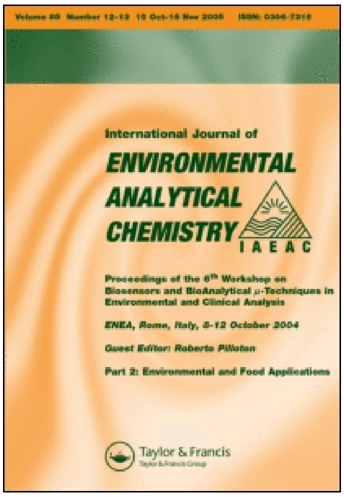

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Interference from 2,3,5,6-3',4'-Hexachlorobiphenyl (CB 163) in the Determination of 2,3,4-2',4',5'-Hexachlorobiphenyl (CB 138) in Environmental and Technical Samples

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LETTER TO THE EDITOR

INTERFERENCE FROM 2,3,5,6-3′,4′-HEXACHLOROBIPHENYL (CB 163) IN THE DETERMINATION OF 2,3,4-2′,4′,5′-HEXACHLOROBIPHENYL (CB 138) IN ENVIRONMENTAL AND TECHNICAL SAMPLES

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(Received 20 January 1990)

Dear Sir,

In *Intern. J. Environ. Anal. Chem.* **66** (1989), 155–161, Roos *et al.*[1] reported on problems encountered in the determination of 2,3,4-2′,4′,5′-hexachlorobiphenyl (CB 138) in environmental samples. These authors indicated that an interference was present for the capillary column gas chromatographic (HRGC) determination of CB 138. This interference was tentatively identified (without standard) as 2,3,5,6-3′,4′-hexachlorobiphenyl (CB 163). We have synthesized CB 163 and can confirm that it co-elutes with CB-138 on the widely used HRGC phase for polychlorinated biphenyl (PCB) analysis, 5% phenyl, 95% methylpolysiloxane (CP-SIL8-CB) even on a 50 m column. However, we managed to separate CB 163 from CB 138 on the very polar phase, bis-cyanopropylphenyl polysiloxane (SP-2330), using a 30 m column. We have quantified CB 163 and CB 138 in various environmental and technical samples and have found CB 163 to CB 138 ratios from 1:10 up to 3:10.

Since the quantification of CB 138 is incorporated in legislations in many countries and international organizations, we believe these findings to be of great importance to the society of analytical chemists involved in PCB analysis.

EXPERIMENTAL

Apparatus

HRGC analysis was performed with a DANI 8520 gas chromatograph equipped with an electron capture detector (ECD) operated in the splitless mode. The

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cleaned-up extracts (saponification[2] followed by acid-silica/silica open column chromatography[3]) were injected on two different fused silica capillary columns. For the 50 m × 0.25 mm (0.26 μm film) CP-SIL8-CB (Chrompack, Milan, Italy) the following GC conditions were used: injector temperature, 90–270 °C ballistically; linear helium carrier gas velocity, 23 cm/s; temperature programme, 0.6 min at 90 °C, then at 10 °C/min to 180 °C, then at 1.5 °C/min to 270 °C; detector, 310 °C. For the 30 m × 0.25 mm (0.20 μm film) SP-2330 (Supelco, Milan, Italy) milder conditions were necessary; injector temperature, 90–240 °C ballistically, linear helium flow 24 cm/s; temperature programme, 0.6 min at 90 °C, then at 10 °C/min to 150 °C, then at 1.5 °C/min to 240 °C; detector, 310 °C. For both columns the ECD make-up gas was 5% methane in argon at 60 ml/min.

Data were acquired and processed on a Chromstation/2 system (Spectra Physics, Milan, Italy).

HRGC-MSD (mass selective detection) analysis for confirmation was conducted on a HP-5970 MSD coupled to an HP-5890 GC (Hewlett-Packard, Milan, Italy). The MSD was run in either full scan mode (140–400 m/z every 0.25 sec) or single ion monitoring (SIM) mode (100 ms dwell time, 70 eV electron impact (EI), 2400 V multiplier, 280 °C source, perfluorobutylamine for mass calibration at m/z: 69, 219 and 502). The GC was equipped with the same SP-2330 column as for GC-ECD analysis or with a 30 m × 0.25 mm (0.25 μm film) SE-54 column (Hewlett-Packard, Milan, Italy): Carrier gas, He, 20 cm/s; splitless injector temperature, 260 °C; temperature programme, 90 °C, at 10 °C/min to 120 °C, then at 1.5 °C/min to 260 °C.

Synthesis

CB 163 was prepared via diazo coupling of 1,2,4,5-tetrachlorobenzene and 3,4-dichloroaniline.[4] A 95.6% purity was achieved after vacuum distillation, triple Florisil open-column chromatography and preparative thin-layer chromatography on tetrabutylammonium phosphate-impregnated silica plates.[5] Identity was checked with HRGC-MSD and ^{1}H-nuclear magnetic resonance on a 500 MHz Bruker instrument. (ppm, TMS): 7.34 (H-6′, dd, J = 8.25, 2.03 Hz); 7.62 (H-2′, d, J = 2.01 Hz); 7.78 (H-5′, d, J = 8.26 Hz); 7.96 (H-4, S).

Chemicals

Glassware, solvents and chemicals were pesticide grade or cleaned as usual for trace analysis. Blanks were run on a routine basis for quality assurance. The following individual chlorobiphenyl calibrants were obtained from the Community Bureau of Reference (BCR) of the EEC in Brussels: 2,4-4′-trichlorobiphenyl (CB 28), 2,5-2′,5′-tetrachlorobiphenyl (CB 52), 2,4,5-2′,5′-pentachlorobiphenyl (CB 101), 2,4,5-3′,4′-pentachlorobiphenyl (CB 118), 2,3,4-2′,4′,5′-hexachlorobiphenyl (CB 138), 2,4,5-2′,4′,5′-hexachlorobiphenyl (CB 153) and 2,3,4,5-2′,4′,5′-heptachlorobiphenyl (CB 180). Other PCBs potentially interfering in the samples considered, were not quantified but tentatively identified from their EI spectrum and their well-known elution patterns of technical mixtures: 2,5-4'-trichlorobiphenyl (CB 31), 2,5-2',4'-tetrachlorobiphenyl (CB 49), 2,3,5-2,4'-pentachlorobiphenyl (CB 90), 2,3,6-2',5'-pentachlorobiphenyl (CB 95), 2,4,5-2',4'-pentachlorobiphenyl (CB 99), 2,3,4-3',4'-pentachlorobiphenyl (CB 105), 2,3,6-2',3',4'-hexachlorobiphenyl (CB 132), 2,4,5-2',3',6'-hexachlorobiphenyl (CB 149), 2,3,4,6-3',4'-hexachlorobiphenyl (CB 158), 2,3,5,6-3',4',5'-heptachlorobiphenyl (CB 193). Other PCBs used in a column performance mixture were synthesized as described elsewhere.[6] As internal (syringe) standards 2,4-dichlorobenzyl heptyl ether and 2,4-dichlorobenzyl tetradecanyl ether were used.[7]

Samples

Certified mackerel oil and cod-liver oil were obtained from BCR[8] together with a sewage sludge analysed by the BCR PCB working group (to be certified). Human milk fat previously analysed for PCBs[9] was from Italian mothers. All these samples have a known concentration of several PCB congeners. The determination of these congeners in parallel with CB 163 served as a quality control.

RESULTS AND DISCUSSION

The EI spectrums of pure standards of CB 163 (top) and CB 138 (bottom) are shown in Figure 1. The presence of chlorine in the *ortho* position of both phenyl rings in CB 138 facilitates the formation of a halonium ion intermediate with a mass cluster around m/z 325. This *ortho* effect does not occur for CB 163. When a mixture of the standards CB 138 and CB 163 was injected onto the HRGC-MSD equipped with a SE-54 column, a single slightly non-symmetric GC peak was obtained in the full-scan mode. In SIM at 325 m/z (monitoring CB 138 only), the retention time shifted downwards 3 sec (data not shown). However, the resulting GC resolution of 0.16 was not sufficient for quantitation, so another GC column (SP-2330) was employed. In Figure 2 parts of the GC-ECD chromatograms obtained after injections on the SP-2330 columns (left) and the CP-SIL 8-CB column (right) of a standard mixture without (upper) and with (lower) addition of CB 163, are shown. It is evident that only the former very polar phase is able to separate CB 163 from CB 138.

In order to verify that the columns were operated under near optimal conditions a cleaned-up extract of a used oil contaminated with PCB was injected and the key separations were examined. The relevant parts of the chromatograms on CP-SIL 8-CB and SP-2330 are shown in Figures 3 and 4, respectively. A baseline resolution of CB 52, CB 153 and CB 180 from any PCB interference was achieved with CP-SIL 8-CB. The pairs CB 31/CB 28, CB 90/CB 101 and CB 149/CB 118 were sufficiently separated (resolution better than 1.0) to avoid interference when peak heights were used for quantification. The CB 138/CB 163 peak was nearly baseline separated from CB 158. To the authors' knowledge resolution of these PCB congeners in environmental samples better than those shown in Figure 2 is rarely achieved. When the analyses for this study were performed the CP-SIL 8-

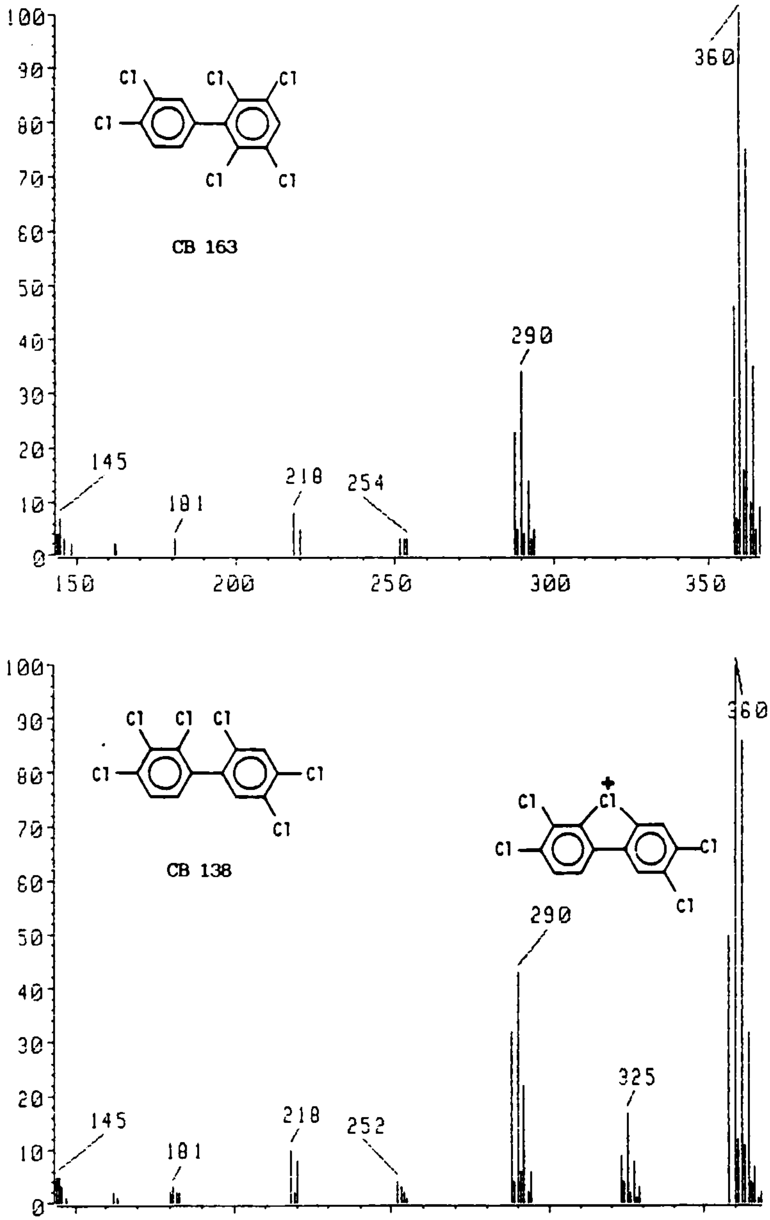

Figure 1 EI spectrums of CB 163 (top) and CB 138 (bottom).

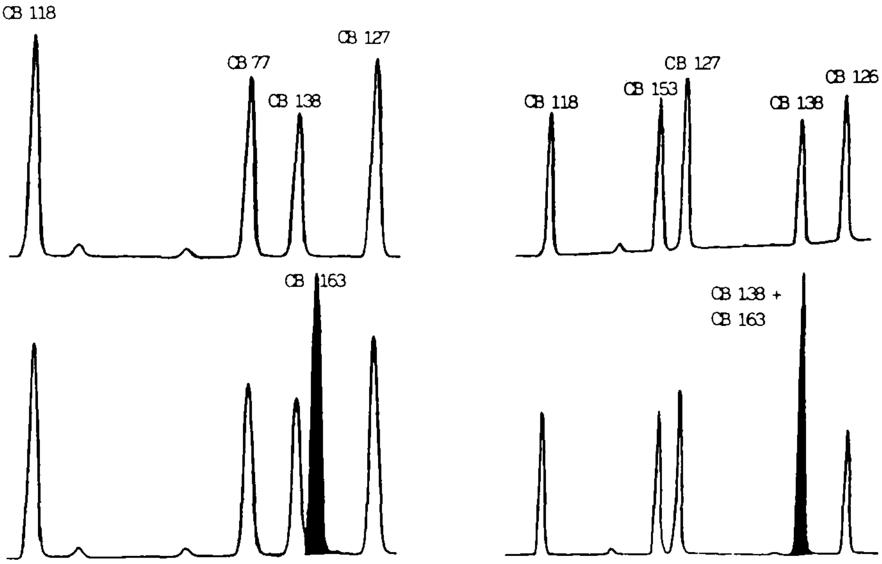

Figure 2 Gas chromatograms of a standard mixture with addition of CB 163 (bottom) and without addition of CB 163 (top) on a SP-2330 (left) and a CP-SIL 8-CB (right) column.

CB column had been used in analysis of environmental samples for more than 6 months with a number of injections exceeding 500. Separation problems have never been encountered as opposed to data reported by Duinker *et al.*[10].

As shown in Figure 4, the very polar SP-2330 column gave baseline separation of CB 118 from other PCB interference. Also CB 28, CB 52 and CB 101 seemed sufficiently separated from interference, whereas the distorted peak shape of CB 153 and CB 180 indicated a major interference. CB 101 was resolved from CB 99 and CB 95, but a minor contribution from CB 90 could not be excluded. The problematic CB 138 was separated from the interference of CB 163.

The quantitative results of the analysis of environmental samples are shown in Table 1. There seems to be a reasonable agreement (within the standard deviation) between results from the different columns and the previously obtained results for all PCB congeners other than CB 138. As is evident from Table 1 and Figure 5, all environmental samples contained CB 163 in the proportion of 22–33% relative to CB 138. The results on CB 138 obtained by the CP-SIL 8-CB column are therefore too high.

In the more complex technical samples there was an overall good agreement between the results from the two different columns (Table 2). SP-2330 tended to give higher results for CB 101, CB 153 and CB 180 possibly due to interferences as shown in Figure 4. Even though CB 163 was present at the level of 9–18% results from the CP-SIL 8-CB column (where CB 138 and CB 163 are co-eluting) were not higher than from the SP-2330 column. Whether this is due to the presence of another interfering PCB isomer or due to analytical uncertainty which is in the range of 10% needs to be investigated further, e.g. by multidimensional gas chromatography as described by Duebelbeis *et al.*[11]

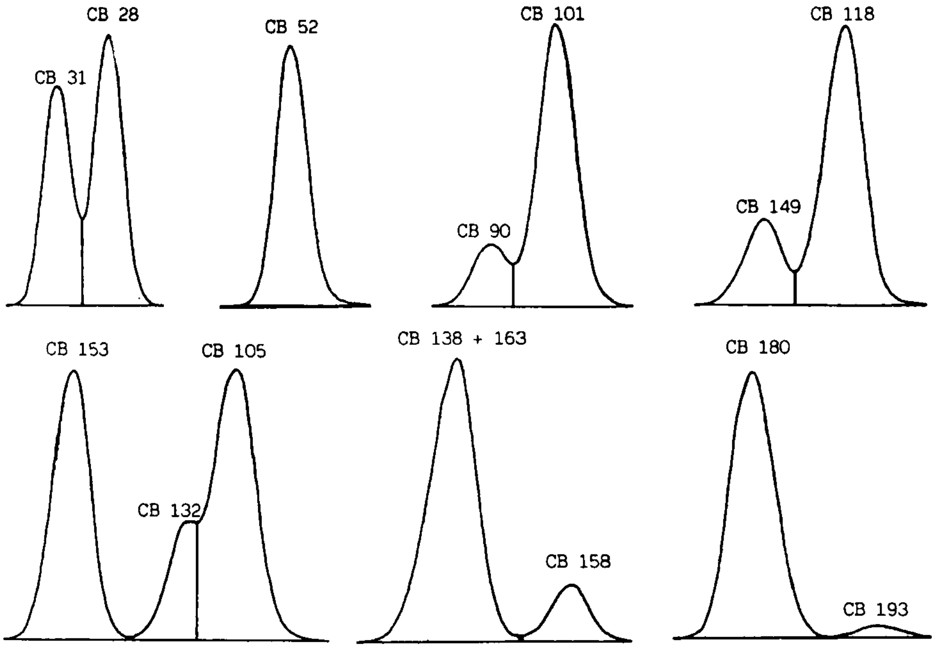

Figure 3 Critical separations on CP-SIL 8-CB after the injection of a used oil extract.

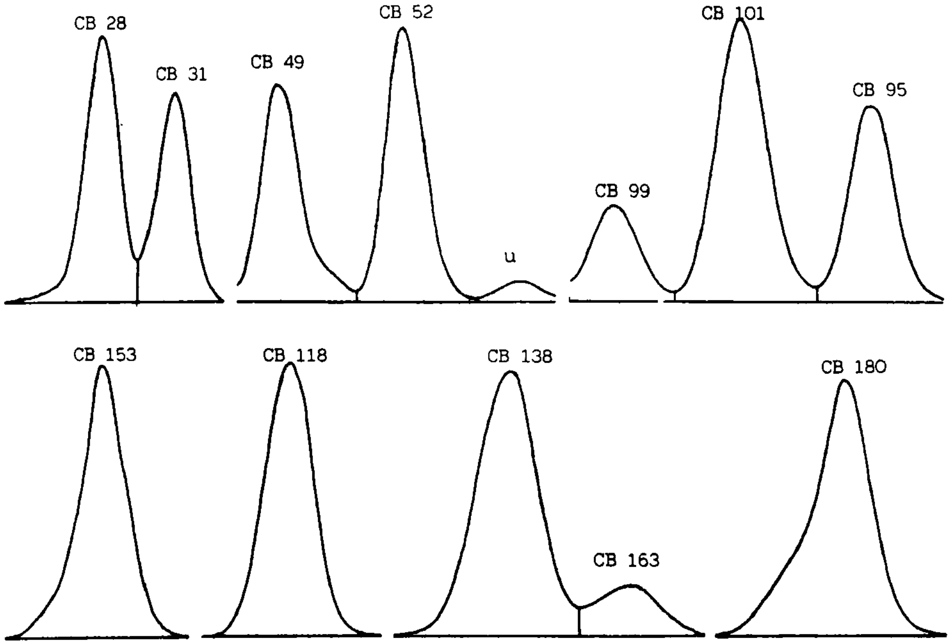

Figure 4 Critical separations on SP-2330 after the injection of a used oil extract.

able 1 Concentrations of eight PCB cogeners in environmental samples determined on two different GC columns

atrix	*Columns*	*IUPAC PCB numbers*							
		28	*52*	*101*	*118*	*138*	*163*	*153*	*180*
ackerel oil	SP-2330[a]	17[f]	57	159	140	209	70	308	79
g/g d.w.	CP-SIL 8-CB[b]	17	57	161	144	265	—	337	68
	Certified[c]	23 ± 6	62 ± 12	165 ± 13	143 ± 30	274 ± 38	—	318 ± 32	73 ± 17
od liver oil	SP-2330	58	129	374	435	618	156	891	338
g/g d.w.	CP-SIL 8-CB	54	152	362	430	791	—	867	244
	Certified	68 ± 12	149 ± 28	370 ± 30	456 ± 48	765 ± 66	—	938 ± 59	282 ± 33
ewage sludge	SP-2330	99	46	151	94	231	53	313	300
g/g d.w.	CP-SIL 8-CB	95	53	130	78	288	—	307	329
	Certified[d]	100 ± 20	78 ± 16	134 ± 21	97 ± 21	—	—	288 ± 36	313 ± 49
uman milk fat	SP-2330	17	7	n.d.	54	117	26	187	122
g/g d.w.	CP-SIL 8-CB	15	7	n.d.	56	146	—	197	113
	Larsen[e]	21 ± 4	23 ± 10	10	56 ± 14	153 ± 44	—	178 ± 34	111 ± 35

[a] Injected on a 30 m SP-2330 fused silica capillary column.

[b] Injected on a 50 m CP-SIL 8-CB fused silica capillary column.

[c] The certified value +SD from Community Bureau of Reference (BCR).

[d] Mean value ±SD from the BCR PCB working group (to be certified).

[e] Mean value ±SD of triplicate determinations by Larsen *et al.* (1989).

[f] Mean value of two replicates.

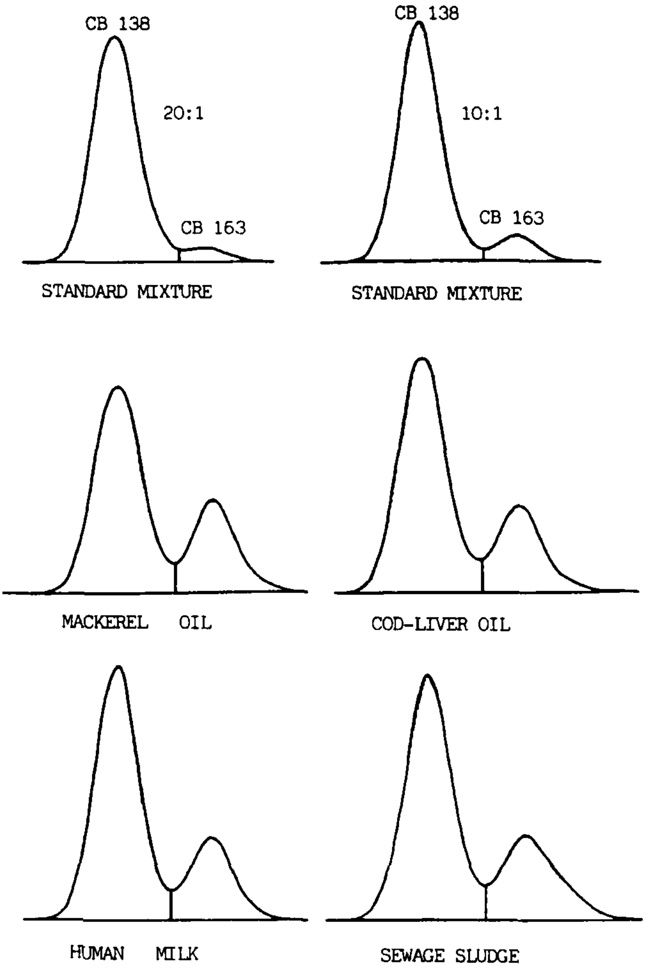

Figure 5 Separation of CB 163 from CB 138 on SP-2330 after injections of standards and environmental sample extracts.

Table 2 Concentrations of eight PCB cogeners in technical samples determined with two different GC columns[a]

Matrix	*Column*	*IUPAC PCB numbers*							
		28	*52*	*101*	*118*	*138*	*163*	*153*	*180*
Askarel	SP-2330	0.5	1.17	5.40	2.89	9.85	1.77	9.23	4.23
% w/w	CP-SIL 8-CB	0.3	1.31	5.23	2.81	9.78	—	9.12	4.14
Aroclor-1254	SP-2330	0.4	0.79	6.75	13.3	7.22	0.63	4.10	0.5
% w/w	CP-SIL 8-CB	0.3	0.93	6.27	13.7	6.81	—	3.54	0.48
Used oil	SP-2330	0.61	1.00	1.70	1.88	1.52	0.18	0.85	0.30
μg/g d.w.	CP-SIL 8-CB	0.47	0.96	1.50	1.72	1.48	—	0.75	0.10

[a]For explanation, see Table 1.

The HRGC-MSD analysis with the SP-2330 column of the environmental and technical samples confirmed the presence of a mass cluster around m/z 325 in the first eluting (CB 138) peak and the absence of this cluster in the later eluting (CB 163) peak (data not shown). Due to the low concentration levels in the samples, the rather low sensitivity of the MSD and matrix interference it was not possible to conduct quantitative analysis by the MSD. High resolution mass spectroscopy (HRMS) is then required. Monitoring of the low-intensity mass fragments around m/z 325 by HRGC-HRMS may not be reproducible enough for precise quantitative work, but provides the opportunity to determine CB 138 without CB 163 interference.

Operating the mass spectrometer in the negative chemical ionization mode was tried by Roos *et al.*[1] without obtaining improved selectivity. A new approach to congener- and isomer-specific PCB analysis was recently demonstrated by Duebelbeis *et al.*[11] in the combination of on-line partial dechlorination and multidimensional GC. Further investigations of the key separations of PCB congeners are needed with this technique.

CONCLUSION

A PCB isomer co-eluting with CB 138 on the widely used HRGC phase CP-SIL 8-CB was found in environmental (22–33% of CB 138) and technical (9–18% of CB 138) samples. This PCB isomer has the same EI spectrum as CB 163 with the same retention time on the GC phase SP-2330. The presence of CB 163 in all investigated samples renders previous results on CB 138 questionable.

Even though CB 163 can be separated from CB 138 on the very polar SP-2330, other PCB congeners may co-elute with CB 138. Especially in the analysis of complex technical mixtures containing a large number of PCB congeners interference may bias CB 138 results. CB 138 can be isolated from any interference by means of multidimension gas chromatography. However, this technique is not easily incorporated in routine analysis. The present results confirm the requirement in GC-ECD analysis of PCBs that analysis must be performed on two different GC phases. Normal procedure has been to use a non-polar (e.g. CP-SIL 8-CB) and a medium polar (e.g. CP-SIL 19-CB) column. The present work suggests that the second phase should be very polar (e.g. SP-2330).

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