APPLICATION PARAMETERS OF A RECENTLY DEVELOPED AND PUBLISHED PCB DETERMINATION METHOD FOR THE ROUTINE USE IN ENVIRONMENTAL ANALYSIS

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The composition of environmental PCB-mixtures varies among the sample types according to local contamination, metabolism by animals etc. For appraising the effects of single congeners on biota, an effective separation and quantification method is necessary. Many papers about this problem were published: effective separation of technical mixtures[1], separation on HT-5 column[2], determination and quantification in environmental samples[3,4,5].

In 1995 we presented a GC-MS method[6] which enables us to identify and to quantify each CB according to its retention time and structure dependent fragmentation pattern produced by electron impact ionization.

The aim of this paper is the presentation of the data which is the prerequisite for the application of our method in routine environmental and foodstuff analysis.

Material and Method

The single CBs, OCs and the PCB-mixtures were purchased from Ehrenstorfer, Amchro, Promochem and Alltech (all Germany). The instrumentation used was a Finnigan MAT SSQ 710 MS coupled to a Varian 3400 GC equipped with a split-splitless capillary injector 1075. Detailed information about the working conditions were already published[6]. The GC-columns used were two HT-5 from SGE (Australia) purchased from Axel Semrau (Germany), length 2x25 m, coupled by a glass press-fit connector from ICT (Germany).

Results and Discussion

The separation of the PCB-mixtures, the identification of the congeners according to their retention times and fragmentation behavior were described[6]. For the quantification, groups of congeners were formed within the degrees of chlorination, one quantification-congener with a median $[M]^+/\Sigma<[M]^+$ value was chosen in each group.

After that, additionell congeners present in Clophen A30/A40/A60 and Aroclor 1254/1260 were examined for their $[M]^+$, $[M\text{-}1Cl]^+$ and $[M\text{-}2Cl]^+$ values. No deviations from the general principles of fragmentation occured which we had established[6]. Thus, for the analysis of environmental samples these congeners were appointed to the suitable groups. In Tab. 1 all CBs and additionally eight OCs (α,β,γ-Hexachlorocyclohexane = HCH, Hexachlorobenzene = HCB, Octachlorostyrole = OCS, p,p'-DDT, p,p'-DDE-and p,p'-DDD) - mostly present in environmental samples - are listed. Three internal standards were added to the samples, two of them (TBB = Tetrabrombenzene, HBB = Hexabrombenzene) were mainly chosen for correction, the third one (DBrN = Dibromnaphthalene) was previously used instead of TBB. For each CB the quantification-congener is listed (Tab. 1, row Quan./Cong.) in order to show the corresponding subgroup membership of the single CB. The two main identification m/z-channels are listed in Tab. 1, too.

ANA (po)

Number	CB/OC	IS	Degree of CL	Quan./Cong.	Mass 1	Mass 2
1	4	TBB	2		222.0	224.0
2	6	TBB	2		222.0	224.0
3	8	TBB	2		222.0	224.0
4	a-HCH	TBB			180.9	218.9
5	19	TBB	3	18	256.0	258.0
6	HCB	TBB			283.8	248.8
7	TBB	TBB			393.7	395.7
8	**18**	**TBB**	**3**	**18**	**256.0**	**258.0**
9	17	TBB	3	18	256.0	258.0
10	b-HCH	TBB			180.9	218.9
11	24	TBB	3	28	256.0	258.0
12	27	TBB	3	28	256.0	258.0
13	DBrN	DBrN			285.9	287.9
14	y-HCH	TBB			180.9	218.9
15	15	TBB	2		222.0	224.0
16	32	TBB	3	28	256.0	258.0
17	16	TBB	3	18	256.0	258.0
18	26	TBB	3	28	256.0	258.0
19	25	TBB	3	28	256.0	258.0
20	31	TBB	3	28	256.0	258.0
21	53	TBB	4	52	291.9	289.9
22	**28**	**TBB**	**3**	**28**	**256.0**	**258.0**
23	51	TBB	4	52	291.9	289.9
24	45	TBB	4	52	291.9	289.9
25	20/33	TBB	3	28	256.0	258.0
26	22	TBB	3	28	256.0	258.0
27	46	TBB	4	52	291.9	289.9
28	**52**	**TBB**	**4**	**52**	**291.9**	**289.9**
29	49	TBB	4	52	291.9	289.9
30	47/48	TBB	4	52	291.9	289.9
31	44	TBB	4	52	291.9	289.9
32	42	TBB	4	52	291.9	289.9
33	96	TBB	5	118	325.9	323.9
34	64	TBB	4	66	291.9	289.9
35	41/71	TBB	4	52	291.9	289.9
36	OCS	TBB			379.7	307.8
37	37	TBB	3	28	256.0	258.0
38	40	TBB	4	52	291.9	289.9
39	95	TBB	5	92	325.9	323.9
40	74	TBB	4	66	291.9	289.9
41	91	TBB	5	92	325.9	323.9
42	**155**	**HBB**	**6**	**155**	**361.8**	**357.8**
43	70	TBB	4	66	291.9	289.9
44	**66**	**TBB**	**4**	**66**	**291.9**	**289.9**
45	**84/92**	**TBB**	**5**	**92**	**325.9**	**323.9**
46	**101**	**TBB**	**5**	**101**	**325.9**	**323.9**
47	56/60	TBB	4	66	291.9	289.9
48	99	TBB	5	101	325.9	323.9
49	83/119	TBB	5	92	325.9	323.9
50	136	HBB	6	153	361.8	357.8
51	86/97	TBB	5	101	325.9	323.9
52	p,p'-DDE	TBB			317.9	246.0
53	87/115	TBB	5	92	325.9	323.9
54	85	TBB	5	101	325.9	323.9
55	110	TBB	5	118	325.9	323.9
56	151	HBB	6	138	361.8	357.8

Table 1,I: Quantitation- Masses, -Congeners (boldtype) and IS of the CBs and OCs (Abbreviations see Text)

Number	CB/OC	IS	Degree of CL	Quan./Cong.	Mass 1	Mass 2
57	135	HBB	6	138	361.8	357.8
58	144/147	HBB	6	138	361.8	357.8
59	82	TBB	5	92	325.9	323.9
60	149	HBB	6	138	361.8	357.8
61	134	HBB	6	138	361.8	357.8
62	107	TBB	5	118	325.9	323.9
63	**118**	**TBB**	**5**	**118**	**325.9**	**323.9**
64	179	HBB	7	189	393.8	397.8
65	132/146	HBB	6	138	361.8	357.8
66	114	TBB	5	118	325.9	323.9
67	p,p'-DDD	TBB			235.0	237.0
68	176	HBB	7	189	393.8	397.8
69	**153**	**HBB**	**6**	**153**	**361.8**	**357.8**
70	141	HBB	6	138	361.8	357.8
71	105	TBB	5	118	325.9	323.9
72	137	HBB	6	138	361.8	357.8
73	130	HBB	6	138	361.8	357.8
74	178	HBB	7	180	393.8	397.8
75	160/163/164	HBB	6	155	361.8	357.8
76	p,p'-DDT	TBB			235.0	237.0
77	**138**	**HBB**	**6**	**138**	**361.8**	**357.8**
78	158	HBB	6	155	361.8	357.8
79	175/189	HBB	7	180	393.8	397.8
80	129	HBB	6	138	361.8	357.8
81	183	HBB	7	180	393.8	397.8
82	202	HBB	8	194	431.8	427.8
83	**126**	**TBB**	**5**	**126**	**325.9**	**323.9**
84	185	HBB	7	180	393.8	397.8
85	174	HBB	7	180	393.8	397.8
86	128	HBB	6	138	361.8	357.8
87	201	HBB	8	194	431.8	427.8
88	159	HBB	6	153	361.8	357.8
89	177	HBB	7	180	393.8	397.8
90	167	HBB	6	155	361.8	357.8
91	171	HBB	7	180	393.8	397.8
92	197	HBB	8	194	431.8	427.8
93	200	HBB	8	194	431.8	427.8
94	HBB	HBB			549.5	551.5
95	172	HBB	7	180	393.8	397.8
96	156	HBB	6	155	361.8	357.8
97	157	HBB	6	155	361.8	357.8
98	**180/191**	**HBB**	**7**	**180**	**393.8**	**397.8**
99	193	HBB	7	189	393.8	397.8
100	199	HBB	8	194	431.8	427.8
101	170	HBB	7	180	393.8	397.8
102	196/203	HBB	8	194	431.8	427.8
103	169	HBB	6	155	361.8	357.8
104	190	HBB	7	189	393.8	397.8
105	208	HBB	9	209	465.7	461.7
106	207	HBB	9	209	465.7	461.7
107	195	HBB	8	194	431.8	427.8
108	**189**	**HBB**	**7**	**189**	**393.8**	**397.8**
109	**194**	**HBB**	**8**	**194**	**431.8**	**427.8**
110	205	HBB	8	194	431.8	427.8
111	206	HBB	9	209	465.7	461.7
112	**209**	**HBB**	**10**	**209**	**497.7**	**499.7**

Table 1,II: Quantitation- Masses, -Congeners (boldtype) and IS of the CBs and OCs (Abbreviations see Text)

The identification m/z-channels were chosen out of the $[M]^+$-isotope cluster and depending on high sensitivity and no or diminutive signal interferences.The interferences originate not only by the matrix but also by the fragment ions produced from coeluating higher chlorinated CBs. For a more secure determination the m/z-channels for $[M-1Cl]^+$ and/or $[M-2Cl]^+$ were measured additionally.

Di-CBs

For the Di-CBs the m/z 226.0 ($[M]^+$) was chosen additionally. No overlapping with fragment ions of higher degrees of chlorination occur because of different retention times.

Tri-CBs

The distinctive marks of the Di-CBs are also valid for the Tri-CBs where m/z 221.0 ($[M-Cl]^+$) was examined too.

Tetra-CBs

For the Tetra-CBs m/z 255.0 ($[M-Cl]^+$) was measured additionally. A distinction between their $[M]^+$ and the fragment ions of the Penta- and Hexa-CBs is possible. The isotope-ratio of m/z 289.9 to m/z 291.9 derived from the Tetra-CBs ($[M]^+$) differs from the ratio produced by the Hexa-CBs ($[M-2Cl]^+$), (Tetra-CBs: 0.7, Hexa-CBs: 2.0). The $[M-1Cl]^+$ Penta-CB fragment ion m/z 288.9 does not occur in the pattern of the Tetra-CBs. By means of these m/z-channels a selective and simultaneous determination of Tetra-, Penta- and Hexa-CBs in one GC-peak has become possible[6].

Penta-CBs

The difference between the Penta-CBs and the Hexa-CBs becomes visible by examining the $[M-1Cl]^+$ Hexa-CB fragment ion with m/z 322.9, this fragment is not produced by the Penta-CBs. It is also possible to measure the $[M-2Cl]^+$ Hexa-CB fragment ion with m/z 287.9, one of the most often formed ions of Hexa-CBs $[M-2Cl]^+$ and which is missing at the Penta-CB cluster. There is no overlapping between Penta-CBs and Hepta-CBs because of different retention times.

Hexa- and Hepta-CBs

The distinction between Hexa- and Hepta-CBs is possible because of different ratios of the ions m/z 287.9 to m/z 288.9 to m/z 289.9. These fragment ions result from Hexa-CBs ($[M-2Cl]^+$) and Hepta-CBs ($[M-3Cl]^+$) with ratios of 8:1:10 (Hexa-CBs) and 1:10:1.5 (Hepta-CBs). Furthermore, the m/z 356.8-channel (Hepta-CB, $[M-1Cl]^+$) was considered. This fragment ion is not present in the Hexa-CB cluster. Additionally, the m/z-channel 356.8 can be applied to differentiate between Hepta- and Octa-CBs $[M-2Cl]^+$. The ratios between this channel and m/z 357.8 for Hepta- and Octa-CBs are different (Hepta-CBs: 7.4, Octa-CBs: 0.07). There is no overlapping between Hepta-and Nona-CBs GC peaks

Octa- and Nona-CBs, Deca-CB

For Octa- and Nona-CB one more ion out of the $[M]^+$-pattern was considered: m/z 433.8 for the Octa- and m/z 463.7 for the Nona-CBs. No further ion besides the $[M]^+$ (m/z 497.7, m/z 499.7) were measured for the Deca-CB. No problematic overlappings occur in these three degrees of chlorination.

Time-windows

Six time-windows were established for the optimal identification and quantification of the CBs. Several m/z-channels were measured in two or more windows according to the different retention times of the CBs. The m/z-channels for the OCs - investigated also in the routine analysis - and the internal standards (IS) were added. Times were given in minutes and hundredth minutes. The degrees of chlorination, dechlorination, name of the OCs or IS are placed in parentheses.

Window 1 (Time 10.00 - 13.80)

180.9 (HCH), 218.9 (HCH), 221.0 (Tri-1CL), 222.0 (Di), 224.0 (Di), 226.0 (Di), 248.8 (HCB-1Cl), 256.0 (Tri), 258.0 (Tri), 283.8 (HCB), 285.9 (DBrN), 287.9 (DBrN), 312.8 (TBB-1Br), 314.8 (TBB-1Br), 393.7 (TBB), 395.7 (TBB).

Window 2 (Time 13.81 - 19.10)

221.0 (Tri-1Cl), 222.0 (Di), 224.0 (Di), 255.0 (Tetra-1Cl), 256.0 (Tri), 258.0 (Tri), 288.9 (Penta-1Cl), 289.9 (Tetra), 291.9 (Tetra), 307.8 (OCS-2Cl), 322.9 (Hexa-1Cl), 323.9 (Penta), 325.9 (Penta), 357.8 (Hexa), 361.8 (Hexa), 379.7 (OCS).

Window 3 (Time 19.11 - 21.10)

246.0 (DDE-2Cl), 256.0 (Tri), 288.9 (Penta-1Cl), 289.9 (Tetra), 291.9 (Tetra), 317.9 (DDE), 322.9 (Hexa-1Cl), 323.9 (Penta), 325.9 (Penta), 357.8 (Hexa), 361.8 (Hexa), 397.8 (Hepta).

Window 4 (Time 21.10 - 23.95)

235.0 (DDD+DDT), 237.0 (DDD+DDT), 288.9 (Penta-1Cl), 322.9 (Hexa-1Cl), 323.9 (Penta), 325.9 (Penta), 356.8 (Hepta-1Cl), 361.8 (Hexa), 390.8 (Octa-1Cl), 393.8 (Hepta), 397.8 (Hepta), 427.8 (Octa), 431.8 (Octa).

Window 5 (Time 23.96 - 25.70)

322.9 (Hexa-1Cl), 323.9 (Penta), 325.9 (Penta), 356.8 (Hepta-1Cl), 357.8 (Hexa), 361.8 (Hexa), 390.8 (Octa-1Cl), 393.8 (Hepta), 397.8 (Hepta), 427.8 (Octa), 431.8 (Octa), 549.5 (HBB), 551.5 (HBB).

Window 6 (Time 25.71 - 40.00)

322.9 (Hexa-1Cl), 356.8 (Hepta-1Cl), 357.8 (Hexa), 361.8 (Hexa), 390.8 (Octa-1Cl), 393.8 (Hepta), 397.8 (Hepta), 427.8 (Octa), 431.8 (Octa), 433.8 (Octa), 461.7 (Nona), 463.7 (Nona), 465,7 (Nona), 497.7 (Deca), 499.7 (Deca).

Conclusions

Our presented method permits for the first time the precise qualitative and quantitative determination of single PCB-congeners in environmental samples by means of only few single CBs[6]. In technical mixtures 133 CBs were detected[6], in environmental samples between 66 and 97[7,8] CBs were quantified. Differences in PCB pattern between species[7] and within species[8] were discovered as well as seasonal changes in Σ PCB and pattern of fish dependent on environmental conditions, too[7]. This method is very suitable for environmental analysis and is used in different laboratories with success. The determination of the toxic Non-ortho CBs is possible without any additional separation steps. Furthermore, the investigation of foodstuff is possible. CB-138 which is important for these kind of analysis is totally separated from CB-158, CB-160, CB-163 and CB-164, coeluating congeners on SE-54 columns. Therefore,realistic values for this CB can be determined.

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