REPORT 84.77 1984-08-13

Pr.nr. 505.0400

Determination of some individual chlorobiphenyls in eel-fat with capillary gas-chromatography: Collaborative study

L.G.M.Th. Tuinstra, A.H. Roos and
G.A. Werdmuller

Verzendlijst: direkteur, sektorhoofden, direktie VKA (v.d. Meijs, Mol, Kloet, v. Venetië), afd. Normalisatie/Harmonisatie (Humme), projektbeheer, circulatie, deelnemers ringtest (15x), afd. Organische Contaminanten/Bestrijdingsmiddelen, LAC stuurgroep Visverontreiniging (15x), LAC Stuurgroep Zuivelverontreiniging (20x).

Determination of some individual chlorobiphenyls in eel-fat with capillary gaschromatography: Collaborative study.

L.G.M.Th. Tuinstra, A.H. Roos and G.A. Werdmuller

State Institute for Quality Control of Agricultural Products (RIKILT), Wageningen, The Netherlands.

Collaborators: H.C. Compaan, J. Dornseiffen, G.F. Ernst, M.Th.J. Hillebrand, J.A. Jans, M.A.T. Kerkhoff, A.J. van Munsteren, L.J. Poortvliet, A.P.J. van der Putten, J. Quirijns and R.C.C. Wegman.

8477.0

Abstract

A method for the determination of six individual chlorobiphenyls in eel-fat, based on saponification of the sample and determination with capillary gaschromatography, was studied collaboratively. Eleven laboratories submitted analytical results in duplo of six individual chlorobiphenyls on two samples naturally contaminated eel-fat. The reproducibility coefficient of variation is about 14% at the 1 mg/kg level per clorobiphenyl compound and about 23% at the 0,1 mg/kg level. For each compound the mean recovery is about 90% with a standard deviation varying from 7 to 9%.

Introduction

One of the conclusions of the recent PCB seminar in The Hague, The Netherlands (28-30 September 1983) (1) was that a more generalized use of capillary gaschromatography for the determination of PCB congeners will produce more reliable and accurate data. This was in line with earlier expectations in the Netherlands.

In 1982 a preliminary interlaboratory study was carried out with cleaned sample extracts of butterfat, chickenfat and eel-fat (2), to obtain experience with PCB analysis on capillaries. The results were discussed in a workshop with particular attention to the used gaschromatographic conditions. For future work special attention was paid to extraction and clean-up procedures. In this report results will be reported of a collaborative study of individual chlorobiphenyls in eel-fat, in which six defined chlorobiphenyls had to be identified and quantified after analysing according to a detailed method (annex 1).

Collaborative study

Description of samples

The participating laboratories received a set of the following samples:

Two sealed ampoules standard solution with the chlorobiphenyl compounds 2,4-4' trichlorobiphenyl (PCB 28), 2,5-2'5' tetrachlorobiphenyl (PCB 52), 2,4,5-2'5' pentachlorobiphenyl (PCB 101), 2,3,4-2'4'5' hexachlorobiphenyl (PCB 138), 2,4,5-2'4'5 hexachlorobiphenyl (PCB 153) and 2,3,4,5-2'4'5' heptachlorobiphenyl (PCB 180), each 0,1 µg/ml in isooctane/pentane (4/1) (code A).

The numbering of the chlorobiphenyls is according to the rules of IUPAC (3). For each ampoule containing 5 ml standard solution the weight was given to control loss of weight after receipt of the samples.
A practice sample swine fat spiked with the PCB's 28, 52, 101, 138, 153 and 180, each at the 0,5 mg/kg level (code B).
Two unknown samples eel-fat, naturally contaminated respectively with a low and high chlorobiphenyl content (code C and D).

Description of Study

The collaborators were instructed to check first the weight of the sealed ampoules with standard solution. By way of an acknowledgement of receipt new ampoules could be asked for in case the ampoules were not received in good condition (e.g. more than 1% weight difference). They were instructed to provide the GC system with a new septum and glassliner in the injection system and to analyse the practice sample (code B) first, including a reagent blanc and a recovery experiment to become familiar with the procedure. In case results were obtained differing more than 20% from the indicated content of the spiked chlorobiphenyls, contact should be made with the organizing laboratory. When no problems were met the laboratories were asked to analyse the unknown eel-fat samples (code C and D) each two times, together with a reagent blanc and a recovery experiment with standard solution added to the chemicals. For quantification the second standard ampoule (code A) should be used. No correction for recovery should be applied. Results should be reported on enclosed forms in mg/kg for the chlorobiphenyl determinations, recovery in % and blanc values in ng in the total final extract. Also instrumental parameters and chromatograms had to be returned, eventual together with remarks and comments. In the chromatograms the compounds of interest had to be indicated with an arrow.

Method

Though the method studied had been described earlier for milk fat (4), a more detailed procedure (annex 1) was made available to the collaborators.

However the gaschromatographic conditions with respect to the polarity of the capillary column, the injection volume, injection temperature and the temperature program was described in more general advising, rather than prescribing terms. The collaborators were free to set instrumental parameters according to own experiments with the practice sample especially with respect to separations of the six chlorobiphenyl compounds of interest. A splitless injection technique according to Grob (5) was also advised. As internal standard hexabromobenzene was advised for quantification purposes.
After saponification and clean-up of the samples the analyses had to be carried out in the linear range of the electron capture detector and within recorder paper scale. This was necessary as the quantification procedure prescribes use of peak height, not peak area.

Results and discussion

Eleven of the 14 collaborators completed the analyses. Three collaborators could not complete the analyses within time schedule due to other priorities.
The several gaschromatographic conditions used are presented in table 1.
The linearity of the gaschromatographic system was tested, with the exception of collaborator 5 and 6. As test compounds organochlorine pesticides were used by collaborator 2 and 4, chlorobiphenyl compounds by collaborator 1, 3, 7, 8, 9 and 10. Collaborator 11 did not report the test compound. Collaborator 1 and 8 reported that the linearity was moderate. Nobody reported correction for non-linear behaviour of the electron capture detector.
As a general rule the linearity of a gaschromatographic system should be tested with the compounds of interest. Naturally the analyses should be carried out in the linear range. In case linearity is less acceptable it is recommendable to analyse samples close to the concentration level in the standard by concentrating or diluting the sample and of course to work in the tested range.
Most collaborators used a capillary column with comparable polarity namely a CP Sil 7, CP Sil 8 CB or SE 54 column. Two collaborators used a more polair CP Sil 19 CB column and one collaborator a more apolair CP Sil 5 CB column.

From the received chromatograms, and with no clear relation to the polarity of the capillary column, it was observed in the chromatograms that the separation of the chlorobiphenyl compounds 28, 52 and 101 was not so pronounced as for the other chlorobiphenyl compounds 138, 153 and 180.

The linear gas velocity used by the collaborators was in most cases in agreement with the advised velocity for capillary columns (0,2-0,3 mm inner diameter), which should be at 220°C for nitrogen 10-15 cm/sec, for helium 25-30 cm/sec and for hydrogen 40-45 cm/sec.

Eight collaborators used helium and three nitrogen as carrier gas. Nobody used hydrogen. Collaborator 6 and 8 used a too high linear gas velocity. In the case of collaborator 6 using nitrogen at 33 cm/sec inferior separation of all chlorobiphenyls was observed in the chromatograms.

A high injection temperature is necessary to achieve instantaneous evaporation of the sample and a low initial column temperature in order to achieve the "solvent effect". In general initial oven temperatures and injector temperatures are in agreement with this rule of thumb. The injector temperature varies from 200 to 275°C. Most collaborators used a temperature of 240-250°C. Peak height of late eluting chlorobiphenyl compounds is higher at higher injector temperatures, as also observed in a preliminary interlaboratory study organized by EC-Community Bureau of Reference (6). The collaborators using 200°C as injector temperature should test the effect of higher temperatures on the response of the chlorobiphenyl compounds.

All collaborators, except collaborator 5, used the splitless injection technique according to Grob (5). Collaborator 5 used the split-mode as nonreproducible response with splitless injection was observed.

The test sample swine fat spiked with the chlorobiphenyl compounds 28, 52, 101, 138, 153 and 180 had to be analysed first by the laboratories to become familiar with the whole method. Collaborator 8 omitted the analyses of this sample to save time. None of the other collaborators reported problems with the method of analyses. So it can therefore be concluded, that everybody found results within the 20% range given in the description of the study.

Interferences with the chlorobiphenyls of interest in the blanc chemical experiment were reported in ng in the total final extract. The quantities were recalculated by us into mg/kg assuming they were obtained with the described analytical procedure. The results indicate that the interferences from the blanc chemical experiments are neglectable (meaning lower than 0,005 mg/kg per chlorobiphenyl compound for most collaborators) certainly in relation to the content in the eel-fat samples. Only collaborator 6 reported higher blanc values for most chlorobiphenyls up to 0,015 mg/kg.

The results of the recovery experiments with the standard solution of individual chlorobiphenyls added to the blanc chemicals are presented in table 2. Recoveries vary from 74 to 104%. For each compound the mean value is about 90% with a standard deviation varying from about 7 to 9%. The results indicate that recoveries higher than 80% should be obtained. The reported recoveries are not used for correction of the data.

In the analyses of both eel-fat samples, blanc and recovery experiment, hexabromobenzene was advised as internal standard and used by eight collaborators. Also pentachloroaniline, pentachlorobenzene and mirex were used. Collaborator 2 reported problems with the reproducibility of the response of the chlorobiphenyls and hexabromobenzene, in particular for hexabromobenzene and did not use the internal standard for quantification purposes. Each sample was therefore injected twice and the mean response was used in the quantification and reported. On account of this the results were not used for statistical calculation.

Collaborator 3 reported also a problem with the internal standard hexabromobenzene and used therefore mirex, which is not present in Dutch eel-fat. After inspection of the chromatograms it appeared however that the name hexabromobenzene was given to a wrong peak, which varied in response. When the right name to the right peak had been given no problems should have been met.

Collaborators 1, 5, 7, 9 and 11 used a data integrating system for measuring peak height, the other collaborators used a manual method. Collaborator 5 quantified the chlorobiphenyls also using a manual method. Between the manual quantification and the quantification with an integrating system hardly no difference was found. Only the results of the manual quantification are used and reported.

Collaborator 8 analysed the samples both on CP Sil 8 CB and CP Sil 19 CB column. The chromatograms of the CP Sil 19 CB were worse than the results from the CP Sil 8 CB column. Baseline was rising (bleeding, changing flow?); also separations of chlorobiphenyl 28 and 52 were less. Therefore only the results of the CP Sil 8 CB column are used and reported.
Before statistical treatment all the chromatograms of each collaborator were studied. It was observed that the separation for the chlorobiphenyl compounds 28, 52 and 101 in a few laboratories was moderate. For the other chlorobiphenyl compounds each laboratory had a good separation.
In the chromatograms of collaborator 11 probably due to nonreproducible retention times the identification for the chlorobiphenyl compound 28 was not correct. On account of this the results for compound 28 of collaborator 11 for both eel-fat samples were eliminated and not used for statistical calculation.

All analytical data of both samples eel-fat analysed in duplo, are presented in table 3. As said the results are not corrected for recovery and blanc.
After elimination of the results of collaborator 2 for all data and collaborator 11 for PCB 28 a statistical treatment per eel-fat and per chlorobiphenyl compound has been carried out according to ISO 5725, using a computer program. With the Cochran maximum variance test the precision under repeatable conditions in the laboratories and with the Dixon's outlier test the precision between the laboratories were tested. The repeatability, reproducibility and the mean were calculated.
In sample C no stragglers or outliers with the Cochran and Dixon's test were found.
In sample D collaborator 6 and 8 caused a Cochran outlier respectively for the chlorobiphenyl compound 28 and 180.
As the samples were not randomly numbered unknowns and the duplo's probably have been extracted and cleaned at the same time, Cochran outliers were not eliminated and no repeatability coëfficient of variation is calculated, as this CV would show a too optimistic result.

In sample D collaborator 6 caused Dixon stragglers for the chlorobiphenyl compounds 28, 52, 153 and 180. On account of this all the results of collaborator 6 for sample D were rejected where after a second statistical calculation was carried out. Now no outliers and stragglers were found in eel-fat sample D.
In table 4 the number of collaborators (n), rounded off results for mean (x), repeatability (r), reproducibility (R) and also the coëfficient of variation CV(R) for both eel-fat samples after the second statistical calculation are reported. The coëfficient of variation was calculated with the following formula:

$$CV(R) = \frac{R \times 100\%}{2{,}83 \times \text{mean value}}$$

The CV(R) at the 1 to 2 mg/kg level in the eel-fat varies between 13 and 17% and at the 0,1 to 0,4 mg/kg level varies between 21 and 24%. The reproducibility (R) and the CV(R) are influenced by the content of the chlorobiphenyls. The results are to be considered as excellent especially when they are related to the results in the earlier mentioned preliminary interlaboratory study with cleaned extracts (2), in which at the same level the CV(R) was about two times higher.

The data used for final statistical calculation are shown as histograms (figure 1-12).

Conclusion

The described method for the determination of some individual chlorobiphenyls based on saponification and determination with capillary gaschromatography will result in a CV(R) of about 14% at the 1 mg/kg level per chlorobiphenyl compound and about 23% at the 0,1 mg/kg level.

Acknowledgments

The cooperation of the following collaborators is gratefully acknowledged:
Compaan H.C., Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek, afdeling Maatschappelijke Technologie, Delft.
Dornseiffen J., Keuringsdienst van Waren, Amsterdam.
Ernst G.F., Keuringsdienst van Waren, Utrecht.

Hillebrand M.Th.J., Nederlands Instituut voor Onderzoek der Zee, Den Burg, Texel.
Jans J.A., Zuivelcontrole-instituut, Leusden.
Kerkhoff M.A.T., Rijksinstituut voor Visserijonderzoek, IJmuiden.
van Munsteren A.J., Rijks-Kwaliteitsinstituut voor land- en tuinbouwprodukten, Wageningen.
Poortvliet L.J., Boter en Kaascontrolestation "Friesland", Leeuwarden.
van der Putten A.P.J., Keuringsdienst van Waren, Haarlem.
Quirijns J., CIVO Instituten TNO, Zeist.
Wegman R.C.C., Rijksinstituut voor de Volksgezondheid en Milieuhygiëne, Bilthoven.

References

1. Proceedings PCB Seminar, Scheveningen, The Hague, The Netherlands, September 28-30, 1983. Edited by Barros M.C., Könemann H. and Visser R., VROM 84060/6-84, 3247/71.

2. Tuinstra L.G.M.Th., Roos A.H., Keukens H.J., Traag W.A. and Werdmuller G.A. Internal report 83.24 (1983), State Institute for Quality Control of Agricultural Products in Wageningen, The Netherlands (In Dutch).

3. Ballschmiter K. and Zell M. (1980), Fresenius Z. Anal. Chem. 302, 20-31.

4. Tuinstra L.G.M.Th., Traag W.A. and Keukens H.J. (1980), J. Assoc. Off. Anal. Chem. 63, 952-958.

5. Grob K. and Grob G. (1972), Chromatographia 5, 3-12.

6. Tuinstra L.G.M.Th., Roos A.H. and Werdmuller G.A. Internal RIKILT report 84.27 (1984), in English, BCR-ringtest of individual chlorobiphenyls - Summary of results - organized by EC-Community Bureau of Reference, Brussels.

Table 1 Gaschromatographic conditions collaborative study of chlorobiphenyls in eel-fat

Collaborator	Apparatus (type)	Detector (type)	Linearity tested	Column phase	Column length (m)	Column i.d. (mm)	Column film-thickness (µm)	Linear gas velocity (cm/sec)	Gas (type)	Temperature programme	Injector temperature (°C)	Injection volume (µl)	Type of injection
1	HP 5880 A	Ni 63	yes	CP Sil 7	25	0,22	0,45	14	N2	4 min 110°C-20°C/min-220°C	260	6	splitless
2	Sigma 2 B	Ni 63	yes	CP Sil 5 CB	25	0,22	0,11	28	He	4 min 100°C-10°C/min-220°C	200	2	splitless
3	PB 419	Ni 63	yes	CP Sil 8 CB	25	0,25	0,40	26	He	5 min 87°C-32°C/min-215°C	240	1	splitless
4	HP 5730 A	Ni 63	yes	SE 54	25	0,31	0,17	22	He	2 min 90°C- 3°C/min-240°C	250	5	splitless
5	HP 5713	Ni 63	no	CP Sil 19 CB	25	0,31	0,20	23	He	200°C (isothermal)	250	2	split-mode
6	Sigma 2	Ni 63	no	CP Sil 7	25	0,23	0,25	33	N2	4 min 100°C-40°C/min-240°C	240	5	splitless
7	HP 5880 A	Ni 63	yes	SE 54	50	0,33	0,10	26	He	2 min 60°C-10°C/min-8 min 180°C-4°C/min-5 min 220°C-4°C/min-250°C	230	1	splitless
8	Sigma 2000	Ni 63	yes	CP Sil 8 CB	45	0,32	0,12	45	He	2 min 50°C-30°C/min-100°C-3°C/min-250°C	275	1	splitless
9	Varian 6000	Ni 63	yes	CP Sil 19 CB	25	0,22	0,19	-	N2	3 min 50°C-25°C/min-0,5 min 200°C-4°C/min-260°C	200	1	splitless
10	Tracor 550	Ni 63	yes	CP Sil 7	25	0,25	0,45	31	He	4 min 100°C-10°C/min-230°C	240	5	splitless
11	HP 5880	Ni 63	yes	CP Sil 8 CB	25	0,33	0,36	23	He	3 min 80°C- 3°C/min-290°C	200	0,5	splitless

- = not reported.

Table 2 Results of recovery experiments with the standard solution of individual chlorobiphenyls (%)

Collaborator	PCB 28	PCB 52	PCB 101	PCB 153	PCB 138	PCB 180
1	82	83	85	85	81	74
2	88	89	98	101	101	103
3	86	86	95	90	95	87
4	100	100	100	97	100	91
5	82	82	94	84	82	84
6	101	91	87	84	81	83
7	85	88	89	92	93	94
8	78	81	81	86	87	87
9	103	96	101	90	100	89
10	90	90	94	99	96	100
11	92	102	100	101	104	104
mean	90	90	93	92	93	90
st.dev.	8,4	7,0	6,7	6,8	8,6	9,2

Table 3 Duplo results of individual chlorobiphenyls in the eel-fat samples (mg/kg)

Sample C (low level)

Collaborator	PCB 28	PCB 52	PCB 101	PCB 153	PCB 138	PCB 180
1	0,031	0,09	0,12	0,27	0,35	0,11
	0,031	0,092	0,12	0,26	0,35	0,12
2	0,043	0,11	0,16	0,29	0,39	0,14
	0,040	0,10	0,15	0,27	0,37	0,13
3	0,032	0,11	0,17	0,32	0,38	0,13
	0,035	0,12	0,18	0,34	0,41	0,15
4	0,050	0,13	0,15	0,29	0,34	0,16
	0,047	0,12	0,14	0,27	0,32	0,14
5	0,026	0,086	0,10	0,23	0,31	0,11
	0,026	0,082	0,090	0,22	0,31	0,11
6	0,044	0,079	0,11	0,20	0,30	0,10
	0,048	0,086	0,12	0,22	0,35	0,10
7	0,029	0,094	0,12	0,27	0,35	0,15
	0,028	0,092	0,12	0,26	0,33	0,14
8	0,025	0,12	0,12	0,35	0,42	0,12
	0,025	0,12	0,12	0,32	0,38	0,13
9	0,044	0,15	0,16	0,42	0,53	0,20
	0,047	0,15	0,18	0,44	0,60	0,20
10	0,030	0,12	0,15	0,37	0,57	0,17
	0,028	0,10	0,14	0,37	0,49	0,19
11	<0,010	0,084	0,096	0,36	0,50	0,18
	<0,010	0,071	0,078	0,36	0,51	0,19

Sample D (high level)

Collaborator	PCB 28	PCB 52	PCB 101	PCB 153	PCB 138	PCB 180
1	0,19	1,07	1,08	1,63	2,11	0,63
	0,20	1,13	1,15	1,73	2,17	0,65
2	0,32	1,15	1,68	1,77	2,28	0,78
	0,34	1,23	1,74	1,90	2,48	0,86
3	0,16	1,10	1,34	1,64	2,03	0,74
	0,16	1,09	1,34	1,66	2,05	0,74
4	0,28	1,14	1,06	1,53	1,87	0,82
	0,28	1,14	1,12	1,55	1,87	0,83
5	0,21	1,22	0,87	1,40	1,91	0,69
	0,21	1,22	0,85	1,42	1,91	0,69
6	0,54	0,60	0,50	0,59	0,87	0,33
	0,46	0,51	0,50	0,60	0,91	0,33
7	0,17	1,02	0,94	1,44	1,78	0,70
	0,17	1,02	0,95	1,44	1,82	0,73
8	0,17	1,35	1,12	1,85	2,28	0,87
	0,16	1,21	1,00	1,69	2,07	0,69
9	0,25	1,46	1,10	2,05	2,82	1,00
	0,25	1,40	1,05	1,95	2,50	0,90
10	0,18	1,45	1,28	2,00	2,85	0,93
	0,19	1,54	1,26	2,09	2,79	0,87
11	0,57	1,28	1,10	1,63	2,64	0,92
	0,59	1,31	1,13	1,55	2,50	0,88

Table 4 Results statistical evaluation of the eel-fat samples

Sample C (low level)

PCB compound	$\bar{x}$ (mg/kg)	r (mg/kg)	R (mg/kg)	CV(R) (%)	n
28	0,035	0,005	0,026	27	9
52	0,10	0,018	0,067	22	10
101	0,13	0,022	0,084	23	10
138	0,40	0,083	0,27	24	10
153	0,31	0,034	0,20	23	10
180	0,14	0,025	0,098	24	10

Sample D (high level)

PCB compound	$\bar{x}$ (mg/kg)	r (mg/kg)	R (mg/kg)	CV(R) (%)	n
28	0,20	0,012	0,12	21	8
52	1,23	0,13	0,46	13	9
101	1,10	0,11	0,42	14	9
138	2,22	0,28	1,07	17	9
153	1,68	0,16	0,64	14	9
180	0,79	0,15	0,32	14	9

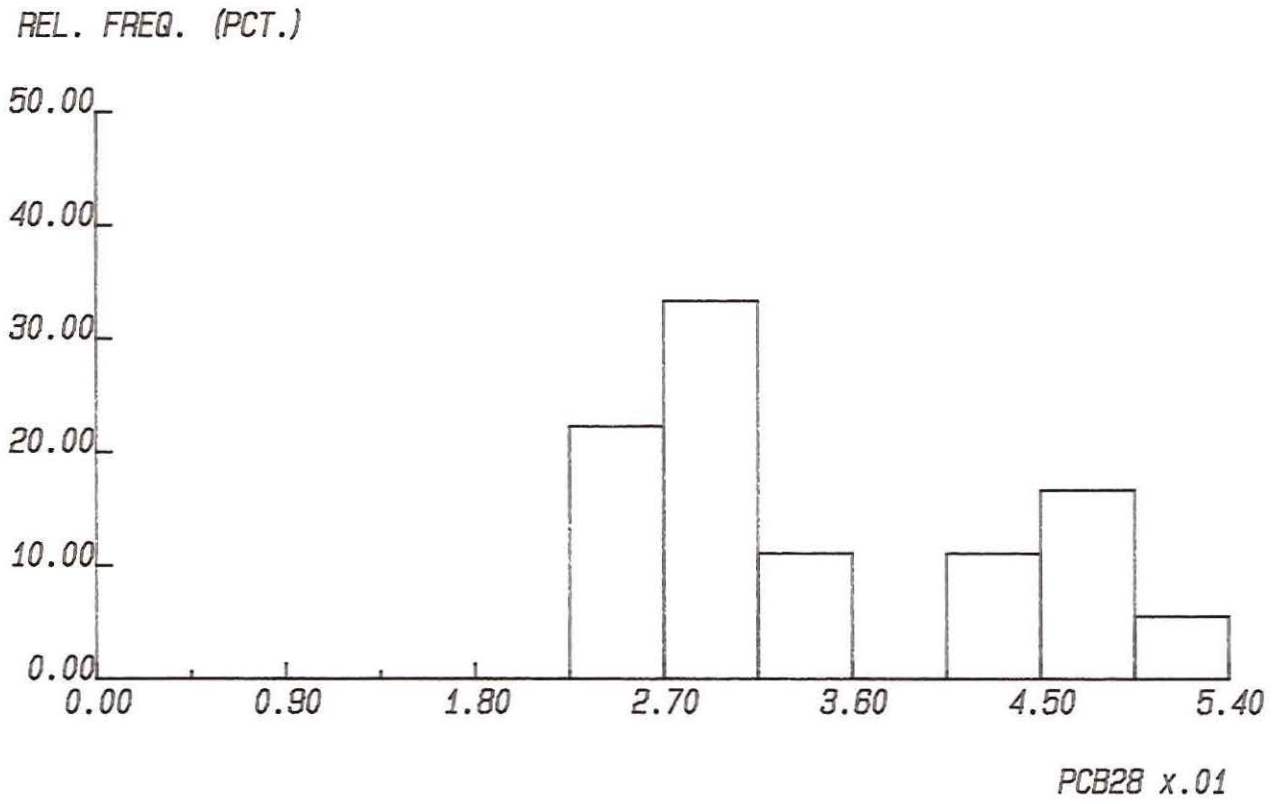

Figure 1. Histogram PCB28 Sample C (Content in mg/kg)

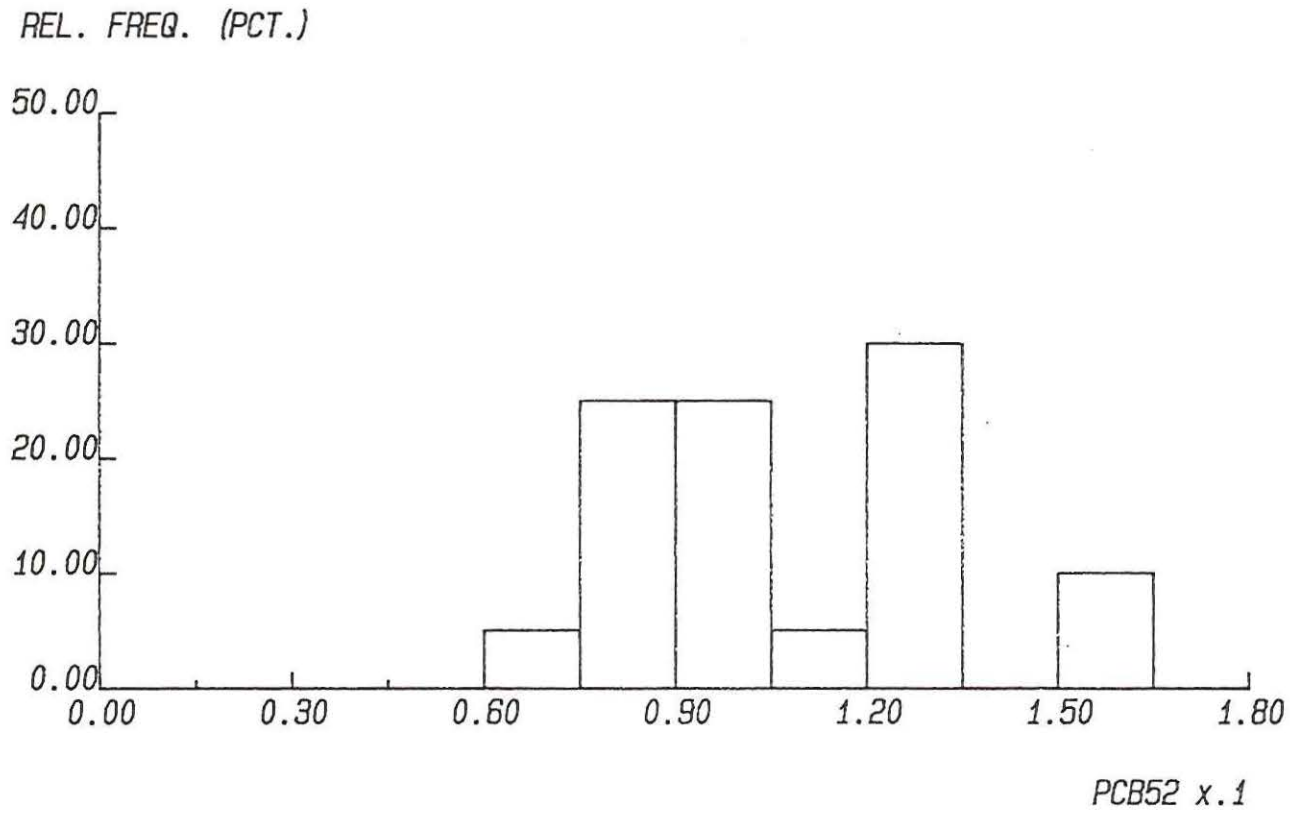

Figure 2. Histogram PCB52 Sample C (Content in mg/kg)

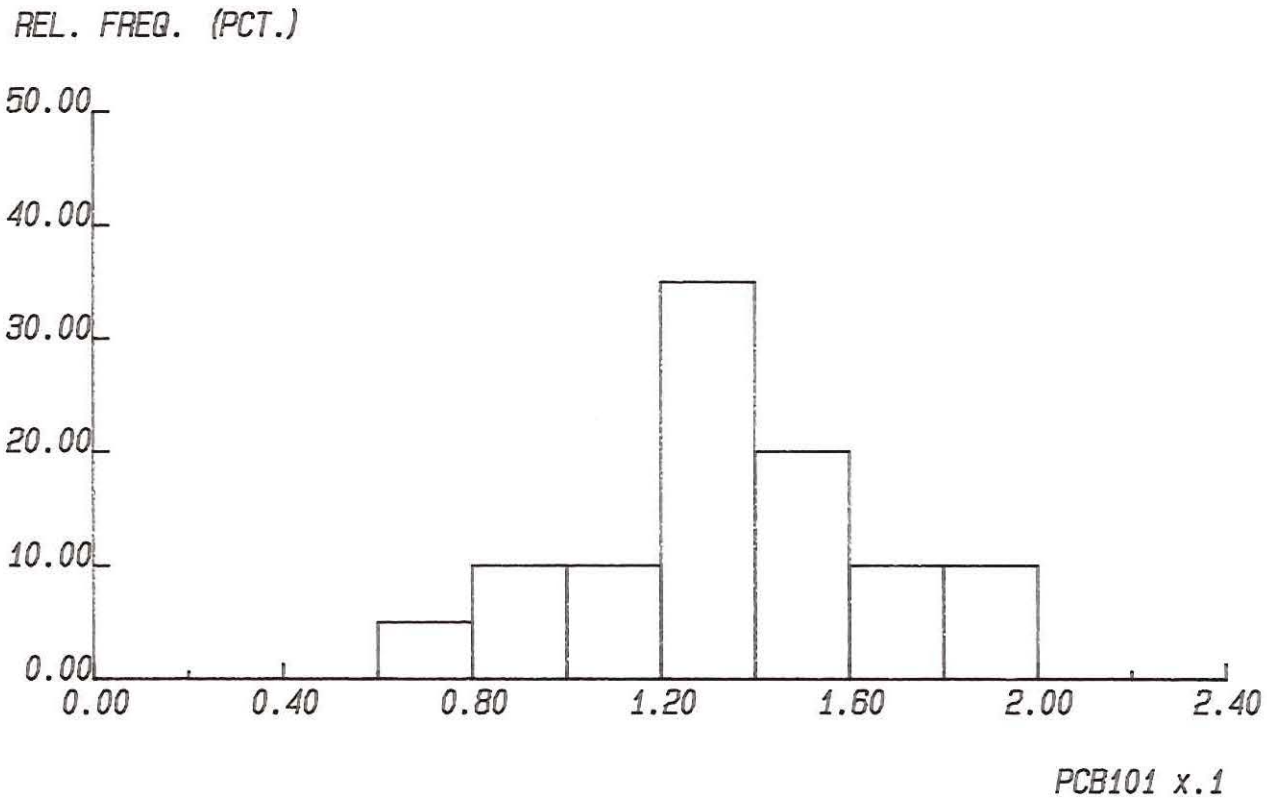

Figure 3. Histogram PCB101 Sample C (Content in mg/kg)

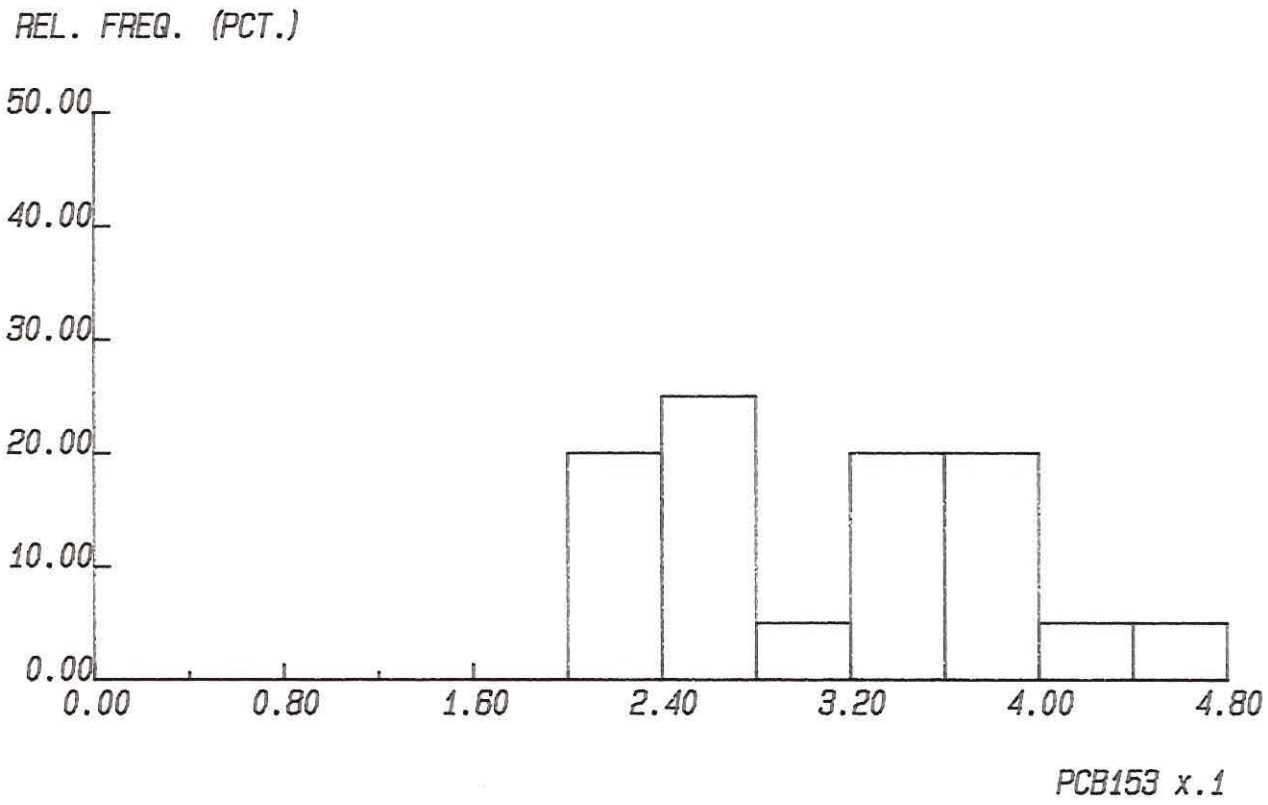

Figure 4. Histogram PCB153 Sample C (Content in mg/kg)

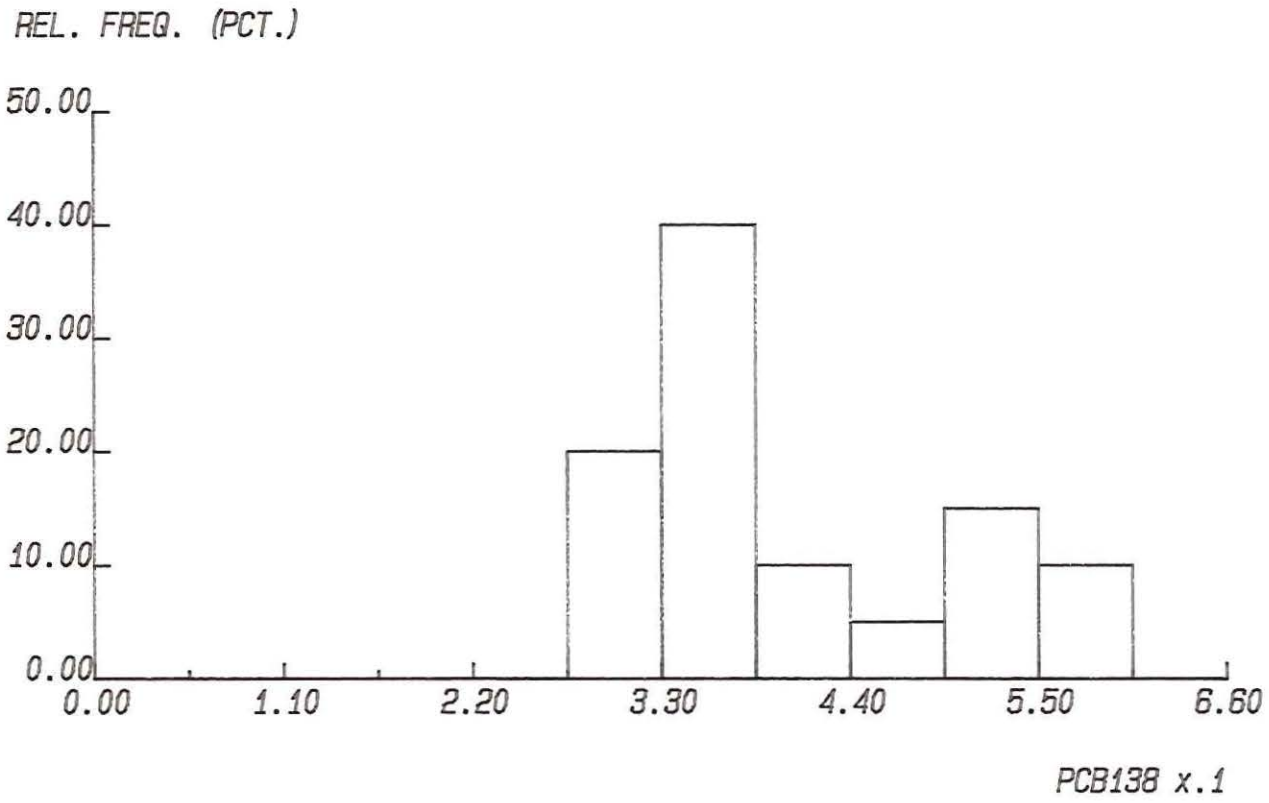

Figure 5. Histogram PCB138 Sample C (Content in mg/kg)

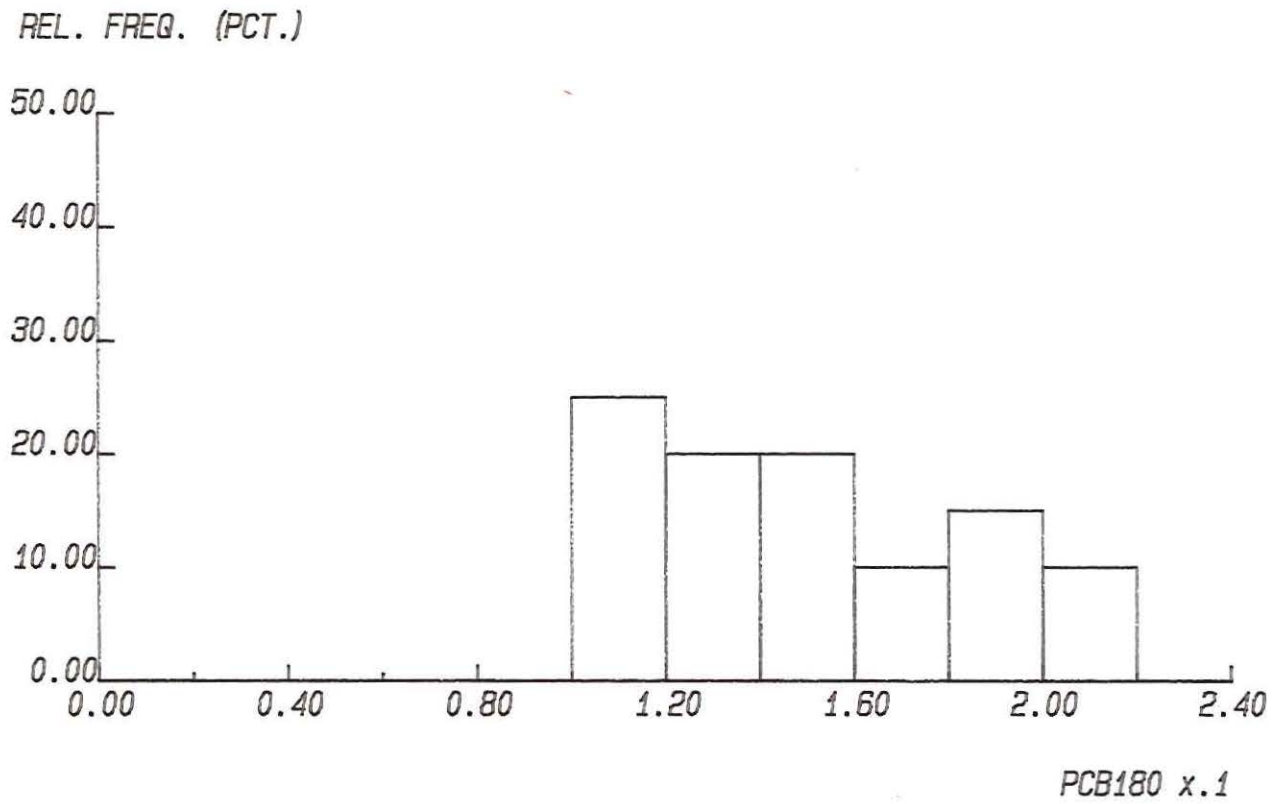

Figure 6. Histogram PCB180 Sample C (Content in mg/kg)

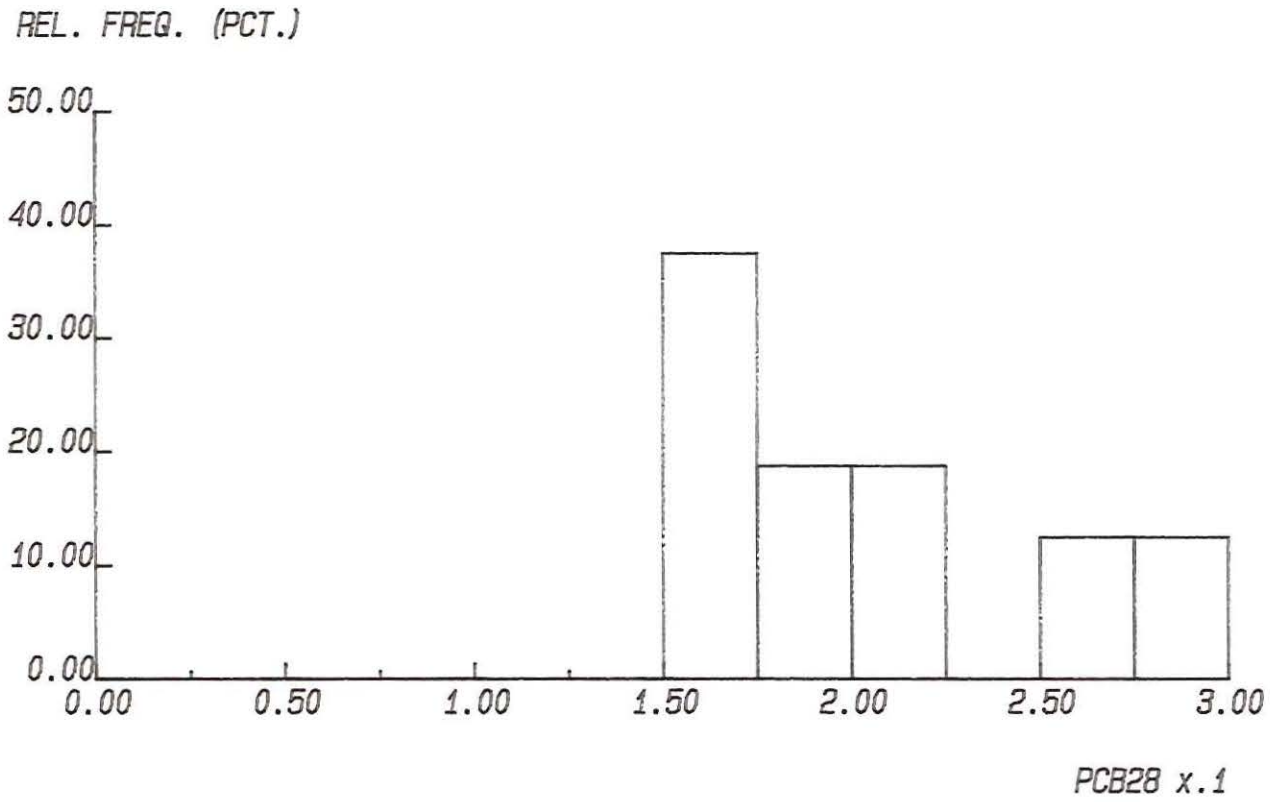

Figure 7. Histogram PCB28 Sample D (Content in mg/kg)

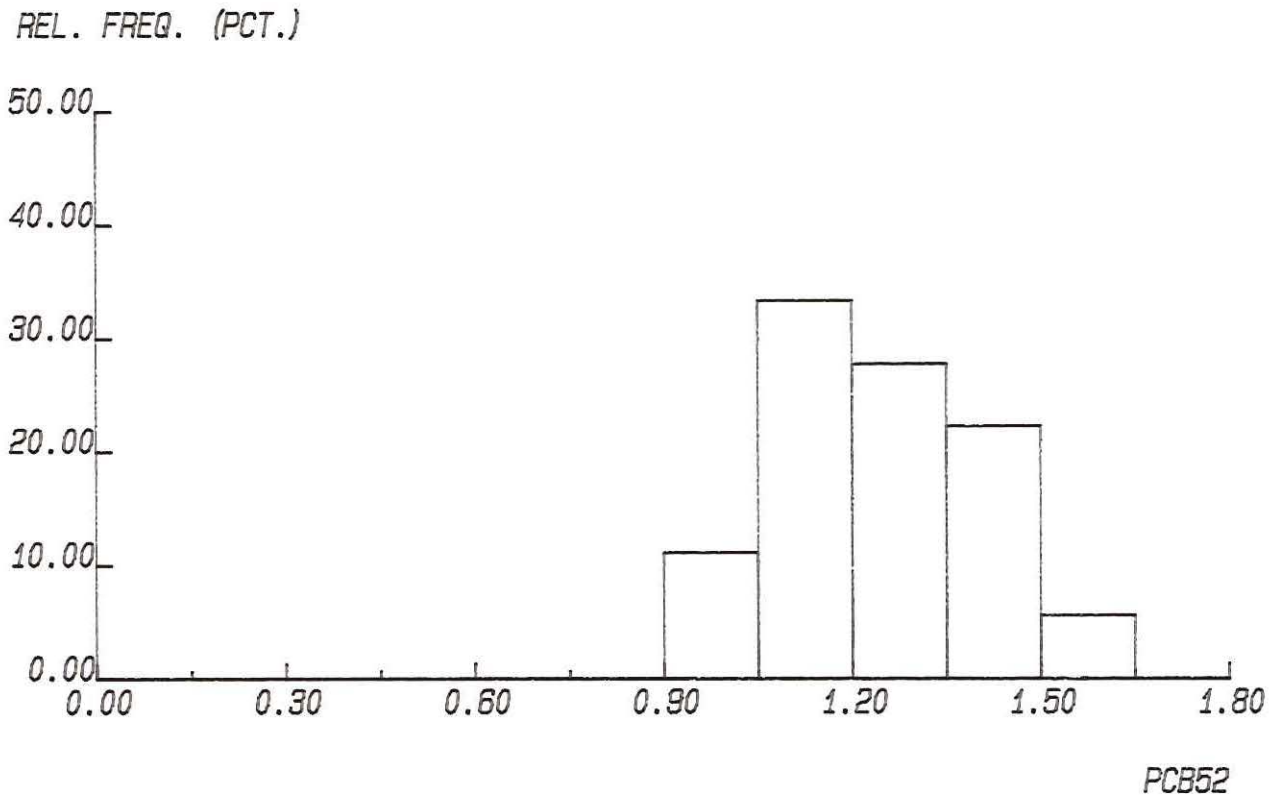

Figure 8. Histogram PCB52 Sample D (Content in mg/kg)

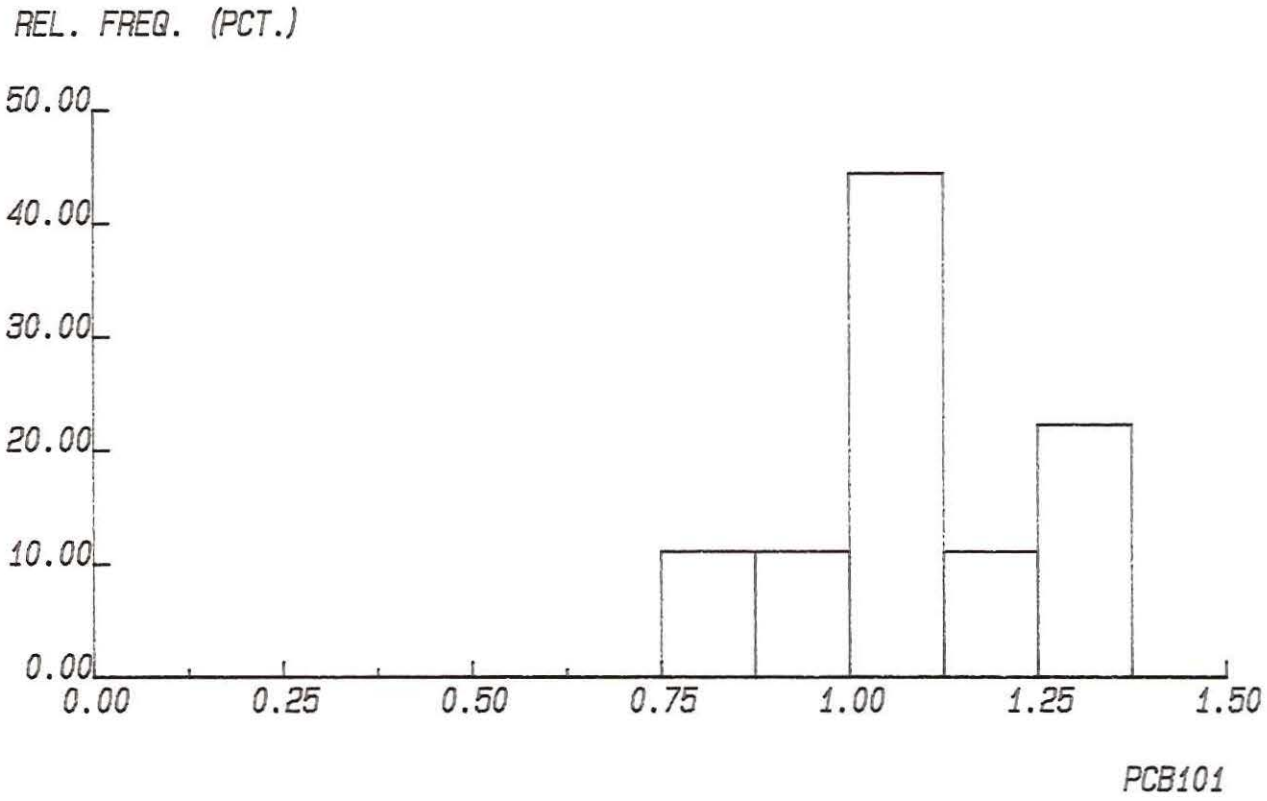

Figure 9. Histogram PCB101 Sample D (Content in mg/kg)

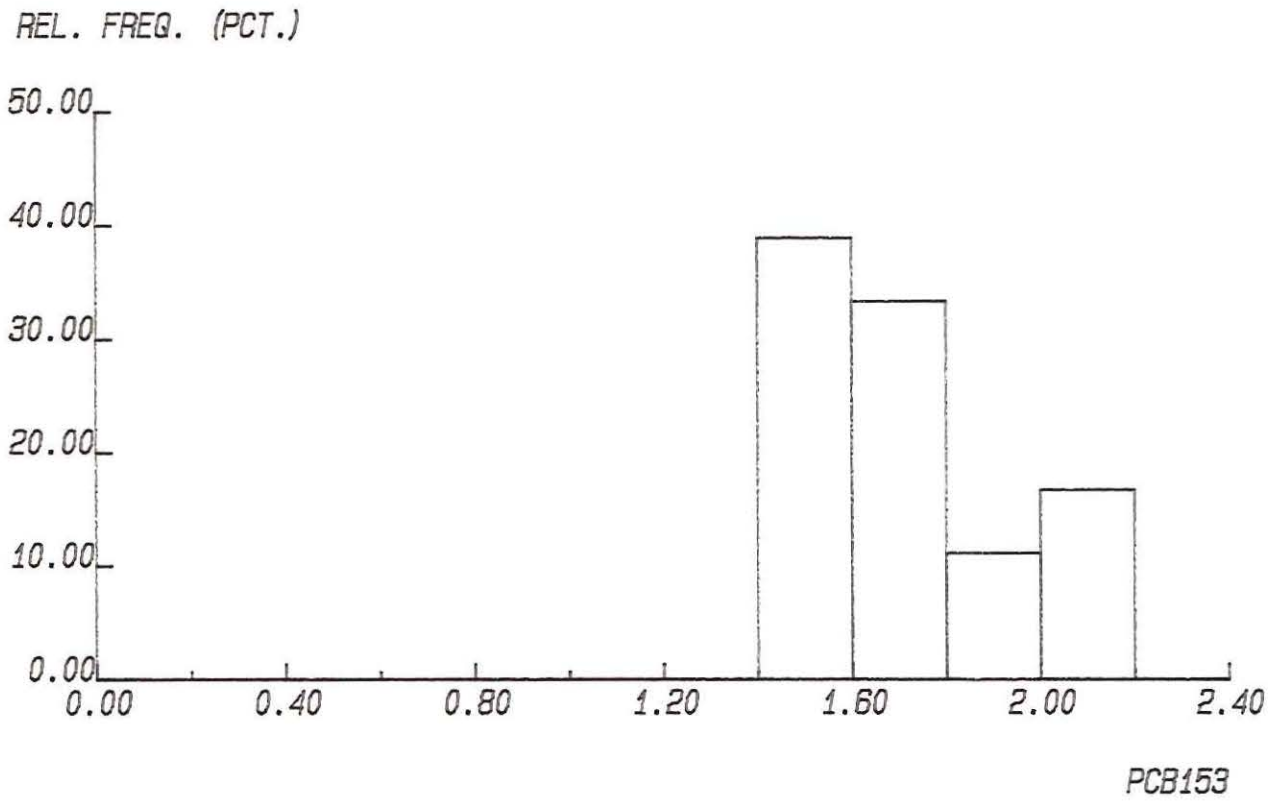

Figure 10. Histogram PCB153 Sample D (Content in mg/kg)

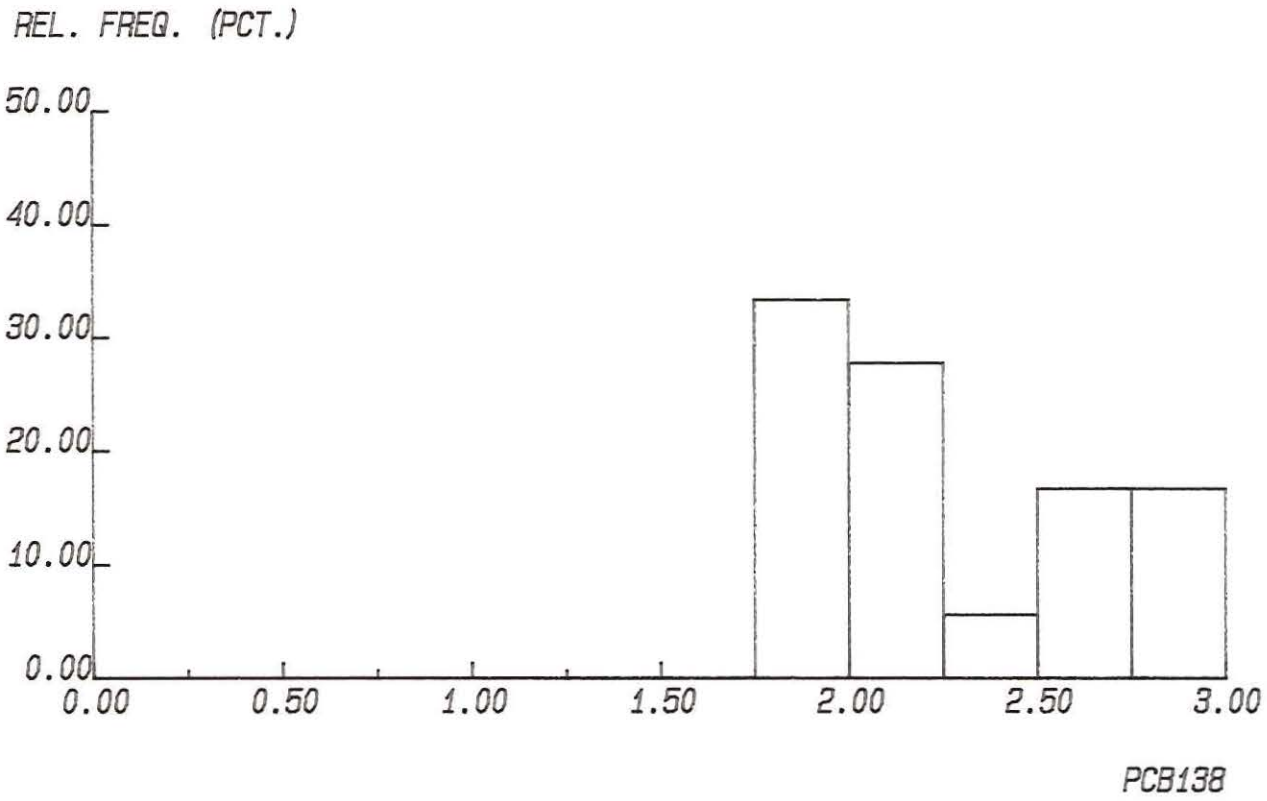

Figure 11. Histogram PCB138 Sample D (Content in mg/kg)

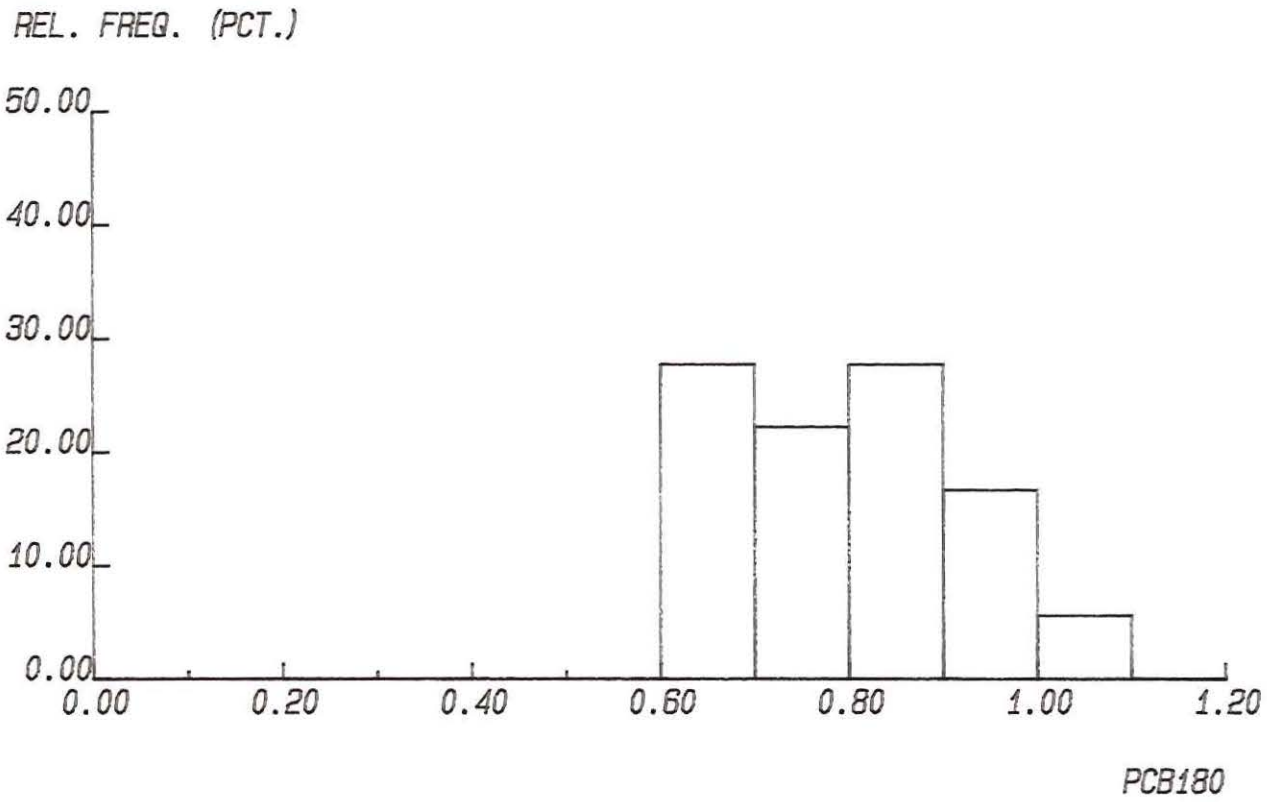

Figure 12. Histogram PCB180 Sample D (Content in mg/kg)

ANNEX 1

QUANTITATIVE DETERMINATION OF SIGNIFICANT INDIVIDUAL CHLOROBIPHENYLS IN FISH OIL WITH GLASS CAPILLARY GAS CHROMATOGRAPHY

Note. The method demands experience in capillary gaschromatography. Special consideration has to be given to the problems associated with carrier gas impurities, septum and column bleeding, injection technique and to inertness problems in the low picogram range.

1. SCOPE AND FIELD OF APPLICATION

The method is applicable to fish oil and allows the determination of specified individual chlorobiphenyls at the 1-10 µg/kg level.

2. PRINCIPLE

The fish oil is saponified. Reaction products solved in pentane are washed with water, dried on sodium sulphate and cleaned on alumina.

The residues are identified and determined in the eluate by capillary gas chromatography using splitless (or cold direct) injection techniques.

3. DEFINITION

The content of significant chlorobiphenyls in fish oil: The contents of substances determined by the procedure described and usually expressed in milligrams of compound per kilogram of oil.

4. REAGENTS

4.1 n-Pentane, suitable for residue analysis. Distil, if necessary, over a Raschig column of at least 50 cm length.

4.2 i-Octane, suitable for residue analysis. Distil, if necessary, over a Raschig column of at least 50 cm length.

4.3 Glass wool, heated at 135°C for 24 h.

4.4 Sodium sulphate, anhydrous, heated at 600°C for 2 h and stored in a well sealed container.

4.5 Alumina, basic, Woelm, activity I. Deactivate with 5% (m/m) of water (4.7). Control elution pattern so that the chlorobiphenyls elute completely and dieldrin and endrin are retained on the column.

4.6 Alcoholic potassium hydroxide solution. Dissolve 35 g of potassium hydroxide in 20 ml of water (4.7) and dilute with ethanol to 1 litre. Prepare the solution on the day of use.

4.7 Water, distilled, suitable for residue analysis.

4.8 Chlorobiphenyl standard solution
(0,1 µg/ml of each compound in i-octane/pentane (4/1 v/v))

2,4,4'-Trichlorobiphenyl	(No 28)
2,5,2'5'-Tetrachlorobiphenyl	(No 52)
2,4,5,2'5'-Pentachlorobiphenyl	(No 101)
2,4,5,2'4'5'-Hexachlorobiphenyl	(No 153)
2,3,4,2'4'5'-Hexachlorobiphenyl	(No 138)
2,3,4,5,2'4'5'-Heptachlorobiphenyl	(No 180)

Note - The concentrations are for guidance only and can be adjusted to suit various requirements.

Chlorobiphenyls are commercially available from Promochem, Wesel, FRG or Analabs, USA.

4.9 Internal standard solution
Mirex (150 ng/ml) or Pentachlorobenzene (100 ng/ml) or Hexabromobenzene (400 ng/ml) in i-Octane.

Note - The concentrations and compounds are for guidance only and can be adjusted to suit various requirements.

5. APPARATUS

5.1 Rotary evaporator with accessories or special concentrator (Kuderna-Danish).

5.2 Chromatographic columns, inner diameter 6,5-7,0 mm, with glass wool (4.3) plug.

5.3 Gas chromatography with ^{63}Ni electron capture detector and pressure controlled capillary inlet system suitable for splitless (or cold direct) injection.

5.4 Glass or fused silica capillary column, at least 25 m length, inner diameter 0,2-0,35 mm; stationary phase: SE 30, SE 54, CP Sil 5 CB, CP Sil 7 or comparable.

6. PROCEDURE

6.1 Saponification and clean-up on alumina
Weigh 1,0 g of oil into a 100 ml flask, add 20 ml of alcoholic potassium hydroxide and saponify on a waterbath for at least 45 min at 70°C inside the flask. Use a condensor on the flask. Add a few drops of water and mix. If the soap solution turns turbid proceed with saponification. Cool the solution and pour it into a separating funnel (250 ml). Add 30 ml of pentane, 20 ml of water and shake for 30 s. Transfer the pentane layer into another separating funnel. Extract the water layer 3 times with 15 ml of pentane and transfer the pentane layers to the second separating funnel. Wash the combined pentane solutions several times with 15 ml of water until neutral. The pentane solution should now be clear. Pass the pentane solution through a chromatographic column containing anhydrous sodium sulphate. Rinse the column with pentane and concentrate carefully to 2 ml at 40°C using a nitrogen flow.

Note - Especially chlorobiphenyls with lower chlorine contents are volatile and may evaporate.

Pour 2,0 g of alumina into a chromatographic column. Prewash with 5 ml of pentane, discard the eluate and place a measuring cylinder under the column. Transfer the sample solution quantitatively onto the column using a pipette, rinse with 2 ml of pentane and elute with 8 ml of pentane and concentrate to 2,0 ml. Add 1,0 ml internal standard and fill up to 10,0 ml with i-octane.

Note - The solvent of the final solution must be the same as used for the standard solution.

6.2 Gas chromatography

The gas chromatographic conditions must be thoroughly optimized to achieve a good resolution of the compounds to be analysed.

The following conditions are for guidance only and must be determined for each instrument and column used.

- carrier gas	- helium or nitrogen
- linear velocity of the carrier gas	- 10-15 cm/s for nitrogen, 25 cm/s for helium
- gas flows in the injection port e.g. septum purge	- depending on injection port design
- detector purge gas	- nitrogen or argon/methane 90/10 (v/v) 20-40 ml/min depending on the detector
- injection port temperature	- around 250°C
- detector temperature	- 300-350°C
- initial temperature	- use 90°C for i-octane
- initial time	- 2-5 min
- program rate	- 10°C/min
- final temperature	- 210-240°C
- final time	- 0-20 min, depending on final temperature
- cool time	- depending on instrument specifications
- closing time of splitter	- 20-200 s after injection, depending on injection port volume

For splitless injection close the splitter, inject 0,5-5 µl of the sample extract with a microliter syringe and reopen the splitter after the optimised time. For cold direct injection inject 0,5-2 µl using a narrow bore syringe. Start the temperature programmer at the time of injection.

Note - It is advisable to run with each series of determinations a standard solution for calibration as well as a blank and a recovery determination.

6.3 Identification and quantitative determination

Identification should be carried out on the basis of relative retention data, e.g. referred to the internal standard. Peak height should be used for quantification. The recoveries of all compounds determined in the separate recovery experiment should be higher than 80%.

Note - It is advisable to use a second column of different polarity or another independent qualitative and quantitative information for confirmation of the results obtained.

The blanc values determined in the separate blank tests should not exceed 5 µg/kg for each compound.

7. EXPRESSION OF RESULTS

Contents of individual compounds are calculated in milligrams per kilogram fish-oil using the internal standard method.