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EVALUATION OF ENVIRONMENTAL POLYCHLOROBIPHENYLS AND DDE IN TERMS OF MIXTURES OF COMMERCIAL PREPARATIONS FROM PEAK HEIGHTS OF PACKED-COLUMN GAS CHROMATOGRAMS USING A PROGRAMMABLE CALCULATOR

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SUMMARY

A method is proposed for evaluating gas chromatograms of multi-component PCB mixtures and superposed single components simultaneously. Apparent concentrations relative to a calibration mixture are assigned to a number of suitable peaks, and the apparent concentrations are related to the true concentrations by a set of linear equations, which are solved by least-squares approximation. The results are provided with an estimate of their confidence intervals.

INTRODUCTION

In samples from birds, fish and wildlife, the content of polychlorobiphenyls (PCBs) is generally limited to the more highly chlorinated compounds, while non-biological materials such as marine sediments may contain the whole range of PCBs. Consequently, the method for the evaluation of PCBs based on a direct comparison of the chromatogram for the sample with that of some appropriate commercial standard, as applied by Zitko¹ and Holden² for fish and by Schmidt *et al.*³ for coastal waters, is not applicable to marine sediments.

Methods whereby weights are assigned to the individual PCB peaks by standardization with *p,p'*-DDE⁴ are useful if the more highly chlorinated PCBs are predominant⁵. Most satisfactory from a theoretical viewpoint are methods whereby the amount of PCB corresponding to an individual peak is calculated by means of a relationship between the detector response and the degree of chlorination of the molecules that generate the peak^{6,7}. For routine work, these methods require a good reproducibility of the detector response and the use of an automatic integrator.

The effect of using mixed standards on the quantitation of PCBs was shown by Beezhold and Stout⁸ for Aroclor 1254 and 1260. The cut-and-weigh method applied, however, seems to be time consuming, even for mixtures of only two products.

In this paper a method based on mixed standards is described that allows frequent re-calibrations of the apparatus and manual evaluation of gas chromatographic data. Its applicability, however, is limited to mixtures in which the gas chromatographic pattern of the underlying commercial products is essentially retained.

EXPERIMENTAL

Gas chromatography

Apparatus. A Beckman GC 5 gas chromatograph equipped with a helium-glow electron-capture detector and a 6 ft. \times 2 mm I.D. glass column containing 5% SE-30 on Chromosorb W, 80–100 mesh was used. The column and inlet temperatures were 220°C and the carrier gas was helium at a flow-rate of 40 ml/min.

Standards. The standards used were Clophen A30, A40, A50 and A60 (Bayer, Leverkusen, G.F.R.), Aroclor 1242, 1248, 1254 and 1260 (Analabs, North Haven, Conn., U.S.A.) and *p,p'*-DDE (Riedel-De Haen, Seelze-Hannover, G.F.R.).

Mixed standards. Solutions in *n*-hexane of single preparations and of various mixtures of either Clophen or Aroclor preparations, including DDE, are referred to as "mixed standards".

Calibration

Calibration is based on a suitable mixture of commercial PCB preparations and *p,p'*-DDE dissolved in *n*-hexane. A series of different total concentrations of this mixture is prepared, in order to obtain calibration graphs for a number of gas chromatographic peaks, which are selected for evaluation. In our laboratory, a mixture that bears some resemblance to sediment samples from the North Sea is used (Fig. 1). One "calibration unit" contains the following concentrations: Clophen A 30, $2.5 \cdot 10^{-4}$; Clophen A 60, $1 \cdot 10^{-4}$; and *p,p'*-DDE, $5 \cdot 10^{-6}$ g/l.

On the gas chromatogram of this mixture, seven peaks were selected for evaluation of peak heights; peak 4 contains DDE. A $2\text{-}\mu\text{l}$ volume of each of five concentrations (1, 2, 3, 5 and 8 times the calibration unit) are injected.

Calculator

A Hewlett-Packard Model 9820 A programable calculator (173 registers) equipped with 11221 A Mathematics Block is used (an extended memory would be preferable).

Software. Of the 9820 A Math Pac supplied by the manufacturer, the following programs are used as sub-routines: 1–5, N simultaneous linear equations with real coefficients in N unknowns ($N \leq 9$); and 1–3, real matrix inversion ($N \leq 9$).

Mathematical procedure

Calibration graphs are constructed for each of the selected peaks separately by least-squares approximation based on the equation^{9,10}.

$$y_i = b_{11} h_i + b_{12} h_i^2 + b_{13} h_i^3 \quad (1)$$

where h_i is the height of peak i ($i = 1, 2, \dots, m$) and y_i is the apparent concentration assigned to peak i , expressed in calibration units. Values of y_i are assigned to the corresponding peaks of samples by interpolation. Here, "corresponding peaks" are defined as peaks of identical retention time, irrespective of their chemical composition.

For a mixture composed of n standardized commercial preparations, the ap-

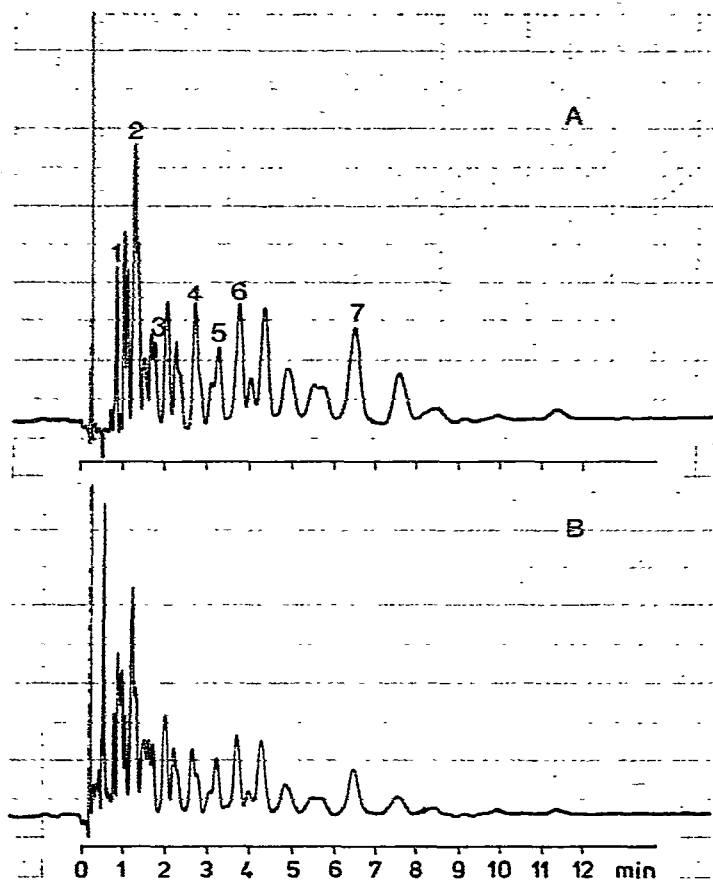


Fig. 1. Gas chromatograms of PCB mixtures and DDE. (A) Calibration mixture; selected peaks numbered 1-7; peak 4 contains DDE. (B) PCB in North Sea sediment.

parent concentrations, y_i , are related to the concentrations x_k ($k = 1, 2, \dots, n$) of the individual preparations by a set of linear equations:

$$y_i = \sum_k a_{ik} x_k \quad (2)$$

These equations have to be modified in order to allow for departures from linearity, errors in the evaluation of peak heights and variations in the composition of environmental PCB mixtures due to partial degradation or the presence of products other than the standards. If the number of evaluated peaks, m , exceeds the number of components, n , the method of least squares can be applied^{9,10}:

$$\sum_l v^2 = \sum_l \left(\sum_k a_{lk} x_k - y_l \right)^2 = \text{minimum} \quad (3)$$

Assuming equal accuracy of the y_i , minimizing with respect to variations of the x_k leads to the following system of equations:

$$\left. \begin{aligned} \sum_i a_{i1}^2 x_1 + \sum_i a_{i1} a_{i2} x_2 + \dots + \sum_i a_{i1} a_{in} x_n &= \sum_i a_{i1} y_i \\ \sum_i a_{i1} a_{i2} x_1 + \sum_i a_{i2}^2 x_2 + \dots + \sum_i a_{i2} a_{in} x_n &= \sum_i a_{i2} y_i \end{aligned} \right\} \quad (4)$$

On solving eqns. 4, some concentrations x_k may appear negative. With PCBs, this result is regarded as an indication that the corresponding preparations are absent. The calculation is repeated for the remaining PCB preparations, omitting all of the terms that bear the indices k concerned. Occasionally, several repetitions are needed. Negative DDE values are neglected.

The coefficients a_{ik} are obtained in a similar manner by relating the apparent concentrations y_{ij} , calculated for a sufficient number (r) of gas chromatograms j ($j = 1, 2, \dots, r$) of mixed standards, to the known concentrations x_{jk} :

$$y_j = \sum_k x_{jk} a_k \quad (5)$$

Peaks with identical retention times ($i = \text{constant}$) are treated separately, and the sum of the squares of the errors is minimized with respect to variations in a_k :

$$\sum_j v^2 = \sum_j \left(\sum_k x_{jk} a_k - y_j \right)^2 = \text{minimum} \quad (6)$$

The usual procedure leads to the following equations:

$$\left. \begin{aligned} \sum_j x_{j1}^2 a_1 + \sum_j x_{j1} x_{j2} a_2 + \dots + \sum_j x_{j1} x_{jn} a_n &= \sum_j x_{j1} y_j \\ \sum_j x_{j1} x_{j2} a_1 + \sum_j x_{j2}^2 a_2 + \dots + \sum_j x_{j2} x_{jn} a_n &= \sum_j x_{j2} y_j \end{aligned} \right\} \quad (7)$$

Data concerning DDE enter the calculation together with those of the corresponding PCB peak (or shoulder). For this peak, the number of equations, n , increases by one.

Estimation of errors

The calculation is completed by estimating the accuracy of the final result. Errors enter the calculation with the y_i values, which are assumed to be of equal precision for all of the selected peaks.

Unlike the usual practice in least-squares approximations, where the coefficients follow from mathematical reasoning, the coefficients of eqns. 4 are derived from experiment, and their errors must be accounted for. The law of error propagation can be applied to eqns. 2:

$$s_{y_i}^2 = \sum_k \left(\frac{\partial y_i}{\partial x_k} \cdot s_{x_k} \right)^2 + \sum_k \left(\frac{\partial y_i}{\partial a_{ik}} \cdot s_{a_{ik}} \right)^2 \quad (8)$$

where standard deviations are denoted by s with the appropriate index ($s^2 = \text{variance}$). Because

$$\frac{\partial y_i}{\partial x_k} = \bar{a}_{ik} \text{ and } \frac{\partial y_i}{\partial a_{ik}} = \bar{x}_k$$

the mean variance of y becomes

$$s_y^2 = \frac{1}{m} \sum_i \sum_k \bar{a}_{ik}^2 s_{x_k}^2 + \frac{1}{m} \sum_i \sum_k \bar{x}_k^2 s_{a_{ik}}^2 \quad (9)$$

where the bars refer to mean values. This equation can be simplified to

$$s_y^2 = s_y'^2 + s_y''^2 \quad (9a)$$

In order to save programming space, the second term on the right-hand of eqn. 9 is applied in the following form:

$$s_y''^2 = \sum_k \bar{x}_k^2 \left(\frac{1}{m} \sum_i s_{a_{ik}}^2 \right) = \sum_k x_k^2 \bar{s}_{a_k}^2 \quad (10)$$

whereby the original number of variances is reduced to n averages. The first term on the right-hand side of eqn. 9 is the variance of y , which would be obtained if the a_{ik} were the true values, and it can therefore be computed in the usual way, starting from eqn. 3:

$$s_y'^2 = \frac{1}{f} \sum_i v^2 \quad (11)$$

where f is the number of degrees of freedom ($f = m - u$), m being the number of peaks and u the number of non-zero concentrations x_k that are actually obtained on solving eqns. 4. To calculate the second term on the right-hand side of eqn. 9, the standard deviations of the a_{ik} must be known. Applying eqn. 6, the variance of y_j is obtained from

$$s_{y_j}^2 = \frac{1}{f} \sum_j v^2 \quad (12)$$

with $f = r - n$, where r is the number of mixed standards used to solve eqns. 7 (the index j will be retained as a distinguishing symbol).

To arrive at the standard deviations of x_k and a_{ik} , eqns. 4 and 7 are written in matrix notation:

$$A'Ax = A'y \quad (4a)$$

$$X'Xa = X'y \quad (7a)$$

where $A'A$ and $X'X$ are the symmetrical matrices formed by the coefficient-products of eqns. 4 and 7, respectively; their inverses, $(A'A)^{-1} = Q$ and $(X'X)^{-1} = R$ with the elements q_{ki} and r_{ki} , are the matrices of the weight reciprocals. The diagonal elements of Q and R represent sums of squares; their roots transform the standard deviations of y into those of x_k and a_{ik} :

$$s_{x_k} = s_y \sqrt{q_{kk}} \quad (13)$$

$$s_{a_k} = s_{y_j} \sqrt{r_{kk}} \quad (14)$$

In practice, the total PCB concentration, $x_T = \sum_{k^*} x_{k^*}$, is important (symbols marked with an asterisk refer to PCB only). The standard deviation of this function of the unknowns is obtained by the following equation⁹:

$$s_{x_T} = s_{y^*} \sqrt{\sum_{k^*} \sum_{l^*} q_{k^* l^*}} \quad (15)$$

where the diagonal elements are replaced by the sums of all the elements of Q^* . On calculating s_{x_T} , data derived from the DDE peak are omitted (note, however, that $s'_{y^*} = s'_{y^*}$). It must be remembered that Q and Q^* depend on the set of coefficients used to solve eqns. 4, i.e., on the particular PCB combination in the sample. Limits of confidence are introduced by

$$S = s t(f) \quad (16)$$

where $t(f)$ is the tabulated value of the Student t -distribution for the number of degrees of freedom, $f = m - u$. As f is directly related to the PCB combination, it is convenient to tabulate the values of $t \sqrt{q_{kk}}$ for DDE and $t \sqrt{\sum_{k^*} \sum_{l^*} q_{k^* l^*}}$ for all possible combinations (for four types of PCB, there are 15 combinations).

RESULTS AND DISCUSSION

The coefficients a_{ik} for seven peaks of the four Clophen preparations and DDE, obtained under the gas chromatographic conditions specified, are listed in Table I, together with the standard deviations. The values are based on a set of standard mixtures selected at random. A more uniform distribution of the experimental errors might be obtained by calculating the matrix R in advance in order to check the proper choice of the standard mixtures. The use of overlapping peaks resulting in non-linearity, which increases the apparent error, cannot be entirely avoided, however. Such an effect may be the cause of the high standard deviations observed with peak

TABLE I
COEFFICIENTS AND STANDARD DEVIATIONS

Coefficients a_{ik} (10^4 g^{-1}) and standard deviations $s_{a_{ik}}$ (10^4 g^{-1}) for Clophen A30 ($k = 1$), A40 ($k = 2$), A50 ($k = 3$) and A60 ($k = 4$) and DDE ($i = 4$; $k = 5$).

<i>i</i>	<i>k</i>		1		2		3		4		5	
	a_{ik}	$s_{a_{ik}}$	a_{ik}	$s_{a_{ik}}$	a_{ik}	$s_{a_{ik}}$	a_{ik}	$s_{a_{ik}}$	a_{ik}	$s_{a_{ik}}$	a_{ik}	$s_{a_{ik}}$
1	0.382	0.009	0.008	0.006	0.007	0.025	0.010	0.020	0	0		
2	0.376	0.008	0.168	0.005	0.020	0.022	0.031	0.018	0	0		
3	0.384	0.016	0.827	0.021	0.202	0.045	0.078	0.038	0	0		
4	0.017	0.007	0.196	0.005	0.448	0.020	0.109	0.016	17.396	0.179		
5	0.019	0.016	0.349	0.011	1.219	0.048	0.929	0.038	0	0		
6	0.008	0.012	0.155	0.008	0.636	0.034	0.979	0.027	0	0		
7	0.005	0.010	0.015	0.007	0.114	0.030	0.975	0.024	0	0		

TABLE II
THEORETICAL AND CALCULATED CONCENTRATIONS OF CLOPHEN MIXTURES

Apparent concentrations, γ_i										Clophen and DDE concentrations (10^{-4} g l^{-1})							
γ_1	γ_2	γ_3	γ_4	γ_5	γ_6	γ_7	Theoretical (a) or calculated (b)		A60	A50	A40	A50	A60	Total	$\pm S_{95}$	DDE	$\pm S_{95}$
3.00	3.23	5.21	2.29	7.26	5.07	3.14	a	7.50	2.05	3.00	3.00	3.00	3.00	15.55		0	
							b	7.57	1.72	3.23	2.77	15.29	1.2	0.004	0.046		
0.10	0.77	2.76	1.04	3.69	3.26	3.04	a	0	3.02	0	3.03	6.05	0	0			
							b	0.34	2.87	0	2.95	6.16	0.72	0.009	0.026		
2.88	3.10	4.47	0.63	1.06	0.50	0.16	a	7.50	2.05	0	0	9.55	0	0			
							b	7.45	1.90	0.15	0.07	9.57	0.67	0.003	0.025		
0.16	0.85	3.38	2.37	7.95	5.31	3.28	a	0	3.08	3.00	3.00	9.08	0	0			
							b	0.38	2.81	3.49	2.84	9.52	1.4	-0.004	0.054		
1.96	2.39	4.49	0.83	1.39	0.66	0.25	a	5.00	3.08	0	0	8.08	0	0			
							b	5.02	3.07	0.05	0.16	8.30	0.55	0.006	0.021		
0	0.46	2.18	1.94	5.45	3.70	2.40	a	0	2.05	2.00	2.00	6.05	0	0			
							b	0	1.89	2.28	2.11	6.28	0.47	0.018	0.026		
0	0.11	0.68	2.02	6.50	4.79	3.32	a	0	0	3.00	3.00	6.00	0	0			
							b	0	0	2.97	3.03	6.00	0.33	0.021	0.029		
1.89	1.93	2.40	1.62	3.74	1.67	0.31	a	5.00	0	3.00	0	8.00	0	0			
							b	4.87	0	2.89	0	7.76	0.78	0.014	0.029		

3 (Fig. 1). In spite of strong overlapping, the coefficients of peak 4 show rather low errors. The value of a_{45} , the coefficient for DDE, was determined separately.

In order to test the reproducibility of the method and the reliability of the error estimation, some of the standard mixtures were re-analyzed. Table II shows that most of the results for individual PCB preparations deviate by less than 10% from the theoretical values; for total PCBs, the deviations are less than 5%. The errors are well within the calculated 95% confidence limits for total PCBs as well as for DDE.

TABLE III
AROCLOR MIXTURES CALCULATED AS CLOPHEN AND DDE

Given Aroclor mixtures (10^{-4} g l $^{-1}$)				Clophen calculated (10^{-4} g l $^{-1}$)						DDE calculated (10^{-4} g l $^{-1}$)	
1242	1248	1254	1260	A30	A40	A50	A60	Total	$\pm S_{95}$	Amount	$\pm S_{95}$
6.0	4.0	4.0	2.0	4.71	4.66	2.82	3.27	15.46	4.1	0.015	0.16
0	3.0	3.0	3.0	0.41	2.51	1.35	4.88	9.14	3.9	0.025	0.15
8.0	4.0	0	0	6.71	5.40	0	0.04	12.15	1.6	0.005	0.06
0	6.0	3.0	0	0.45	5.59	2.64	0.34	9.01	0.81	0.008	0.03
0	6.0	0	3.0	1.27	3.94	0	3.46	8.66	5.5	0.004	0.20

As larger errors may be expected with independent samples, some mixtures of Aroclor preparations were evaluated, using coefficients based on Clophen standards. The results in Table III in fact show large deviations as regards the individual preparations and the confidence limits, but the total PCB concentrations are surprisingly close to the theoretical values. The errors appear higher because Aroclors show different gas chromatographic patterns, leading to altered coefficients. Any variation in the coefficients must result in a higher value of $\sum v^2$ and s'_y . If coefficients of different commercial products are available, it may be possible to find out of which particular brand a sample is composed by selecting the set of coefficients that gives the best fit, *i.e.*, the lowest value of s'_y . To analyze samples that contain products of varying origin (such as marine sediments), it may be convenient to use standard mixtures of more than one commercial brand in order to secure a more general applicability of the coefficients.

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