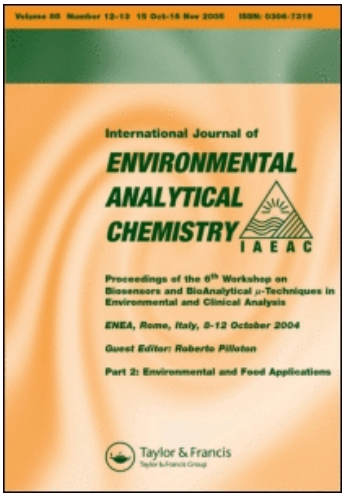

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Quantitative Determination of Specified Chlorobiphenyls in Fish with Capillary Gas Chromatography and its use for Monitoring and Tolerance Purposes†

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A gel permeation (GPC) clean-up procedure of fish samples in combination with capillary gas chromatography is used for the determination of individual chlorobiphenyls. The described method is compared with a more universal method based on saponification. Starting from a tolerance for PCB's expressed as a technical Aroclor, tolerance for some specified individual chlorobiphenyls are derived. It is shown that these tolerances for specified individual chlorobiphenyls can be used for monitoring and tolerance purposes. Rejections obtained with the Dutch PCB tolerance agree with rejections obtained with individual tolerances.

INTRODUCTION

The river Rhine, important for the water supply of the Netherlands, is a highly polluted river. One cannot be surprised, therefore, to measure high PCB levels in fat fish (eel) caught in the river Rhine and some of its tributaries.[1] From the Dutch fish monitoring program[2] it is indeed known that the presence of considerable amounts of PCB's overshadow the presence of other chlorinated pesticides. Fishery products may be

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considered as one of the main sources of PCB's in the average Dutch food.[3] The fish monitoring program is mainly directed to the Dutch coastal waters and the Yssel Lake. Recently an extra surveyance program was carried out to confirm the situation in some suspected areas in connection with the river Rhine and its tributaries.

METHOD

Reagents

a) Solvents. Ethyl acetate, toluene, chloroform, methanol and iso-octane freshly distilled in glass.
b) Internal standard solution in iso-octane. Mirex concentration, 0.15 μg/ml.
c) Chlorinated biphenyls (see Table I). For GC analysis, dilute 5 ml to 20 ml with iso-octane and inject 5 μl. For GPC analysis, evaporate 2 ml to dryness, dissolve in 4 ml toluene-ethyl acetate (1:1) and inject 500 μl. The numbering of chlorobiphenyls is according to the rules of IUPAC.[4]

Apparatus

a) Ultra Turrax.
b) Gel permeation chromatograph consisting of a constant flow pump Waters M45, automatic sampler Waters Wisp 170 B, RI detector, UV detector wavelength 254 nm, three-way valve (Chromatronix), controlled by a mechanical timer. Gel permeation column thermostated at 40°C, length 0.45 m, I.D. 0.015 m. Packing material Bio Beads SX 3, eluent toluene-ethyl acetate (1:1), flow 1.0 ml/min.
c) Gas chromatograph. Tracor 550 with ^{63}Ni electron capture detector and provided with splitless capillary injection system.
d) Capillary column. Material fused silica, length 25 m, I.D. 0.2 mm; stationary phase CP-Sil 5 ($\sim$SE 30), film thickness 0.14 μm plate number for component 138 > 60.000 at 220°C isotherm and capacity factor > 6 (Chrompack, Middelburg, The Netherlands).

   Gas flows: pressure controlled mobile phase (He), linear velocity 20–30 cm. sec^{-1}. Make up gas (nitrogen or argon/methane), ca. 20 ml/min. Detector purge, 30 ml/min. Temperatures: injector, 210°C; detector, 300°C; column oven programmed from 80 to 220°C at 40°/min; initial hold 4 min; final hold about 65 min.
e) Automatic injection device (Varian 8000).
f) Data system. Spectra Physics 4000.

TABLE I

Composition of standard chlorobiphenyl mixture

Code number	Structure	Concentration (μg/ml)
28	2,4-4'*)	0.04
52	2,5-2'5'*)	0.04
49	2,4-2'5'	0.04
44	2,3-2'5'	0.04
72	2,5-3'5'	0.04
70	2,5-3'4'	0.04
95	2,3,6-2'5'	0.04
101	2,4,5-2'5'*)	0.04
97	2,4,5-2'3'	0.04
87	2,3,4-2'5'	0.04
151	2,3,5,6-2'5'	0.04
149	2,3,6-2'4'5'	0.04
118	2,4,5-3'4'	unknown
153	2,4,5-2'4'5'*)	0.04
141	2,3,4,5-2'5'	0.04
138	2,3,4-2'4'5'*)	0.04
187	2,3,5,6-2'4'5'	0.04
128	2,3,4-2'3'4'	0.04
185	2,3,4,5,6-2'5'	0.04
180	2,3,4,5-2'4'5'*)	0.04
170	2,3,4,5-2'3'4'	0.04
201	2,3,4,5-2'3'5'6'	0.04
194	2,3,4,5-2'3'4'5'	0.04
206	2,3,4,5,6-2'3'4'5'	0.04

*)Monitoring compounds.

Extraction (5)

Weigh 5 g of homogenised fatty fish (eel) sample, add 3 ml water, 20 ml methanol and 10 ml chloroform. Macerate 30 sec with Ultra Turrax. Add again 10 ml chloroform and blend again during 30 sec. Now add 10 ml water and blend again 30 sec. Centrifuge 20 min at 1800 g. Transfer the lower chloroform layer over Na_2SO_4 into a round bottom flask of 100 ml and evaporate the chloroform. Dissolve the fish oil in such a volume of toluene/ethyl acetate (1:1) that the fish oil concentration equals 200 mg/ml solvent.

Clean-up (6, 7)

Activate the GPC system and let stabilize for 30 minutes at a flow rate of 0.5 ml/min. Increase the flow up to 1.0 ml/min.

Determine the retention time of the PCB compounds on the GPC column by injecting a standard mixture of PCB's. Tune the timers in such a way that the PCB fraction is completely collected.

Inject 500 μl of the sample into the GPC system after filtration through a microfilter (e.g. Millipore FHLP 0.5 μm).

Determination

The gas chromatographic separation starts with injection, under splitless conditions, of a 5 μl sample in the capillary. The solvent (toluene-ethyl acetate, 1:1) is allowed to elute. After 3 min, the splitter is opened to fore-flush possibly higher boiling (contaminating) compounds from the injector. After 4 min, the oven temperature programmer is started. Samples can be injected automatically or manually. In the latter case, opening and closing the splitter, and starting the temperature programmer and computer must also be carried out manually.

Relative retention times, with regard to Mirex, are used for identification.

Quantification is done by comparing peak areas from sample components with appropriate chlorobiphenyl standard areas.

RESULTS AND DISCUSSION

Since their discovery in the middle of the sixties it has been proven that PCB's are present in every organism all over the world. Since that time many publications were devoted to identification and quantification of PCB's. One of the quantification methods is based on a comparison of peaks with corresponding retention times in the sample and some technical formulation(s). The number of peaks selected by various authors covers a range from the minimum value of one to up to more than ten peaks.[8,9]

From our work on the analysis of PCB's in a great variety of samples it has appeared to be impossible to relate the peak pattern in the sample with the peak pattern in technical formulations. Especially with capillary gas chromatography the lack of resemblance is obvious. This lack is due to differences in stability, accumulation, and metabolism of the various chlorobiphenyls.

Another way of quantification of PCB's is their conversion into decachlorobiphenyl by perchlorination.[10] One drawback of this method is that the original PCB pattern is lost. It is impossible to determine if an Aroclor 1260 or 1242 was the main source of contamination. Another, even more important, drawback is the fact that too high values can be

found. In the literature[11,12] more and more evidence is given that with certain types of samples the determined DCB value is much too high, because of the presence of certain compounds which—upon perchlorination—give rise to formation of decachlorobiphenyl. Too high values can be expected in sewage sludge and concentrated feeding stuff.

To overcome these problems a method for the determination of individual chlorobiphenyls has to be used as Jensen[13] stated already in 1974. The high separation power required can be obtained by using capillary columns (4, 14). Especially the recent introduction of fused silica columns makes this technique feasible on a routine scale; coupling in gas chromatographs and direct coupling with the source of a mass spectrometer is no longer a problem.

To extract the fish oil from fish samples the method of Bligh and Dyer[5] is used because this method extracts all the oil from the fish.

Clean-up of fish oil samples is carried out in our lab by two methods. The first one is based on saponification and considered by us as a universal method for all types of samples. Normally the sample is extracted with hexane or pentane and saponified. In the case of fish it is possible to add the homogenous fish sample to the alcoholic KOH solution and to extract and saponify in one step. Good recoveries are obtained and the time of analysis is reduced. After a final clean-up on a small basic alumina column the sample is ready for injection on the capillary column. For a detailed description of the method, see[15].

The second method, used for this study, is based on clean-up by gel permeation chromatography.[5,6] Both methods give identical results as is shown by comparing Figure 1a and 1b. The most striking difference is the solvent peak. The GPC method is preferred in cases of higher contaminated samples.

With the compounds given in Table I we can quantitate about 70% of the total PCB contant in fish samples. Quantification of identified chlorobiphenyls is done by comparing peak areas of sample and standard. For the compounds in the sample for which no standards were available the content was estimated as follows. From the analysis carried out with a Finnigan 4000 mass spectrometer[16] the number of chlorine atoms of each unknown PCB peak is known. From the chlorobiphenyls in our possession (64) we knew the response in relation to the number of chlorine atoms present in the chlorobiphenyls. So the mean response factor for chlorobiphenyls with an equal number of chlorine atoms was known. Using this response factor the quantity of unknown chlorobiphenyls (with known number of chlorine atoms) was calculated.

In Table II for 33 eel samples taken from suspected areas, the sum of total chlorobiphenyls is shown in column 2. 11 samples exceed the official

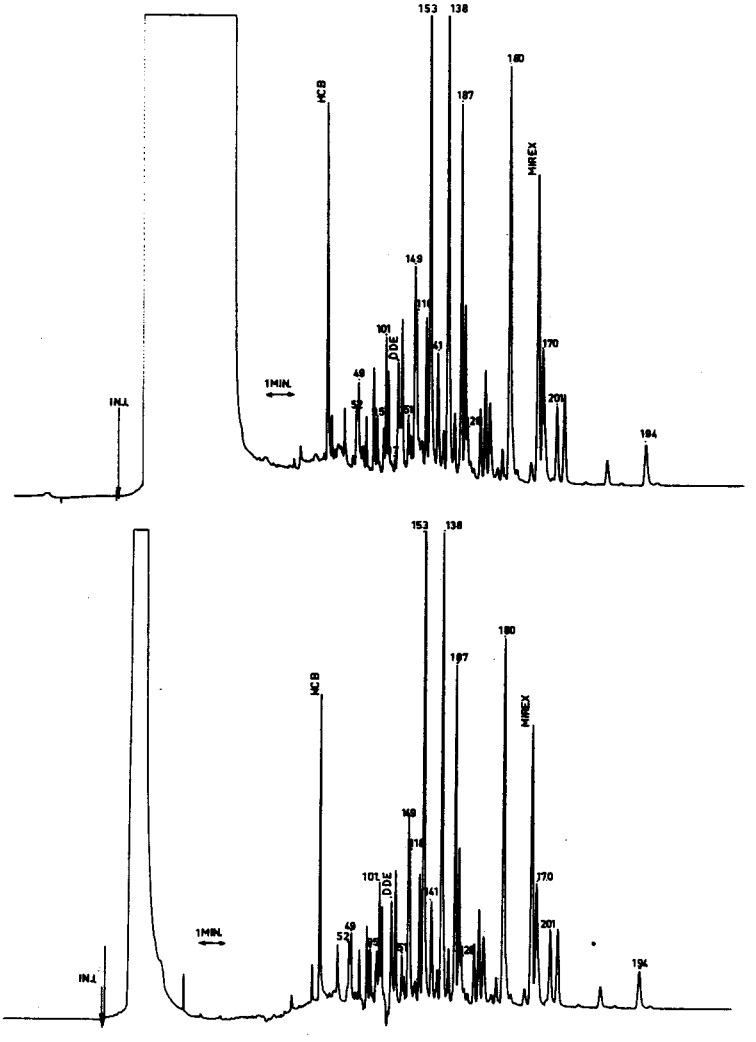

FIGURE 1 Comparison of clean-up procedures for PCB's in fish. (a) clean-up with GPC; (b) same fish cleaned by saponification procedure.

tolerance of 5 mg/kg total PCB's which was set in June 1981. The determination of the total PCB content is relatively difficult and time consuming. An attempt was made, therefore, to simplify the procedure while maintaining the same expressiveness.

On the basis of results from chlorobiphenyl content measurements in other matrices,[17,18] and from carry-over experiments[19,20] we selected 6

TABLE II

The total PCB content and the content of 6 chlorobiphenyls in mg/kg in eel samples

Nr.	Total PCB	2,4-4′ nr. 28	2,5-2′5′ nr. 52	2,4,5-2′5′ nr. 101	2,3,4-2′4′5′ nr. 138	2,4,5-2′4′5′ nr. 153	2,3,4,5-2′4′5′ nr. 180
1	0.57	0.012	0.042	0.023	0.065	0.091	0.021
2	—	<0.005	0.010	0.005	0.009	0.013	0.007
3	—	0.009	0.014	0.013	0.019	0.024	0.014
4	1.6	0.018	0.080	0.086	0.18	0.23	0.10
5	4.7	0.059	0.16	0.27	0.44	*0.51*	0.21
6	0.35	0.012	0.023	0.017	0.037	0.048	0.015
7	2.0	0.062	0.079	0.083	0.21	0.24	0.12
8	*5.6*	0.14	*0.29*	0.27	0.29	0.34	0.16
9	3.1	0.067	*0.21*	0.21	0.25	0.35	0.12
10	*5.2*	0.087	*0.24*	0.25	0.46	*0.51*	0.24
11	*7.4*	0.14	*0.36*	0.40	*0.62*	*0.71*	0.29
12	0.6	0.008	0.038	0.031	0.064	0.091	0.024
13	4.1	0.053	0.16	0.18	0.32	0.37	0.17
14	*6.1*	0.054	*0.21*	0.31	*0.53*	*0.59*	0.43
15	*7.4*	0.097	*0.38*	*0.52*	*0.66*	*0.63*	0.26
16	*8.3*	0.064	0.11	0.28	*0.96*	*1.2*	*1.1*
17	0.2	<0.005	0.010	0.009	0.027	0.038	0.016
18	4.2	0.036	*0.22*	0.23	0.45	0.46	0.29
19	*12*	0.11	*0.43*	*0.82*	*1.2*	*1.2*	0.72
20	3.3	0.034	0.11	0.19	0.40	0.42	0.25
21	0.3	0.007	0.014	0.007	0.036	0.038	0.017
22	*5.1*	0.065	0.17	0.29	*0.51*	*0.53*	0.32
23	3.1	0.045	0.097	0.16	0.33	0.34	0.20
24	0.7	0.014	0.012	0.037	0.064	0.065	0.040
25	0.7	0.007	0.019	0.034	0.10	0.098	0.045
26	0.3	<0.005	0.011	0.015	0.037	0.039	0.021
27	0.8	0.007	0.020	0.030	0.092	0.093	0.050
28	*8.8*	0.15	*0.40*	*0.50*	*0.73*	*0.69*	0.50
29	1.6	0.023	0.051	0.086	0.17	0.16	0.098
30	4.5	0.022	0.10	0.17	*0.54*	*0.51*	0.47
31	*5.8*	0.049	0.19	0.29	*0.58*	*0.56*	0.39
32	*9.3*	0.067	*0.24*	*0.48*	*0.87*	*0.85*	0.65
33	1.1	0.013	0.042	0.063	0.081	0.11	0.053

individual chlorobiphenyls. These are present in one or more technical Aroclor mixtures, have a moderate or high accumulation factor, are commercially available (or easily synthesised), and have adequate resolution from neighbouring chlorobiphenyls. Furthermore evidence is obtained with several types of samples showing matrix interferences generally to be absent. In Table III those 6 compounds are shown as well as the maximum percentage that they make up in technical Aroclors

TABLE III
Maximum percentage of six monitoring compounds and their derived tolerances

Compound	Maximum percentage in technical Aroclors	Derived tolerances (mg/kg)
28	10 (IN AROCLOR 1242)	0.5
52	4 (IN AROCLOR 1254)	0.2
101	8 (IN AROCLOR 1254)	0.4
138	10 (IN AROCLOR 1260)	0.5
153	10 (IN AROCLOR 1260)	0.5
180	12 (IN AROCLOR 1260)	0.6

previously analysed.[16] In Table II in columns 3–8 the contents for the 6 chlorobiphenyls are given for all eel samples studied.

As a starting point for our simplified procedure we consider the situation in the U.S.A. where the PCB content in a sample is expressed as (a mixture of) technical Aroclor(s). According to that view it does not matter if the peak pattern in the sample resembles that of Aroclor 1221 or Aroclor 1260. Only the content in that sample expressed as an Aroclor mixture is important.

If we assume that only one technical Aroclor mixture is present in the sample, we can derive the maximum allowable content for each of the individual monitoring compounds. These are given in the third column of Table III. In practice, of course, contamination occurs not only from one technical mixture, but always from several mixtures. This will always lead to a lower concentration of the compound of interest in the said mixture (i.e., the source of contamination). This implies that in that case the derived individual tolerances as calculated in Table III are too high. In other words, when concentrations are found exceeding the values in the third column of Table III, this can only be explained by the fact that the sum of the individual chlorobiphenyls originating from technical Aroclor mixtures exceeds the Dutch tolerance of 5 mg/kg.

In Table II we can compare the number of rejections based on the tolerance for total PCB content with the number of rejections based on one or more exceedings of the tolerance for individual chlorobiphenyls. One can conclude that rejections obtained with the total PCB content agree with rejections obtained with the individual tolerances, and vice versa, even though there are four borderline cases (no. 5, 9, 18 and 30; note number of significant figures in Table III) in the latter case.

In Figure 2 for compounds 52, 138 and 153, the three most interesting cases, the situation is clarified, in histograms, compared to the total PCB content.

Compound 28 does not exceed the individual tolerance in the samples, probably because compound 28 has a much lower stability than have the other compounds. Compound 180 does not appear in the histogram for the following reason. In the environment, the contamination with Aroclor 1254 is much more important than the contamination with Aroclor 1260.

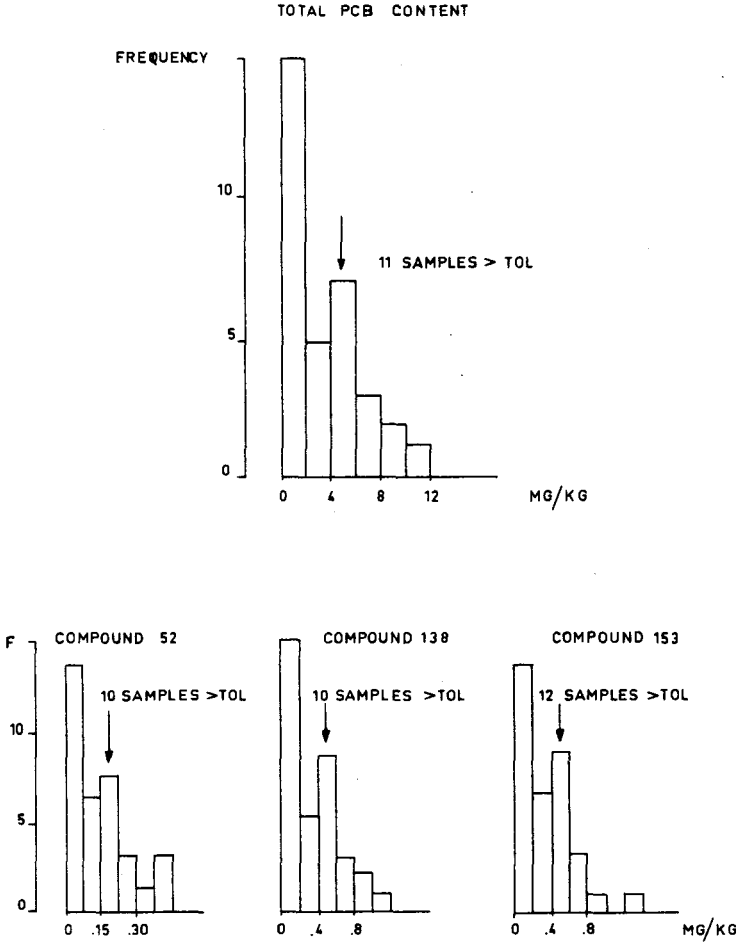

FIGURE 2 Histograms of total PCB content, the contents of 52 (2,5-2′5′ tetrachloro-); 128 (2,3,4-2′4′5′ hexachloro-) and 153 (2,4,5-2′4′5′ hexachlorobiphenyl) in eel samples ($N = 33$). By arrow are indicated the tolerance for total PCB content and individual tolerances, respectively.

Aroclor 1254 contains only 1.5% of compound 180; therefore, very high contents for compound 180 are not to be expected. This is in contrast with compounds 138 and 153 which have the same stability as compound 180, but are present in Aroclor 1254 in much higher percentages (6–10%). Of course when a local contamination with Aroclor 1260 occurs the content of compound 180 in samples will increase. The situation for compound 101 is in between that for 180, and 153 and 138.

Mass spectrometry of the analysed fish samples has revealed that no mono- and dichlorobiphenyls are present.

CONCLUSION

Extraction and clean-up of PCB's from fish samples can be carried out in several ways, e.g. by GPC or saponification. Especially the latter is suitable for very low levels. The use of capillary GC in routine methods can no longer be considered as impracticable. The determination of certain specified chlorobiphenyls is possible without interference from other compounds. The suggested simplified procedure for fish samples, determining only 6 individual chlorobiphenyls leads to the same results as with a total PCB content determination proving that the derived individual tolerances for these 6 compounds are a good alternative.

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