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Comparative analysis of thirty polychlorinated biphenyl congeners on two capillary columns of different polarity with non-linear multi-level calibration

EVA STORR-HANSEN

Ministry of Environment, National Environmental Research Institute, Division of Environmental Chemistry, Mørkhøj Bygade 26H, DK-2860 Søborg (Denmark)

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ABSTRACT

The elution patterns of polychlorinated biphenyls (PCBs) are different on capillary columns of different polarities. Congeners which elute as single peaks on one type of column may co-elute with other congeners on another type of column. This paper describes quantitative results from the analysis of calibration standards and various sample extracts from seals using a capillary dual-column gas chromatography system with electron-capture detection, where the two capillary columns are operated in parallel with a glass T-split. Non-linear multi-level calibration graphs and choice-of-fit functions are discussed with respect to the quantitative analysis of thirty PCB congeners. The differences in the results of the analyses on the two columns are evaluated, and an approach for preparing a single quantitative report from the two sets of analytical results is proposed.

INTRODUCTION

The analysis of specific polychlorinated biphenyl (PCB) congeners in environmental samples by capillary gas chromatography–electron-capture detection (GC–ECD) has been widely reported [1–4]. The precise identification and quantitation of specific PCB congeners is required as the toxicity of the congeners varies considerably [2]. The burden of the most toxic congeners, the non-*ortho* co-planar PCBs and their mono- and di-*ortho* derivatives, must be determined with the best precision possible in environmental samples.

The most common methods use a single capillary column for the separation, identification and quantitation of the PCB congeners. However, the complete separation of all congeners on a single capillary column has not yet been reported. Identification based solely on retention time (t_R) on a single column with ECD may still leave interfering compounds to be falsely identified as PCBs. Confirmation analysis of PCBs is frequently performed by analysing the sample on a second column of a different polarity [4–9]. Dual-column chromatography, where the two columns are installed in parallel in a GC oven, enables confirmation analysis to be routinely performed [5,8].

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Comparable analytical results are expected from the two columns when the analyte elutes as a single-component chromatographic peak on both columns. In this instance, either of the two columns may act as a confirmation column for the other. However, many PCBs do not elute in single-component peaks, and possible co-eluting PCBs have only been reported for the DB-5 column [1–3]. The problems of co-elution increase with the number of PCB congeners analysed [9], and the resolution of the PCB congeners must be verified for each of the two columns used. This applies in particular to less abundant congeners, and to congener clusters containing both major and minor analytes.

There are relatively few reports of quantitation procedures for dual-column chromatography [4,7–9]; the two analyses are usually performed separately [4,7,9]. The quantitation procedure reported by Erickson [4] used the average value if the results were within 20%. The lower value was taken when the results differed by more than 20%. Co-eluting or unresolved PCBs were summed.

Analytical systems in which the two columns are operated simultaneously allow the determination of interferences for each PCB congener and eliminate variability from repeated injections of the sample [5,8]. The determination of technical Aroclor mixtures (total PCBs) and organochlorine pesticides in various samples has been reported as an average of the results from the two columns, but the quantitation procedure was not discussed further [8].

The precision of the quantitation procedure depends on the mode of calibration. Many electron-capture detectors display non-linear response curves [6,10]. As PCB congeners in environmental samples show a wide range of concentrations, calibration is a possible source of error in the quantitative analysis of PCBs.

EXPERIMENTAL

Chemicals

PCB congeners were selected on the basis of toxicity and persistency [3,5,11] for a study of chlorinated organic micropollutants in marine mammals. In the following, the PCB congeners are referred to by their IUPAC numbers [4]; the molecular structures of the PCB congeners in the calibration mixture are listed in Table 1. The PCB standards were purchased from Ultra Scientific, Promochem, Cambridge Isotope Laboratories and Community Bureau of Reference, EEC. The purity was greater than or equal to 99% and the PCBs were not purified further before use. PCB-15, -44, -136, -70 and -95 were generous gifts from Dr. L. G. Hansen (University of Illinois, Urbana-Champaign, IL, USA).

Pentachlorobenzene, hexachlorobenzene and *p,p'*-DDE were found in the PCB fraction from the clean-up of samples and were included in the calibration standard mixture. PCB-53 and PCB-155 were used as internal standards, as recommended by Wells *et al.* [12], as these compounds are not found in technical PCB formulations and are thus unlikely to occur in the environment. Octachloronaphthalene (OCN) was used as a late eluting internal standard. Any one of the three internal standards could be used for quantitation, but PCB-155 was usually preferred as it eluted in the middle of the PCB chromatogram. In total the mixture contained 36 compounds, of which three were internal standards and two were recovery surrogate standards (PCB-3 and PCB-198) [12].

TABLE I

PCB CONGENERS IN CALIBRATION STANDARD MIXTURE

Pentachlorobenzene, hexachlorobenzene, *p,p'*-DDE and octachloronaphthalene are included in the mixture, but not discussed further here. PCB-149 (2,2',3,4',5',6-H_xCB) was added in later preparations of the mixture. M_oCB = Monochlorobiphenyl; D_iCB = dichlorobiphenyl; T_rCB = trichlorobiphenyl; T_e = tetrachlorobiphenyl; P_eCB = pentachlorobiphenyl; H_xCB = hexachlorobiphenyl; H_pCB = heptachlorobiphenyl; O_cCB = octachlorobiphenyl.

Structure	IUPAC No.	1-*ortho*[a]	2-*ortho*[b]	ICES[c]	Other purpose
4-M_oCB	PCB-3				x[d]
4,4'-D_iCB	PCB-15				
2,4,4'-T_rCB	PCB-28			x	
2,4',5-T_rCB	PCB-31			x	
3,4,4'-T_rCB	PCB-37				x[e]
2,2',3',5-T_eCB	PCB-44				
2,2',5,5'-T_eCB	PCB-52			x	
2,2',5,6'-T_eCB	PCB-53				x[f]
2,3',4',5-T_eCB	PCB-70				
3,4,4',5-T_eCB	PCB-81				x[e]
2,2',3,5',6-P_eCB	PCB-95				
2,2',4,5,5'-P_eCB	PCB-101			x	
2,3,3',4,4'-P_eCB	PCB-105	x		x	
2,3,3',4',6-P_eCB	PCB-110				x[g]
2,3,4,4',5-P_eCB	PCB-114	x			
2,3',4,4',5-P_eCB	PCB-118	x		x	
2,2'3,3',4,4'-H_xCB	PCB-128		x		
2,2',3,3',6,6'-H_xCB	PCB-136				
2,2',3,4,4',5-H_xCB	PCB-137		x		
2,2',3,4,4',5'-H_xCB	PCB-138		x	x	
2,2',4,4',5,5'-H_xCB	PCB-153		x	x	
2,2',4,4',6,6'-H_xCB	PCB-155				x[f]
2,3,3',4,4',5-H_xCB	PCB-156	x		x	
2,3,3',4,4',5'-H_xCB	PCB-157	x			
2,3,3',4,4',6-H_xCB	PCB-158		x		
2,3,4,4',5,6-H_xCB	PCB-166		x		
2,3',4,4',5,5'-H_xCB	PCB-167	x			
2,3,3',4,4',5-H_xCB	PCB-170		x		
2,2',3,4,4',5,5'-H_pCB	PCB-180		x	x	
2,3,3',4,4',5,5'-H_pCB	PCB-189	x			
2,2',3,3'4,4',5,5'-O_cCB	PCB-194		x		
2,2',3,3',4,5,5',6-O_cCB	PCB-198				x[d]

[a] Mono-*ortho* co-planar PCBs [2].
[b] Di-*ortho* co-planar PCBs [2].
[c] Compounds recommended by ICES [6].
[d] Recovery surrogate standard.
[e] Non-*ortho* co-planar PCB, less toxic than PCB-77, -126, -169 [2].
[f] Internal standard. Octachloronaphthalene is third internal standard.
[g] Interfering compounds known from DB-5 (PCB-77 and PCB-110) [1,3].

A stock solution of the calibration mixture containing approximately 1000 ng/ml of each compound (PCB-3 = 43 000 ng/ml because of low ECD response) was prepared in iso-octane. Standard calibration mixtures were made by diluting the stock solution by volume.

Samples

A number of samples originating from seals in the Danish part of the Wadden Sea were analysed using the dual-column system.

Sample extraction and clean-up

The sample extraction and clean-up was a modified version of that of Jensen *et al.* [13]. A detailed description of the procedure and the method validation study is in preparation. The main features were as follows: 10 g of homogenized sample were blended three times with dichloromethane–methanol (2:1, v/v) in an Ultra-Turrax blender. The combined extract was shaken with acidified water in a separating funnel. The organic phase was filtered through anhydrous sodium sulphate and the solvent evaporated. The residue was redissolved in hexane and treated with sulphuric acid adsorbed on silica gel. The final clean-up of the extract was by chromatography on basic aluminium oxide, deactivated with 1% (w/w) water. Internal standards were added to the samples and the volume of the extract was adjusted to 1 ml. Extracts of blubber samples were analysed at dilutions 1:200 and 1:20, and extracts of other tissues at dilutions 1:20 and undiluted.

Most of the samples were submitted to chromatography on Carbopack C, which retained the non-*ortho*-substituted co-planar PCBs (PCB-37, -81, -77, -126 and -169). These congeners were eluted from Carbopack C with toluene and analysed by isotope dilution technique and combined GC and mass spectrometry (MS) for the precise determination of the much lower levels of these particular congeners.

Gas chromatographic analysis

Analysis was performed on a HP-5890A (II) gas chromatograph with an HP-7376 autosampler. The GC parameters were as follows: Column 1, J&W DB-5 (5% phenylmethylsilicone), 60 m × 0.25 mm I.D., 0.11 μm d_f; and column 2, J&W DB-1701 (14% cyanopropylphenyl), 60 m × 0.25 mm I.D., 0.15 μm d_f.

A splitless injection of 2 μl of sample was used, with a splitless time of 1 min and an injector temperature of 250°C. Detection was with two ^{63}Ni electron-capture detectors operated at 300°C. The carrier gas was helium, with a column head pressure of 170 kPa, corresponding to a linear flow-rate of approximately 25 cm s at 150°C. The make-up gas was nitrogen at a flow-rate of 50 ml min. The temperature was 90°C for 1 min, then 90 to 180°C at 25°C min, 180°C for 2 min, then 180 to 220°C at 1.5°C min, 220°C for 2 min, then 220 to 275°C at 3°C/min and finally 275°C for 10 min.

The columns were connected to the injector by 1 m of uncoated 530 μm pre-column and a glass T-piece. The collection and processing of data were performed by a Vectra QS/20 PC, with HP Chemstation software.

RESULTS AND DISCUSSION

Qualitative analysis

Chromatograms of the entire calibration mixture and retention times on the two columns have been given previously [5]. The identification of the congeners was on the basis of t_R, where the Chemstation software used the three internal standards to adjust the t_R values of the calibration table to t_R values of the sample [5,14]. A t_R tolerance of 1‰ was used for the internal standards and 0.2‰ for the analytes. The stability of the t_R values and injection volume have been described previously [5].

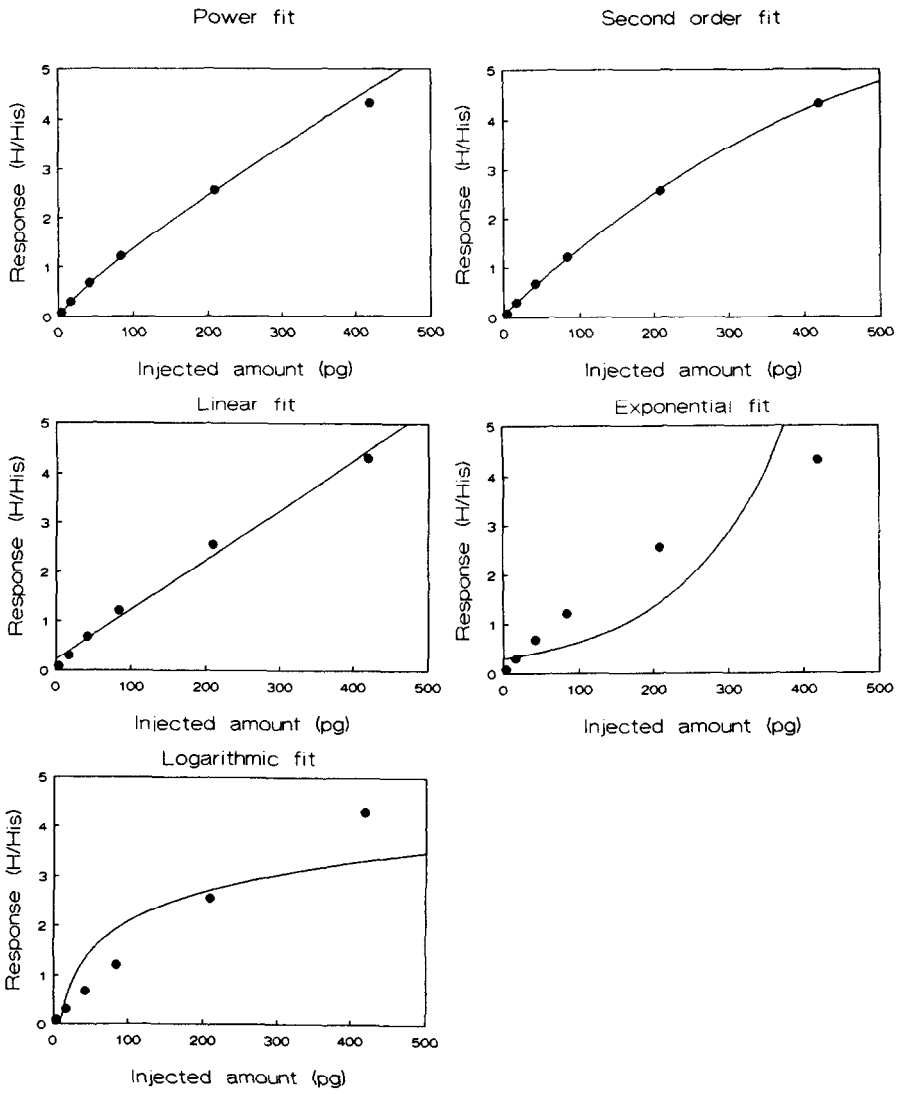

Fig. 1. Response curve fits for PCB-101 showing power, second-order, linear, exponential and logarithmic fits. The *X*-axis units are the injected amounts of PCB-101 in picograms and the *Y*-axis units are the peak heights of PCB-101 relative to the peak height of the internal standard PCB-155 (IS-2).

Quantitative analysis

The instrumental detection limit was calculated as $3SC_s$ (ng/ml), where S is the standard deviation of the response [peak heights relative to the peak height of the internal standard PCB-155 (H/H_{IS})] for three injections of standard calibration mixture with a concentration C_s = 5 ng/ml. The values ranged from 0.1 to 0.5 ng/ml injected. In samples, the detection limit could be higher, corresponding to the degree of dilution of the extracts. Dilutions were required as a result of the large amount of sample used, which was in turn required by the low levels of non-*ortho* co-planar PCBs present in the samples.

The response of both the electron-capture detectors in the system was non-linear and thus multi-level calibration was required for the simultaneous determination of the range of concentrations of individual PCB congeners present in marine mammal samples. The Chemstation software provided five possible mathematical functions to use as calibration graphs (linear, power, exponential, logarithmic and second-order fit) and three ways of handling the origin (as a data point, ignored or connected to the lowest data point by a straight line [14]). The diluted calibration standard solutions (5, 20, 50, 100, 250 and 500 ng/ml) were analysed by the dual-column system, and the various curve fits applied to the data for PCB-101 (Fig. 1). PCB-101 was representative of the remaining PCBs with respect to the appearance of the response graph. The exponential and logarithmic fits were not useful as fit functions. Among the remaining three fits, the power fit (ax^b) yielded values which were too low at the high level of PCB-101, whereas the second-order curve ($ax^2 + bx + c$) fitted the data well. The linear fit overestimated the middle-level concentrations and did not pass through the origin. The three types of fit were compared by calculating the relative error as a percentage:

$$\text{Relative error} = (X_{\text{calc}} - X_{\text{meas}})100/X_{\text{meas}} \quad (1)$$

where X_{calc} is the calculated response of the PCB congener with IUPAC number x, and X_{meas} is the actual measured response for the same congener. For a representative congener, PCB-101 (Fig. 2), the results indicated that the linear fit had a large relative fit error at low concentrations. The power fit and the second-order fit were compara-

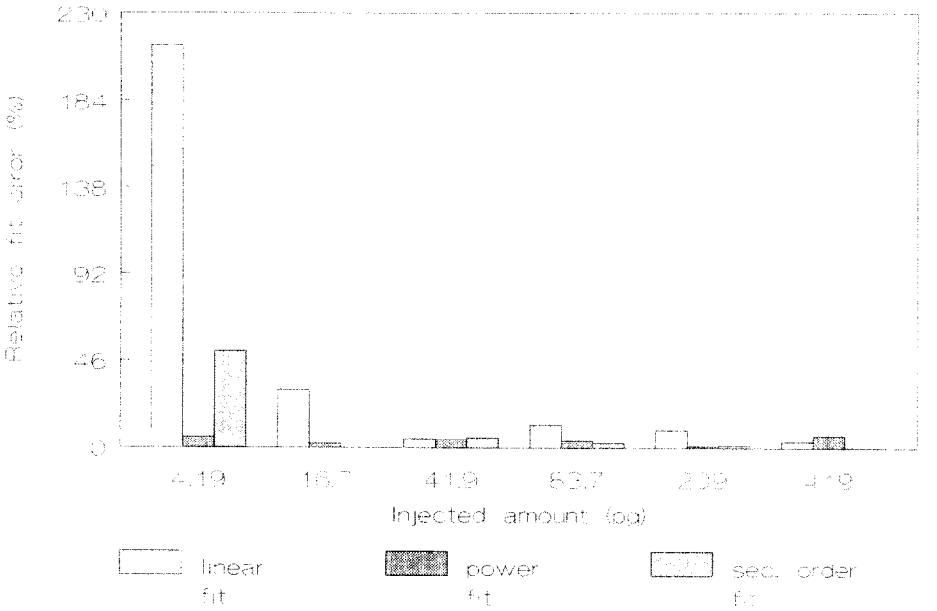

Fig. 2. Relative fit error (%) for the three best fits: power, second-order and linear fit in the concentration range 5–500 pg PCB-101 injected. Power fit is the best choice in the concentration range 5–250 ng/ml (250 pg injected onto each column). This corresponds to a calibrated range from the detection limit to 250 ng/ml.

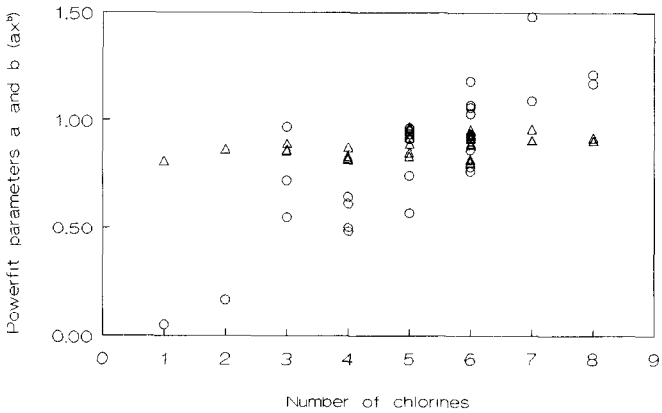

Fig. 3. Coefficient a (○) and power b (△) of the power fit function ax^b of each analysed PCB congener as a function of the number of chlorines. The value of a increases with degree of chlorination, whereas b is fairly uniform for different degrees of chlorination.

ble with respect to relative error, except near the detection limit where the power fit had the lowest relative error. Based on this, the calibrated range was narrowed down from detection limit up to 250 ng/ml, and the power fit method was chosen for all PCB congeners. As a result of the relatively large number of analytes, only four calibration levels were used, namely 5, 20, 100 and 250 ng/ml, because of the limitations of the software in data handling. As the power function passes the origin by definition, the origin was ignored as a data point.

For each PCB congener in the mixture, and for both columns, the best fit of the power fit function, based on the least-squares method, was then calculated during calibration. The fit was characterized by coefficient a and power b (ax^b) in the fit function, and a correlation coefficient r^2. The value of r^2 was in the range 0.998–1.000 for all PCB congeners ($n=30$) for both the DB-5 and DB-1701 columns. The fit parameters for each PCB congener on the DB-5 column were plotted *versus* the degree of chlorination (N_{Cl}) (Fig. 3). The power of the fit function (b) was seen to be fairly uniform for the different degrees of chlorination, whereas the coefficient (a) increased with degree of chlorination. This finding was consistent with literature reports of increasing response factors (RFs) with increasing numbers of chlorines [1,4] from linear or single level calibration.

The values for the coefficient (a) and the power (b) of the fit functions from the two columns were compared for all congeners by a two-tailed Student's t-test. A significant correlation ($p=0.05$) between both the a values from the two columns and between the b values from the two columns were found. This was taken to indicate that similar calibration functions apply on both columns and detectors.

If a larger calibration range was used, the second-order fit was preferable. The fit error near the detection limit was reduced by plotting a straight line from the lowest calibrated point to the origin.

The stability of the RFs was investigated to determine how often recalibration should be performed during larger numbers of analyses. An RF was calculated and

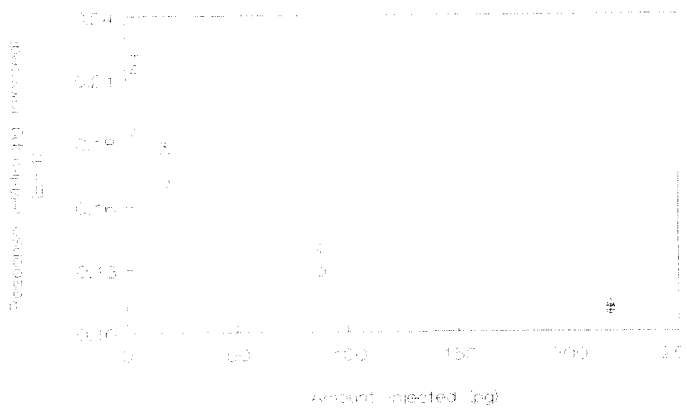

Fig. 4. RFs from three complete calibrations, performed approximately 10 days apart. ○ = June 26th, 1990, △ = July 7th, 1990 and □ = July 19th, 1990. The RFs show a small decrease during the period. A linear range, defined as a range with a maximum of 10% variation in response factors, could only be defined for small concentration ranges (10% is approximately one division at the *y*-axis).

compared for (1) six repeated injections of the same 50 ng/ml solution during 7 h of analysis and (2) a number of sample batch runs over several days.

$$RF = H_x/(H_{IS} \times \text{injected amount}) \quad (2)$$

From the repeated injections of the standard solution, the RF for PCB-101 had a relative standard deviation (R.S.D.) of 0.90%. This indicated a very stable detector response during daily analysis. It was therefore decided to recalibrate once every four samples, to adjust the retention times in the calibration table, rather than to adjust the RFs.

The long-term stability of the detector response in three batches of samples, analysed about 10 days apart (Fig. 4), indicated a change in the RFs during this period. It was concluded that a complete recalibration at all four levels was necessary prior to the analysis of a new batch of samples.

Comparison of quantitative results from DB-5 and DB-1701 columns

After the correction of any blank values, the difference between the Front and Rear signals (DB-5 and DB-1701 columns, respectively), $\Delta_{F\text{-}R}$, was calculated as a percentage for each PCB congener analyte:

$$\Delta_{F\text{-}R} = (X_{DB\text{-}5} - X_{DB\text{-}1701})100/(X_{DB\text{-}5} + X_{DB\text{-}1701}) \quad (3)$$

X_n is the analytical result from column n in ng/ml and $\Delta_{F\text{-}R}$ is a percentage. When $\Delta_{F\text{-}R}$ was positive, the DB-5 result was the larger value and *vice versa*, and the maximum and minimum values were ± 100%, respectively. The validity of $\Delta_{F\text{-}R}$ as an evaluation parameter was tested on standard calibration mixtures and a number of samples of blubber and other tissues from seals.

Calibration standards

The difference in the quantitative results from the DB-5 and DB-1701 columns

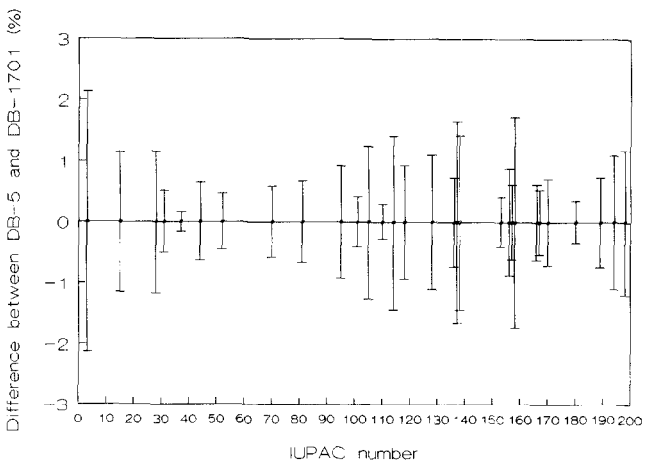

Fig. 5. Difference between analytical results from DB-5 and DB-1701 columns as a percentage ($\Delta_{F/R}$). Mean ± standard deviation of values from four calibration levels for each PCB congener. The two quantitative results are comparable and independent of calibration level.

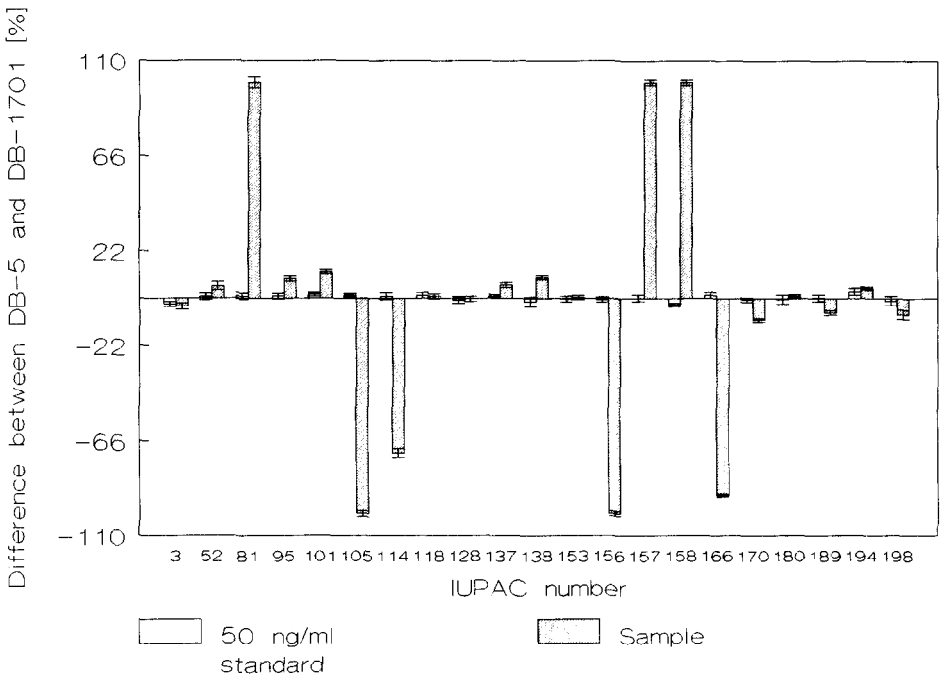

Fig. 6. Difference between analytical results from DB-5 and DB-1701 columns as a percentage ($\Delta_{F/R}$). Mean ± standard deviation of $\Delta_{F/R}$ values for repeated injections of a 50 ng/ml calibration standard compared to repeated injections of a seal blubber extract.

was investigated for the 5, 20, 100 and 250 ng/ml calibration standards. For each PCB congener, $A_{F/R}$ was calculated for all levels, and subsequently the mean value of the four calibration levels was calculated. The results were all within the range $\pm 2\%$, as shown in Fig. 5. This result indicates that the quantitative results from the analysis of calibration standards on the two columns were comparable and independent of the concentration level within the calibrated range for all the PCB congeners studied.

Comparison of standard mixture and blubber extract

The mean and standard deviation for six injections of a 50 ng/ml standard mixture was compared to six injections of a blubber extract (Fig. 6). The variance of the measurement of each congener, indicated by the error bar, was small. Significant differences were observed between the quantitative results for most PCB congeners in the sample and in the standard mixture. The mean values of some congeners in the standard mixture were not zero (PCB-3, -101, -137, -194), as a result of the fact that the calibration was not performed on the 50 ng/ml standard mixture, in contrast to the four calibration levels in Fig. 5.

Based on the maximum standard deviation of $\pm 2\%$ in the calibration standards in Fig. 5, and the observed non-zero mean of $A_{F/R}$ in the uncalibrated standard

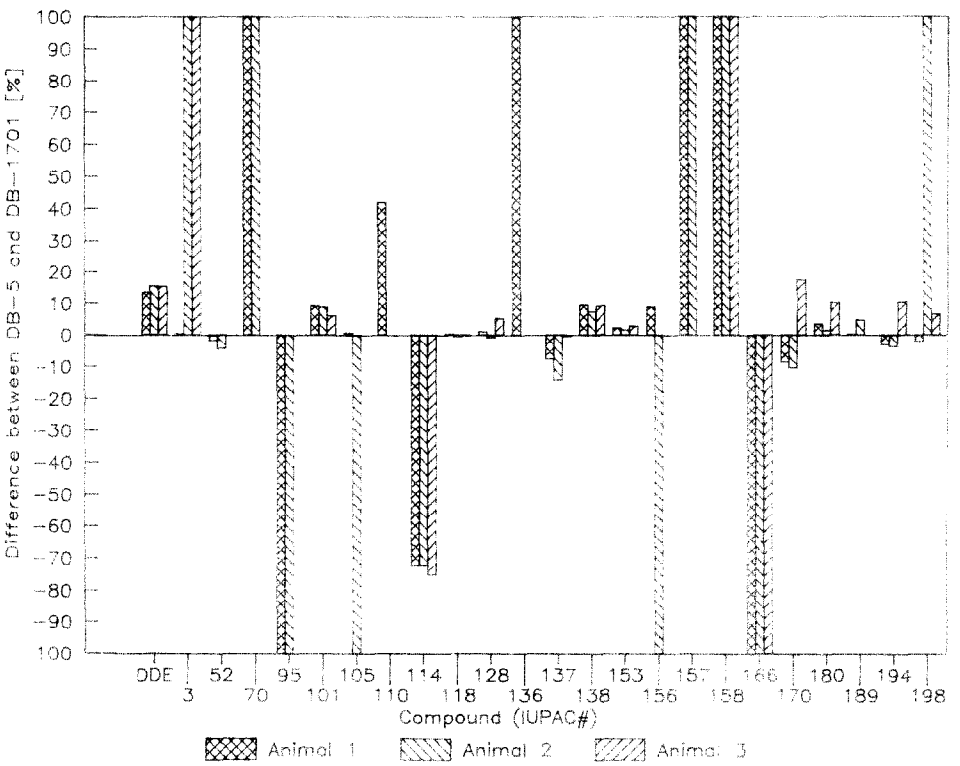

Fig. 7. Difference between analytical results from the DB-5 and DB-1701 columns as a percentage ($A_{F/R}$) in extracts of blubber samples from three seals. PCB-101 is overestimated by about 10% by the DB-5 column in all three samples; PCB-138 is overestimated on the DB-5 column by about 10%.

mixtures in Fig. 6, a maximum value for $\Delta_{F/R}$ of ± 5% was chosen as an estimate for the insignificant difference between the two signals during sample analysis.

Analysis of animal tissues

Blubber samples from three animals and several tissues from one seal were analysed and the values of $\Delta_{F/R}$ in the sample extracts evaluated (Figs. 7 and 8). The blubber extracts were diluted 2–400 times. The tissue samples were analysed in sequences as given in the caption to Fig. 7.

In all samples, PCB-114 had a co-eluting compound on the DB-1701 column as reported previously [5]. PCB-101 was overestimated by the DB-5 column ($\Delta_{F/R}$ = 7–11%) in all samples. This was in accordance with earlier reports, which stated that PCB-90 is a possible co-eluting compound with PCB-101 on the DB-5 column [3,7,15], and that it constitutes about 10% of the peak at the t_R value of PCB-101 on the DB-5 column [16]. PCB-138 was overestimated on the DB-5 column ($\Delta_{F/R}$ = 3–11%). This was in agreement with the results reported by Larsen and Riego [7], in which PCB-163 (2,3,3′,4′,5,6,-H_xCB) was identified to constitute 10–30% of the peak at the t_R value of PCB-138 in various environmental and technical samples analysed on the DB-5 column. Williams and Lebel [9] also reported consistently higher values (about 10%) for PCB-138 on an SPB5 column relative to values found by a more polar SPB35 column. In all samples, *p,p′*-DDE had positive values of $\Delta_{F/R}$ (13–30%).

In the tissue samples, PCB-170 was overestimated by the DB-1701 column

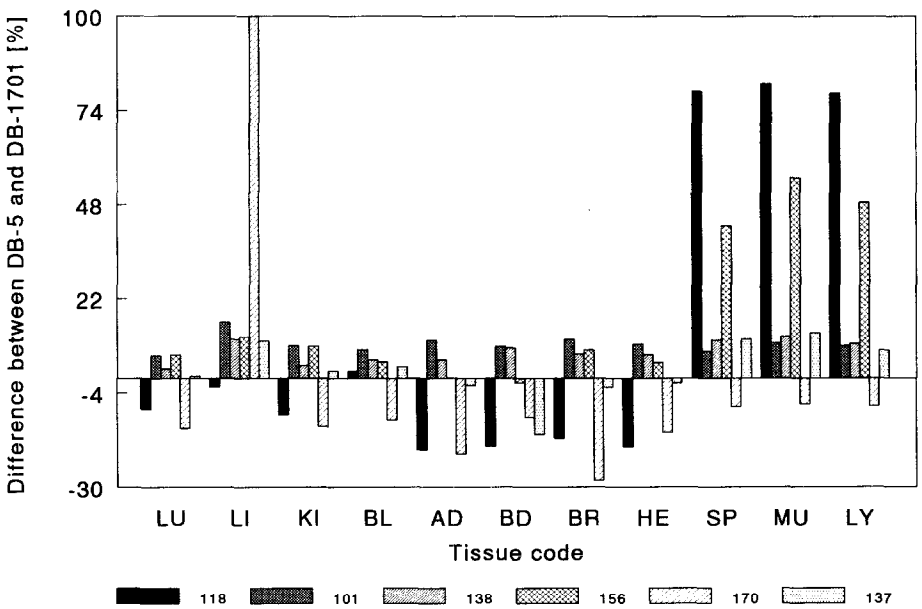

Fig. 8. Difference between analytical results from the DB-5 and DB-1701 columns as a percentage ($\Delta_{F/R}$) for selected PCB congeners, in extracts of various tissues from one seal. Capital letters = tissue code in figure, date of analysis of batches in parentheses: LIver/LUng/KIdney (May 18th, 1990), BLubber (July 6th, 1990), ADrenals/BlooD/BRain/HEart (July 19th, 1990) and SPleen/MUscle/LYmph nodes (July 26th, 1990). The value of $\Delta_{F/R}$ of PCB-118 follows the batch; a possible co-eluting compound on DB-5 was PCB-149 [3,15].

($\Delta_{F/R}$ = −7 to −28%). The value of $\Delta_{F/R}$ for PCB-156 in tissue samples was generally positive ($\Delta_{F/R}$ = −1 to 55%), in contrast to what was found for humane adipose samples analysed on SPB5 and SPB35 columns [9]. The $\Delta_{F/R}$ behaviour of PCB-118 in tissue samples followed the batch. The quantitation of this congener was extremely sensitive to separation from a close eluting congener, possibly PCB-149 (2,2',3,4',5',6-H_5CB) on DB-5 [3,15]. The separation of PCB-118 and PCB-149 was later tested (Fig. 9) and was better on the DB-1701 column than on the DB-5 column; PCB-149 was not present in the original standard calibration mixture, but was included in later preparations of the standard mixture. The analysis of blubber extracts with the new standard preparation revealed PCB-149 to be present at similar levels to PCB-118. Fig. 8 illustrates that the ability to separate CB-149, 118 on DB-5 is lost for the last batch of tissue samples (spleen/muscle/lymph nodes).

PCB-105 was not identified on the DB-5 column in some samples, possibly due to incomplete separation from PCB-153, which was the most abundant PCB congener in all analysed samples. PCB-132 (minor) could influence quantitation on both columns [17], but was not part of the analytical programme. The separation of PCB-132 and PCB-105 seemed most critical on the DB-5 column. PCB-157 co-eluted with PCB-180 (major congener) on the DB-1701 column, and was only detected on DB-5 in all samples analysed ($\Delta_{F/R}$ = 100%). PCB-158 co-eluted with the second most abundant PCB congener PCB-138 on the DB-1701 column, and was only detected on DB-5 ($\Delta_{F/R}$ = 100% in all samples). PCB-167 and PCB-128 were best separated on the DB-1701-column, but only just separated from the baseline. PCB-167 was not reported in any samples on the DB-5 column.

In some blubber extracts, analysed in dilution of 1:20, the internal standard PCB-155 had a co-eluting compound on the DB-1701 column, which rendered it unsuitable as an internal standard. OCN was used as an alternative internal standard for these samples. For the recovery of the standards PCB-198 and PCB-3, co-eluting compounds were observed on the DB-1701 column in one and two of the blubber samples, respectively. As these co-eluting compounds eluted close to and were comparable in intensity to the analytes, the software did not detect the analytes. No trend was observed for the remaining congeners.

Proposed criteria for handling of data from dual-column systems

The first step is to subtract blank values from the sample data. Data should only be reported within the calibrated range. Chromatograms, including calibration standards, should be examined in detail for each analysis to identify critical separations which may influence the identification or quantitation of the target compounds. Dilution factors calculated from the data should correspond to the actual dilution factors for major congeners identified in both dilutions.

In addition to these procedures the following set of criteria for the preparation of a single quantitative report from the dual-column system are proposed. The criteria are based on these presented analytical results in standard calibration mixtures and seal tissues, and a knowledge of the incomplete separation of PCB congener pairs from the literature [3,17,18].

(1) A congener, identified on only one of the two columns, is reported as not detected (n.d.).

(2) If $|\Delta_{F/R}|$ is less than 5%, the difference is regarded as insignificant, based on

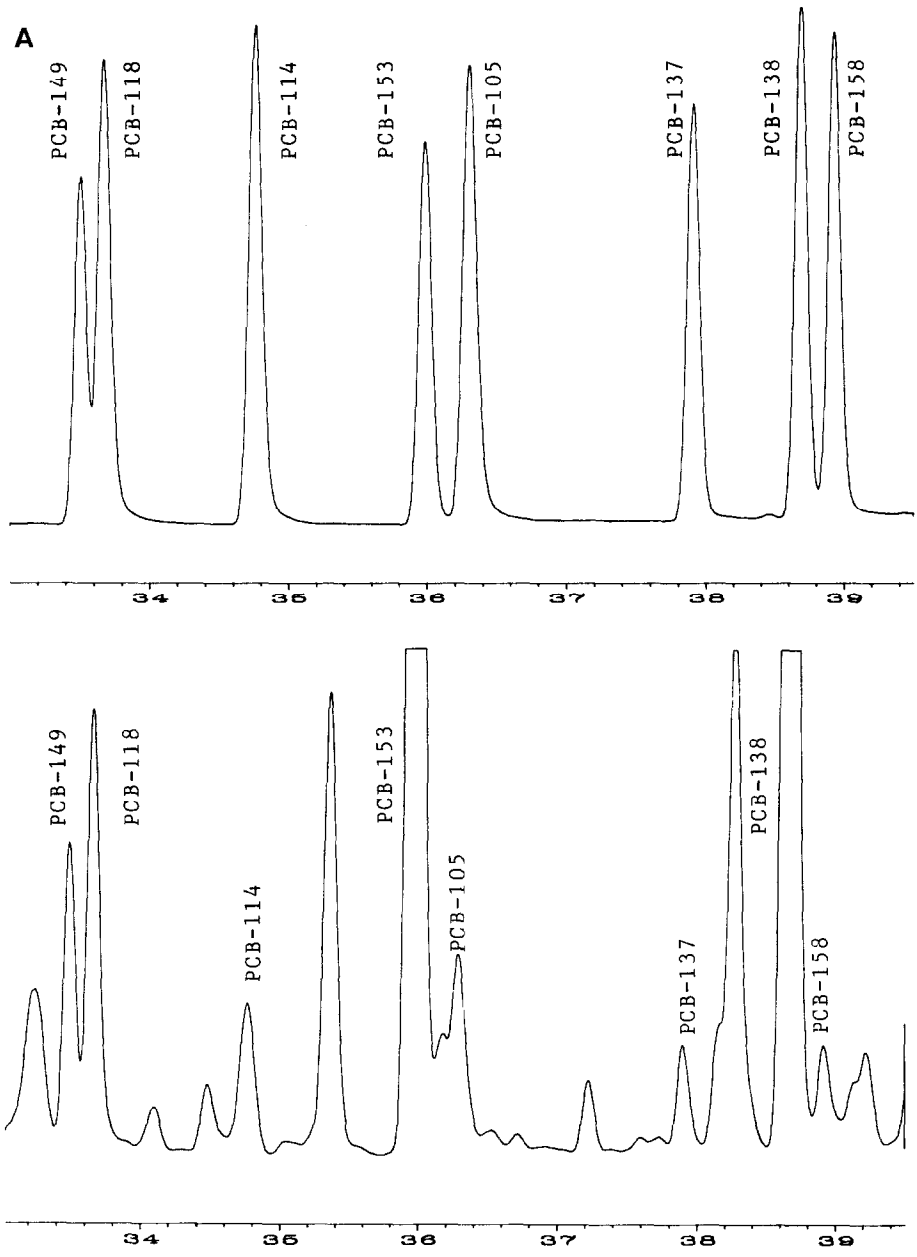

Fig. 9.

(*Continued on p. 388*)

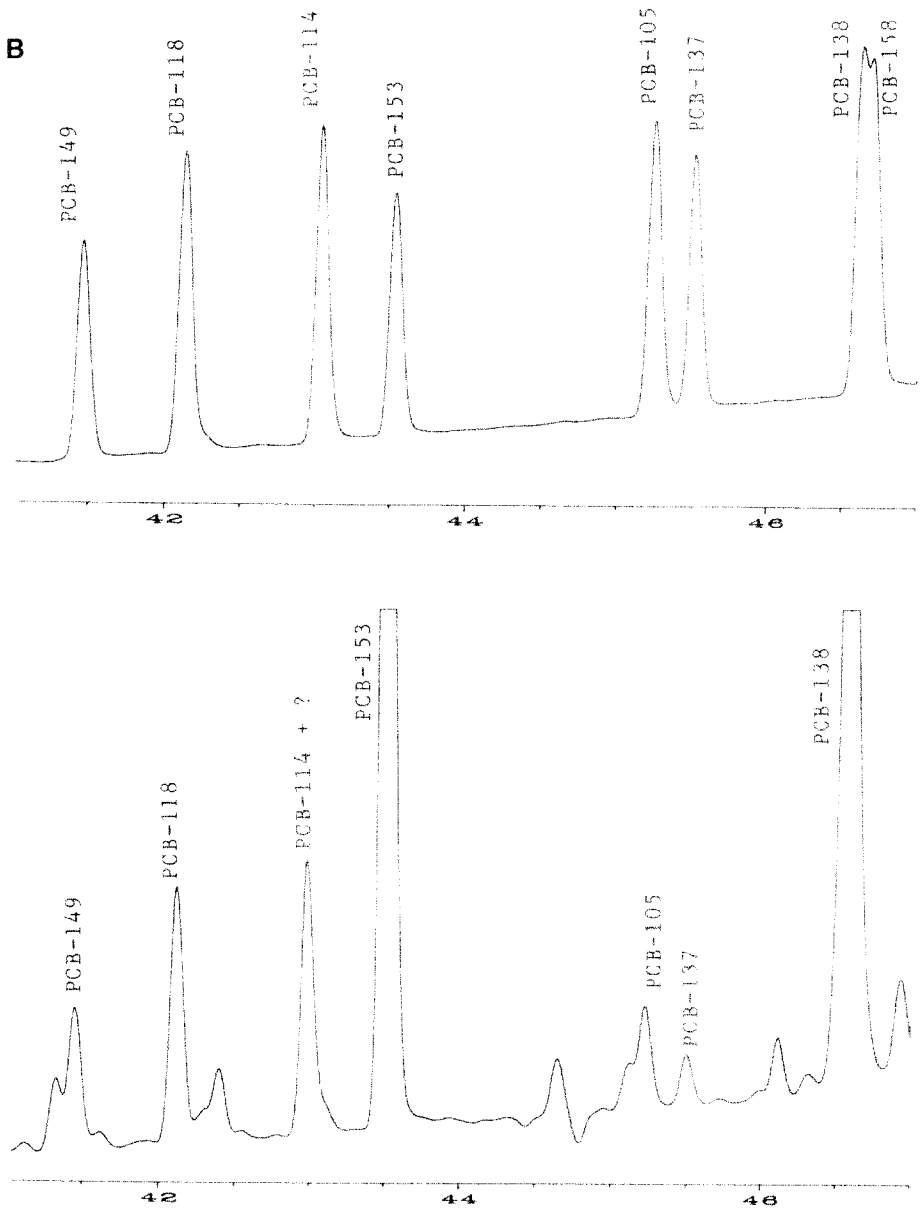
B
PCB-149
PCB-118
PCB-114
PCB-153
PCB-105
PCB-137
PCB-138
PCB-158
42
44
46
PCB-149
PCB-118
PCB-114 + ?
PCB-153
PCB-105
PCB-137
PCB-138
42
44
46

Fig. 9.

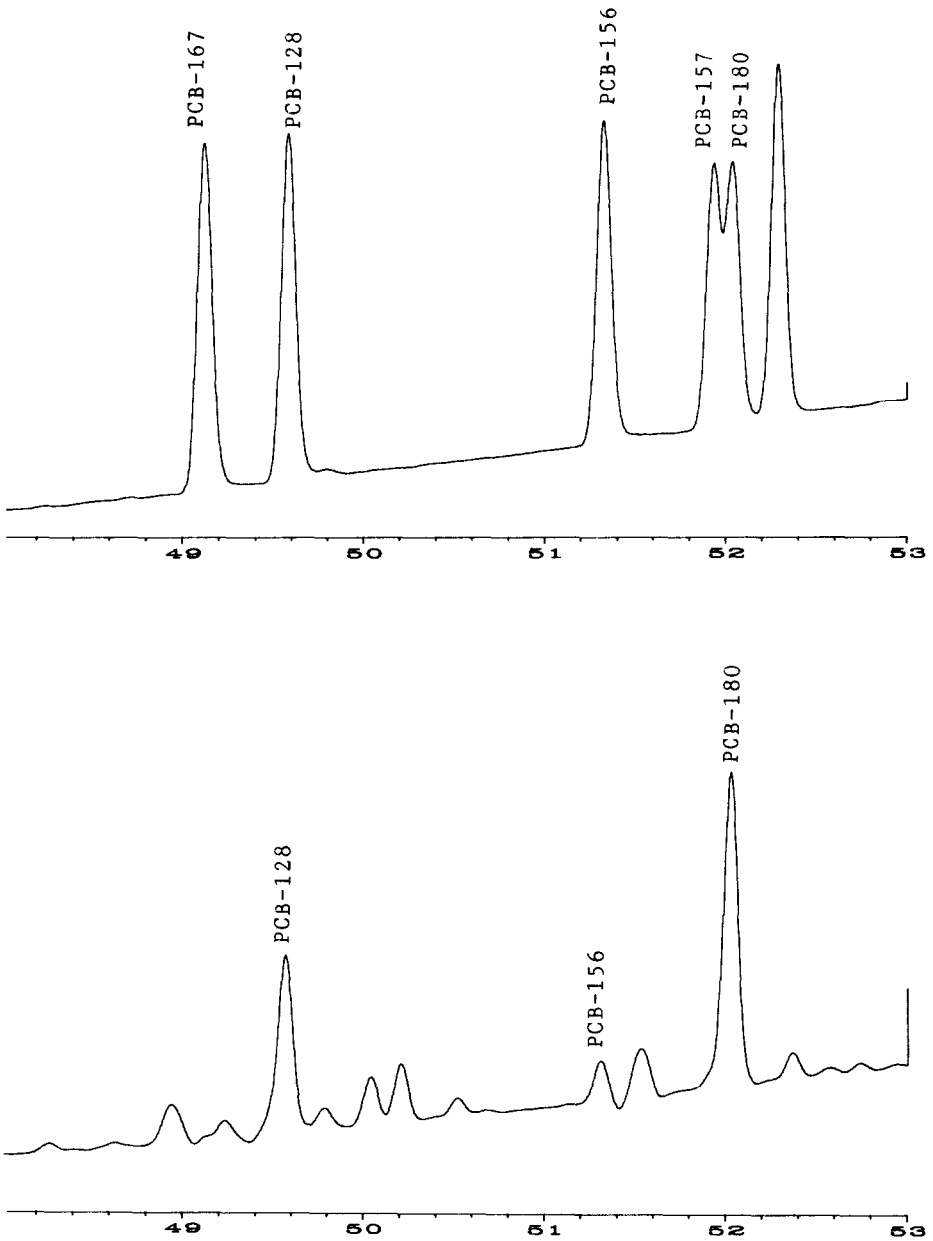

Fig. 9. (A) Critical chromatographic separations of PCB-118 and PCB-149, PCB-153 and PCB-105 and PCB-138 and PCB-158 in a standard mixture (upper panel) and a seal blubber extract (lower panel) on the DB-5 column. (B) Critical chromatographic separations of PCB-118 and PCB-149, PCB-153 and PCB-105, PCB-138 and PCB-158 and PCB-157 and PCB-180 in the same standard mixture (upper panel) and a seal blubber extract (lower panel) as in (A), analysed on the DB-1701 column.

the variation from the calibration standards as discussed earlier. Either of the two results may be used. Erickson [4] proposed the use of the average value.

(3) If the $|d_{F,R}|$ value is larger than 5%, the lowest quantitative result is accepted, based on the assumption that the higher value was caused by an impurity or co-eluting congener. This is consistent with the procedure discussed by Erickson [4]. However, matrix interferences should be considered, for instance, in the analysis of waste oils, which may lower the ECD response of the PCBs.

Excepted from these criteria are the following congeners:

PCB-105 is quantitated on the DB-1701 column, and the result is accepted even if n.d. is reported from the DB-5 column. The close eluting congeners on DB-5 are PCB-153 (major) and PCB-132 (minor). Confirmation analysis may be performed by GC–MS.

PCB-157 is quantitated on the DB-5 column, and the result is accepted even if n.d. is reported from the DB-1701 column. The close eluting congener on the DB-5 column is PCB-180 (major). Confirmation analysis may be performed by GC–MS.

PCB-158 is quantitated on the DB-5 column, and the result is accepted even if n.d. is reported from the DB-1701 column. The close eluting congener on DB-5 is PCB-138 (major). Confirmation may be performed on another polarity column; confirmation by GC–MS not possible as both PCB-138 and PCB-158 are hexachlorinated.

PCB-167 and PCB-128 should both be reported from the DB-1701 column, even if one (PCB-167, minor) is reported as n.d. on the DB-5 column. Confirmation may be performed on another polarity column. Confirmation by GC–MS not possible as both PCB-167 and PCB-128 are hexachlorinated.

If a congener is reported from only one column, the result should be followed by the statement "unconfirmed".

Recommendations for the analysis of marine mammal samples

The separation of PCB-153 and PCB-105 and the possible co-eluting PCB-132 was critical in all samples. The separation of PCB-118 and PCB-149 was also critical in all the presented samples, and PCB-149 was found at levels comparable to PCB-118. There is currently considerable interest in the determination of both PCB-105 and PCB-118, partly based on toxicity, and partly as both congeners are included in the set of congeners recommended by the ICES for marine monitoring [6,10].

It is recommended to include PCB-132 and PCB-149 in the standard calibration mixture as column performance controls, even is these compounds are not analytes in the analytical programme. This use of column performance controls is generally recommended for high-resolution congener-specific PCB analysis.

CONCLUSIONS

The comparative quantitative analysis of thirty PCB congeners on two capillary columns of different polarity was performed. The columns were connected to the injector via a glass T-split, and accordingly the variation due to replicate injections of samples was eliminated. This dual-column system offers a convenient route to improve the quality of PCB analysis by GC–ECD.

Non-linear multi-level calibration was required for the determination of specific PCB congeners in marine mammal samples using this system. Power fit (ax^b) was the best fit for the calibrated range from detection limit up to 250 ng/ml, whereas the second-order fit (ax^2+bx+c) was a better choice for larger calibrated ranges.

The dual-column analysis of blubber and other tissues from seals revealed that PCB-101 and PCB-138 were overestimated by about 10% on the DB-5 column relative to the DB-1701 column. This finding is consistent with earlier reported work, and the DB-1701 column is thus preferred as a quantitative column for these congeners.

The difference in analytical results from the two columns were evaluated as a percentage, $\Delta_{F/R}$. $\Delta_{F/R}$ was useful in the evaluation of the very large amount of data from the dual-column system. A quantitation procedure based on the value of $\Delta_{F/R}$ is proposed. In general, the smallest analytical result was accepted, with exceptions for PCB-105, -158, -157, -128 and -167. These PCB congeners could be identified or quantitated on only one of the two columns, and accordingly other techniques are necessary to confirm the identity for these PCB congeners. A 5% difference between the DB-5 and DB-1701 quantitative results is considered insignificant, based on the overall measurement uncertainty in this automated capillary GC–ECD system.

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