

# Isomer Specific Determination of Polychlorinated Biphenyls in Animal Tissues by Gas Chromatography Mass Spectrometry

W. A. Heidmann

Chemisches Institut, Tierärztliche Hochschule, Bischofsholer Damm 15, D-3000 Hannover 1, FRG

## Key Words

Gas chromatography/mass spectrometry

PCB

Animal tissues

Trace analysis

Isomer determination

## Summary

A procedure was developed which determines the concentration and composition of PCB mixtures in animal tissues by using seven standard congeners to take in consideration the different sensitivities of isomers of different level of chlorination. The selection criterion of these congeners is the abundance in animal tissues. The PCB mixture present in the matrix is not compared with a commercial mixture, rather, the PCB congeners are analysed individually and summed up to give the real concentration. However, because congeners of very small abundances are neglected, some 10% of the PCB mixture is not evaluated. Therefore, a correlation with a high chlorinated commercial mixture (Aroclor 1254 and Clophen A 60) is necessary. The PCB concentrations measured in animal tissues are compared with those received according to a conventional procedure and the PCB composition found are discussed. Finally, an attempt will be made to define a simple measure for the degree of metabolization of a PCB mixture present in animals.

## Introduction

The polychlorinated biphenyls (PCBs) are substances difficult to analyse in environmental samples because these compounds are mixtures which may contain up to 209 congeners. Until recent advances in analytical instrumentation, most of the conventional methods yielded only semiquantitative data with almost no information on the PCB isomer composition. This was due to the fact that for

a long time packed columns were used in gas chromatography (GC) of PCBs. With this technique, a separation of the PCB congeners is not possible, and quantitation of a sample proceeds by comparison with a commercial mixture (e.g. Clophen A 60) [1]. A deviation of the isomer composition from a commercial mixture leads to false quantitative data.

After introduction of capillary columns, the separation of most congeners is possible. Nevertheless, isomer specific analyses seem not to be feasible because a calibration procedure for all isomers has to be done. Therefore, a commercial mixture is used, again as reference, and the calculation of the PCB concentration proceeds by using few selected congeners (see e.g. [2]). With this approach, a too high value will be obtained [3].

Recently, Beck and Mathar [4] suggested a procedure using a set of 6 congeners which are determined individually. The concentrations of these isomers are given rather than one of the PCB mixture. Thus, an approximation of the isomer composition can be made; however, summing up the concentrations of the isomers, a too low concentration of the mixture will be obtained.

In order to get the real content of a PCB mixture present in environmental samples and to obtain data about their composition, a determination of as many PCB congeners as possible is indispensable. Using an electron capture detector (ECD), the response of the PCB isomers is influenced by the number as well as by the biphenyl ring position of the chlorine atoms. Thus, the response of congeners even with the same level of chlorination may vary by the factor of 15 [5]. Therefore, a determination of PCB concentrations via their individual isomers can only be done with reasonable expense if the isomers with similar response will be calibrated via a common surrogate standard without regard to their different level of chlorination [5]. This is a complex task because these PCB congeners may be scattered over the whole chromatogram. The problem may be reduced by using a flame ionisation detector (FID) which shows almost the same response with all isomers of the same level of chlorination; thus, only one calibration for all members of such an isomer group is required [6]. However, its lack of specificity and sensibility precludes the use in trace analyses.

The mass spectrometer (MS) in electron impact mode (EI) is a highly specific GC detector; and when used in selected ion monitoring mode (SIM) sensitive as well. A differentiation between PCB congeners of different level of chlorination is easily achieved. However, the response within an isomer group may still vary by a factor of 2–3 [7, 8]. Yet, when selecting appropriate surrogate standards for PCB congeners of each level of chlorination, possible errors may be minimized.

A MS in negative ion chemical ionization mode (NICI) is less suited. Because of the very low abundance of the molecular ion of the isomers, the level of chlorination cannot be determined [9]. The signal of the molecule mass can be enhanced by mixing the reacting gas with N<sub>2</sub>O [9]. However, this will raise the expense for maintenance considerably and preclude a routine operation.

Recently, Alford-Stevens et al. reported on a determination of the real concentration and the composition of PCB mixtures using a MS in EI mode as detector [8, 10]; the concentration of all PCB congeners found in the sample are determined and summed up. However, in order to identify also unknown compounds as PCB congeners much information is needed; thus, 33 masses have to be monitored at the same time.

This paper suggests a different approach. In order to get a sensitivity high enough to analyse animal tissues, only six masses are simultaneously monitored, two of these are used for monitoring other chlorinated hydrocarbons (CHC). This information is sufficient to achieve correct data without false positives or negatives if exclusively known PCB congeners are examined. The selected PCB congeners should be those whose concentrations are proved to be highest in animal tissues. Because they represent only some 90% of weight of the higher chlorinated commercial mixtures (e.g.

Aroclor 1254, Clophen A 60) this approach necessitates such a mixture as reference in order to determine the total PCB concentration. This value, then, is almost independent of the composition of the PCB mixture present in the sample, and a determination of the composition is also easily achieved.

## Experimental

### Materials

The 7 PCB congeners used as surrogate standards (Table II) were purchased from Promochem (Germany). Stock solution of each congener: 10 µg/ml in isooctane; multicomponent solution I: 1 µg/ml in isooctane. Aroclor 1254; Clophen A 60: α-, β-, γ-HCH; HCB; p-, p'-DDE; p-, p'-DDD; p-, p'-DDT; octachlorostyrene were purchased from Ehrentorfer (Germany). Stock solution of each congener: 1000 µg/ml in isooctane; multicomponent solution II: 25 µg/ml (Aroclor 1254 and Clophen A 60 each), 2.5 µg/ml in isooctane (other CHCs). Hexabromobenzene (98%) was purchased from Ega (Germany). Stock solution: 100 µg/ml. All chemicals are used without further purification.

Hexane (mixture of isomers), dichloromethane, both technical grade, and isooctane (p.A., Merck, Germany) were fractionated through a 1 m column. Sodium sulfate (purum, Merck, Germany) was heated at least 2 hours at 450 °C. Silica gel (no. 7734, Merck) was heated accordingly, after cooling in a desiccator mixed to give silica gel with 10% water (w/w), and shaken for 30 min. This mixture was kept in a tightly closing flask [11]. Sulfuric acid impregnated silica gel (40% w/w) was prepared accordingly using concentrated sulfuric acid rather than water [12]. Quartz wool was heated 24 hours at 750 °C for purification.

Table I PCB congeners monitored. Identity according to [6], congener no. according to [14].

No. (Fig. 1)	Chlorine substitution	Congener no. [14]	Level of chlorination	No. (Fig. 1)	Chlorine substitution	Congener no. level of chlorination [14]	
1	2,2',5,5'/2,2',4,5' 2,2',4,4'	52,49 47	4	15	2,2',3,4',5,5'/2,2',3,3',4, 6'2,2',4,4',4,5'	146,132 153	6
2	2,2',3,5'/2,2',3,4'	44,42	4	16	2,2',3,4,5,5'	141	6
3	2,3,4',6/2,2',3,4	64,41	4	17	2,2',3,4,4',5'/-	138,u	6
4	2,4,4',5/2,3',4',5 2,3',4,4'	74,70 66	4	18	—	u	7
5	2,2',3,5',6	95	5	19	2,2',3,4',5,5',6	187	7
6	2,2',3,4',5	90	5	20	2,2',3,3',4,5,6	173	7
7	2,2',4,5,5'	101	5	21	2,2',3,3',4,5,6'	174	7
8	2,2',4,4',5	99	5	22	2,2',3,3',4',5,6	177	7
9	2,2',3',4,5/2,2',3,4,5' 2,3',4,4',6	97,87 119	5	23	2,2',3,4,4',5,5'/-	180u	7
10	2,2',3,3',6,6'	136	6	24	2,2',3,3',4,4',5'/-	170,u	7
11	—	u	5	25	2,2',3,3',4,5,5',6	198	8
12	2,2',3,5,5',6	151	6	26	2,2',3,3',4,5,6,6'	199	8
13	2,2',3,4',5',6/2,2',3,4 5',6	149,144	6	27	2,2',3,3',4,4',5,6	195	8
14	2,3,3',4,5'/2,3',4,4',5	108,118	5	28	2,2',3,3',4,4',5,5'	194	8

u = unknown

## Instrumentation

Mass spectral data were obtained with a Finnigan MAT 44S operated in EI mode interfaced (open split) to a Varian 3700GC equipped with a Carlo-Erba split-splitless injector. A 25m x 0.27mm glass capillary with a 0.2µm film of OV-1, deactivated and coated according to [13] was used. A MAT Spectro System 2000 data system consisting of a PDP 11/34 mini-computer was used to acquire and process mass spectral data. Software to calculate the concentration and composition of PCB mixtures was written in Fortran IV.

## Preparation of Samples

Grind sample (liver, muscle or egg), weigh max. 10g, containing max. 1g fat, add 1µg hexabromobenzene as internal standard (IS) (10µl of stock solution) and mix with a 3-fold excess sodium sulfate until sample is dry. Transfer to a 60cm x 2cm i.d. chromatographic column filled with a plug of quartz wool, 2g sodium sulfate, 5g sulfuric acid impregnated silica gel, 15g silica gel (10% water), and 2g sodium sulfate. Rinse with 10ml hexane and eluate with 140ml of a mixture of hexane/dichloromethane 80 + 20 (v/v), evaporate in vacuum (min. 200mbar) at 30°C to a small volume, add 200µl isooctane, and evaporate again.

Fat (max. 1g) is dissolved in 20ml of elution mixture. Add 1µg IS, transfer to chromatographic column, and rinse twice with 5ml elution mixture. Eluate with 130ml.

## GC-MS Data Acquisition

The injection port was operated at 300°C, carrier gas pressure was 1.5bar (helium); the source temperature was kept at 200°C, the interface temperature at 220°C. One minute after injection (1–2µl), the split valve was opened and the oven temperature was increased from 120°C to 205°C at a rate of 25°C/min, where the oven temperature was raised further at a rate of 1°C/min to a final temperature of 224°C. Five groups of six masses were monitored to provide data of 28 PCB congeners, 8 other CHCs, and 1 IS (ion sets I–V in Fig. 1; see also Table I). Cycle time used with ion set I: 0.4sec; with ion sets II–V: 0.8sec.

## Calculation of the Amount and Composition of PCB Mixtures

For each level of chlorination  $n$  ( $n = 4–8$ ) a correction factor is calculated:

$$f_n = AS_6/AS_n \quad (1)$$

$AS_6$ ,  $AS_n$  = area of surrogate standard of level of chlorination 6 and  $n$ , respectively (Table II).

There are two surrogate standards for  $n = 4$  and 6; the mean is taken for calculation. Next, the sums of the areas of all congeners  $m$  ( $m = 1–28$ ) of each level of chlorination  $A_{m(n)}$  reduced to the area of IS is calculated (Table I; Fig. 1):

$$SRA_n = (\sum A_{m(n)}) \cdot f_n/A_i \quad (2)$$

$A_i$  = area of IS (Fig. 1).

The total sum of the relative areas is derived from

$$\sum RA = \sum_{n=4}^8 SRA_n \quad (3)$$

and the total sum of the relative areas with consideration of mol weights

$$\sum RAM = \sum_{n=4}^8 (SRA_n \cdot M_n) \quad (4)$$

$M_n = m_6/m_n$  and  $m_6$ ,  $m_n$  resp. = nominal mol weight of the level of chlorination 6 and  $n$ , respectively (Table II).

Calibration is normally done by spiking 2 or 10g of liver or egg with five different amounts (0.05–5µg PCB) of the multicomponent solution II. The amount of PCB is plotted against  $\sum RA$  ( $r = 0.99992$ , limit of detection: 0.08µg).

The mole fraction in percent of the individual PCB congeners is calculated by

$$FRM_m = RA_m \cdot f_n \cdot M_n \cdot 100/\sum RAM \quad (5)$$

$RA_m = A_m/A_i$  and  $A_m$  = area of congener  $m$ , the amount of the PCB congeners by

$$W_m = W_T \cdot RA_m \cdot f_n/\sum RA \quad (6)$$

$W_T$  = total amount of PCB derived from the calibration curve.

Table II PCB congeners selected as surrogate standards.

No. (Fig. 1)	Chlorine substitution	Congener no. [14]	Level of chlorination	Nominal mol weight	Quantitation ion	Correction factor	RSD* [%]
1	2,2',5,5'	52	4	290	291.9	0.406	2.3
4	2,3',4,4'	66	4	290	291.9		
7	2,2',4,5,5'	101	5	324	325.9	0.684	3.3
15	2,2',4,4',5,5'	153	6	358	359.9	1.0	
17	2,2',3,4,4',5'	138	6	358	359.9		
23	2,2',3,4,4',5,5'	180	7	392	393.8	1.65	1.1
28	2,2',3,3',4,4',5,5'	194	8	426	427.8	1.88	2.5

\* relative standard deviation derived from 3 measurements

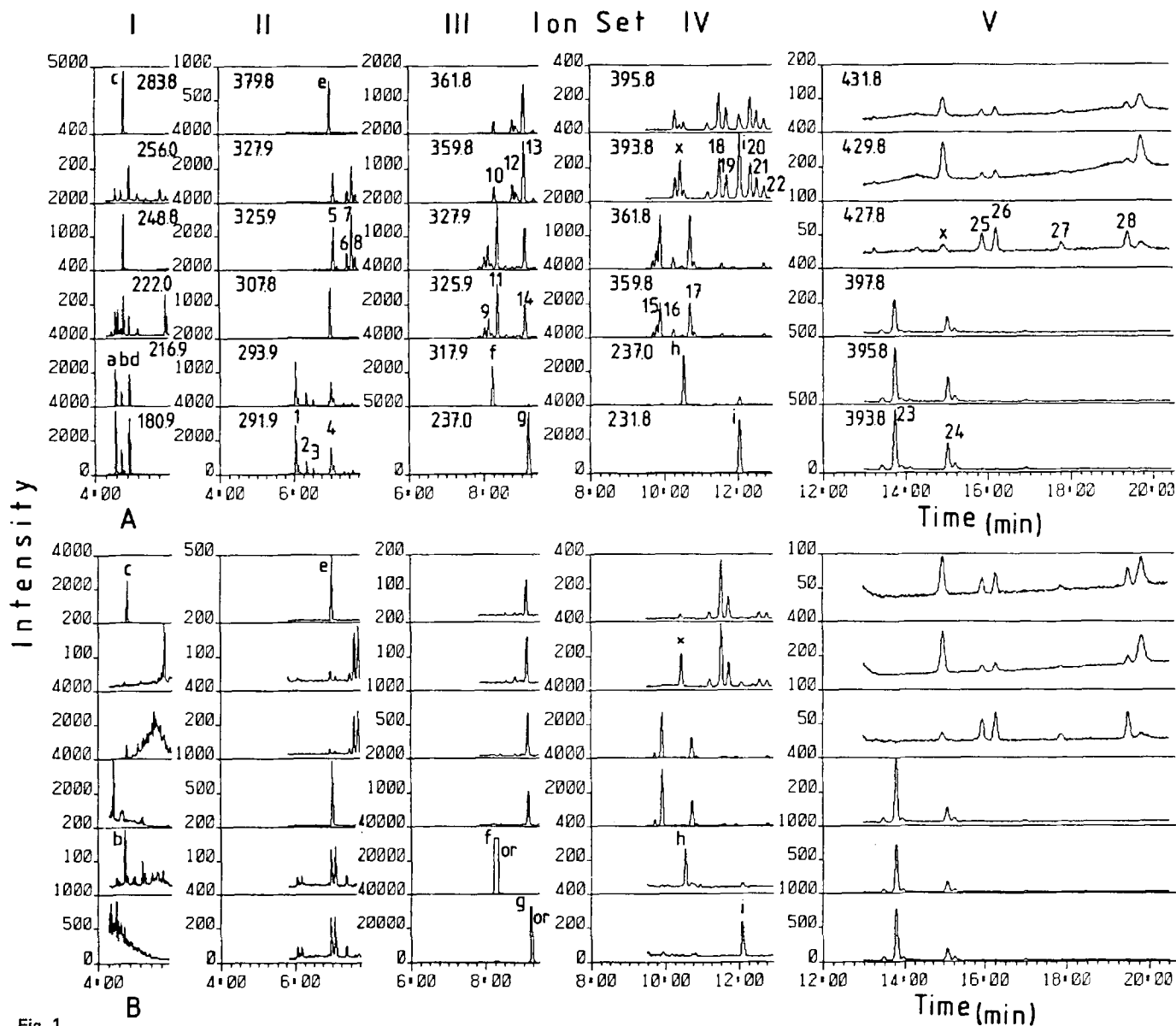


Fig. 1

Mass fragmentogram with ion set I–V and masses monitored.

(A) 1  $\mu$ l of a mixture of Arochlor 1254/Clophen A 60 = 1 + 1 v/v. Concentration 25  $\mu$ g/ml (1.25  $\mu$ g/ml other CHC, 20  $\mu$ g/ml IS)

(B) PCB mixture in liver of a barn owl (Table IV, 7)

Congener no. see Tables I and II. a =  $\alpha$ -HCH, b =  $\beta$ -HCH, c = HCB, d =  $\gamma$ -HCH, e = octachlorostyrene, f = p-, p'-DDE, g = p-, p'-DDD, h = p-, p'-DDT, i = IS

x = interference, or = out of range

Quantification of the PCBs with the aid of congeners no. 15, 17, and 23 proceeds with the expression

$$\text{RAPCB3} = ((A_{15} + A_{17}) \cdot f_6 + A_{23} \cdot f_7) / A_i \quad (7)$$

rather than with  $\Sigma RA$  ( $r = 0.99986$ ), whereas

$$\text{RA60} = \text{RA}_{23} \quad (8)$$

( $r = 0.99969$ ) is used for the estimation of the amount of the PCB mixture with 60% chlorine (PCB 60% Cl), and the expression

$$\text{RA50} = (A_{17} - A_{23} \cdot 2.15) / A_i \quad (9)$$

( $r = 0.99980$ , see [16]) for the one of the mixture with 50% chlorine (PCB 50% Cl). In the latter two cases, the total amount of either mixture is  $W_T/2$ .

## Results and Discussion

Fig. 1 shows the mass fragmentogram of two PCB mixtures: A, a mixture of Arochlor 1254 and Clophen A 60 = 1 + 1 and, B, a highly metabolized PCB mixture in the liver of a barn owl. In the method presented here, the selected isomers are evaluated individually as far as they are not combined to small groups of 2 (in 4 cases of 3) isomers (see Table I). This is necessary in order to minimize analysis time, which will raise considerably if an attempt will be made to separate all isomers. Thus, besides a determination of the total PCB concentration, the fraction and concentration, respectively, of each PCB congener may be evaluated. This seems to be of particular importance because the toxicity of the PCB congeners vary considerably. Additionally, the fraction of the levels of chlorination from 4 to 8

may be calculated by summing up the appropriate isomers (calculation see eq. (1)–(6)).

The clean up described consisting mainly of a chromatography on silica gel and an on column treatment with concentrated sulfuric acid produces very clean solutions. This enables the unequivocal determination of PCBs by selected ion monitoring (SIM) with four masses only without interferences. What is more, the other CHC and the IS can be successfully monitored by one mass for each compound.

In some cases, however, difficulties at the analysis of small amounts of HCH isomers may arise when unpolar, not hydrolyzable interferences are present in the matrix (Fig. 1B). Nevertheless, by monitoring only few masses, very low PCB concentrations and, additionally, the composition may be determined (Table IV, 2).

Own investigations show that the isomers given in Table I constitute some 90% of the concentration of the higher chlorinated PCB mixtures (e.g. Aroclor 1254 = 50% chlorine or Clophen A 60 = 60% chlorine). The same result will be obtained considering the data of the composition of Clophen A 60 given by Schulte and Malisch [6]: the fractions of the isomers in this investigation are summed up and compared with the sum of the fractions of all isomers found in [6]. Therefore, in order to prevent underestimation of the concentration of the PCB mixture to be analysed the method described need a calibration with a commercial mixture of a content of 50% and/or 60% chlorine.

The approach discussed in this paper does not evaluate isomers of a level of chlorination lower than 4 and higher than 8. However, the many PCB determinations carried out in this laboratory showed neither in fish nor in birds and mammals fractions of isomers with 2 to 4 chlorine atoms per molecule greater than only a few percent. Virtually no isomers with 9 or 10 chlorine atoms per molecule were observed. Yet, in ion set I the monitoring of chlorination level 2 and 3 is provided for in order to prevent the risk of serious errors.

In order to cope with different responses of congeners with different levels of chlorination, correction factors are introduced relative to the sensitivity of congeners with the level of chlorination of 6 which is set to 1. These factors are determined by 1 or 2 surrogate congeners for each

level (see eq. (1)). Because even the isomers of the same level of chlorination show a variation in sensitivity it is important to select correct standards in order to minimize errors. Therefore, congeners with highest concentrations in the animal tissues should be selected, and these are the most persistent ones which are simultaneously those with a 4,4' ring position of the chlorine atoms. This fact is confirmed by the data given in Table IV. In addition, in Fig. 1B these isomers predominate.

The selected surrogate congeners (Table II) follow this guideline with the exception of those with the level of chlorination 4 and 5. This is due to the low abundance of congener 2,2', 4,4' Tetra-CB (part of no. 1) in commercial mixtures. Thus, its occurrence in animal tissues is low as well, even though the persistence is high. The isomer no. 8 (2,2', 4,4', 5 Penta-CB) and no. 14 (2,3', 4,4', 5 Penta-CB) could not be supplied and were replaced by no. 7 (2,2', 4,5, 5' Penta-CB). The correction factors obtained by surrogate standards according to eq. (1) are listed in Table II.

The validity of the procedure developed in this research can be demonstrated by analysis of commercial PCB mixtures. Table III shows the fractions of the levels of chlorination analysed by the method described here, and values obtained by other authors. The data are in good agreement although detectors used by the other authors are different from MS. Minor discrepancies may be due to the fact that it was not possible for the author to obtain PCB mixtures of the same lot number used by Albro et al. [15] and Schulte and Malisch [6].

Like Alford-Stevens et al. [8] we found that the precision for PCB congener measurements is almost independent from the retention time (RT) of analyte and IS. Therefore, the method presented here, uses one IS only.

Table IV shows some typical results of PCB determinations in animal tissues. While in birds, especially in birds of prey and in owls, the PCB mixture present is highly metabolized and the congeners with 4,4' ring position of chlorine predominate (see also Fig. 1B), in fish and water fowl the PCB mixture is metabolized to a lesser extent. Remarkably, the PCB concentrations in eggs of rooks are lower than in livers of nestlings, and, additionally, the PCBs in eggs are higher metabolized than in livers of nestlings. Therefore, it may be concluded that in Middle Europe the PCBs

Table III Mole percent of PCB isomer groups in Aroclor 1254 and Clophen A 60

Level of chlorination	Aroclor 1254		Clophen A 60				
	Lit. values [15]; non MS-detector mole%	no. of isom.	Experimental values; MS-detector mole%	no. of isom. <sup>1)</sup>	Lit. values [6]; non MS-detector mole% <sup>2)</sup>	no. of isom. <sup>1)</sup>	
2	0.07	1					
3	0.99	3					
4	18.67	16	19.25	4(10)	1.61	1.21	6(7)
5	51.97	20	54.38	7(10)	20.89	17.91	11(11)
6	21.64	16	23.61	6(10)	50.18	52.20	21(22)
7	5.35	10	2.76	7(9)	24.99	25.51	18(18)
8	1.00	1		4(4)	2.34	3.17	9(9)

1) in parenthesis, all isomers monitored; without parenthesis, no. of groups of isomers (see Tab. I, Fig. 1A)

2) recalculated from weight %

Table IV Typical concentrations and compositions of PCB found in animal tissues. Congener no. see Table I and Fig. 1A

Congener no.	Composition in mole%								congener no.	Composition in mole%							
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8
1	4.05	3.42	0.19	1.62	0.68	0.69	0.42	0.17	21	0.98	2.41	0.95	0.55	4.96	1.58	0.73	0.34
2	1.16	2.60	0.04	0.01	0.03	0.16	0.03	0.02	22	0.59	1.36	0.82	0.66	0.41	1.72	0.64	0.39
3	0.49	1.29	0.11	0.58	0.01	0.06	0.04	0.02	23	4.76	6.72	8.52	8.03	8.63	12.3	16.6	20.8
4	3.85	4.79	2.21	3.82	0.50	1.37	1.49	0.32	24	2.76	3.74	3.51	2.86	3.98	9.37	5.13	8.46
5	6.22	3.77	0.05	0.06	0.78	0.49	0.07	0.05	25	0.30	- b	0.41	0.25	0.77	2.52	1.27	1.73
6	2.47	1.34	0.07	0.04	0.04	0.12	0.15	0.04	26	0.41	- b	0.70	0.60	0.56	2.91	1.68	0.96
7	8.36	5.93	1.07	2.17	1.98	0.72	1.03	0.24	27	0.17	- b	0.21	0.14	0.24	1.31	0.60	0.42
8	1.80	1.44	3.37	3.46	1.46	2.24	1.20	1.21	28	0.38	- b	0.28	0.37	0.53	4.50	1.78	1.99
9	3.89	1.93	3.05	0.24	0.90	0.17	0.07	0.03	level of chlorination in mole%								
10	1.49	0.95	0.02	0.05	0.38	0.07	0.03	0.01	4	9.55	12.1	2.55	6.03	1.21	2.28	1.98	0.52
11	8.54	4.95	1.29	2.17	0.29	0.42	0.23	0.11	5	37.7	24.0	17.7	15.6	7.97	6.22	8.76	2.52
12	1.64	2.78	0.01	0.03	1.72	0.29	0.15	0.17	6	37.6	42.7	57.7	39.7	49.7	41.5	52.3	57.0
13	7.17	5.26	1.20	1.31	4.20	1.59	1.08	0.23	7	13.9	21.3	20.5	17.3	39.0	38.7	31.6	34.9
14	6.46	4.60	8.79	7.45	2.52	2.06	6.00	0.84	8	1.26	- b	1.61	1.39	2.10	11.2	5.34	5.10
15	13.1	19.2	32.7	37.3	20.8	27.0	32.4	36.0	PCB concentration in µg/g								
16	2.01	2.07	0.37	0.58	0.15	0.17	0.37	0.05	PCB		.009	1.33	6.52	.913	.177	1.38	2.15
17	12.2	12.4	23.5	20.4	22.5	12.4	18.3	20.5	PCB (3 isom)		.012	2.49	12.3	1.31	.251	3.07	5.42
18	1.76	3.36	4.48	2.74	13.1	9.46	5.93	3.47	PCB 50% Cl		.002	.648	1.79	.308	- c	- c	- c
19	1.16	2.27	2.11	2.28	1.22	4.27	2.39	1.44	PCB 60% Cl		.008	1.29	5.89	.847	.193	2.82	5.41
20	1.86	1.44	0.08	0.17	6.72	0.03	0.16	0.01									

- 1 mixture Arochlor 1254/Clophen A 60 = 1 + 1 (see Fig. 1A)
- 2 pike, muscle (Lappland, Sweden)<sup>a</sup>
- 3 common tern, nestling, liver (German Bight)
- 4 common tern, egg (German Bight)
- 5 rook, nestling, liver (Northern Germany)
- 6 rook, egg (Northern Germany)
- 7 barn owl, liver (Northern Germany), see Fig. 1B
- 8 barn owl, egg (Northern Germany)

a We are indebted to Dr. M. Olsson (Naturhistoriska Riksmuseet, Stockholm) for leaving the sample to us  
 b traces  
 c negative values (see text)

will be received in the industrialized breeding areas, and will be metabolized to a certain extent in the lower industrialized wintering areas [17].

In addition, Table IV shows clearly that quantification with the aid of only three congeners (no. 15, 17, and 23) will give too high PCB concentrations. This is due to the selected congeners whose abundance in animals tissues may be very high (up to 77 mole %) because of their persistence (see Table IV and Fig. 1B).

When attempting to give a result of the composition and, consequently, the degree of metabolization of a PCB mixture present in environmental samples, one is forced to give the fractions of many isomers or at least the fractions of the levels of chlorination. Either result is little distinct. Thus, an easy measure of the degree of metabolization is needed which necessarily will be of lower accuracy.

Bearing in mind that congener no. 17 is not as persistent as congener no. 23 (see Table IV and Fig. 1B), the ratio of both congeners can be taken as such a measure. In our laboratory, however, we prefer a different presentation. Because in Arochlor 1254 congener no. 23 is present in very low abundance (below 1 mole%), this isomer represents the amount of the PCB mixture with a content of 60% chlorine (PCB 60% Cl). However, in Clophen A 60 the area of isomer no. 17 in the mass fragmentogram is 2.15 times greater than the one of isomer no. 23. Thus, isomer no. 17 represents

the PCB with a content of 50% chlorine (PCB 50% Cl) after subtracting 2.15 times the abundance of isomer no. 23 (see eq. (8) and (9)) [16]. As can be seen from Table IV, the values obtained for PCB 50% Cl may become negative if the degree of metabolization is very high, viz. greater than the one which is in accordance with the composition of Clophen A 60.

### Conclusion

The method presented in this paper shows that when using a MS as GC detector it is possible to determine easily the real concentrations and, additionally, the composition of PCB mixtures present in animal tissues. Thus, the fate of single PCB isomers in nature may be traced down with reasonable effort.

### Acknowledgment

The author wishes to thank Prof. H. A. Rüssel for many helpful discussions, and Mrs. A. Bütke for the prudent management of the laboratory without which this work had not been published. This investigation was supported by a financial aid of the Land Niedersachsen.

## References

- [1] *R. G. Webb, A. C. McCall*, *J. Chromatogr. Sci.* **11**, 366 (1973).
- [2] *E. Schulte, H. P. Thier, L. Acker*, *Dtsch. Lebensm. Rundsch.* **72**, 229 (1976).
- [3] *E. Schulte, R. Malisch*, *Fresenius Z. Anal. Chem.* **319**, 54 (1984).
- [4] *H. Beck, W. Mathar*, *Bundesgesundheitsbl.* **28**, 1 (1985).
- [5] *S. D. Cooper, M. A. Moseley, E. D. Pellizari*, *Anal. Chem.* **57**, 2469 (1985).
- [6] *E. Schulte, R. Malisch*, *Fresenius Z. Anal. Chem.* **314**, 545 (1983).
- [7] *G. D. Martelli, M. G. Castelli*, *Biomed. Mass Spectrom.* **8**, 347 (1981).
- [8] *J. E. Gebhart, T. L. Hayes, H. L. Alford-Stevens, W. L. Budde*, *Anal. Chem.* **57**, 2458 (1985).
- [9] *E. D. Pellizari, M. A. Moseley, S. D. Cooper*, *J. Chromatogr.* **334**, 277 (1985).
- [10] *J. E. Gebhardt, T. L. Hayes, H. L. Alford-Stevens, W. L. Budde*, *Anal. Chem.* **57**, 2464 (1985).
- [11] *H. Steinwandter*, *Fresenius Z. Anal. Chem.* **312**, 342 (1982).
- [12] *C. M. Smith, D. L. Stalling, J. L. Johnson*, *Anal. Chem.* **56**, 1830 (1984).
- [13] *G. Schomburg, H. Husmann, H. Borwitzky*, *Chromatographia* **12**, 651 (1979).
- [14] *K. Ballschmiter, M. Zell*, *Fresenius Z. Anal. Chem.* **302**, 20 (1980).
- [15] *P. W. Albro, J. T. Corbett, J. L. Schroeder*, *J. Chromatogr.* **205**, 103 (1981).
- [16] *A. Büthe, W. A. Heidmann*, in "Rückstandsanalytik von Wirkstoffen in tierischen Produkten", H. A. Rüssel, Georg Thieme Verlag, Stuttgart, 1986, in press.
- [17] *W. A. Heidmann*, *Seevögel* **6**, special edition, 63 (1985).

Received: March 14, 1986  
Accepted: March 26, 1986  
D