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QUALITATIVE AND QUANTITATIVE ANALYSES OF POLYCHLORINATED BIPHENYLS BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A model is presented of the relationship between the relative responses of flame-ionization and electron-capture detectors and the structure of polychlorinated biphenyls (PCBs). The model permits the calculation of detector responses for all PCBs and opens the possibility of detailed structural analyses.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are chemically and microbiologically resistant environmental contaminants, and are therefore incorporated into various biological life cycles. Through the food chain they accumulate in the higher members of the chain and may cause undesirable effects.

The analysis of PCBs is not easy owing to the large number of possible components, *i.e.*, 209 plus biphenyls. Quantitative analyses are routinely based on a comparison of the gas–liquid chromatographic (GLC) pattern of the sample with the PCB profiles of commercial products, and results are based on one or several prominent peaks. This method may give realistic values provided that the PCB pattern in the sample is similar to that found in a commercial PCB sample, or alternatively similar to mixtures of several commercial PCBs. Unfortunately, it is not possible in practice to verify the assumption of identical, or even similar, PCB patterns owing to the complexity of the chromatograms from biological samples; for this reason, quantitative figures for PCBs reported on the basis of this method should be regarded as only approximate.

A more satisfactory method consists in determining the amounts of all of the individual components and summing them to give the total amount of PCBs. At present glass capillary column GLC with an electron-capture detector (ECD) provides the best specificity and sensitivity, and is the method of choice. It is, however, necessary to calibrate the detector against standards for each PCB to be determined. It is known that the ECD response is dependent on the structure of the PCB and not only on the number of chlorine atoms[1,2]. Only a limited number of PCBs have been synthesized, and their ECD responses have not been systematically studied.

A model is presented here that permits the calculation of ECD calibration factors for all PCBs. With this model it becomes possible to obtain both quantitative and detailed qualitative information on the components of PCBs.

EXPERIMENTAL

Twenty-one pure PCBs and DDE (purity >99%) were obtained from Ana-labs, North Haven, Conn., U.S.A. The GLC equipment consisted of a Perkin-Elmer F22 two-channel gas chromatograph fitted with a flame-ionization detector (FID) and an (ECD. The FID channel was used with a 25 m × 0.25 mm I.D. OV-101 glass capillary column (Perkin-Elmer) and the ECD was connected to a 20 m × 0.25 mm I.D. OV-1 (Jaeggi, Trogen, Switzerland) column. The oven temperature was kept at 22° and injector and detector were held at 290° and 300°, respectively. Argon–methane was used as the carrier gas with the ECD channel and nitrogen with the FID channel. Peak areas were measured with a Spectra-Physics SP 4000 data system.

Mixtures of baseline-separated PCBs and DDE in n-hexane were prepared such that the ratio between the highest and lowest concentrations of a given PCB relative to DDE was 16. The amount of sample injected by the splitless technique was 1 μl. Concentrations of PCBs analysed with use of the ECD ranged from 20 to 100 mg/l, and were chosen so as to minimize column adsorption without saturation of the detector.

The samples analysed with use of the FID were 5–10 times more concentrated. The relative detector calibration factors, KF_{rel}, were determined using DDE as an internal standard. When the PCB in question had a relative retention time (RRT) close to unity, a second PCB was used as an intermediate internal standard. Detector calibration lines were calculated by linear regression.

RESULTS AND DISCUSSION

The approach was to measure the ECD and FID responses for a given PCB relative to DDE as an internal standard. The calibration factor, KF, is defined as the ratio between the amount of compound and the area under the corresponding peak on the chromatogram:

$$KF = \text{amount/area} = X/A \tag{1}$$

The relative response is $KF_{rel} = \dfrac{X_{PCB}/A_{PCB}}{X_{DDE}/A_{DDE}}$

These calibration factors were measured for 21 PCBs using the FID and ECD and are listed in Table I. Each KF_{rel} value was determined by linear regression based on 6–18 points. It was established that the constant term b in the expression $X_{rel} = KF_{rel} \cdot A_{rel} + b$ in all instances was less than 1% of the highest value of X. All regression lines therefore passed through the origin. A more easily accessible empirical parameter is obtained by dividing relative responses for the FID and ECD:

$$KF_{relFID}/KF_{relECD} = \frac{X_{PCB}/A_{PCB}}{X_{DDE}/A_{DDE}} \text{ (FID)} \cdot \frac{X_{DDE}/A_{DDE}}{X_{PCB}/A_{PCB}} \text{ (ECD)}$$

and if X_{PCB}/X_{DDE} is kept constant:

$$KF_{relFID}/KF_{relECD} = \frac{A_{PCB}/A_{DDE}\ (ECD)}{A_{PCB}/A_{DDE}\ (FID)} \qquad (2)$$

The ratio between the relative areas obtained with the two detectors is identical with the ratio between the relative calibration factors. Thus this ratio may be obtained for all well separated peaks without knowing the identity of the peaks, and without a knowledge of the corresponding amounts of compounds.

For both types of detector there is a linear relationship between area and amount of compound[3]. It was ascertained that all measurements with the ECD were made within the linear range of the detector. As the PCBs and the internal standard are contained in the same volume, we can substitute concentration for amount:

$$KF_{relFID}/KF_{relECD} = Constant \cdot \frac{[PCB]/[DDE]\ (ECD)}{[PCB]/[DDE]\ (FID)} \qquad (3)$$

The expression on the right-hand side is mathematically equivalent to the expression for an equilibrium constant. In order to develop a model for relative responses it is therefore reasonable to search for linear free energy relationships involving the logarithm of KF_{relFID}/KF_{relECD}.

As can be seen from Table I, the KF_{relFID} values increase gradually with the number of chlorine atoms, from 0.910 for the dichloro isomer to 2.18 for the decachloro isomer. On the other hand, the KF_{relECD} values are more variable. Although there is a general tendency towards lower values with an increasing number of chloro substituents, there are very large differences between isomers that have the same number of chlorine atoms. For example, for the tetrachloro-PCBs, the highest KF_{relECD} value is 14.0 and the lowest value is 2.41. The ratio between KF_{relFID} and KF_{relECD} therefore reflects primarily the behaviour of KF_{relECD} values, and it becomes necessary to consider the processes taking place in the ECD.

By means of negative ion mass spectrometry it was shown that phenoxy anions are formed in the ECD during analysis of PCBs[4]. The anions may be formed by reaction with trace amounts of oxygen in the carrier gas according to the equation

$$(4)$$
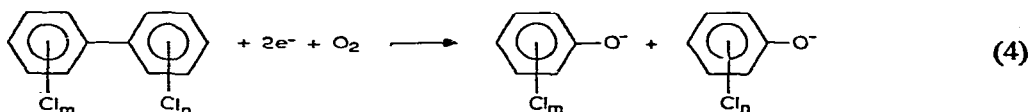
The detector signal is proportional to the number of electrons being captured per second, and this depends on the stability of the phenoxy anions and on the strength of the C–C biphenyl bond.

The following Hammett-like equation was applied:

$$\log (KF_{relFID}/KF_{relECD}) = a_o + a_1 \Sigma\sigma_{o,m,p} + a_2\Sigma\sigma_{o,m} \qquad (5)$$

TABLE I

FID AND ECD RESPONSE FACTORS FOR POLYCHLORINATED BIPHENYLS, RELATIVE TO DDE

Structure (ring 1-ring 2)	FID, 25-m OV-101				ECD, 20-m OV-1 column				$KF_{FID}/KF_{ECD} \times 10^4$	Log (KF_{FID}/KF_{ECD})	
	RRT	KF_{rel}*	Points	r	RRT	KF_{rel}*	Points	r		Found	Calculated
26	0.453	0.910	9	0.9932	0.564	16.9	13	0.9967	538	2.73	2.84
234	0.639	0.814	13	0.9997	0.715	3.24	14	0.9998	2515	3.40	3.54
236	0.569	0.782	12	0.9991	0.652	8.16	12	0.9974	960	2.98	3.07
25-3	0.601	1.01	9	0.9897	0.680	15.8	12	0.9922	639	2.81	2.65
2345	0.826	0.975	15	0.9994	0.857	2.41	6	0.9878	4046	3.61	3.77
2356	0.715	0.948	9	0.9993	0.766	3.96	11	0.9981	2394	3.38	3.29
24-25	0.688	1.17	9	0.9985	0.750	12.9	8	0.9983	907	2.96	3.21
24-34	0.831	1.19	13	0.9998	0.859	4.34	8	0.9987	2742	3.44	3.54
25-25	0.678	0.984	9	0.9980	0.748	14.0	11	0.9993	703	2.85	2.80
23-25	0.723	0.910	12	0.9985	0.795	10.3	9	0.9974	883	2.95	2.80
245-23	0.987	1.19	18	0.9983	0.973	4.75	10	0.9976	2505	3.40	3.54
234-23	1.02	1.20	10	0.9980	1.00	1.46	6	0.9931	8235	3.92	3.54
245-25	0.904	1.11	13	0.9980	0.928	2.96	9	0.9972	3750	3.57	3.54
234-25	1.00	1.17	10	0.9992	1.00	3.25	7	0.9986	3604	3.56	3.54
236-236	1.02	1.17	18	0.9982	1.00	7.48	9	0.9986	1563	3.19	3.19
245-245	1.28	1.32	16	0.9956	1.21	1.49	9	0.9942	8859	3.95	3.84
234-245	1.42	1.31	16	0.9960	1.32	1.41	11	0.9985	9291	3.97	3.84
23456-25	1.68	1.38	13	0.9946	1.51	0.946	6	0.9891	14581	4.16	4.18
2345-2345	3.29	1.55	14	0.9984	2.71	0.792	9	0.9963	19570	4.29	4.06
23456-2345	3.97	-	11	0.9987	3.31	-	6	0.9955	20807	4.32	4.18
23456-23456	4.84	2.18	8	0.9918	3.89	1.01	10	0.9961	21584	4.33	4.45

* KF = amount/area.

where $\sigma_o = 0.68$, $\sigma_m = 0.37$ and $\sigma_p = 0.23$. The second term is the sum of substituent constants for chlorine atoms in the PCB molecule, and this term reflects the stability of phenoxy anions. The σ_o constant is taken from the ionization of phenols[5], and the other two constants are ordinary substituent constants.

The last term contains σ constants for o- and m-positions relative to the central C–C bond, and reflects the inductive substituent effects on this bond. By applying eqn. 5 to data from Table I, it was found necessary to divide the PCBs into two classes, $viz.$, those compounds containing the same number of chlorine atoms in both rings in class 1, and all of the others in class 2.

For compounds in class 1 it is reasonable that both of the rings in the PCB molecule contribute to the detector signal. The following regression equation was obtained:

$$\log (KF_{relFID}/KF_{relECD}) = 2.19 + 2.27 \, \Sigma\sigma_{o,m,p} - 1.98\Sigma\sigma_{o,m} \qquad (6)$$

where $R = 0.9324$ (9 points). In this equation the sum of σ-values is taken for the total number of chlorine atoms in the PCB molecule.

For PCBs in class 2 the following equation was obtained:

$$\log (KF_{relFID}/KF_{relECD}) = 2.01 + 3.87 \, \Sigma\sigma_{o,m,p} - 3.26 \, \Sigma\sigma_{o,m} \qquad (7)$$

where $R = 0.8956$ (12 points). In eqn. 7 the σ values are summed for only the most heavily substituted ring in the PCB molecule.

Calculated values for $\log (KF_{relFID}/KF_{relECD})$ are compared with the experimental values in Table I, and it can be seen that the agreement is reasonable.

Qualitative analyses of PCBs can be performed by comparing calculated with experimental values, and the number of structural possibilities is thus narrowed. There is, however, a certain degree of overlapping of theoretical $\log (KF_{relFID}/KF_{relECD})$ values for isomers containing the same number of chlorine atoms if they do not belong to the same class (1 or 2). Pairs of components containing different numbers of chlorine atoms may also overlap if they contain the same number of chlorine atoms in their most heavily substituted rings. In these instances further information on the structures of the PCBs can be obtained by comparing measured relative retention times with those calculated on the basis of additivity of retention indices (RI) for the two substituted rings[6]. Relative retention times in Table I measured on the OV-101 capillary column follow closely the theoretical values based on measurements on packed columns with the same stationary phase[6] (see Table II).

By means of mass spectrometry the number of chlorine atoms in a given PCB can be determined[7], and this information, together with that provided by GLC according to the present method, may allow unique structural assignments to individual PCBs. The type of information available can be illustrated by considering in detail the results for tetrachlorobiphenyls. Calculated and experimental values for retention times and relative detector responses are given in Table II. Based on a closeness criterion of $\Delta RI \geqslant 10$ (ref. 6) and $\Delta\log(KF_{relFID}/KF_{relECD}) \geqslant 0.2$, unique structures can be assigned to 29 isomers, while there are two or three possibilities for each of the remaining 13 isomers.

TABLE II

CALCULATED AND EXPERIMENTAL VALUES FOR TETRACHLOROBIPHENYL ISOMERS

No. of chlorine atoms in position, used for calculation			Log (KF_{relFID}/KF_{relECD})		RI (calculated) (unique isomers in italics)	RRT	
o-	m-	p-	Calculated	Found		Calculated	Found
0	4	0	2.63		*2046*		
1	3	0	2.72		*1983*, *2021*		
2	2	0	2.80	{2.85 / 2.95	1920, *1944*, *1958*, 1996	{0.678 / 0.726	0.678 / 0.723
1	2	0	2.88		*1931*, 1997, 2007		
3	1	0	2.90		*1881*, 1919		
4	0	0	2.99	*1842*	*1842*		
0	3	1	3.04		*2095*		
2	1	0	3.06		1899, 1965, *1975*		
1	2	1	3.13		1993, *2032*, *2070*		
2	1	1	3.21	2.96	*1930*, 1968, 1993	0.691	0.688
2	2	0*	3.29	3.38	*1955*	0.722	0.715
3	0	1	3.31		*1891*		
0	2	1	3.36		2044, *2110*, *2120*		
0	2	2	3.45		*2144*		
1	1	2	3.54	3.44	2042	0.831	0.831
1	1	1	3.54		1943, *1979*, *2009*, *2019*, *2045*, *2055*		
2	0	2	3.63		1940		
2	0	1	3.72		*1849*, *1915*, *1925*		
1	2	1*	3.77	3.61	*2039*	0.827	0.826
2	1	1*	3.95		*1953*		

* Only one ring substituted.

Having established the identity of PCBs, quantitative analyses can be made on the basis of calculated KF_{relECD} values. For samples containing a reasonable number of individual PCBs the deviations of KF from theory will tend to cancel out, and reliable values for the total amount of PCB can be obtained.

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