183, 193) and 5 CB congeners were obtained from J. deBoer, The Netherlands Institute for Fisheries Research (33, 56, 82, 85 and 157). Technical PCB mixtures were obtained in 2,4,4-trimethylpentane from Supelco (Milan) as the Aroclor lots LA12790 (A1232), LA13646 (A1242), LA13647 (A1248), LA13614 (A1254), LA13576 (A1260), LA12791 (A1262) and a mixture of four Aroclors (A1016, A1232, A1248 and A1262, 1:1:1:1).

The GC columns were obtained from Chrompack (SIL-8, SIL-13), J&W (DB-17) and Scientific Glass Engineering (HT-5, BPX-70) as fused silica wall coated open tubular columns: 15% diphenyl dimethylsiloxane (0.20 μm SIL-13, 50 m × 0.25 mm), 50% diphenyldimethylsiloxane (0.25 μm DB–17, 60 m × 0.25 mm), 5% diphenyldimethylsiloxane (0.25μ SIL–8, 25 m× 0.25 mm) series coupled by use of a glass press-fit connector (MEGA, Milan) with 1,10-dicarba-*closo*-dodecarborane dimethylsiloxane (0.1 μm HT-5, 25 m × 0.2 mm) and a 70% cyanopropyl silphenylene dimethyl siloxane copolymer (0.25 μm BPX-70, 50 m × 0.22mm). The DB-17 column was only available in a length of 30 m. In order to obtain sufficient separation power two DB-17 columns were series coupled by means of a glass press-fit connector (MEGA, Milan).

Gas chromatography-electron capture detection

GC analysis was performed with a pressure controlled Hewlett-Packard 5890A II gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector (ECD) and a HP7673A auto-sampler. The electron capture detector (ECD) was operated at 300 °C, purged with 60 ml/min of 10% methane in argon. The hydrogen carrier gas flow was 45 cm/s at 90°C and held constant by the pressure controlled inlet throughout the whole temperature programme. Detection limits were 0.2–2 pg depending on the column bleed. Aliquots (1.0 μl) of the standards in 2,4,4-trimethylpentane were on-column injected at 90°C. The Aroclors were injected at concentrations around 8 ng/μl (total PCB). The investigated columns were installed in the GC oven together with a 2 m × 0.53 mm fused silica pre-column using a glass press-fit connector (MEGA, Milan).

The GC oven temperature programme was as follows: initial temperature 90°C, held for 2 min, then increasing at a rate of 20°C/min to 170°C, held isothermal for 7.5 min, then increasing at a rate of 3°C/min to 275°C (only 260°C for BPX-70) which was held isothermal until the end. The given conditions result in GC runs of around 50 min and represent a good compromise between speed of analysis and efficiency of separations. Optimizations for specific critical separations were obtained with a slower temperature programme of a total run time around 90 min: initial temperature 90°C, held for 2 min, then increasing at a rate of 20°C/min to 130°C then increasing at a rate of 2°C/min to 275°C (only 260°C for BPX-70) which was held isothermal until the end.

Chromatographic data were acquired on an HP Vectra i386/i387 personal computer with the Chemstation Hewlett-Packard 3365 software.

Gas chromatography-mass spectrometry

A Finnigan Mat ITS-40 ion trap mass spectrometer interfaced to a Varian 3400 gas chromatograph and a Compaq/386 computer was used for the mass spectrometric analysis of the technical PCB mixtures. Full scan spectra were run in the electron impact (EI) mode from 50 to 550 amu every second. Selected ion traces were software reconstructed as follows (m/z): Mono-CBs 186+188, di-CBs 222+224+226, tri-CBs 254+256+258, tetra-CBs 290+292+294, penta-CBs 324+326+328, hexa-CBs 358+360+362, hepta-CBs 394+396+398, octa-CBs 430+432+434, nona-CBs 464+466+468, and deca-CB 498+500+502. Injections were performed manually (2 μl) in the splitless mode (split open after 2 min) at 280°C. The detection limit obtained with the ITS-40 was around 50 pg (full spectrum of CB 77 at a signal to noise ratio of 10) depending on the GC column bleeding. The Aroclors were injected in 2,4,4,-trimethylpentane at concentrations of 0.8 μg/μL (total PCB) and the individual PCB standards at concentrations around 1-2 ng/μl. The Varian 3400 gaschromatograph was equipped with the same columns as for GC-ECD analysis and run under identical conditions with the only exception being that the carrier gas was helium (at 24 cm/s). This was necessary as the ion trap needs a specific pressure of helium to work properly. The use of helium in stead of hydrogen as carrier gas did not alter the gas chromatographic elution of the CB congeners significantly.

Peak assignments

The identification of the peaks for each column followed the same procedure as previously described [11,14]. Briefly described, it was based on *i*) co-injections of authentic reference compounds and *ii*) electron impact mass spectrometric data on peaks for which no reference compounds were available for this study, derived from GC/ITS-40 analysis of the Aroclor mixtures (A1016-A1262).

The mass spectrometric data were used in the following way:

1) Full scan chromatograms established the degree (or degrees) of chlorination of individual peaks. Confirmations of partial structures where *o,o*'-substitution was involved were based on the relative intensity of the [M-Cl];+ fragment ion[6,16].
2) Selected ion chromatograms obtained for each chlorination class were constructed and, when possible, peaks were identified with reference compounds. The selected ion chromatograms on the polar phases investigated in this paper were compared to selected ion chromatograms obtained with a SE-54 stationary phase assigned from published chromatographic data (obtained with all 209 PCBs available as authentic reference compounds)[8,17]. Selected ion chromatograms are much simpler than ECD chromatograms and change only marginally from column to column[14]. Using the knowledge of the quantitative composition of the Aroclor mixtures[2,18], and the structural principles for CB retention on polar phases[11], the peaks for which no standards were available could be assigned in the selected ion chromatograms.
3) Finally, the retention data from the selected ion chromatograms were unified for each column and transferred to ECD chromatograms of an Aroclor mixture representing a broad range of chlorination degrees (A1016, A1232, A1248 and A1262, 1:1:1:1).

RESULTS AND DISCUSSION

For all chlorination classes the selected ion chromatograms obtained with the SIL-13 column were very similar to the reference chromatograms of SE54 or SIL-8, and no problems in peak assignments were encountered. Thus, the tentative peak assignments on SIL-13 (CBs: 5, 7, 9, 12, 17, 19, 22, 24, 25, 27, 32, 34, 41, 43, 45, 46, 51, 59, 63, 67, 71, 83, 85, 90, 91, 97, 99, 102, 107, 130, 131, 132, 134, 135, 144, 146, 147, 155, 160, 174, 175, 176, 177, 178, 179, 193, 197, 198, 200) have a very high level of confidence. The DB-17 and the SIL-8/HT-5 columns produced selected ion chromatograms somewhat similar to those of SIL-8 or SIL-13. Tentative peak assignments on DB-17 and SIL-8/HT-5 (CB: 5, 7, 9, 12, 22, 32, 41, 51, 85, 102, 131, 135, 144, 146, 174, 175, 176, 197, 198, 200) are also of a high level of confidence. BPX-70, however, produced rather different selected ion chromatograms and the tentative peak assignments for the low intensity peaks which sometimes co-elute with higher chlorinated congeners and therefore have some MS indifference (CBs: 24, 34, 45, 63, 67, 83, 130, 131, 134, 135, 144, 197, 198) may not be so certain as the tentative assignments of the high-intensity peaks or the low-intensity peaks which elute clearly separated (CB: 5, 7, 9, 12, 17, 19, 22, 25, 27, 32, 41, 46, 51, 59, 71, 85, 91, 97, 99, 131, 132, 146, 155, 174, 175, 176, 177, 178, 179, 193, 200).

The ECD chromatograms of the A1016-A1262 mixture obtained with the four columns

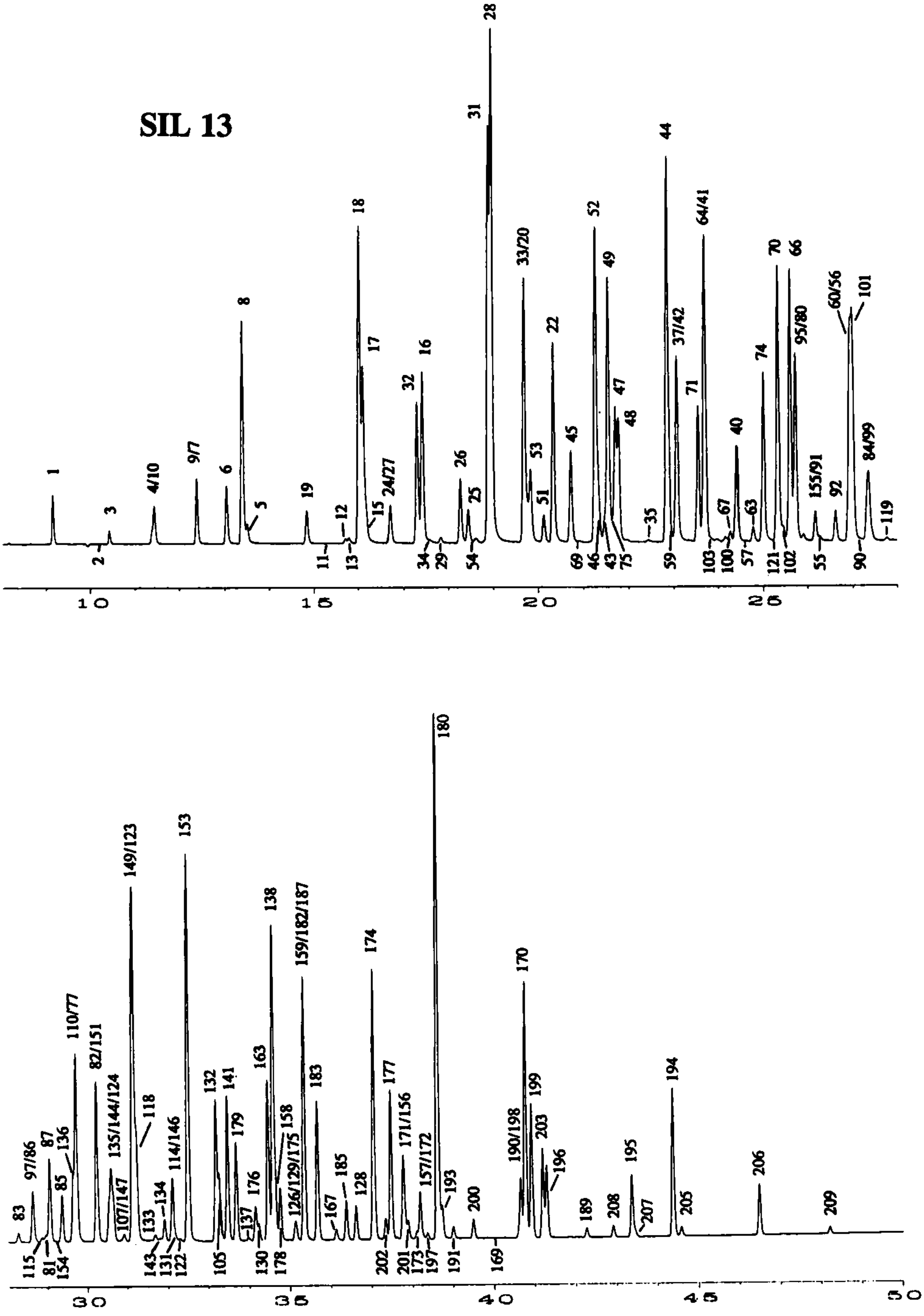

Figure 1. ECD-chromatogram of an Aroclor mixture (A1016, A1232, A1248, A1260 1:1:1:1) on the SIL-13 column.

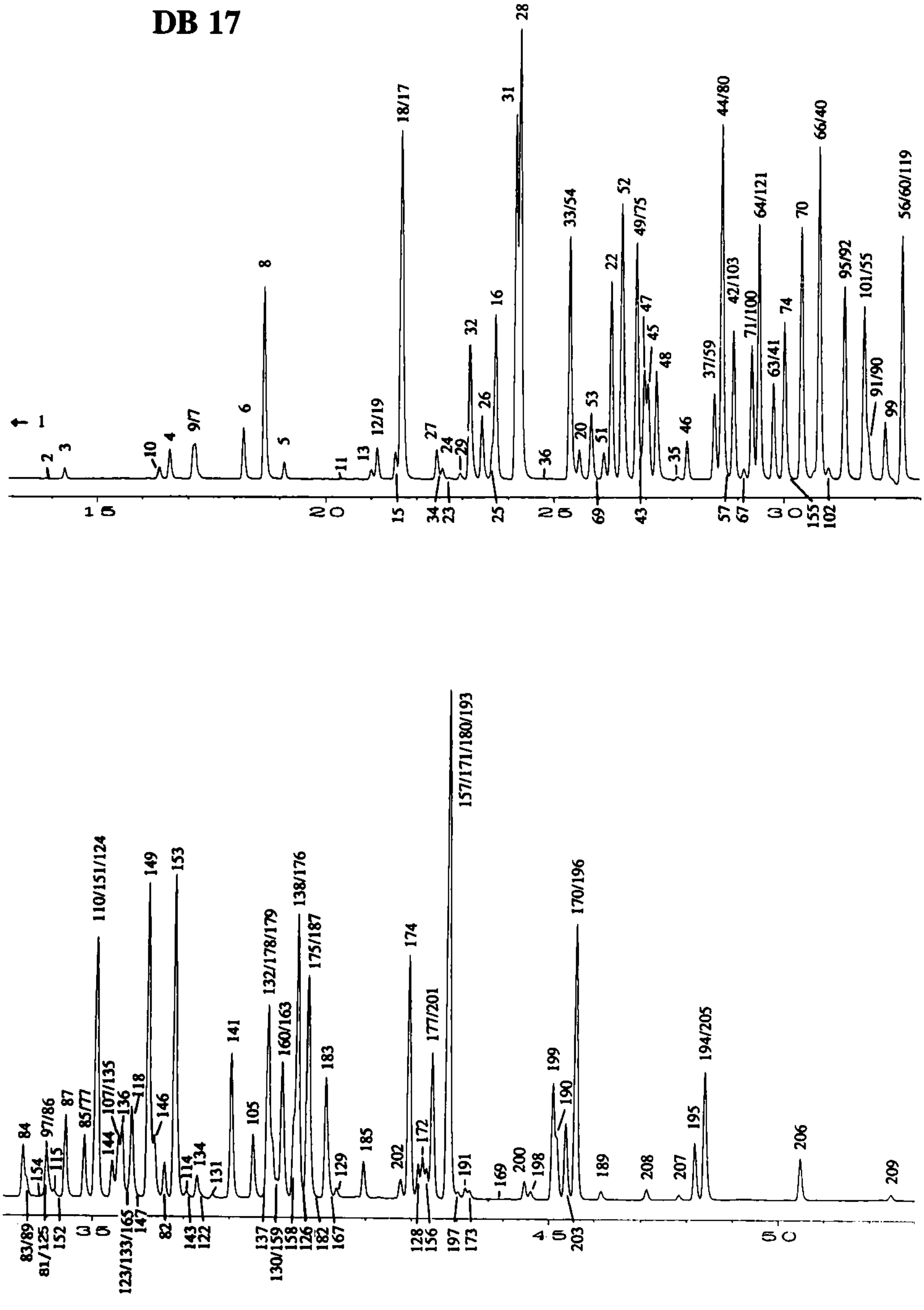

Figure 2. ECD-chromatogram of an Aroclor mixture (1016, A1232, A1248, A1260 1:1:1:1) on the DB-17 column.

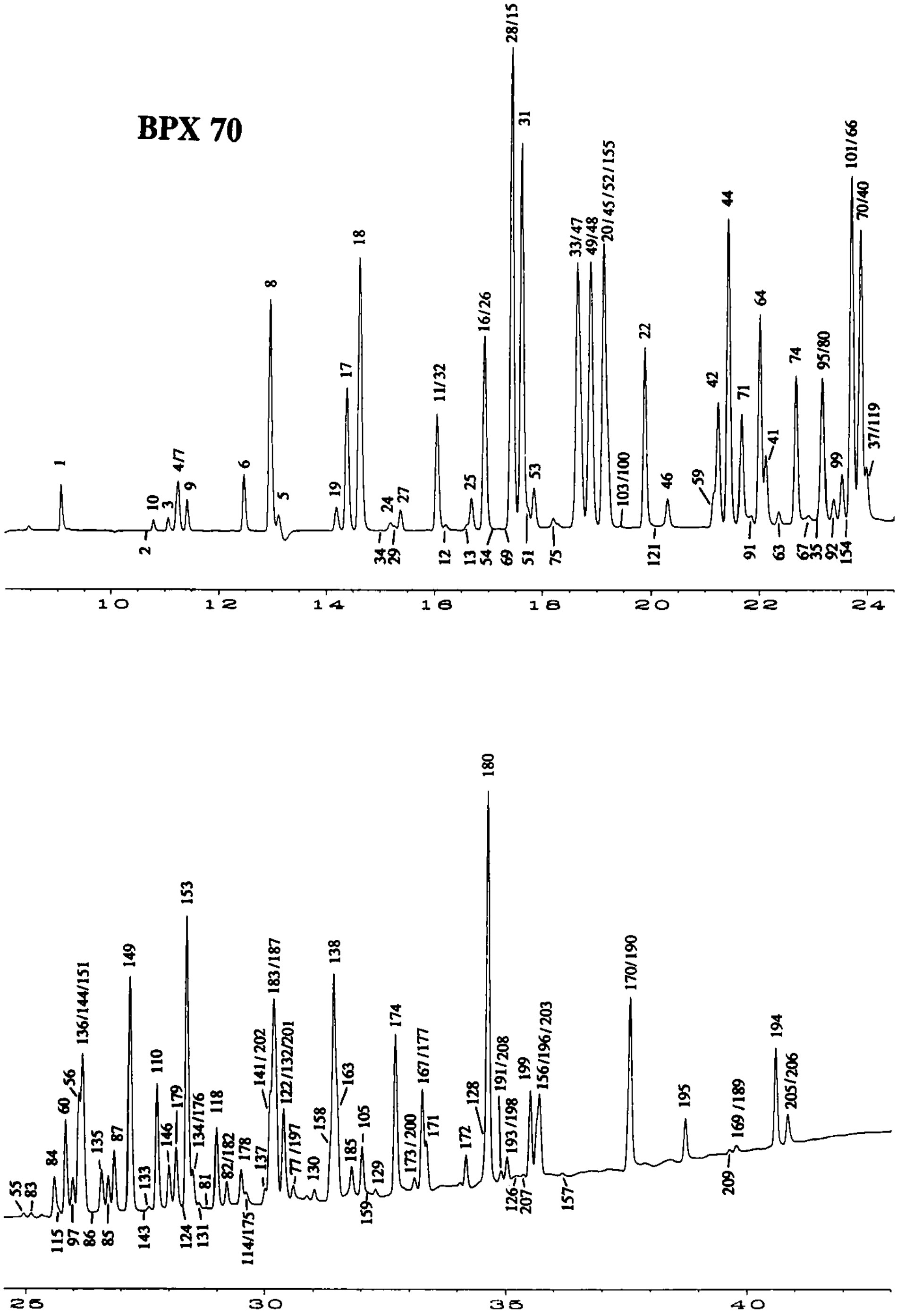

Figure 3. ECD-chromatogram of an Aroclor mixture (1016, A1232, A1248, A1260 1:1:1:1) on the BPX-70 column.

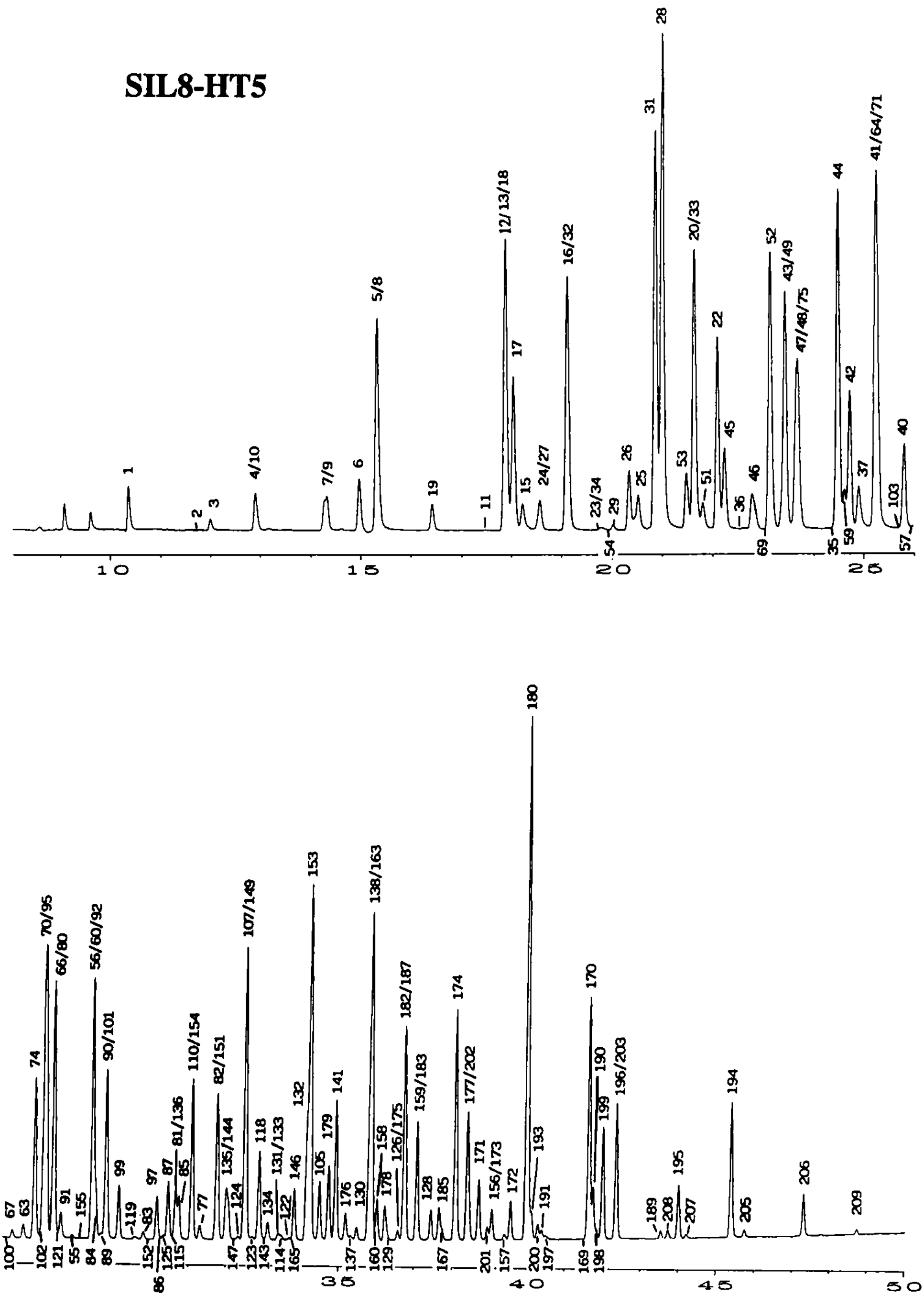

Figure 4. ECD-chromatogram of an Aroclor mixture (A1016, A1232, A1248, A1260 1:1:1:1) on the SIL-8/HT-5 column.

are shown in Figures 1–4. None of the investigated columns managed to separate all the congeners present in the technical mixtures. However, the only six congeners (CBs: 60, 81, 124, 144, 149 and 160) which until now have been impossible to separate from co-elutants on any high temperature resistant stationary phase[9-14] could be separated on at least one of the investigated columns.

A reasonable criterion for a sufficient separation proves to be less than 10% interference from a co-eluting congener. It is well-known that this criterion is met for symmetric peaks when the separation is just visible and when the peak heights are used for quantification[19]. Interfering CBs by HRGC-ECD analysis according to this criterion are listed in Table 1 (toxic congeners [20] are listed in bold). Although MS detection may deteriorate GC separations due to dead volumes in the ion source, CBs with different numbers of chlorine substituents may be resolved better by selected ion monitoring MS analysis than HRGC-ECD analysis. Critical separations for which the interference can be reduced to less than 10% by HRGC-MS are listed in brackets in the table. Several GC oven temperature programmes were run for each column. For some co-eluting CBs the change in temperature programme leads to an improvement of the separation. These congeners are marked in the table with a "#" sign.

Effects of diphenyl in the stationary phase on CB retention

The substituion of diphenyl groups into the dimethylsiloxane polymer as in SIL-13 (15%) and DB-17 (50%) leads to an alteration in the elution order of the CBs. A close comparison of the selected ion chromatograms from a previous investigation[11] (SIL-5 and SIL-8) and the present investigation (SIL-13 and DB-17) revealed some structural principles for the effect on CB retention of diphenyl in the stationary phase. It has previously been found that one determining factor for retention is the degree of *ortho*-substitution i.e. by increasing the polarity of the stationary phase through substitutions with cyanopropyl groups into the dimethylsiloxane polymer the retention time increases more for CBs with none or a few *ortho*-substitutions than for CBs with many *ortho*-substitutions. The same effect is obtained by increasing the amount of diphenyl in the stationary phase from 0% to 5%. However, when the amount of diphenyl in the stationary phase is increased further through 15% to 50% the determining factor is no longer the *ortho*-substitution but the presence of *vicinal* non-substituted carbon atoms in the biphenyl skeleton which increase retention on SIL-13 and DB-17. This can be seen when the rôle of the substitution pattern in the two rings is studied closer: Increasing the amount of diphenyl in the stationary phase from 0% to 5% changed the retention time (relative to CB-209) in the following way (2-, 3-, and 4- represent the *ortho-*, *meta-* and *para*-position, respectively):

mono-CBs.	increased in the order: 2->>3->4-
di-CBs.	decreased in the order: 2,4-~2,5-~2,6->3,5->2,3->3,4-
tri-CBs.	increased in the order: 2,3,4->3,4,5-and
	decreased in the order: 2,4,6->>2,3,6-~2,4,5->2,3,5-
tetra-CBs.	increased in the order: 2,3,4,5->>2,3,5,6-~2,3,4,6-

Table 1. Co-eluting congeners on four narrow-bore GC columns with polar stationary phases.

PCB;	SIL-13	DB-17	SIL-8/HT-5	BPX70
Monochlorobiphenyls				
1	-	-	-	-
2*	-	-	-	-
3	-	-	-	-
Dichlorobiphenyls				
4	10#	-	10	7
5	-	-	8	-
6	-	-	-	-
7	9	9#	9#	4
8	-	-	5	-
9	7	7#	7#	-
10	4#	-	4	-
11*	-	-	-	32
12*	-	19	18	-
13*	-	-	18	-
15	17	-	-	28
Trichlorobiphenyls				
16	-	25	32	26
17	-	18	-	-
18	-	17	(13*)	-
19	-	(12*)	-	-
20	33	-	33	45,52,155*
22	-	-	-	-
23*	no data	-	34*	no data
24	27	-	27	-
25	-	16	-	-
26	-	-	-	16
27	24	34*	24	-
28	-	-	-	(15)
29	-	-	-	-
31	-	-	-	-
32	-	-	16	(11*)
33	20#	54*	20	47.
34*	-	24,27	23*	-
35*	-	-	-	80*,95
36*	no data	-	-	no data
37	42	59	-	119
Tetrachlorobiphenyls				
40	-	66	-	70
41	64	63	64/71	-
42	(37)	103*	-	-
43	49#	47,49,75	49	no data

CBs which have been indicated as priority congeners[20] due to toxicity are highlighted.
*=Congener found at insignificant concentrations in technical mixtures.
#=Separation can be improved by optimizing temperature programme.
()=Interference is reduced to less than 10% by use of MS.

Table 1 (continued). Co-eluting congeners on GC columns with polar stationary phases.

PCB;	SIL-13	DB-17	SIL-8/HT-5	BPX70
44	-	80*	-	-
45	-	-	-	(20),52,155*
46	52	-	-	-
47	-	-	48,75#	(33)
48	-	-	47,75#	49
49	43#	43,75*	43	48
51	-	-	-	(31)
52	-	-	-	(20),45,155*
53	-	-	-	-
54*	-	(33)	-	-
55	-	101	-	-
56	60,101#	60,119	60,92	-
57*	-	44	-	no data
59	44	(37)	-	42
60	56,101#	56,119	56,92	-
63	-	41	-	-
64	41	121*	41,71	-
66	-	40	80*	101
67	100*	-	-	-
69	-	-	-	-
70	121*	-	95,102	40
71	-	100*	41,64	-
74	-	155*	-	-
75	49	43,49	47#,48#	-
77	110	85	-	197
80*	95	44	66	95
81*	-	86,97,125*	136	-
Pentachlorobiphenyls				
82	151	-	151	182*
83	-	84#,89*	-	-
84	99#	-	(56,60),92#	-
85	-	(77)	136#	-
86*	97	(81),97,125*	97	-
87	-	-	115	-
89*	no data	83	-	no data
90*	-	91,101	101	no data
91	155*	90,101	-	-
92	95	-	(56,60),84#	-
95	80*	92	(70),102*	80*
97	86*	(81*),86*,125*	-	-
99	84#	-	-	-
100	(67)	(71)	-	103*
101	(56#,60#)	(55)	90*	(66)
102*	(70)	-	(70,95)	no data
103*	-	(42)	-	100*
105	132#	-	-	-
107	147*	135,144	149	no data

CBs which have been indicated as priority congeners[20] due to toxicity are highlighted.
*=Congener found at insignificant concentrations in technical mixtures.
#=Separation can be improved by optimizing temperature programme.
()=Interference is reduced to less than 10% by use of MS.

Table 1 (continued). Co-eluting congeners on GC columns with polar stationary phases.

PCB#	SIL-13	DB-17	SIL-8/HT-5	BPX70
110	(77)	124*,151	154*	-
114	146	-	-	175
115	-	-	87	-
118	149	-	-	-
119	-	(56,60)	-	(37)
121*	(70)	(64)	(66,80*)	-
122*	-	134#	-	132,201
123*	149	133*,165*	-	no data
124*	135,144	110,151	-	153
125*	no data	(81*),86*,97	-	no data
126*	129,175	-	175	-
Hexachlorobiphenyls				
128	-	-	-	180
129	175,126*	-	178	-
130	176#	159	-	-
131	114,146	-	133*	-
132	-	179	153#	(122*),201
133*	-	(123),165*	131	-
134	-	-	-	176
135	124*,144	(107)	144	-
136	110#	-	(81*)	144,151
137	-	-	-	-
138	-	176	160#*,163#	158,163
141	-	-	-	183,187,202
143*	-	-	-	-
144	(124*),135	-	135	136,151
146	(114)	-	-	-
147*	(107#)	(118)	-	no data
149	(123*)	-	107*	-
151	(82)	(110,124*)	(82)	136,144
152*	no data	-	-	no data
153	-	-	-	-
154*	-	-	(110)	-
155*	(91)	(74)	-	(20,45,52)
156	171	-	173	196#,203#
157	172	171,180,193	-	-
158	-	-	-	138
159*	182*,187	130	183	-
160*	163	163	138#,163#	no data
163	(160*)	160*	138#,160*#	138
165*	no data	(123),133*	-	no data
167	-	183	185#	177
169*	-	-	-	189#

CBs which have been indicated as priority congeners[20] due to toxicity are highlighted.
*=Congener found at insignificant concentrations in technical mixtures.
#=Separation can be improved by optimizing temperature programme.
()=Interference is reduced to less than 10 % by use of MS.

Tabel 1 (continued). Co-eluting congeners on GC columns with polar stationary phases.

PCB#	SIL-13	DB-17	SIL-8/HT-5	BPX70
Heptachlorobiphenyls				
170	-	196	-	190
171	(156)	(157),180,193	-	-
172	(157)	-	-	-
173	-	-	(156)	200
174	-	-	-	-
175	(126*,129)	187	126*	(114)
176	-	(138)	-	(134)
177	-	201	202	(167)
178	-	-	-	-
179	-	(132)	-	-
180	-	(157),171,193	-	(128)
182*	187	-	187	(82)
183	-	-	(159)	187
185	-	-	-	-
187	(159),182*	175	182*	183
189	-	-	-	169*#
190	198	-	-	170
191	-	-	-	208
193	180	(157),171,180	180#	198
Octachlorobiphenyls				
194	-	205*	-	-
195	-	-	-	-
196	-	(170)	203	203
197	-	-	-	(77)
198	(190)	-	(190)	(193)
199	-	-	-	-
200	-	-	-	(173)
201	(156,171)	(177)	-	(122*,132)
202	-	-	(177)	(141)
203	-	-	196	196
205*	-	194	-	206
Nonachlorobiphenyls				
206	-	-	-	(205*)
207*	(195)	-	-	-
208	-	-	-	(191)
Decachlorobiphenyl				
209	-	-	-	-

CBs which have been indicated as priority congeners[20] due to toxicity are highlighted.
*=Congener found at insignificant concentrations in technical mixtures.
#=Separation can be improved by optimizing temperature programme.
()=Interference is reduced to less than 10% by use of MS.

Increasing the amount of diphenyl in the stationary phase further through 15% to 50% changed the retention time (relative to CB-209) in the following way:

mono-CBs: decreased in the order: 2->>3->4-
di-CBs: increased in the order: 2,6->2,3->3,4-and
decreased in the order: 3,5->2,5-~2,4-
tri-CBs: increased in the order: 2,3,6-~2,3,4-and
decreased in the order: 2,4,6->2,3,5->2,4,5-~3,4,5-
tetra-CBs: remained unchanged: 2,3,4,5-~2,3,5,6-~2,3,4,6-

This shift in retention mechanism is interesting. One explanation may be a shift in the sterical structure of the stationary phase upon increasing its concentration of diphenyl. With a low percentage of diphenyl in the dimethylsiloxane polymer the diphenyl groups rotate and vibrate freely and are completely accessible for retention of the CB molecules. When the percentage of diphenyl is high their rotation and vibration are moderated by neighbor diphenyl groups and the stationary phase is forced into a more ordered structure. It is perceivable that in such a (hypothetical) ordered structure of packed layers of phenyl groups the retention of CB molecules with their large chlorine substituents may be partially sterically hindered. Furthermore, it is likely that CB molecules with the presence of *vicinal* non-substituted carbon atoms in the biphenyl skeleton are less sterically hindered than others as they may slide in between the packed layers of phenyl groups in the stationary phase.

The effect on the retention time of the diphenyl in the stationary phase can be illustrated by some examples of separation of critical pairs: The CB-77 (3,4-3',4'-tetra) and the CB-110 (2,3,6-3',4'-penta) have identical substitution pattern in one ring so the retention effect of the other ring can easily be studied. These two congeners are separated on SIL-5 (0% diphenyl) with CB-77 eluting first. With 5% diphenyl in the stationary phase (SIL-8) the 2,3,6-substituted ring in CB-110 is pulling more downwards than the 3,4-substituted ring in CB-77, which results in co-elution. When the amount of diphenyl in the stationary phase is increased to 15% the effect is reversed and the 2,3,6-substituted ring in CB-110 is pulling upwards but not significantly more than the 3,4-substituted ring in CB-77 and therefore co-elution is also seen on SIL-13. However, when the amount of diphenyl in the stationary phase is increased to 50% the resulting upwards pull from the 2,3,6-substituted ring in CB-110 becomes significantly stronger than the upwards pull from the 3,4-substituted ring in CB-77, and separation is again obtained on DB-17 with CB-77 as the first eluting peak. Another example of essentially the same phenomenon is the retention behavior of CB-118 (2,4,5-3',4'-penta) and CB-149 (2,4,5-2',3',6'-hexa). On SIL-5 (0% diphenyl) these two congeners co-elute. With 5% diphenyl in the stationary phase (SIL-8) the 2,3,6-substituted ring in CB-149 is pulling downwards more than the 3,4-substituted ring in CB-118, which results in separation with CB-149 eluting first. When the amount of diphenyl in the stationary phase is increased to 15% the effect is reversed and the 2,3,6-substituted ring in CB-149 is pulling upwards just slightly more than the 3,4-substituted ring in CB-149, and therefore the two peaks approach each other on SIL-13. However, when the amount of diphenyl in the stationary phase is increased to 50% the resulting upwards pull from the 2,3,6-substituted ring in CB-149 becomes significantly stronger than the upwards pull from the 3,4-substituted ring in CB-118, and separation is again obtained on DB-17 but this time with CB-118 as the first eluting peak.

Effects of cyanpropyl in the stationary phase on CB retention

The substitution of cyanopropyl groups into the dimethylsiloxane polymer as in BPX70 (70%) leads to considerable changes in the elution order of the CBs. A close comparison of the selected ion chromatograms from BPX70 with those from a previous investigation[11] (SIL-5, SIL-19 and SIL-88) revealed some structural principles for the effect on CB retention of cyanopropyl in the stationary phase. The data from BPX70 confirms that the determining factor is the degree of *ortho*-substitution i.e. by increasing the polarity of the stationary phase through substitutions with cyanopropyl groups into the dimethyl siloxane polymer the retention time increases more for CBs with none or a few *ortho*-substitutions than for CBs with many *ortho*-substitutions. A secondary factor is the presence of *vicinal* non-substituted carbon atoms in the biphenyl skeleton which increase the retention when the amount of cyanopropyl groups in the stationary phase is increased.

In our previous investigation of stationary phases for CB separations[11] we did not note the secondary effect of *vicinal* non-substituted carbon atoms. However, this effect becomes clear when the rôle of the substitution pattern in the two rings is studied closer. Increasing the amount of cyanopropyl in the stationary phase changed the retention time (relative to CB-209) in the following way (2-,3-, and 4- represent the *ortho*-, *meta*- and *para*-position, respectively):

mono-CBs.	4-increased, 3-approx. constant, 2-decreased
di-CBs.	increased in the order: 3,4->2,3 and decreased in the order: 2,4->2,5->2,6-~3,5-
tri-CBs.	increased in the order: 3,4,5->2,3,4-and decreased in the order: 2,4,6->>2,4,5-~2,3,5->2,3,6-
tetra-CBs.	decreased in the order: 2,3,5,6-~2,3,5,6->2,3,4,5-

Performance of the GC columns

In evaluating the performance of a GC column for PCB separation an important parameter is the total number of separated congeners detected with the most common detector, the ECD. However when a more selective detector as the MS is used CBs with a different number of chlorine substituents may be resolved even though they co-elute. The use of MS in PCB analysis is becoming increasingly popular and therefore the total number of separated congeners detected with MS must be noted. Finally, the ability of separating priority CBs and indicator CBs as mentioned in the introduction may be a criterion for selecting a GC column. The performance with this respect of the four GC columns investigated in the present paper is discussed in the following.

SIL-13. The substitution of 15% diphenyl into the dimethylsiloxane polymer does not result in increased bleeding when ECD or MS is used as detector. As seen in Figure 1 there is a total of 22 peaks in the chromatogram of this column containing one or more co-eluting CBs. A total of 91 congeners elute as clearly resolved peaks and can be analyzed by GC-ECD in technical mixtures without interference. By GC-MS the number is 106. A further number of 11 congeners can be resolved by a slower temperature programme resulting in a total run period of 90 min. Out of the 36 priority congeners 19 can be analyzed by GC-ECD and 23 by GC-MS. Among the seven indicator CBs five can be analyzed by GC-ECD and six by GC-MS. SIL-13 is the only published thermo-stabile column to date, which allows for interference-free analysis of the priority congener CB-196 and of CB-132 (a potential interference for the priority congeners CB-153 and CB-105). SIL-13 is also the only published column able to resolve CB-43 from interference. This can be used to prove the presence of this congener at significant concentrations in technical mixtures (Figure 5). Finally, the important indicator congener CB-138 is resolved on SIL-13 and altogether this column seems to be an interesting alternative to the widely used 5% diphenylpolysiloxane columns such as SE54 and SIL-8.

DB-17. With the development of thermo-stabile chemically bonded phases it has become feasible to perform routine analysis of PCBs on heavily modified polysiloxane phases. The substitution of 50% diphenyl into the dimethylsiloxane polymer results in increased bleeding when EI-MS is used as detector but not with ECD. This bleeding may interfere when ultra trace analysis is required. The OV-17 type of columns (here DB-17) has been a popular choice for confirmation of PCB analysis on SE54. Complete retention data for PCBs were not available on this stationary phase, so for the CB congeners to be confirmed the analyst was obliged to accept the result obtained with the column giving the lowest result notwithstanding that there might have been co-elution on both columns. With the retention data from the present investigation the OV-17 type columns can be used for confirmational analysis on a sound basis. As seen in Figure 2, the total number of resolved CBs present at significant concentrations in technical mixtures is 92 by GC-ECD and 111 by GC-MS. An additional number of four CBs can be resolved by a slower temperature programme with a total run period of 2 hours. Out of the 36 priority congeners 20 can be analyzed by GC-ECD and 24 by GC-MS. Among the seven indicator CBs four can be analyzed by GC-ECD and five by GC-MS. DB-17 is the only published thermo-stabile column to date, which allows for interference-free analysis of CB-20 and CB-182. These two congeners have often been mistaken from CB-33 and CB-187, respectively. With the present data from the DB-17 column it is proven that CB-20 is only a minor compound compared to CB-33 and that CB-182 is not present in technical mixtures at significant levels (Figure 5).

BPX-70. The incorporation of the aromatic group silphenylene into the siloxane backbone is introduced *i)* to delocalize the increase in the vibrational/rotational energy produced during temperature programming of the phase and *ii)* to reduce cyclic siloxane degradation products. The benefit from these effects is an increase in the maximum operating temperature of the phase and a reduction in the column bleed which is otherwise observed for polar stationary phases. As expected the background noise (bleed) in the GC-ITS analysis was

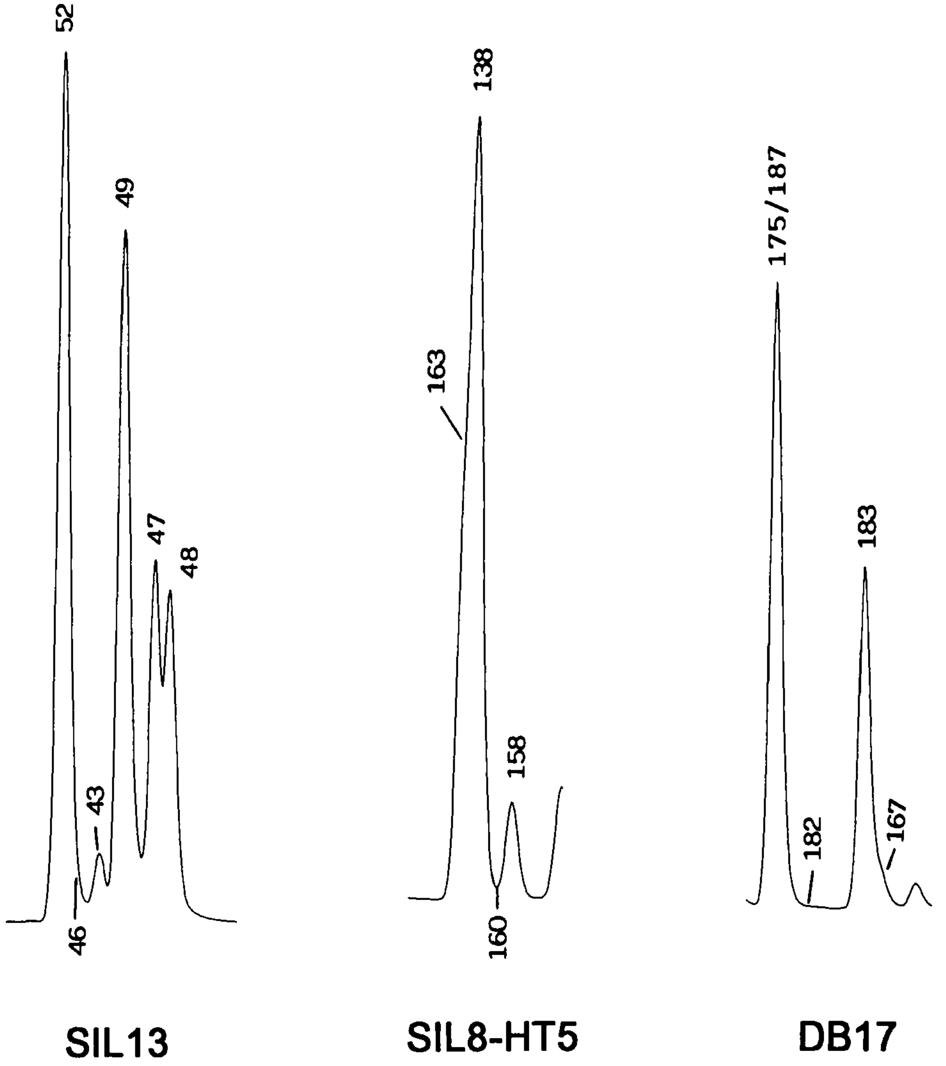

Figure 5. Critical separations of an Aroclor mixture (A1016, A1232, A1248, A1260 1:1:1:1) on the SIL-13, SIL-8/HT-5 and DB-17 columns run with slow temperature programmes.

significantly lower than previously seen for SIL-88. However an increase in the background signal by GC-ECD was seen at elevated temperatures (Figure 3). This column bleed may interfere with trace level analysis of PCBs. In order to minimize the bleed the BPX-70 column was run at a milder temperature programme than the other columns in the present investigation. The substitution of 70% cyanopropyl into the silphenylene siloxane polymer drastically changed the elution profile of PCBs. Several lower chlorinated CBs moved upwards in the chromatogram and caused co-elutions with higher chlorinated CBs. For this reason, BPX70 is not a very good choice for PCB analysis. As seen in Figure 3, the total number of resolved CBs present at significant concentrations in technical mixtures is 80 by GC-ECD and 100 by GC-MS. An additional number of two CBs can be resolved by a slower temperature programme with a total run period of 90 min. Out of the 36 priority congeners 13 can be analyzed by GC-ECD and 19 by GC-MS. Among the seven indicator CBs 2 can be analyzed by GC-ECD and five by GC-MS. Despite the mediocre overall performance of BPX-70 for PCB analysis this phase has some specific advantages over other stationary phases. CB-52 and CB-138 are the only indicator congeners not completely resolved with GC-MS on BPX70. However the co-elutants are minor compounds found as a right and a left shoulders (CB-163/CB-138/CB-158 and CB-20/CB-52/CB-45). The interference on the peak height of CB-52 and CB-138 from their two co-elutants is therefore not very high and a reasonably good measurement of all seven indicator congeners can be obtained by GC-MS with this column. Furthermore, BPX-70 is the only published thermo-stabile column to date which allows for interference-free analysis of CB-41, CB-60 and the priority congener CB-75.

SIL-8/HT-5. Series coupling of HT-5 with SIL-8 in one GC oven has previously been shown to improve PCB separations significantly[12,14]. This combination was optimized for separation of a set of toxic congeners listed by Safe, 1990[12,21]. Unfortunately this combination was not able to resolve all seven indicator congeners from co-elutants. As there is no single column able to do this task it was decided to optimize a series combination for GC-ECD analysis of the seven indicator CBs based on the knowledge of the retention mechanisms of the columns described earlier in this paper and in a previous paper[11]. Again, our choice fell again on the temperature resistant columns SIL-8 and HT-5, but this time with a relative magnification of the retention mechanisms of the carborane-siloxane co-polymer (HT-5) obtained by shortening the SIL-8 column. This shorter version of SIL-8/HT-5 offers the interference-free analysis of all seven indicator CBs in technical PCB mixtures when run at slow temperature programmes. CB-101 and CB-138 does not elute as baseline separated peaks. However, for the former of these CBs the potential co-elutant (CB-90) is not found at significant concentrations in technical PCB mixtures and for the latter the potential co-elutant (CB-163) is minor and appearing as a barely visible shoulder (Figure 5). Quantitative measurements have proven that CB-163 does not interfere significantly on the peak height of CB-138 with this shorter version of SIL-8/HT-5. As seen in Figure 4, the total number of resolved CBs present at significant concentrations in technical mixtures is 98 by GC-ECD and 107 by GC-MS. An additional number of ten CBs can be resolved by a slower temperature programme with a total run period of 90 min. Out of the 36 priority congeners 27 can be analyzed by GC-ECD and 29 by GC-MS. Compared with the previous, longer version of SIL-8/HT-5 the shorter version (run with a slow temperature programme) has on one hand gained separation for 22 congeners (CBs: 53, 84, 85, 101, 114, 122, 123, 128, 130, 136, 138, 141, 146, 149, 157, 160, 163, 172, 176, 179, 187, 201) but on the other hand lost separation for 12 congeners (47, 48, 70, 81, 82, 95, 132, 126, 136, 151, 159, 183). This short version of SIL-8/HT-5 is the only published column to date, which allows for interference-free analysis of CB-160. This congener has often been mistaken for CB-163.

With the present data (Figure 5) it is proven that CB-160 is not present in technical mixtures at significant concentrations.

CONCLUSION

The analysis of PCBs as single congeners is a very complicated task. The present investigation provides complimentary data to a previous investigation of commercially available, chemically and thermally stabile, narrow-bore columns representing a broad range of GC phase compositions[11-14] for the separation of PCBs. No single column is able to resolve all 209 congeners, neither the 150 congeners present in technical mixtures, nor the 32 congeners given special priority owing to their toxicity and occurrence in the environment[4] and most surprisingly, not even the set of seven indicator congeners incorporated in the legislation of some European countries and international bodies.

It is clear that for unambiguous determination of CB congeners parallel analysis on at least two columns is essential.

With the data from the present and previous investigations[11-14] it has become possible to analyze separately each CB congener present in technical mixtures at significant concentrations on at least one column. The choice of columns in future PCB analysis may therefore be made on the basis of the set of congeners to be quantified. The best two-column combination seems to be the short SIL-8/HT-5 (with a slow temperature programme giving a total run period around 90 min) in parallel with DB-17, which allow for the interference-free analysis of 134 CBs (32 priority CBs) with GC-ECD and 141 (2 priority CBs) with GC-MS. We have been using this combination in our laboratories for routine dual-column, dual-ECD analysis in a single GC oven over a 12-month period with more than 1000 injections of environmental samples without noticeable deteriorations of the column performance. In addition to the high number of CBs ten organochlorine pesticides can be analyzed in the same extracts free of CB-interference[22].

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