Journal of Chromatography, 204 (1981) 413–419
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,286

DETERMINATION OF INDIVIDUAL CHLORINATED BIPHENYLS IN AGRICULTURAL PRODUCTS BY AUTOMATED CAPILLARY GAS CHROMATOGRAPHY

DETERMINATION IN CATTLE FEED AND ITS RELATION TO MILK RESIDUES

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SUMMARY

Polychlorinated biphenyls were determined by glass capillary gas chromatography with electron capture detection, with automatic splitless injection. Using a saponification step as clean-up procedure, the detection limit for individual polychlorinated biphenyls can be improved up to the sub-ppb* level for vegetabele material and up to the ppb level for fatty material. Quantification is carried out by comparison of separated chlorobiphenyl peaks with the corresponding individual chlorobiphenyl standards.

The results of monitoring programs of milkfat and cattle feed are summarized. Accumulation factors for several chlorobiphenyls, determined in an experiment with lactating cows, are used to show that contamination of the milkfat is not caused by contaminated cattle feed.

INTRODUCTION

The gas chromatographic (GC) determination of polychlorinated biphenyls (PCBs) in all kinds of samples is commonly accomplished by injecting the sample extract onto a packed column and comparing the chromatogram of the sample with the chromatogram of a technical Aroclor 1254 or 1260 mixture. Sometimes the total peak area of the sample is compared with the total peak area of an Aroclor mixture, or only a few similar peaks are used for quantitation. Especially with the last method, the results obtained on the same sample by different laboratories will differ considerably, because of the differences in the GC conditions (*e.g.*, stationary phase, column temperature). Within one laboratory, when analysing one type of sample, the quantitation (or better, estimation) is adequate for establishing trends. However, more frequently we are interested in relationships between different commodities in order to understand accumulation factors in food chains.

* Throughout this article, the American billion (10^9) is meant.

0021-9673/81/0000–0000/$02.50

The poor match between the peak patterns from the sample and the technical standard mixture has frequently been noted in the literature (*e.g.*, refs. 1–4). This implies that the significance of the quantitation of PCBs in different commodities giving different peak patterns will be, at least, questionable. For this reason we chose a method suitable for determining individual PCBs in all kinds of samples (fish, milk, dairy products, soil, vegetables, etc.), which also has a very low detection limit. In this paper attention is focused on the determination of PCBs in concentrated cattle feed and its relation to milkfat.

EXPERIMENTAL

Extraction of fatty material from cattle feed

Eighty grams of concentrated cattle feed were extracted for 24 h with 200 ml pentane in a stoppered flask using a shaking machine. A 100-ml volume of the pentane extract was then concentrated to 5 ml.

Fat extraction from milk[5]

A 200-ml volume of milk was centrifuged at 2000 *g*. The separated cream layer was placed into a beaker and enough anhydrous Na_2SO_4 added to give agranular mixture on stirring. A 50-ml volume of pentane was added with stirring. The pentane layer was then decanted through anhydrous Na_2SO_4 and glass wool. The pentane extraction was repeated several times until a yellow colour was no longer observed in the pentane. The solvent was then evaporated to obtain fat.

Extraction of PCBs

The PCBs in milkfat and in cattle feed extract were isolated by saponification of the fatty material as follows. A 5-ml volume of the cattle feed extract or 2.0 g of milkfat was placed into a flat-bottomed flask, 20 ml of alcoholic 0.6 *N* KOH were added and the mixtured allowed to saponify on a water-bath at 70°C for at least 30 min. A few drops of water were added followed by mixing. If the soap solution became turbid the saponification was allowed to continue. On completion of saponification the mixture was cooled and poured into a separator. A 30-ml volume of pentane and 20 ml water were added and shaken for 30 sec. The pentane layer was transferred into a separator.

The water layer was extracted three times with 15-ml portions pentane. The combined pentane fractions were washed several times with water until the water was neutral. The pentane layer should be clear. The pentane extract was then passed through anhydrous Na_2SO_4 and concentrated carefully to 2 ml on a water-bath (50°C) with a slow stream of nitrogen. Lower chlorinated biphenyls are volatile and may evaporate.

Clean-up

A 2-g amount of basic alumina (deactivated with 5% water) was placed in chromatographic tubing and washed with 5 ml pentane. The prewash was discarded and a measuring glass placed under the column. The sample extract (2 ml) was transferred quantitatively to the column, rinsing with 2 ml pentane, and eluted with 8 ml pentane. The eluate was concentrated to about 1 ml and made up with isooctane to 5.0 ml.

Gas chromatography

A Tracor 550 or Packard-Becker 429 gas chromatograph was used with a ^{63}Ni electron capture detector and a capillary injection system according to Grob and Grob[6]. The capillary column (25 m × 0.25 mm I.D.) was coated with CP-Sil 7, film thickness 0.4 μm (Chrompack, Middelburg, The Netherlands). Flow-rates: pressure-controlled mobile phase (He or H_2), linear velocity 30–40 cm/sec; make up (N_2 or Ar–CH_4), *ca.* 20 ml/min; detector purge, *ca.* 30 ml/min. Temperatures: injector, 210°C; detector, 300°C; column oven programmed from 100 to 220°C at 40°C/min, initial hold 4 min, final hold *ca.* 65 min, cooling time 4 min. Data system: Perkin-Elmer PEP 1 or Spectra-Physics 4000. Injection system: Precision Sampling 4200 or Varian 8000 autosampler. In case of the former, closing and opening of the splitter is remotely controlled by the three timers on the injection device as described elsewhere[7]. With the latter, the Spectra-Physics data system controls the timing of the splitter. Splitless injection was carried out automatically using 5 μl of the extract. Three minutes after injection the splitter opens automatically to flush the injector. The splitter closes 2 min before injecting the next sample.

Quantitation

To clarify the problems mentioned in the Introduction, the results for a cattle feed sample (Fig. 1) were obtained by comparison with a technical Aroclor 1260 mixture (Fig. 2). When peaks 6, 11, 17, 20, 22 and M (an unknown chlorobiphenyl) were used for calculation, the following results for each peak were obtained: 3.3; 7.5; 1.6; 3.1; 3.2 and 17.5 μg/kg in the cattle feed. However, when peaks 24, H, L (present

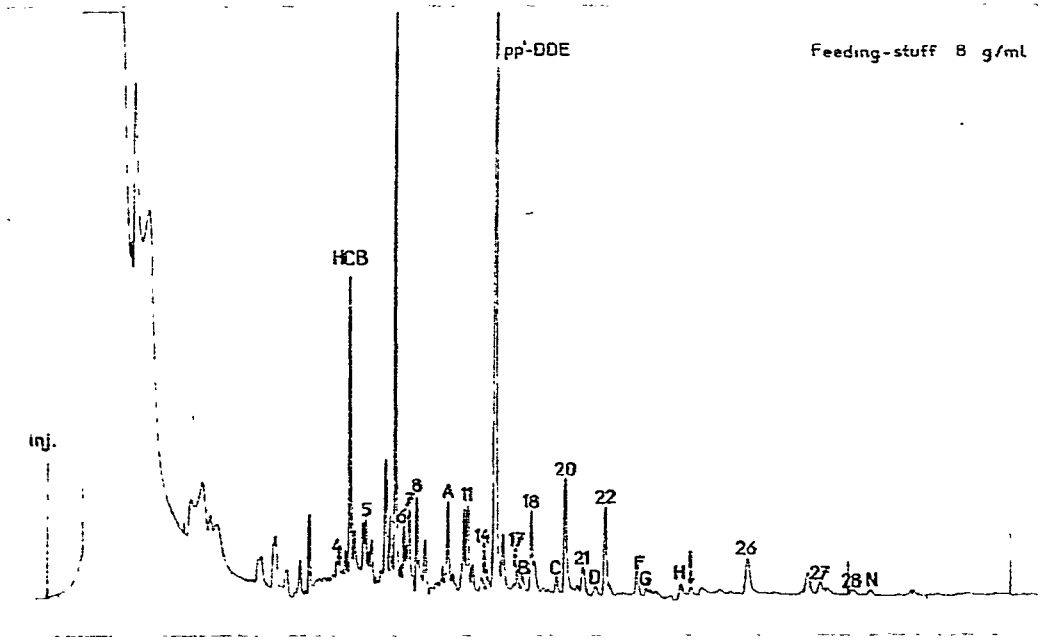

Fig. 1. Chromatogram of a cattle feed extract (8 g/ml) analysed on a CP-Sil 7 capillary (for structures see Table I).

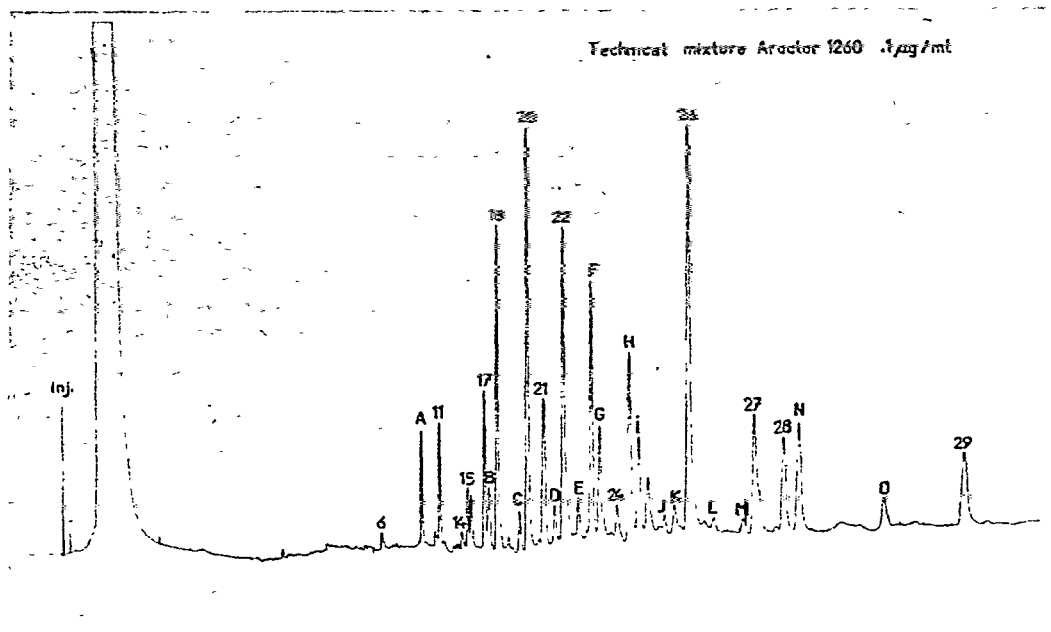

Fig. 2. Chromatogram of technical Aroclor 1260 mixture (0.1 µg/ml) analysed on a CP-Sil 7 capillary (for structures see Table I).

in the Aroclor 1260 mixture, but not in the sample) were employed the PCB content in the sample was calculated to be zero. Although this analysis was carried out on a capillary column, the same kind of problem will occur with a packed column.

The same type of problem also occurs when analyzing the same commodity: *e.g.*, in several fish species we have found not only big differences between the PCB patterns in the sample and in Aroclor 1254, but also between the PCB peak patterns of the different species[8]. Therefore, we decided to use an independent method of calculation by way of the individual chlorobiphenyls. In Fig. 3 the chromatogram of a standard mixture of chlorobiphenyls is shown (for structures and concentrations see Table I). Currently, peaks are identified by their retention times; mass spectrometrical confirmation will be added in the future.

RESULTS

Thirty-seven samples of cattle feed, taken at random from different producers, have been analysed. As is seen from Fig. 1, not all peaks have been identified, but this is not critical. In analysing 165 milk samples, the results of which will be published elsewhere[9], it was shown that in most samples only 17 individual compounds were detectable; of these, only 9 were present on average at a level higher than 2 µg/kg, calculated on a fat basis. Therefore, when analysing cattle feed, attention was focused especially on these compounds.

In cattle feed, the detection limit for polychlorinated biphenyls with two or more chlorine atoms in one phenyl ring is *ca.* 0.1 ppb. Only 4,4'-dichlorobiphenyl has

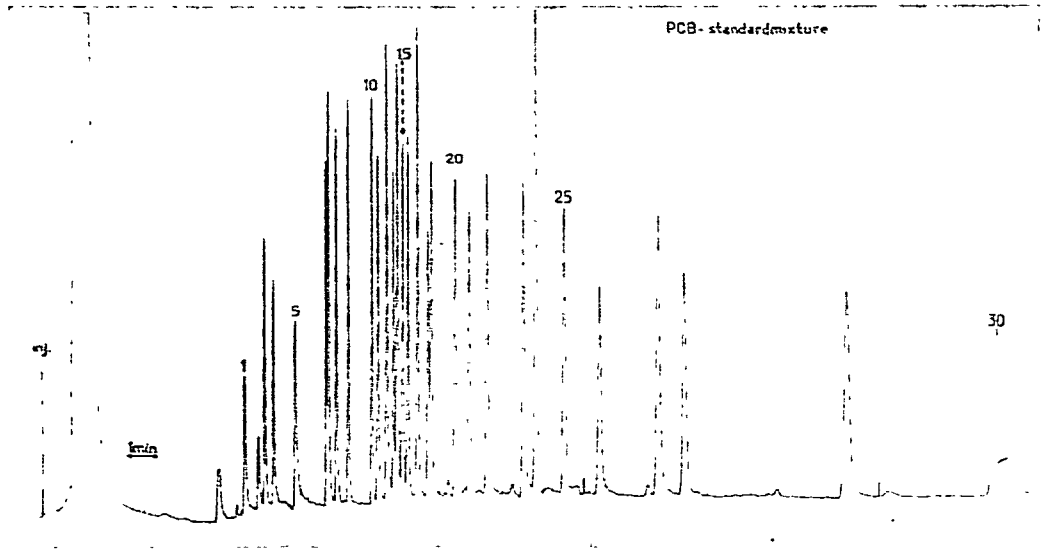

Fig. 3. Chromatogram of standard mixture of chlorinated biphenyls analysed on a CP-Sil 7 capillary (for structures see Table I).

TABLE I

COMPOSITION OF STANDARD MIXTURE OF PCB COMPOUNDS IN ISOOCTANE

Peak number	*Structure*	*Concn. (μg/ml)*	*Peak number*	*Structure*	*Concn. (μg/ml)*
1	2	2	16	2,4,5,2′,4′,6′	0.2
2	4	2	17	2,3,5,6,2′,5′	0.2
3	2,2′	2	18	2,3,6,2′,4′,5′	0.2
4	2,4	0.2	19	2,3,4,2′,4′,6′	0.2
5	4,4′	1	20	2,4,5,2′,4′,5′	0.2
6	2,5,2′,5′	0.2	21	2,3,4,5,2′,5′	0.2
7	2,4,2′,5′	0.2	22	2,3,4,2′,4′,5′	0.2
8	2,3,2′,5′	0.2	23	2,3,4,2′,3′,4′	0.2
9	2,4,6,2′,5′	0.2	24	2,3,4,5,6,2′,5′	0.2
10	2,4,6,2′,4′,6′	0.2	25	2,3,5,6,2′,3′,5′,6′	0.2
11	2,4,5,2′,5′	0.2	26	2,3,4,5,2′,4′,5′	0.2
12	2,4,6,3′,4′	0.2	27	2,3,4,5,2′,3′,4′	0.2
13	2,4,5,2′,3′	0.2	28	2,3,4,5,2′,3′,5′,6	0.2
14	2,3,4,2′,5′	0.2	29	2,3,4,5,2′,3′,4′,5′	0.2
15	2,3,6,2′,3′,6′	0.2	30	2,3,4,5,6,2′,3′,4′,5′	0.2

a detection limit of 1 ppb. In Table II the mean contents for the more important individual chlorobiphenyls in cattle feed are shown, together with the coefficient of variation (C.V.).

In another experiment, Aroclor 1260 was administered, together with the feed, to nine cows to establish the accumulation factor of individual chlorobiphenyls[10]. The accumulation factor for a given compound is defined as the ratio between the concentration in the milkfat and the concentration in the cattle feed on a dry matter

TABLE II

PERCENTAGE OF THE CONTAMINATION OF MILKFAT ACCOUNTED FOR BY CATTLE FEED

Compound	*Mean content of cattle feed*		*Accumulation factor* feed → milkfat*		*Theoretical content of milkfat*		*Actual mean content of milkfat*		*Contribution from the cattle feed contamination*	
	μg/kg	*C.V.*			*μg/kg*	*C.V.*	*μg/kg*	*C.V.*	%	*C.V.*
2,4	0.56	12	0.13	(19)	0.073	22	8.1	4	0.9	23
4,4′	3.50	19	0.14	(31)	0.49	36	22	10	2.2	38
2,5,2′,5′	0.28	14	1.7	(35)	0.48	38	2.1	2.6	23	38
2,3,2′,5′	0.35	25	1.2	(17)	0.42	30	0.9	4.3	47	31
2,4,5,2′,5′	0.24	16	0.44	(14)	0.11	21	3.1	3.5	3.4	22
2,3,5,6,2′,5′	0.12**	8	0.41	(3.2)	0.05	9	0.6	6.5	8.2	11
2,3,6,2′,4′,5′	0.32	11	0.46	(3.0)	0.15	11	2.5	5.3	5.9	13
2,4,5,2′,4′,5′	0.36	14	4.5	(3.2)	1.6	14	13.1	2.1	12	15
2,3,4,5,2′,5′	0.11**	6	0.48	(4.3)	0.05	7	0.6	8	8.8	11
2,3,4,2′,4′,5′	0.32	13	3.6	(3.6)	1.25	13	10.9	2.1	11	14
2,3,4,2′,3′,4′	0.13**	8	4.1	(4.2)	0.53	9	1.2	3.2	44	10
2,3,4,5,2′,4′,5′	0.15**	14	4.1	(3.0)	0.62	14	6.4	3.0	10	15
2,3,4,5,2′,3′,4′	0.10**	2.6	3.8	(3.0)	0.39	4	1.8	3.5	22	5

* C.V. value in parentheses.

** In most samples this compound is not detectable. This results in a mean content around the detection limit and causes a low C.V. Furthermore, this has consequences for the results in the last column. In fact the contribution will be lower than calculated.

basis. The values obtained from this experiment, together with the C.V., are shown in Table II, second column. By multiplying the mean content in feed by the accumulation factor[10], the expected mean content of a given chlorobiphenyl in milkfat is obtained (next column), assuming that the cattle feed is the only source for the contamination of milkfat with PCBs. However, the actual measured value for a compound in milkfat[9] (Table II) clearly shows that, in general, only a small part of the milkfat contamination with PCBs can be accounted for by contaminated cattle feed (last column). This conclusion is in agreement with the finding that there are only very small differences between the PCB contents of summer milk and winter milk[9].

ACKNOWLEDGEMENT

Some of the compounds used for quantitation were a generous gift from Professor C. A. Wachtmeister of Stockholm University (2,3,6,2′,4′,5′-hexachlorobiphenyl; 2,3,4,5,2′,4′,5′-heptachlorobiphenyl; 2,3,4,5,2′,3′,4′-heptachlorobiphenyl).

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