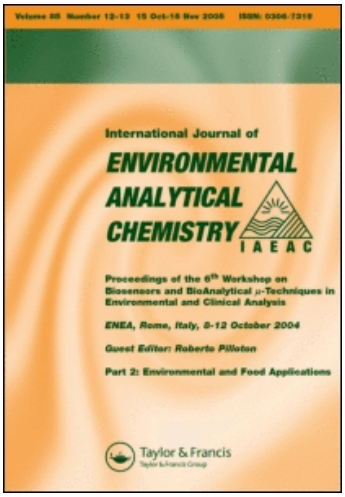

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Limitations in the Use of Perchlorination as a Technique for the Quantitative Analysis of Polychlorinated Biphenyls

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To cite this Article De Kok, A. , Geerdink, R. B. , Frei, R. W. and Brinkman, U. A.TH.(1982) 'Limitations in the Use of Perchlorination as a Technique for the Quantitative Analysis of Polychlorinated Biphenyls', International Journal of Environmental Analytical Chemistry, 11: 1, 17 — 41
To link to this Article: DOI: 10.1080/03067318208071559
URL: http://dx.doi.org/10.1080/03067318208071559

Intern. J. Environ. Anal. Chem., 1982, Vol. 11, pp. 17–41
0306-7319/82/1101-0017 $06.50/0

Printed in Great Britain

Limitations in the Use of Perchlorination as a Technique for the Quantitative Analysis of Polychlorinated Biphenyls

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(*Received July 20, 1981*)

Quantitative analysis of mixtures of polychlorinated biphenyls (PCBs) is frequently done by means of perchlorination of all individual PCBs to decachlorobiphenyl, with subsequent determination on a gas chromatograph equipped with an electron-capture detector. The potential of this perchlorination technique has been evaluated by comparing results so obtained with those of the conventional pattern-comparison method, using both non-biological and biological samples. With the former type of sample (paper, paper board, printing inks, sewage sludge) the PCB content as calculated from perchlorination data almost invariably is much higher—typically 2-30-fold—than that calculated on the basis of the pattern-comparison technique. With biological samples, large discrepancies also occur but much less frequently.

In an attempt to interpret these findings, the behaviour—under perchlorination conditions—of related types of compounds, such as polychlorinated terphenyls and naphthalenes, polybrominated biphenyls, hydroxylated biphenyls and organochlorine pesticides, has been studied. Evidence is presented to show that the presence of these types of compounds generally cannot explain the high results obtained for PCBs after perchlorination. With some recycled paper samples, high-performance liquid chromatographic (HPLC) fractionation and subsequent gas chromatography-mass spectrometry has allowed the isolation and identification of several hydrogenated terphenyls, which appear to be the main interfering compounds. This has been confirmed during a study of the commercial hydrogenated terphenyl-containing product HB-40.

Dechlorination of PCB mixtures and HPLC analysis, with UV detection, of the biphenyl formed is discussed as a promising alternative to perchlorination.

KEYWORDS: perchlorination, dechlorination, PCBs, PBBs, hydrogenated terphenyls, paper samples, sewage sludge, biological samples.

INTRODUCTION

Since 1929, polychlorinated biphenyls (PCBs) have been used extensively in a wide variety of industrial products because of their chemical and thermal stability and further favourable physico-chemical properties. They have been used both in closed systems (transformers and capacitors), nominally closed systems (hydraulic and heat-transfer fluids) and in open-ended applications (plasticizers in rubbers and resins, carbonless copy paper, ink and dye carriers and adhesives). Here, it should be added that, since about 1971, their usage has virtually been in closed systems only. A comprehensive review on the production, properties and usage of PCBs since their introduction has recently been published by Brinkman and De Kok.[1] A wealth of information on the chemistry of PCBs can be found in a book by Hutzinger *et al.*[2]

The properties which make PCBs desirable for industrial usage also make them an undesirable industrial pollutant. The PCBs, and particularly those containing a large number of chlorine substituents, are known to be highly resistant to chemical, metabolic and photochemical breakdown. They can therefore, after being taken up by living organisms, strongly accumulate in the higher levels of the food chain; other accumulation sites are soil, sediments, sewage sludge, landfills and also recycled paper. Because of the toxic and also supposed mutagenic and carcinogenic nature of these persistent pollutants, over the last decade much attention has been devoted to the analysis of PCBs in a wide variety of environmental samples.

Although high-performance liquid chromatography (HPLC) has been used successfully for the characterization of industrial PCB mixtures,[3,4] real environmental samples are mostly analysed by means of gas chromatography (GC) combined with electron-capture detection (ECD). Quantitation of PCBs, which are always present as complicated mixtures of at least several tens of individual compounds, is generally done (1) directly by matching the sample GC pattern with that of (an) appropriate commercially available PCB mixture(s) and comparing the total peak area or, more frequently, the height of an arbitrary number of peaks, or (2) via perchlorination of all individual PCBs to the fully chlorinated decachlorobiphenyl (DCB), which has a very low detection limit of about 0.1 pg in GC-ECD. High-temperature (170–180°C) perchlorination with reagents such as $SbCl_5$, $SbCl_5$-I_2 and a mixture of S_2Cl_2 and anhydrous $AlCl_3$ in sulphuryl chloride has repeatedly been propagated. It eliminates separation and interference problems caused by co-elution of e.g. organochlorine pesticides or polychlorinated naphthalenes and simultaneously increases sensitivity by the conversion of many individual

PCBs into a single, highly EC-sensitive, end-product. It has also been emphasized, however, that perchlorination—apart from causing a complete loss of PCB peak pattern and, thus, of PCB mixture identification—may lead to gross errors due to the partial conversion of other environmental contaminants into products having GC characteristics closely analogous to those of DCB, or even into DCB itself.

In recent years, in our laboratory, for a large number of non-biological samples much higher PCB contents have been found when using the perchlorination instead of the pattern-comparison method. This urged us to evaluate more closely the merits of both methods of analysis. During this work, the influence of type of sample—e.g., biological versus non-biological—was also investigated. For a selected number of samples, a relatively simple dechlorination technique[5] was used as reference method. As for interferences, initially the behaviour—under perchlorination conditions—of hydroxylated PCBs, polychlorinated terphenyls (PCTs) and naphthalenes (PCNs), polybrominated biphenyls (PBBs) and other related classes of compounds was studied. At a later stage, a more detailed investigation was carried out for recycled paper samples in order to identify the compounds actually responsible for the extremely high results of PCB analyses carried out via the perchlorination procedure.

EXPERIMENTAL

Materials

The commercially available PCB mixtures Aroclor 1221–1268 and the PCT mixtures Aroclor 5432, 5442 and 5460 (formerly produced by Monsanto, St. Louis, Mo., U.S.A.) as well as the PCN mixtures Halowax 1031–1051 (formerly produced by Koppers, Pittsburgh, Pa., U.S.A.) were purchased from Analabs (North Haven, Conn., U.S.A.). The PBB mixture fireMaster BP-6 (formerly produced by Michigan Chemical Corporation, St. Louis, Mich., U.S.A.) and technical decabromobiphenyl were supplied by Borg-Warner Chemicals (Amsterdam, The Netherlands) and Péchiney Ugine Kuhlmann (Paris, France), respectively. (Chloro)hydroxybiphenyls were purchased from RFR Corporation (Hope, R.I., U.S.A.); biphenyl and decachlorobiphenyl were obtained from Aldrich Europe (Beerse, Belgium) and the three tetra-decachloroterphenyl isomers from Analabs. Samples of the commercial (hydrogenated) polyphenyl mixture HB-40 produced by Monsanto were obtained as gifts from G. Sundström (Wallenberg Laboratory, University of Stockholm, Sweden) and Monsanto. Technical-

quality organochlorine pesticides, and related compounds such as DDE and DDD, were obtained from various sources.

For perchlorination antimony pentachloride "for synthesis" from Merck (Darmstadt, G.F.R.) was used and for dechlorination lithium aluminium hydride from Baker (Deventer, The Netherlands). Anhydrous diethyl ether (Baker) was redistilled over $LiAlH_4$ and stored on molecular sieve 5A. Ethanol and *n*-dodecane from Merck and tetrachloromethane from Baker were of normal analytical-grade quality, and were used as received.

Clean-up of sample extracts was done on Woelm B-Super I aluminium oxide (Woelm, Eschwege, G.F.R.) which was activated overnight at 200°C and subsequently deactivated with 5% (w/w) of water, and on conventional-grade silica (Kieselgel 60, 70–230 mesh) from Merck, which was activated overnight at 200°C and used as such. Sodium sulphate (Merck) was calcined overnight at 500°C. Quartz wool (Chrompack, Middelburg, The Netherlands) was subjected to soxhlet extraction with hexane.

For HPLC analyses ChromAR hexane from Mallinckrodt (St. Louis, Mo., U.S.A.) was used as solvent. For GC work, Nanograde hexane from Mallinckrodt was selected; it could be re-used after recycling by distillation over a 45% sodium dispersion in paraffin (Fluka, Buchs, Switzerland) to remove electron-capturing contaminants. Both grades of hexane were used for preparing standard solutions of the haloaromatic compounds studied. For the dissolution of the hydroxylated PCBs, the addition of some diethyl ether was often required.

Apparatus

GC measurements were performed on a Packard-Becker (Delft, The Netherlands) Model 419 or a Pye (Philips, Eindhoven, The Netherlands) Model GCV gas chromatograph, equipped with a ^{63}Ni EC and a flame-ionisation detector. The glass columns (1 or 2 m × 2 mm I.D.) were packed with 4% or 1% OV-101 on Chromosorb W HP (80–100 mesh). For pattern analyses a 2-m column and 4% OV-101 were used and for perchlorination analyses a 2-m column and 1% OV-101, or a 1-m column and 4% OV-101. For pattern comparison, analysis was done isocratically at 230°C; for perchlorination, programmed runs were made from 200°C to a final temperature of 300°C, with a rate of 5°C/min. Injector and detector temperatures were 280° and 300°C, respectively. Nitrogen was used as carrier gas and as purge gas for the EC detector, both at a flow-rate of 30 ml/min. Injection volumes were between 1 and 5 μl.

Gas chromatography-mass spectrometry (GC/MS) measurements were carried out on a Finnigan (Sunnyvale, Calif., U.S.A.) Model 9500 gas

chromatograph coupled with a Finnigan 3200 quadrupole mass spectrometer with electron-impact ionization (EI) at 70 eV. The ion-source temperature was maintained at 250°C. Chromatograms were run on a glass capillary column (25 m × 0.25 mm I.D.) coated with SE-30. The helium flow-rate was about 1 ml/min. The oven temperature was programmed from 160 to 260°C at a rate of 8°C/min. Injections were done with a solid injector; the injector temperature was 250°C.

The HPLC system consisted of an Orlita (Giessen, G.F.R.) Model FE 034 sRC reciprocating pump, a Valco (Houston, Texas, U.S.A.) six-port injection valve with a 100-μl loop, a guard column filled with molecular sieve 5A, a stainless-steel column (25 cm × 3 or 4.6 mm I.D.) pre-packed with 5 μm LiChrosorb SI 60 (Merck) or SI 100 (Brownlee, Santa Clara, Calif., U.S.A.) silica, and a Pye-Unicam (Philips) Model LC 3 variable-wavelength UV detector. Hexane dried on molecular sieve 5A was used as mobile phase. Chromatograms were run at ambient temperature.

Sample preparation

The treatment of the various samples prior to the PCB isolation depends on the type of sample. Sewage sludge from waste-water treatment plants is first freeze-dried and subsequently homogenized in a mortar. Wet heron fat, liver or brains, fish liver and whole fish samples (after boning) are dried by adding Na_2SO_4 and then homogenized in a mortar. Paper and paper board samples are cut into small pieces. With the printing inks, drying pastes and varnishes no pre-treatment is required.

The isolation of PCBs from the pretreated sample is accomplished by refluxing an accurately weighed amount of, generally, between 1 and 10 g for 1 h in a 300 ml erlenmeyer flask with 50–200 ml of a 2% ethanolic NaOH solution. The solution is then filtered over a plug of quartz wool into a 300 ml separatory funnel, and 25–75 ml of water are added. Next, the PCBs are extracted with 3 × 50 ml of hexane (recovery over 95%). The combined hexane phases are concentrated to about 5 ml via distillation, dried over Na_2SO_4 and transferred quantitatively to a calibrated centrifuge tube. The extract is finally concentrated to 2 ml under a stream of dry nitrogen. As an alternative, the weighed amount of sample can be subjected to soxhlet extraction with 100–200 ml of hexane during 8 h; concentration proceeds as described above.

Clean-up

A glass column (15 cm × 3 mm I.D.) provided with a glass frit is packed with 2 g of suitably deactivated (5% water) aluminium oxide—which, to

our experience, is preferable to Florisil—and washed with a few ml of hexane. Then 2 ml of concentrated sample extract in hexane are applied onto the top of this column and the PCBs are quantitatively eluted (95–100%) with 6 ml of hexane which are collected in a calibrated centrifuge tube. Apart from any required concentration or dilution, the extract is now ready for GC analysis in the pattern-comparison mode.

Clean-up over the aluminium oxide column provides an efficient separation of the PCBs from many common organochlorine pesticides and their main (bio)degradation products. However, the ubiquitous p,p'-DDE (which partly originates from conversion of p,p'-DDT during the NaOH treatment) is also eluted from the aluminium oxide column with 6 ml of hexane. Separation of DDE from Aroclor 1254 and 1260 can efficiently be done by an additional clean-up over a glass column packed with 1 g of highly activated silica which has been pre-washed with a few ml of hexane. Quantitative elution of the PCB fraction from this column takes place with 6 ml of hexane; under these conditions, DDE is still retained on the column. With mixtures containing Aroclor 1242, the procedure causes a distinct loss of late-eluting—i.e., low-chlorinated—PCBs. This result is in good agreement with data reported by Kveseth and Bresik.[6]

Perchlorination

An aliquot of the purified PCB extract in hexane is transferred to a glass reaction tube (25 cm × 11 mm I.D.; 17 mm O.D.), which can be sealed with a PTFE-lined, so-called Scott, screw cap. Subsequently, 0.5 ml of CCl_4 is added to the extract and after mixing the hexane is azeotropically evaporated under a gentle stream of dry nitrogen to about 0.2 ml. The addition of CCl_4 (2 ml) and evaporation (to 0.1 ml) is repeated twice in order to ensure complete removal of the hexane. After the addition of 0.2 ml of $SbCl_5$ the tube is closed and placed in an oil bath which is kept at a temperature of 170–180°C. Here, perchlorination takes place during a 15–18 h (overnight) reaction. The tubes are then allowed to cool down to room temperature and opened. Excess reagent is destroyed by the addition of 5–10 ml of 6 N HCl, the first drops of which should be added very carefully. Extraction of the fully chlorinated end-product of the reaction, decachlorobiphenyl (DCB), is done—in the same tube—by shaking the tube for 1 min on a Whirli mixer, with 2 × 5 ml of hexane. The combined hexane phases are transferred to a calibrated centrifuge tube using a pasteur pipette. After suitable concentration or dilution of the extract, should this be required, the DCB content can be determined by means of GC-ECD.

If the DCB-containing extract is too heavily contaminated to allow direct GC analysis, additional clean-up over aluminium oxide can be recommended. The extract is applied to the top of a 5% deactivated aluminium oxide column (cf. above) and eluted with hexane. The first 5-ml fraction contains the DCB.

Recoveries for Aroclor 1242, 1254 and 1260 after perchlorination were found to be 85–95, 95–105 and 95–105%, respectively; this is in good agreement with literature data.[7,8]

Dechlorination

An aliquot of the purified PCB extract is dechlorinated with $LiAlH_4$ in dodecane and in the presence of a small amount of diethyl ether, at 170–180°C, using the same reaction tubes as with perchlorination. The end-product of the reaction, biphenyl, is determined by means of HPLC in the system silica/dry hexane, using UV detection at 248 nm. All details of the dechlorination and chromatographic procedure are given in ref. 5.

RESULTS AND DISCUSSION

Pattern comparison vs. perchlorination for real samples

Sewage sludge. In our laboratory, discrepancies between PCB results obtained via the perchlorination and the pattern-comparison technique were first noted during analyses of sewage sludge from waste-water treatment plants. Results are shown in Table I for sludge samples from four different plants in The Netherlands, which are located in industrialized as well as (semi)-rural areas. In all cases, the PCB sample patterns were a composite of the patterns of Aroclor 1242, 1254 and 1260, with the optimum ratio 1242:1254:1260 generally being 2:1:1 or 2:2:1. Here as well as in the studies discussed below, quantitation was done by comparing the heights of, typically, 4–8 peaks per Aroclor standard used. Comparison of the total PCB content of the samples as μg of DCB per gram of sample (for conversion factors, see Table I) with the DCB content determined by perchlorination reveals that the latter method invariably yields higher results, with the perchlorination/pattern-comparison ratio (PE/PC ratio) varying from 2 to 11. Inspection of the DCB peak of selected samples by means of GC/MS invariably showed it to be over 99% pure. In two instances, the occurrence of high PE/PC ratios was confirmed in an inter-laboratory study with the State Institute for Quality Control of Agricultural Products (Wageningen, the Netherlands). It seems superfluous to add that the large PE/PC ratios cannot be attributed

TABLE I

Comparison of PCB levels[a] in sewage sludge samples as determined by pattern comparison (PC) and perchlorination (PE)[b]

Waste-water treatement plants[c]		Pattern comparison (μg PCB/g)			Perchlorination (μg DCB/g)	PE/PC
		A1242	A1254	A1260		
AW	I	6.6	2.7	2.7	58	2.8
	II	4.1	1.8	1.8	40	2.2
	III	4.3	2.1	2.1	30	2.1
	IV	3.2	3.0	2.6	40	2.8
	V	2.2	1.3	0.7	26	3.6 (4.1)[d]
	VI	2.5	1.6	0.9	38	4.5 (8.3)[d]
	VII	1.7	0.8	0.4	33	6.5
AN	I	3.7	1.0	1.0	38	3.8
	II	4.8	2.3	2.3	30	1.9
	III	1.7	0.6	0.6	54	10.8
	IV	3.3	0.9	0.9	61	6.8
BW	I	1.4	0.5	0.5	23	5.5
	II	1.5	0.7	0.4	21	4.7
GA	I	0.7	0.6	0.3	6.5	2.4
	II	0.8	0.5	0.3	11.8	4.4

[a]Calculated on dry-weight basis.
[b]Conversion factors from PCBs to DCB: A1242, 1.91; A1254, 1.54; A1260, 1.35.
[c]AW and AN, industrialized area; BW, semi-rural area; GA, rural area.
[d]Determined at State Institute for Quality Control of Agricultural Products.

to (relatively small) errors due to imperfect peak-pattern matching of the chromatogram of sample extract and standard mixture.

Paper etc. Conclusions similar to those for sewage sludge were reached with regard to the determination of PCBs in (recycled) paper and paper board samples. Here, in all but two cases, the PCB pattern was closely analogous to that of Aroclor 1242 (cf. Figure 1) which agrees with literature findings. The data, which are summarized in Table II show PE/PC ratios of up to 25; for only four out of the seventeen samples tested, agreement between the two techniques is good (PE/PC ratio, 1.0–1.7). The introduction of an additional clean-up step by shaking the hexane extract of the paper samples with a few millilitres of concentrated sulphuric acid did not effect a noticeable change of the results.

Again, our conclusions were confirmed during an interlaboratory study (Table III). In our laboratory, GC was done on packed columns, whereas capillary GC was used in the laboratory of the National Institute of Public Health (Bilthoven, The Netherlands). It is evident that, despite the rather too widely divergent results for the recycled paper sample No. I,

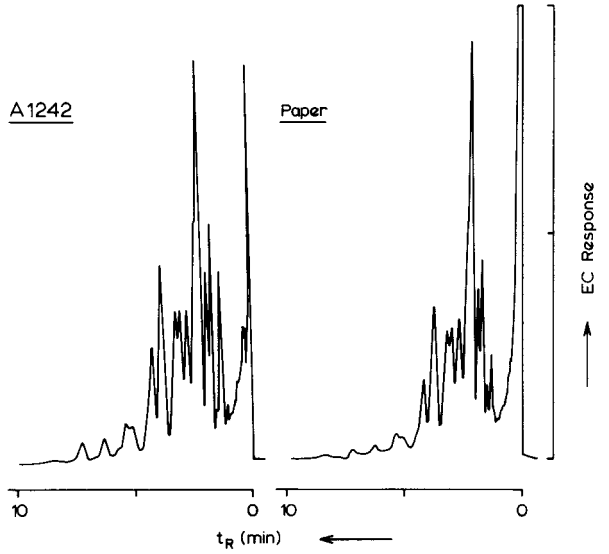

FIGURE 1 GC-ECD chromatograms of a recycled paper (IV) extract and the corresponding PCB standard, Aroclor 1242. For experimental details, see text.

there is essential agreement with regard to the main point of interest, viz., the frequent occurrence of high PE/PC ratios with paper samples.

The data in Table II indicate that fair amounts of PCBs are still present in recycled paper samples, despite the dramatic decline of production of PCBs for use in open or nominally closed systems. As a possible source of contamination, we therefore analysed a number of arbitrarily collected printing inks and auxiliary products used in printing-offices such as drying pastes and varnishes. Analysis by means of the pattern-comparison method revealed the absence of PCBs from all 8 samples examined. In most instances, perchlorination failed to give meaningful results due to charring, which was probably caused by the high carbon content of the products. With two samples, however, the perchlorination procedure could be carried out successfully; for these, very high DCB levels of 215 μg/g (printing-ink) and 12 μg/g (drying-paste) were found. The results were confirmed by analysis via standard addition of known amounts of Aroclor 1242.

Birds and fish. In order to study the influence of type of sample, a number of heron and fish samples were analysed. With these, the PCB pattern almost always showed a satisfactory correspondence to that of an Aroclor 1254:Aroclor 1260 (1:1) mixture. From the data in Table IV it is evident that with the fish samples agreement between the perchlorination

TABLE II

Comparison of PCB levels in paper and paper board samples as determined by pattern comparison (PC) and perchlorination (PE)[a]

Sample		Pattern comparison (μg PCB/g)		Perchlorination (μg DCB/g)	PE/PC
		A1242	A1254		
Toilet paper	I	0.16		0.59	2.0
	II	0.07		0.29	2.2
	III	0.32		6.0	9.8
	IV	5.28		49.3	4.8
	V	1.90		11.0	3.0
Recycled paper	I	0.16	0.11	7.2	15.0
	II	0.67	0.32	48	27
	III	0.10		4.8	25
	IV	1.27		44.5	18.2
	V	0.77		37.5	25.5
	VI	0.55		12.0	10.9
	VII	0.53		3.4	3.4
	VIII	2.55		5.4	1.1
Paper board	I	1.75		3.7	1.1
	II	1.0		21.9	11.5
	III	7.2		14.4	1.0
	IV	0.88		2.1	1.2

[a]For conversion factors from PCBs to DCB, see Table I.

TABLE III

Comparison of PCB levels in three paper samples as determined by pattern comparison (PC) and perchlorination (PE)[a] in an interlaboratory study[b]

Sample	Pattern comparison (μg A1242/g)		Perchlorination (μg DCB/g)		PE/PC	
	NIPH	FU	NIPH	FU	NIPH	FU
Toilet paper	0.37	0.33	3.3	3.0	8.9	9.1
Recycled paper I	0.43	0.58	0.87	1.77	2.0	3.0
II	1.10	0.84	17.3	19.7	15.7	23.5

[a]For conversion factor from PCBs (A1242) to DCB, see Table I.
[b]NIPH = National Institute of Public Health; FU = Free University.

TABLE IV
Comparison of PCB levels[a] in fish samples as determined by pattern comparison (PC) and perchlorination (PE)[b]

Fish sample	Pattern comparison (μg PCB/g) A1242	A1254	A1260	Perchlorination (μg DCB/g)	PE/PC
Cod liver I	0.47	1.29	1.29	4.0	0.9
II	—	0.74	0.74	2.6	1.2
Mackerel	0.14	0.12	0.12	0.7	1.1
Hake liver	0.86	0.81	0.81	4.9	1.2
Sprat	0.29	0.93	0.63	2.9	1.0
Eel	0.50	1.46	1.35	4.4	0.9

[a]Calculated on product basis.
[b]For conversion factors, see Table I.

and pattern-comparison data is excellent. PE/PC ratios are between 0.9 and 1.2. With the heron samples (Table V), a less consistent picture emerges. For the majority of the organs examined, the PE/PC ratio is between 1.0 and 2.0. High ratios of between 2 and 15 were, however, observed with all brain samples and a single liver sample.

Figure 2 shows GC chromatograms of the extract of a sprat sample and the Aroclor mixture used as standard. The efficient removal of p,p'-DDE—and the loss of some low-chlorinated PCBs—owing to the introduction of a clean-up step over active silica can also be read from this figure.

Miscellaneous Recently, capillary GC has been used[9] by us for the determination of PCBs in a contaminated soil sample. Analysis by means of pattern comparison yielded a value of 12 ppm of PCB (calculated as Aroclor 1254). Upon perchlorination, the sample was found to display a PE/PC ratio of 6.7.

Finally we report that high PE/PC ratios of 3 (3), 5 (3), 8 (7) and 17 (9) have also been found during an interlaboratory study with the State Institute for Quality Control of Agricultural Products on the PCB content of four animal feed samples; the values within brackets are those calculated in our laboratory.

Blank values. Using the procedure outlined in the Experimental section, blank values of the total perchlorination procedure were from 5 to 30 ng of DCB per analysis. This is rather similar to the values reported by Trotter and Young,[10] and by Tuinstra *et al.*[11] It is interesting to add our

TABLE V

Comparison of PCB levels[a] in heron samples as determined by pattern comparison (PC) and perchlorination (PE)[b]

Heron sample	Pattern comparison (μg PCB/g) A1242	A1254	A1260	Perchlorination (μg DCB/g)	PE/PC
Sc					
fat around bowels		11.5	11.5	65	1.9
subcutaneous fat		17.4	17.4	62	1.2
liver	0.4	0.4	0.4	2.1	1.1
brain		6.0	3.3	29.3	2.1
KI					
fat around bowels		126	126	361	1.0
subcutaneous fat		102	102	365	1.2
liver		6.2	6.2	27.5	1.5
brain		2.8	2.8	24.4	3.0
KII					
fat around bowels		228	228	876	1.3
subcutaneous fat		211	211	981	1.4
liver		8.1	8.1	22.4	1.0
brain		1.8	1.8	79	15.0
Do					
fat around bowels		10.2	10.2	47	1.6
subcutaneous fat		21.0	21.0	104	1.7
liver		0.7	0.7	8.2	3.9
brain	1.3	0.4	0.4	17.6	4.8

[a]Calculated on product basis.
[b]For conversion factors, see Table I.

experience with ethanol purchased from Baker (Baker Analyzed reagent; Deventer, the Netherlands). Perchlorination of samples which had been pre-treated with this brand of ethanol invariably gave unusually high DCB values. Further research showed these to be due to the presence of (an) interfering compound(s) in the alcohol yielding 300–350 ng of DCB per ml of ethanol. The DCB peak was unambiguously identified as such by means of GC/MS. The nature of the interfering compound(s) has, however, as yet not been elucidated.

Perchlorination of possibly interfering compounds

In order to discover the nature of classes of compounds which interfere in the determination of PCBs via perchlorination, several types of—mainly chlorine-containing—aromatic compounds were studied. The results of

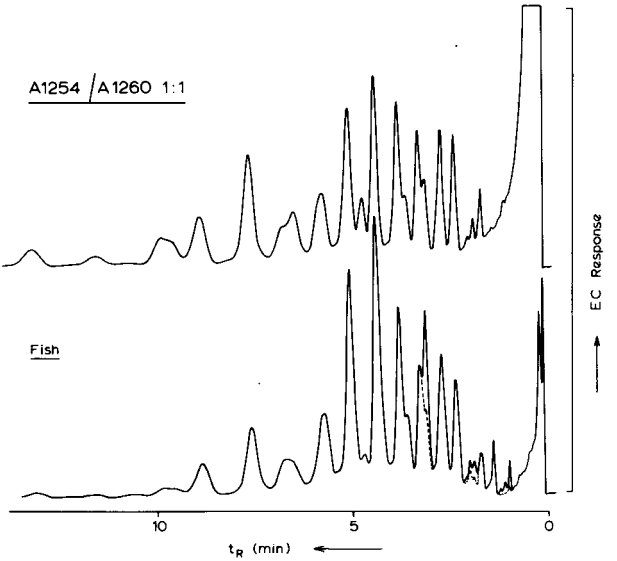

FIGURE 2 GC-ECD chromatograms of a fish (sprat) extract and an Aroclor 1254/1260 (1:1) standard. The dashed trace in the lower chromatogram indicates the influence of the activated silica clean-up step: p,p'-DDE (t_R, 3 min) is completely eliminated. For further details, see text.

experiments carried out under the conditions described in the Methods section are summarized in Table VI.

Benzenes. No noticeable formation of DCB was observed in the perchlorination of benzene or substituted benzenes such as chlorobenzene,

TABLE VI
Conversion of various types of (halogenated) aromatic compounds into DCB[a]

Class of compounds	Conversion into DCB (%)
(Chloro) hydroxybiphenyls	0
Biphenyl	25–30
Hydrogenated biphenyls	5–25
Substituted benzenes	0
Organochlorine pesticides	0
PCNs	0
PCTs	0–3
PBBs	15–20

[a]Perchlorination conditions: as used for PCBs.

toluene, phenol and aniline. Perchlorination of 2-hydroxybiphenyl and 3-chloro-4-hydroxybiphenyl, a known metabolite of PCBs, did not lead to any formation of DCB either. The latter result is in agreement with data published by another group of workers.[12]

Biphenyls. According to expectation, biphenyl itself is converted into DCB upon perchlorination although the recovery of typically 25–30% is low and, besides, varies rather widely with reaction conditions. No doubt, this is due to extensive degradation of the biphenyl nucleus under the vigorous conditions employed. As a consequence of the above, with samples containing—or suspected to contain—biphenyl a selective separation procedure prior to the perchlorination step has to be introduced. Here, one can recommend the use of HPLC in the system silica/dry hexane which will be discussed in more detail in connection with dechlorination (see below).

Perchlorination of (partly) hydrogenated biphenyls, *i.e.*, phenylcyclohexane and bicyclohexyl, gave a rather interesting result. Next to a fair amount of hexachlorobenzene, 5–25% of DCB was formed. In other words, the high-temperature treatment with $SbCl_5$, not unnaturally, effects a partial breakdown of the molecule; however, rather surprisingly a significant proportion of the saturated ring(s) is converted into fully chlorinated unsaturated ring(s).

Pesticides. The combined action of treatment with NaOH, selective elution on basic alumina and perchlorination with $SbCl_5$ caused 10 well known organochlorine pesticides and their degradation products (see Table VII) to be separated from the PCBs or to be converted into reaction products other than DCB. Besides, these did not present separation problems during GC analysis but for one exception: perchlorination of p,p′-DDE caused it to be converted into a variety of products, one of which elutes only slightly ahead of DCB. However, even when using a packed instead of a capillary column, and when DDE is present in high amounts relative to DCB, resolution is sufficient to obtain a well shaped DCB peak sitting on the tail of its predecessor. Interferences due to DDE can of course be eliminated by introducing the clean-up step over a highly active silica column discussed above.

TABLE VII
Summary of pesticides and their degradation products studied

Hexachlorobenzene	Endrin	p, p′-DDT
Heptachlor	α-Endosulfan	p, p′-DDE
Aldrin	β-Endosulfan	p, p′-DDD
Dieldrin	γ-HCH	

The above results agree with literature data.[13, 14] These also quote α-HCH, γ-HCH, and heptachlor epoxide as pesticides which do not interfere in the determination of PCBs via procedures including a treatment with NaOH.

PCNs, PCTs. Among the classes of compounds often quoted as main interferences during PCB analysis, PCNs and PCTs are prominent. In the present study, Halowax PCN mixtures were shown to be completely degraded during perchlorination. That is, no octachloronaphthalene was formed; not unexpectedly, neither was any DCB. Should the perchlorination conditions be chosen such that (partial) conversion of the PCNs into octachloronaphthalene does occur, then separation of the latter compound from DCB can easily be achieved by GC as well as reversed-phase HPLC (ref. 15). The Aroclor PCT mixtures were mainly converted into the three isomeric tetradecachloroterphenyls which display extremely high retention as compared to DCB and, thus, do not interfere. This result, which will be discussed in more detail elsewhere,[16] agrees with literature findings. It was also observed, however, that a small proportion of DCB—typically between 0 and 3%—was formed, the actual percentage being somewhat dependent on the reaction conditions and the Aroclor PCT mixture used. The occurrence of a small peak due to hexachlorobenzene suggests that the formation of DCB is due to breakdown of the terphenyl skeleton. The perchlorination of small amounts of PCBs, possibly present as contaminants in PCTs, should, however, not be fully excluded.

PBBs. If conversion into DCB is considered, PBBs can well be said to be more closely related to PCBs than any other class of compounds. Overnight perchlorination of decabromobiphenyl (DBB) at the conventional temperature of 170°C yielded 15–20% of DCB and smaller proportions of a number of fully halogenated compounds having the general structure $C_{12}Cl_{10-x}Br_x$ with $x>0$. Further experiments showed the degree of conversion into DCB to be strongly dependent on reaction temperature and time (see Table VIII), with the temperature effect being the more important. At a temperature of 200°C, the conversion into DCB is—with an 80–85% recovery—virtually independent of reaction time.

Hutzinger *et al.*[17] have reported that, during perchlorination of fireMaster BP-6 under the conditions normally employed by them, well defined derivatives could not easily be obtained. For this mixture—the major constituents of which are 2,4,5,2′,4′,5′-hexabromobiphenyl (73%) and 2,3,4,5,2′,4′,5′-heptabromobiphenyl[18, 19]—we obtained results similar to those for DBB. At 200°C, the mixture yields DCB as sole fully halogenated end-product. At 170°C, however, the cluster of mixed chlorinated/brominated biphenyl peaks observed is characteristically

TABLE VIII
Perchlorination of decabromobiphenyl as a function of reaction time and temperature

Temperature	% conversion into DCB after		
	4 h	16 h	64 h
140°C	0.5	0.5	3.3
170°C	1.7	17	74
200°C	83	82	80

different from that obtained with DBB, as is nicely illustrated in Fig. 3. The chromatogram of perchlorinated DBB displays—next to the DCB peak—four such peaks with $x=1$, 2, 3 and 4, respectively (note the constant retention increment between the subsequent peaks in the temperature-programmed GC run) while that of perchlorinated fireMaster BP-6 displays only two additional peaks (with $x=1$ and 2). This mutual difference suggests an influence of the position of the bromine substituents in the biphenyl nucleus on the ease of substitution of bromine by chlorine atoms upon treatment by $SbCl_5$. 3,5,3′,5′-Tetrabromobiphenyl and 3,4,5,3′,4′,5′-hexabromobiphenyl—which both have four unoccupied ortho positions, and no and two para positions occupied by bromine atoms, respectively—where therefore perchlorinated under the same conditions as

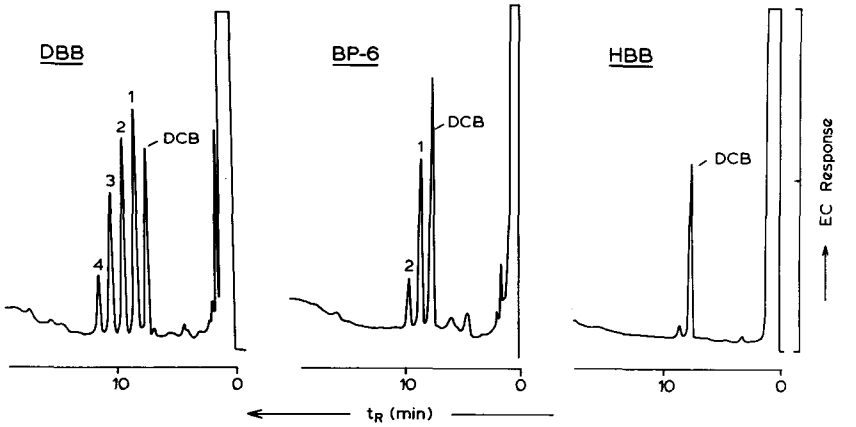

FIGURE 3 GC-ECD chromatograms of perchlorinated samples of decabromobiphenyl (DBB), fireMaster BP-6 and 3,4,5,3′4′5′-hexabromobiphenyl (HBB). The peak numbers for the peaks eluting after DCB denote the number (x) of bromine atoms in the perchlorinated products having the general formula $C_{10}Cl_{10-x}Br_x$. For further explanation, see text.

were DBB and fireMaster BP-6, i.e., for 16 h at 170°C. For both compounds this resulted in virtually complete conversion into DCB; that is, peaks due to $C_{12}Cl_{10-x}Br_x$ hardly show up (see Fig. 3). It can thus be concluded that bromine atoms in positions meta or para to the central C–C bond in the biphenyl nucleus are far more easily substituted by chlorine atoms than are bromine atoms in the ortho position.

A convenient accidental consequence of the presence of the cluster of $C_{12}Cl_{10-x}Br_x$ peaks is the possibility of recognizing the presence of PBBs in real samples, provided the perchlorination reaction is carried out at an appropriate temperature, *i.e.*, 170°C. The presence of the clusters can also be profitable in structure identification, because the number of $C_{12}Cl_{10-x}Br_x$ peaks with $x>0$ equals the number of ortho-substituted positions in the original PBB.

Other interfering compounds

The data presented in the previous section may be summarized by stating that no satisfactory explanation can as yet be given for the high DCB levels obtained after perchlorination. In a further attempt to solve this problem we focussed our attention on the analysis of some recycled paper samples, for which very high PE/PC ratios had been found (cf. Table II). Since "suspected" peaks were notably absent from our GC chromatograms, analysis by means of adsorption HPLC on silica with dry hexane as mobile phase was studied as an alternative. This choice was prompted by the fact that the said system yields[3,4] promising separations of commercial PCB mixtures. Inspection of a number of HPLC chromatograms of paper samples revealed the presence of two partly resolved peaks with retention times slightly larger than that of biphenyl (which, by the way, was absent from all these samples). Such peaks were conspicuously absent from chromatograms of several biological samples which had PE/PC ratios close to 1.0 (cf. Figs. 3 and 4 in ref. 5). Besides, with the paper samples, the heights of the said peaks seemed to vary in much the same way as did the *absolute* difference between PCB levels calculated via perchlorination and pattern comparison.

For a recycled paper sample with a PE/PC ratio of 25 the HPLC column effluent containing the suspected compounds was collected, using a very concentrated extract of a 150 g paper sample for injection (Fig. 4). Upon analysis of this fraction by means of capillary GC/MS six major peaks were identified, *viz.* three diphenylcyclohexanes and three cyclohexylbiphenyl isomers. Selective ion detection of $m/z=236$ clearly showed the presence of the partly hydrogenated terphenyl isomers. The mass spectrum of one isomer is given in Fig. 5.

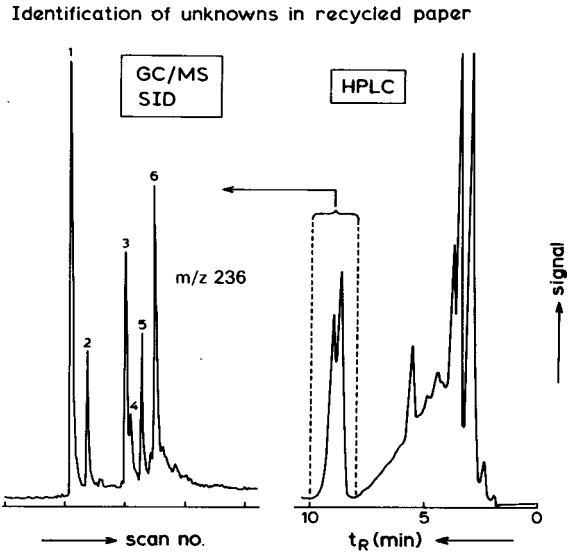

FIGURE 4 (Right) HPLC-UV (248 nm) chromatogram of a recycled paper extract showing the "suspected-peak" fraction analysed by means of GC/MS. System: LiChrosorb SI 60 silica/dry hexane; flow-rate, 1.6 ml/min. (Left) Single-ion detection chromatogram (m/z = 236) of the suspected-peak fraction.

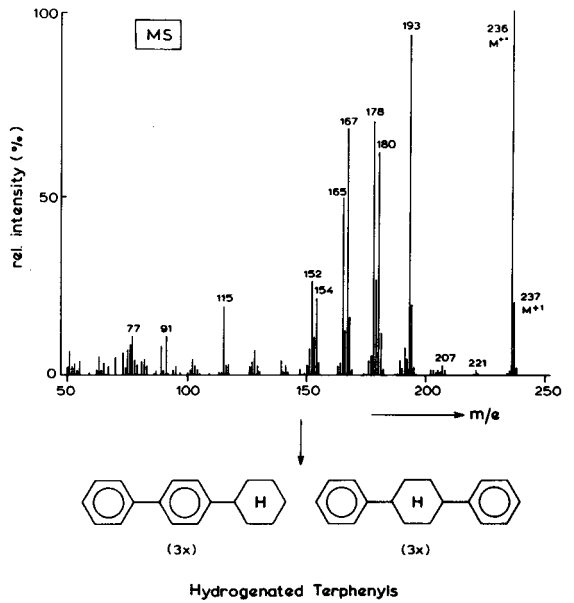

FIGURE 5 General structure of the hydrogenated terphenyl isomers identified in the suspected-peak fraction of a recycled paper extract (cf. Fig. 4), and mass spectrum of one such isomer.

As final proof of the interfering role played by the hydrogenated terphenyls, perchlorination of the pertinent hexane fraction—which does not contain any PCBs, because these all elute prior to biphenyl[3]—was shown to yield a considerable proportion of DCB. This fits in with the previously recorded behaviour of hydrogenated biphenyls under perchlorination conditions (cf. Table VI). Obviously, in both cases the ring system tends to break down partly, yielding the fully chlorinated unsaturated hydrocarbons as end-product.

Hydrogenated terphenyls. Literature data on the production and usage of (hydrogenated) polyphenyls are rather scarce. The patent literature describes the use of such compounds as, e.g., dye solvent for pressure-sensitive copy paper,[20-22] constituent of dielectric fluids for transformers,[23] plasticizer for polyvinylchloride,[24] and nuclear-reactor coolant.[25] A commercially available complex mixture of polyphenyls and hydrogenated polyphenyls—marketed under the trade-name HB-40 (Monsanto)—is mentioned in the literature,[26-28] and designated[29] as a technical product used or intended to be used as a PCB substitute. This 40% hydrogenated mixture of principally *o*-, *m*- and *p*-terphenyl was used, at least in the early 1970s, as a coolant in a nuclear reactor in Canada. Recently, low levels of three hydrogenated terphenyls have been identified in surface drinking water.[30]

Two samples of HB-40, provided by G. Sundström and Monsanto, were shown to have a closely analogous composition by means of HPLC and were used indiscriminately in our study. HPLC was carried out in the system silica/dry hexane, with UV detection at the wavelength of maximum absorption, 202 nm (Fig. 6). If detection was done at 250 instead of at 202 nm (cf. insert in Fig. 6), the general chromatographic pattern remained the same. However, the intensity of the early eluting peaks at $t_R = 1$–4 min decreased dramatically (approx. 50-fold) compared with the modest, e.g., about 2–5-fold, reduction of the peaks at $t_R = 8$–11 and 20–30 min. For further work, three HPLC fractions were collected, viz. Fraction A (t_R, 0–6 min), which should comprise any PCBs present in HB-40; Fraction B (t_R, 6–11 min), in which will elute the main peaks showing up in Fig. 6; these display retention times marginally higher than those of biphenyl†; Fraction C (t_R, 18–30 min), which might contain—for the reason given above—late eluting "suspected" peaks. Each of the fractions was subjected to GC/MS, and GC-ECD and GC-FID

†The silica column used in the environmental studies in both this paper (Figs. 4 and 7) and in ref. 8 showed an activity (and selectivity) distinctly different from that used for the analysis of HB-40 (Fig. 6). In all instances, however, "suspected" peaks invariably eluted *after* biphenyl.

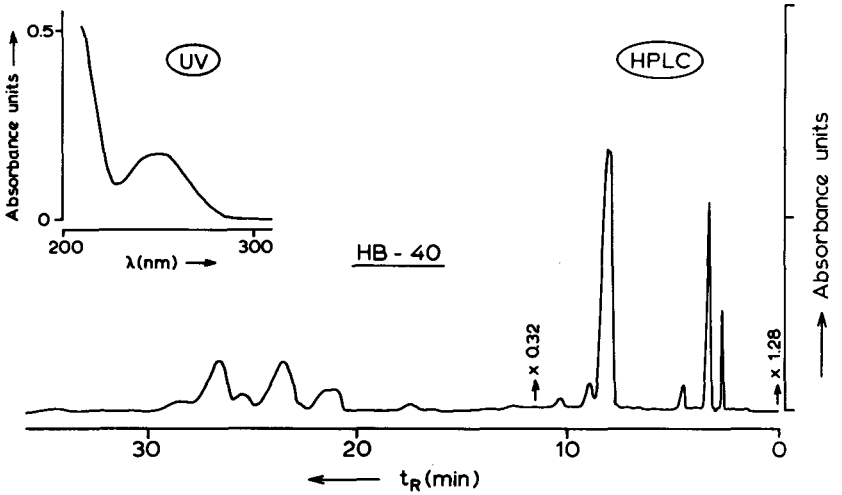

FIGURE 6 HPLC-UV (202 nm) chromatogram and UV absorption spectrum (insert) of the commercial hydrogenated terphenyl mixture HB-40. HPLC system: LiChrosorb SI 100 silica/dry hexane; flow-rate, 2,5 ml/min. UV spectrum: 5 μg/ml solution of HB-40 in hexane. On the basis of a molecular weight of 236, ε values of 23,500 (202 nm) and 7,500 (250 nm) were calculated.

chromatograms were run of HB-40 itself. In the latter two instances, temperature-programmed runs such as are normal in PCB analysis, were used.

GC-FID showed all of Fractions A and B, and at least a large part of Fraction C to co-elute with the more highly chlorinated Aroclor mixtures. As for GC-ECD, even when analyzing a 500 ppm solution of HB-40 in hexane, a completely uninterrupted baseline signal showed up. The combined results demonstrate that (1) even traces of PCBs are notably absent from the sample; (2) (hydrogenated) polyphenyls will elute completely unnoticed, i.e., without disturbing peak patterns, during GC-ECD of PCBs. GC/MS of Fraction A revealed the presence of three major constituents, all of which were terphenyls with two fully hydrogenated rings. The principal peaks observed with Fractions B and C could be assigned to the six isomeric terphenyls hydrogenated in a single ring. Isomers having a hydrogenated terminal ring showed less retention (Fraction B) in the HPLC system than did those having a hydrogenated central ring (Fraction C). Further, and relatively minor, constituents of Fractions B and C included two quaterphenyls, each with three fully hydrogenated rings (Fraction B), and *o*-terphenyl (Fraction C). Biphenyl, bicyclohexyl and phenylcyclohexane were shown to be virtually absent from HB-40 (Fraction A) by both HPLC and GC/MS.

Finally, Fractions A, B and C were subjected to perchlorination with $SbCl_5$ at 170–180°C for 15–18 h and analysed by means of capillary GC on a CP™Sil-5 column. In all instances DCB as well as tetradecachloroterphenyls were found to have been formed. In a typical experiment, processing of 98 μg of HB-40 yielded 8, 7 and 6 μg of DCB in Fractions A, B and C, respectively. Next to this total amount of 21 μg of DCB, 26 μg of perchlorinated terphenyls were found. This result unequivocally demonstrates the interfering role which (hydrogenated) polyphenyls can play in PCB analysis.

Dechlorination/HPLC

For obvious reasons the existence of an alternative to the pattern-comparison technique is desirable. We have therefore studied the use of dechlorination coupled with subsequent analysis by means of HPLC. A relatively simple procedure has been developed[5,31] in which all individual PCBs are converted into biphenyl via reduction with $LiAlH_4$ in the presence of a small amount of diethyl ether. Dechlorination can be carried out in the same reaction tubes as used for perchlorination, provided dodecane is used as solvent and all hexane present is evaporated prior to reaction. Reaction is complete (recovery, 90–100% for standard Aroclor mixtures) in 15 h at 170–180°C. The biphenyl formed is determined by means of HPLC in the system silica/dry hexane. The detection limit for UV detection at 248 or 205 nm is about 0.5 ng.

The dechlorination/HPLC technique has been applied to the analysis of PCBs for a selected number of the various types of samples studied in this paper. Typical chromatograms are shown in Fig. 7. According to expectation, the hydrogenated terphenyl peaks show up in the paper, but not in the eel sample. More importantly, a "suspected" peak is also present in the chromatogram of the heron liver sample selected which (cf. Table V) showed a relatively high PE/PC ratio of 3.9. The results summarized in Table IX confirm and extend those previously reported by our group.[5] That is, the dechlorination/HPLC technique invariably gives results which satisfactorily agree with those of pattern comparison. They thereby provide additional proof that the perchlorination/GC data indeed are incorrect. The range of values of the ratio dechlorination/pattern comparison (0.8–1.4) is one that may well be expected with such a comparison of techniques for different types of samples and PCB levels, the more so since with pattern comparison, a rather crude matching technique has been employed (cf. above), while in the case of dechlorination the results have been calculated on the basis of 100% recovery.

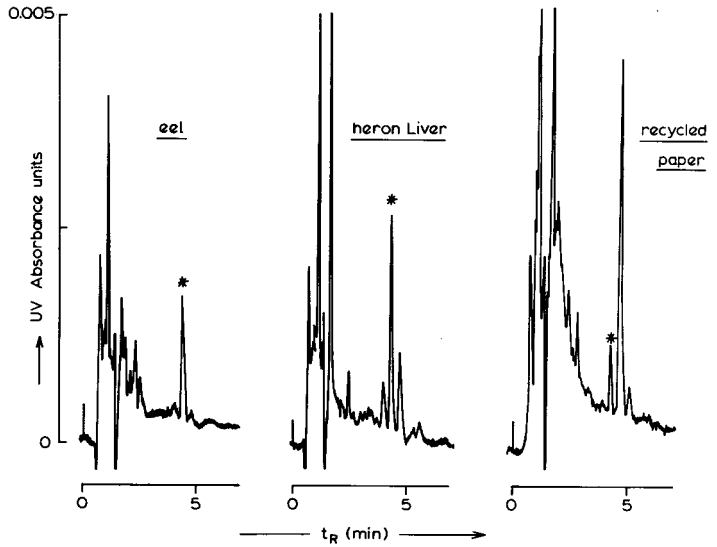

FIGURE 7 HPLC-UV (248 nm) chromatograms of dechlorinated PCB-containing extracts of an eel, a heron liver and a recycled paper sample. Asterisk denotes the biphenyl peak, HPLC system: LiChrosorb SI 60 silica/dry hexane; flow-rate 2.2 ml/min.

TABLE IX

Comparison of perchlorination/pattern comparison (PE/PC) ratios and dechlorination/pattern comparison (DE/PC) ratios for the determination of PCBs in various types of samples[a]

Sample		PE/PC	DE/PC
Toilet paper	I	2.0	0.8
	III	9.8	1.0
	IV	4.8	1.4
	V	3.0	0.8
Recycled paper	V	25.5	1.2
	VI	10.9	0.8
	VII	3.4	0.9
Fish	sprat	1.0	1.2
	eel	0.9	1.0
Heron	Sc-fat	1.9	0.8
	KI-fat	1.0	1.1
	KII-fat-1	1.3	1.0
	fat-2	1.4	1.3
	liver	1.0	0.8
	brain	15.0	1.3
	Do-fat	1.6	1.0
	liver	3.9	1.0

[a]Part of data taken from ref. 8.

CONCLUSION

Using samples of divergent nature and with varying PCB content—i.e., (recycled) paper and paper board (0.05–7 ppm), printing inks and varnishing pastes (<0.1 ppm), and sewage sludge (1.5–12 ppm)—it has been demonstrated that determination of PCBs via perchlorination/GC often yields grossly inaccurate, i.e., too high, results. That is, DCB contents calculated on the basis of this method typically are 2–30-fold higher than those calculated on the basis of the well established pattern-comparison technique. For selected samples, the absence of impurities from the DCB peak obtained after perchlorination has been demonstrated by means of GC/MS. Besides, in several instances the occurrence of high PE/PC ratios has been confirmed in interlaboratory studies. With samples of a biological nature—i.e., heron (2–450 ppm) and fish (0.4–3.3 ppm) samples—the two analytical procedures generally yield satisfactorily agreeing results; however, exceptions have been noted, with PE/PC ratios of up to 15. Good agreement between perchlorination and pattern-comparison data for biological samples has also been reported for fresh chub and sturgeon, eggs and chicken fat (PE/PC ratio, 0.95–1.3; ref. 6), and human milk (PE/PC ratio, 0.7–2.0; refs. 32 and 33).

The combined evidence presented in the above paragraph forces one to conclude that, for many types of sample, perchlorination with $SbCl_5$ is not a reliable technique of PCB analysis. This statement has been fully confirmed during dechlorination experiments performed with various types of samples. These showed that dechlorination/HPLC and pattern-comparison/GC invariably yield closely analogous results with dechlorination/pattern comparison ratios of between 0.8 and 1.4.

As for the nature of the compounds interfering in the perchlorination procedure, a study on the behaviour, under perchlorination conditions, of hydroxylated PCBs, PCNs, PCTs, PBBs, substituted benzenes, organochlorine pesticides, etc. has demonstrated that occasionally small amounts of DCB may originate from the partial conversion of some of these compounds. Significant concentration levels of, e.g., biphenyl and PBBs (cf. Table VI) have, however, been found to be absent from all samples selected for such a study. It seems safe to state, therefore, that these compounds certainly cannot be thought to be the sole, or even main, interferences during perchlorination of PCBs. This is the more true if one realises that in order to explain PE/PC ratios of 10 on the basis of the presence of an interfering compound with a conversion efficiency (into DCB) of some 10%, the concentration level of the said compound has to be about 100-fold higher than that of the PCBs.

With selected paper samples, the combined use of HPLC and GC/MS

has led to the isolation and identification of a series of hydrogenated terphenyls which, upon perchlorination, yield substantial amounts of DCB and, thus, may be stated to be the main interfering compounds with such-like samples. As a confirmation, perchlorination of the commercial product HB-40—which principally consists of hydrogenated terphenyls and does not contain either PCBs or biphenyl—has been shown to cause the formation of a high proportion of DCB. Here, it should be emphasized that these results do not provide any evidence that the high PE/PC ratios obtained with paper (and other) samples are caused by the actual presence of HB-40.

Finally, as regards the determination of PCBs in environmental samples, it is evident that pattern comparison via (capillary) GC is the preferred method of analysis, especially if modified into the powerful individual-peak response method and/or combined with GC/MS. As for an independent check method, dechlorination/HPLC seems to be a promising alternative[5] to perchlorination/GC, sharing its advantages such as speed, ease of reaction, rapid quantitation and elimination of separation problems, but not the fatal drawback of frequently yielding highly inaccurate results. The sensitivity of dechlorination/HPLC is, however, about 10-fold lower than that of perchlorination/GC. It may well be expected, therefore, that attempts will be made to rescue perchlorination from oblivion. To our opinion, in such work modification of the clean-up procedure to eliminate compounds interfering in the perchlorination step will be the main point of interest. Preliminary studies on the use of a $KMnO_4/H_2SO_4$ treatment have met with some success. For 10 sewage sludge samples PE/PC ratios of 2.1–4.6 were found to decrease to 0.9–1.2 upon introducing the oxidation step. In all but one case, the PCBs themselves survived the oxidation essentially unchanged, with recoveries (measured by pattern comparison) of 70–100%.

Acknowledgements

We thank L. G. M. T. Tuinstra (State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands) and R. C. C. Wegman (National Institute of Public Health, Bilthoven, The Netherlands) for their kind cooperation during the collaborative studies, and M. A. T. Kerkhoff (National Institute for Fishery Research, IJmuiden, The Netherlands) for providing us with the fish samples, and R. M. Kok (Academic Hospital, Free University) for providing GC/MS facilities.

The help of H. Ent in optimization studies of the clean-up procedure is gratefully acknowledged.

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