AN ACCURUTE GC/ECNI-MS-SIM QUANTITATION METHOD FOR POLYCHLORINATED TERPHENYLS IN THE PRESENCE OF HIGH PCB EXCESSES

Vetter W[1], Rosenfelder N[1]

[1]University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, D-70593 Stuttgart, Germany

Introduction

Polychlorinated terphenyls (PCTs) are a class of industrial organohalogens produced and used in parallel with polychlorinated biphenyls (PCBs)[1]. Chemically they emerge from three connected phenyl units. Based on biphenyl, the third ring can be attached in *ortho*-, *meta*- or *para*-position (**Figure 1**).

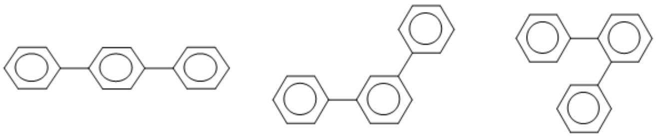

Figure 1: Backbone of *o*-, *m*-, and *p*-terphenyl

The degrees of chlorination theoretically span from mono- to tetradecachlorination, and the theoretical variety is >8,000 congeners[2]. PCT producers were located in the USA, Japan, and in Europe (Germany, Italy, France) [3]. It was estimated that the PCT production amounted to about 5% of the PCBs [1]. In contrast to PCBs, comparably little is known about the concentrations and the fate of PCTs in the environment. Both the GC elution and the mass range of PCTs severely overlap with PCBs. Therefore, environmental data on PCTs are scarce. PCT levels were reported for marine food from Spain [4], aquatic biota and sediment [5-7], as well as in terrestrial samples and human tissue [3]. However, it was mentioned that results from one study are difficult to compare with those of other studies [3]. Consequently, de Boer *et al*. noted that the determination of PCTs by GC/MS is difficult where errors are easily made [1]. Large errors of 30–40% were reported to occur in GC/MS selected ion monitoring (SIM) quantification of PCTs [6]. In this study, we developed a GC/MS-SIM method which allows for the accurate determination of PCTs in the presence of PCBs in environmental samples. The method takes into account the presence of two orders of magnitude higher abundant PCB concentrations which overlap with PCTs. A mathematical algorithm was used to proportion the contribution of PCBs and PCTs in peaks resulting from response to the ions chosen for quantification.

Materials and methods

Samples, chemicals and methods. A blubber sample extract of a monk seal (*Monachus monachus*) from Mauritania (Africa, 1997) was used for the measurements. The chemicals and the clean-up procedure were reported elsewhere [8]. Reference standards (individual PCB congeners) were from Dr. Ehrenstorfer (Augsburg, Germany) and LGC Promochem (Wesel, Germany).

GC/ECNI-MS analysis. Analyses were run with a 7890/5975C GC/MS (Agilent Technologies, Waldbronn, Germany). Transfer line and ion source temperature were set to 300 °C and 150 °C. Methane 5.5 was used as the reagent gas at a flow rate of 40 mL/min. Sample solutions (1 µL) were injected by means of an Agilent 7673 GC/SFC automatic injector operated in pulsed splitless mode. An HP-5MS column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) was installed in the GC. The flow of the carrier gas (helium 5.0) was set at 1.2 mL/min. The GC oven program started at 50 °C (hold time for 2 min), then at 10 °C/min to 300 °C (hold time 29 min). Quantitation of PCTs was performed in the selected ion monitoring (SIM) mode. PCTs were determined in the following seven time windows (quantification ion underlined): (i) from 8-19 min: *m/z* 334, 336, 338 for triCTs and 368, <u>370</u>, 372 tetraCTs; (ii) from 19-22 min: *m/z* 368, <u>370</u>, 372 for tetraCTs and *m/z* 404, <u>406</u>, 408 for pentaCTs; (iii) from 22-25 min: *m/z* 404, <u>406</u>, 408 for pentaCTs and *m/z* 438, <u>440</u>, 442 for hexaCTs; (iv) from 25-28 min: *m/z* 438, <u>440</u>, 442 for hexaCTs and *m/z* 472, <u>474</u>, 476 for heptaCTs; (v) from 28-31 min: *m/z* 472, <u>474</u>, 476 for heptaCTs and *m/z* 508, 510, 512 for octaCTs; (vi) from 31-34 min: *m/z* 508, 510, 512 for octaCTs and *m/z* 540, 542, 544 for nonaCTs; (vii) from 34-55 min: *m/z* 540, 542, 544 for nonaCTs and *m/z* 574, 576, 578 for decaCTs.

Results and discussion

PCT isomers span over the same retention time range as PCBs bearing two more chlorine substituents. Unfortunately, the masses of the molecular ions of these co-eluting groups of PCTs and PCBs generally show some overlap. As an example, M^+ of tetraCTs (*m/z* 366) is 8 u higher than M^+ of hexaCBs at *m/z* 358, and the dominant isotope peak of tetraCTs $[M+2]^+$ at *m/z* 368 is higher by 10 u. Due to the much higher abundance of PCBs in environmental samples [6], the interference of tetraCTs by hexaCBs is still immense (**Figure 2**). For instance, the interference of a tetraCT by hexaCBs at *m/z* 368 is 1.2%, 10.7%, and 54.5% for tetraCT : hexaCB ratios of 1:1, 1:10, and 1:100, respectively. The latter scenario, i.e. PCTs : PCBs ~ 1:100 is rather common for environmental samples. Note, however, that the individual GC isomer profiles of both compound classes are different. Thus, the interference of each PCT peak by PCBs will be different and may range from zero (no interference) to > 100% (in case of the interference of a minor tetraCT peak with a major hexaCB). Therefore, any simple adoption of SIM-mode is associated with a high error [6]. The interference of *m/z* 370 by hexaCBs can also not be ignored, while *m/z* 372 is only found in the mass spectra of tetraCTs (but not in hexaCBs). Yet, quantitation of tetraCTs by *m/z* 372 cannot be recommended due to two reasons. First, this ion amounts only to 3.8% to M^+ of tetraCTs so that its stability and sensitivity is low. Second, GC/MS quantitation should take advantage of a verification ion which could be only *m/z* 374 which is only 0.35% of M^+. Therefore, quantitation by means of *m/z* 368 or *m/z* 370 would be favourable. In this case, the interference of each PCT congener must be calculated as shown in the following section.

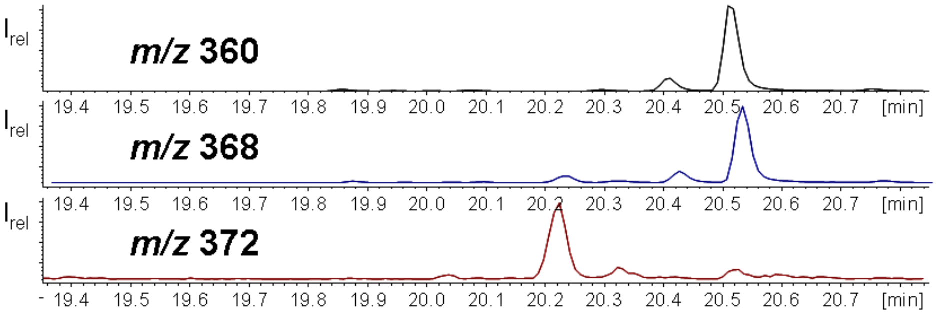

Figure 2: GC/ECNI-MS ion traces for a monk seal blubber sample from Mauretania. Using *m/z* 368, the peaks mainly originate from response to PCBs (see similar ion traces of *m/z* 360 and *m/z* 368).

Developing a calculation algorithm of the interference of a given PCT by a PCB

The interference of a PCT by a PCB peak can be calculated by determining both the area and the (measured) ratio of the selected lower mass ion (example: *m/z* 368 in the case of tetraCTs and hexaCBs) and the 2 u higher mass (example: *m/z* 370). The following basics can be listed:

(i) the abundance of the lower mass ion is the sum of the contributions of the PCT (a) and the PCB (b)
(ii) the abundance of the higher mass is the sum of the contributions of the PCT (c) and the PCB (d).

This leads to equation 1:

$$R = a + b / c + d \qquad (1)$$

with R being the measured ratio of the abundance of lower to higher mass
a and c being the proportion of lower and higher mass of any PCT
b and d being the proportion of lower and higher mass of any PCB

Moreover, the isotope ratio (IR) of lower to higher mass is known for the PCT (IR_{PCT}) and the PCB (IR_{PCB}) (**Table 1**). Thus, "a" can be substituted with "$IR_{PCB} \cdot c$" and "b" can be substituted with "$IR_{PCT} \cdot d$". Insertion into equation 1 leads to equation 2:

$$R = (IR_{PCT} \cdot c + IR_{PCB} \cdot d) \; / \; (c + d) \tag{2}$$

multiplication with (c + d) leads to equation 3:

$$R \cdot c + R \cdot d = (IR_{PCT} \cdot c + IR_{PCB} \cdot d) \tag{3}$$

sorted for d and c, respectively, leads to equation 4:

$$c\,(R - IR_{PCT}) = (IR_{PCB} - R)\,d \tag{4}$$

resolved for c leads to equation 5:

$$c = (IR_{PCB} - R)\,d \,/\, (R - IR_{PCT}) \tag{5}$$

Equation 5 will provide the contribution of the PCT to the higher mass ion. Multiplication of c with the total area of the higher recorded mass (example: *m/z* 370) will provide the area arising from the CT (example: tetraCT). Likewise the contribution of the PCB results from the total peak area minus the contribution of the PCT. Measuring the total peak areas of the lower and higher mass, calculation of R, and insertion into equation 5 (with d = area of the higher recorded mass – c) will provide the proportions of PCTs and PCBs of a given peak.

Table 1: Masses and isotope ratios (IR) of lower to higher mass of PCTs and PCBs

	m/z 368/370	*m/z* 404/406	*m/z* 438/440	*m/z* 472/474	*m/z* 506/508
	tetraCT	pentaCT	hexaCT	heptaCT	octaCT
IR_{PCT}	2.0345	3.0382	2.2981	1.8473	
	hexaCB	heptaCB	octaCB	nonaCB	decaCB
IR_{PCB}	17.2871	19.9672	10.5563	7.1490	

Using this method, any PCT peak interfered by PCBs with two orders of magnitude excess or more can be determined. Using this approach, we studied the tetra- to heptaCTs (and hexa- to nonaCBs) in a seal blubber sample from Africa (**Tables 2-4**). Screening tetraCTs (*m/z* 368 and 370) gave response for eight peaks (1-8, **Table 2**). Six of them (T1-T6) contained PCTs while two originated from PCBs alone. The contribution of the six PCTs to the respective peaks ranged from 11 - 92%. The dominant tetraCT congener in the sample, T4, contributed only 15% to the peak recorded at its retention time if 20.6 min. As can be seen from these measurements, a direct quantification by *m/z* 370 would have led to an error of > 50%.

Likewise nine abundant peaks were obtained for *m/z* 404, i.e. the mass recorded for pentaCTs, which mostly originated from PCTs (**Table 3**). The major pentaCTs, P5 and P9 were detected without interference by PCBs.

Furthermore, all four peaks detected by means of *m/z* 438 partly arose from hexaCTs (H1-H4), which contributed between 11 and 28% to the peak area (**Table 4**). Finally, heptaCTs were not detected in this sample. However, heptaCTs and even octaCTs were detected in samples from North and Baltic Seas [9].

Table 2: Areas and relative contributions of tetraCTs and hexaCBs to peaks

No.	**t_R [min]**	**Area *m/z* 368**	**Area *m/z* 370**	**R (368/370)**	**Contribution of PCT to *m/z* 370**	**Contribution of PCB to *m/z* 370**	**% of PCT**
1/T1	19.944	7280	570	12.68[1]	173	400	30.2%
2/T2	20.163	7890	2400	3.28	2210	196	91.8%
3	20.304	31500	32100	0.98	-	n.d.	0%
4	20.388	9040	6250	1.45	-	n.d.	0%
5/T3	20.494	45100	2900	15.54	332	2570	11.4%
6/T4	20.602	306000	20500	14.94	3150	17300	15.4%
7/T5	20.836	9550	893	10.69	386	507	43.2%
8/T6	21.011	9520	673	14.15	138	535	20.6%
	Sum				6390		

Table 3: Areas and relative contributions of pentaCTs and heptaCBs to peaks

No./ No. PeCT	t_R [min]	Area *m/z* 404	Area *m/z* 406	R (*m/z* 404/406)	Contribution of pentaCT to *m/z* 406	Contribution of heptaCB to m/z 406	% of peak area from PCT
1/P1	21.26	6060	720	8.46	487	233	68%
2/P2	21.35	1860	1700	1.10	1700	n.d.	~100%
3/P3	21.41	4330	3270	13.22	1300	1970	40%
4/P4	21.50	15400	1530	10.04	897	633	59%
5/P5	21.61	149000	96100	1.55	96100	n.d.	~100%
6/P6	21.70	7640	3490	2.19	3490	n.d.	~100%
7/P7	22.00	4870	1430	3.42	1390	36	98%
8/P8	22.19	5220	5010	1.04	5010	n.d.	~100%
9/P9	22.79	69900	46500	1.50	46500	n.d.	~100%
	sum				4080		

Not unexpected, the major interferences were observed for tetraCTs because the interferents -- hexaCBs -- represent the dominant PCB isomer group in most marine mammals. The interference of pentaCTs by heptaCBs was surprisingly low (**Table 3**). The interference of hexaCTs was again relatively high.

Table 4: Areas and relative contributions of hexaCTs and octaCBs to peaks

No./ CT	t_R [min]	Area *m/z* 438	Area *m/z* 440	R (438/440)	Contribution of hexaCT to *m/z* 440	Contribution of octaCB to *m/z* 440	% of peak from PCT
1/H1	22.975	32800	3390	9.66	367	3025	10.8%
2/H2	23.07	33600	3920	8.58	936	2980	23.9%
3/H3	23.59	9030	1050	8.61	248	801	23.6%
4/H4	23.922	27600	3350	8.25	933	2410	27.9%
	Sum				2480		

All in all fourteen PCTs (six tetraCTs, four pentaCFTs, and four hexaCTs) were identified in the seal blubber sample from Mauretania whose contribution could be corrected for interference by PCBs. For each peak the interference by PCBs was different. Using the calculation algorithm, the contribution of the individual PCTs could be calculated. Based on an equal response factor for the individual PCTs, the main contribution to the PCT pollution of the monk seal blubber sample originated from pentaCTs followed by tetraCTs and hexaCTs. For a better quantitation, efforts should be undertaken to synthesize individual PCT standards.

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