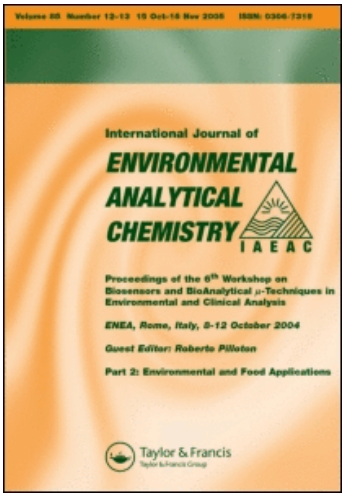

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An Evaluation of Chromatographic Methods for the Analysis of Polychlorinated Terphenyls in Environmental Samples

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Gas chromatography (GC) with packed and capillary columns, high-performance liquid chromatography (HPLC) and thin-layer chromatography have been used to characterize various commercially available mixtures of polychlorinated terphenyls (PCTs). Besides, UV absorption and mass spectrometry data have been collected, and the behaviour of PCTs upon perchlorination to the tetradecachloroterphenyls has been studied.

Using GC, the PCT content of a number of paper and sewage sludge samples has been determined. The application of perchlorination/GC as method of analysis often yields unreliable, *i.e.*, too high, results compared with the direct GC pattern-comparison method. In the case of paper samples, hydrogenated terphenyls appear to be the principal interfering compounds.

INTRODUCTION

Polychlorinated biphenyls (PCBs) and polychlorinated terphenyls (PCTs), which have been known for nearly a century share certain physical properties such as high heat capacity, chemical stability and excellent electrical properties. These make–or made–them highly desirable for a number of industrial uses.[1] Application of PCTs has, however, always been much more limited than that of PCBs.

Compared with PCBs, PCTs have received relatively little attention in the literature. They are, to our knowledge, the chief subject of only a single report,[2] published in 1977 and are summarily dealt with in a number of others.[3,4] It seems advisable, therefore, to open the present paper with a short overview on commercial PCT mixtures, their analysis and the determination in real samples. Next, the paper discusses the merits

of various chromatographic methods of analysis inclusive of the perchlorination of PCTs to the isomeric tetradecachloroterphenyls (TDCTs) with subsequent determination by means of gas chromatography (GC). Here special attention is devoted to any degradation of PCTs (into PCBs), which may adversely affect the determination of PCBs via perchlorination/GC. Finally, the preferred analytical procedures are applied to the determination of PCTs in several types of samples.

PERCHLORINATED TERPHENYLS

Since 1929, mixtures of chlorinated terphenyls have been commercially manufactured in several countries; relevant information is presented in Table I. Because, in the early seventies, concern arose over the environmental effects of the chemically similar PCBs, and because the uses

TABLE I

Summary of commercially manufactured PCT mixtures

Manufacturer		Trade-name
Monsanto	U.S.	Aroclor 5432, 5442 and 5460
Kanegafuchi	Japan	Kanechlor C
Mitsubishi–Monsanto	Japan	Aroclor series
Bayer	G.F.R.	Leromoll 112-90 and 141 Clophen-Harz W
Caffaro	Italy	Cloresil A, B and 100
Prodelec	France	Phenoclor Electrophenyl T-60
?	France	Terphenyl Chlore T60

of PCTs were mainly dispersive, production of all PCT mixtures mentioned in the table was terminated in the period 1970–1980. Information on production data of PCTs is very scarce. It is known that Monsanto produced about 115 million pounds of PCTs in the period 1959–1972 and that, in 1971–1972, the production of Aroclor 5460 was about three times as high as that of Aroclor 5432. The total domestic production of PCTs in Japan (from 1954 to 1972) was about 6 million pounds. No production or sales data have been published by any of the European manufacturers. For the reader, it may be interesting to know–as a means of comparison–that, in the period 1959–1972, Monsanto's PCB production amounted to some 760 million pounds, and that the domestic 1959–1972 production of PCBs in Japan was 130 million pounds.

For general information on the properties and usage of PCTs, the reader is referred to refs. 1 and 2. As for individual PCTs, the synthesis of 21 chlorinated *p*-terphenyls and a single chlorinated *o*-terphenyl has been reported by Chittim *et al.*,[5] who also record melting points, UV spectral data and chromatographic characteristics, and have subsequently studied their photodegradation.[6,7] Relevant data on the isomeric TDCTs are discussed in the pertinent section below.

The analytical techniques used for the determination of PCTs and PCT residues are quite similar to those for PCBs. That is, GC with electron-capture detection (GC-EC) has been used almost exclusively. (A single paper reports on the application of TLC.[8]) However, as expected, the retention times in GC are much longer for the PCTs than for the PCBs, and this may well have caused early workers to overlook the presence of PCTs in PCB-containing samples. Rather surprisingly, up till now GC has almost always been done on packed columns, using a wide variety of stationary phases, and capillary GC has hardly been considered. PCT levels are usually determined by means of pattern comparison with Aroclor 5460 or, in a single case,[9] Clophen Harz W as standard. In papers by Japanese authors, Kanechlor C–which has a degree of chlorination similar to that of Aroclor 5460 (cf. refs. 10 and 11)–is used. Perchlorination of all individual PCTs to the fully chlorinated TDCTs–with subsequent analysis of GC–was first reported by Hutzinger and coworkers[12,13] and has since been discussed as a convenient method of analysis of PCT-containing samples by several groups of workers[14–16] (also see Table II below). Dechlorination of PCTs has occasionally been proposed[17–19] as an alternative to perchlorination and, recently, it has been applied[20] for the first time to an environmental sample. GC/MS has been carried out with Aroclor PCT mixtures[21–23] and TDCTs[12,13] only; relevant results will be included in the discussion below. Rote and Harris[24] have calculated the theoretical probability of the occurrence of ions of different masses in the molecular ion cluster for the PCTs, using isotopic abundance ratios.

Table II presents a literature survey of studies dealing with the analysis of PCT-containing environmental samples. PCT levels are seen to vary widely dependent on parameters such as sample type and sampling site. Relatively high concentrations occur in human and animal fat tissue, and in paper (board) and food packaging materials. In samples taken from aquatic environments (water, sludge, waterfowl, fish) in Japan[41] PCT levels are, as a rule, negligibly low. Generally, PCT levels are distinctly lower than are PCB levels. As an exception, Doguchi and Fukano[10] found PCT levels (av., 5 ppb) in human blood of 27 volunteers to be almost invariably higher than the corresponding PCB levels (av., 3.2 ppb). Since

TABLE II

Literature survey on the analysis of PCT-containing samples

Sample type	Conc. (range or average) in ppm	No. of samples	Refs.
Eggs/fat of herring gulls	1.4/0.1		25/26*
Eggs/fat of cormorants	n.d/n.d		25/26*
Food packaging materials	<1.0, 84.5%; 1–10, 11.4%; >10, 4.1%	445	27*
Foods	<0.01, 94.5%; 0.01–0.05, 5.5%	73	27*
Cheese	n.d.	40	28*
Paperboard	0–163	100	29*
Silo wall scrapings, silage and milk	qualitatively identified		30*
Water	0.07		9
Oysters/eel	0.12/0.4		9
Human fat	0–0.8	4	9
Human fat	0–4.61	99	31
Mother's milk	0–0.007	10	31
Fish	n.d.	29	31
Food/water/sludge	n.d./n.d./n.d.	10/9/14	31
Food wrappers	?		8*
Influent/effluent/activated sludge from sewage plants	0–0.26/n.d./0.09–0.23		32
Human fat	0.1–2.1	20	33*
Human fat	0.03–1.9	70	34
Mother's milk	0–0.001	11	34
Human blood	0.0007–0.0196	27	10
Soil	0–13	45	35
Cooling/drainage/sanitary sewer water	<0.1/<0.1/0–7.5	1/2/3	35
Ditch sediment/sewage sludge	5.1–6.7/5.0	2/1	35
Human fat	0.04–9.20	30	36*
Human blood	0.0011–0.0094	10	36*
Bird livers	0–1.2	71	37*
Dogs/cats	0.05–8.9/0.04–0.14	6/3	39
Fish/wild birds	n.d./0–2.2	156/35	39
Food products	0–0.01	110	39
Ink/plastic/paper/paint	n.d./n.d./0–0.2/n.d.	35/5/7/3	39
Soil	n.d.	3	39
Wrapping material	0–135	129	39
5 other sample types	0–1.0 (two 61 and 500)	44	39
Sludge	0–0.217		40
Fish	0–0.014	107	41
Food (diet), average daily intake	0.05 μg		38
Wild dog tissue	0.034 (female), 0.62 (male)		42
Snake tissue	0.009		42
Oils/lubricants/plastics/paper	n.d.	45	43
Paints/varnish/waxes/etc.	n.d.	55	43
Garbage samples	0.05–10.5	21	43
Fat/flesh of fish	0.05–0.90/0.005–0.80		44*

TABLE II cont.

Sample type	Conc. (range or average) in ppm	No. of samples	Refs.
White-tailed eagle and grey seal	2.8–17.2/0.50–1.0 (as Σ TDCT)	3/3	45*
River water/sediment	n.d./0–3.90 (terphenyls)		46
Pooled samples of human fat	qualitatively identified	2	47
Mother's milk	n.d.	100	48
Sediment	PCTs detected, conc.?		49
Human adipose	0.89–1.3	17	11
Liver, milk and blood	0.0001–0.162	6/24/24	11
Children's and mother's blood	0–0.025	17/16	11
Fetus skin, liver and fat	0–0.050	12/14/4	11
Pigeon fat	0–0.7		50
Cow liver	0.17 and 0.06 (averages)		50

*In these papers perchlorination was applied next to the GC pattern-comparison method for confirmation of the presence of PCTs. In refs. 37 and 45 perchlorination was used as the only quantitation method.

the amount of PCTs present in food[31] generally is very low, the authors conclude[36] that PCTs either have an extremely long period of biological half life or mainly accumulate in the human body via routes other than ingestion of PCT residues in food. Lastly, it is interesting to note that only one group of workers has reported[45] the detection of low-chlorinated PCTs in the environment. Here, one should realise that in GC low-chlorinated PCTs elute in the same retention-time range as do PCBs. In other words, if no PCT-PCB pre-separation is carried out these PCTs will as a rule be obscured by the PCB peak pattern.

For data on the toxicological effects of PCTs, the reader is referred to the review papers in ref. 1.

EXPERIMENTAL

Chemicals

The commercial PCT mixtures Aroclor 5432, 5442 and 5460 (formerly produced by Monsanto, St. Louis, MO., U.S.A.) and the ortho, meta and para isomers of terphenyl and tetradecachloroterphenyl were purchased from Analabs (North Haven, CO., U.S.A.). Bayer (Leverkusen, G.F.R.) supplied the PCT mixtures Leromoll 141 and 112-90 and Clophen-Harz W; Cloresil 100 was a gift from Caffaro (Milan, Italy). Biphenyl and decachlorobiphenyl (DCB) were obtained from Aldrich (Beerse, Belgium) and hexachlorobenzene from Riedel-De Haen (Seelze-Hannover, G.F.R.). The hydrogenated polyphenyl mixture HB-40 was supplied by Monsanto.

For perchlorination reactions antimony pentachloride "for synthesis" from Merck (Darmstadt, G.F.R.) and tetrachloromethane (analytical-grade

quality) from Baker (Deventer, the Netherlands) was used. For dechlorination reactions lithium aluminium hydride and anhydrous diethyl ether from Baker, and *n*-dodecane "for synthesis" from Merck were used. Diethyl ether was redistilled over $LiAlH_4$ and stored on molecular sieve 5A.

Nanograde and ChromAR hexane from Mallinckrodt (St. Louis, MO., U.S.A.) were used for GC and HPLC analyses, respectively. Hexane was recycled after use by distillation over a 45% sodium dispersion in paraffin (Fluka, Buchs, Switzerland).

All other materials used in the GC and HPLC analyses of PCTs and PCT-containing samples are the same as those mentioned before in studies on PCBs, and are reported in ref. 51. Details on thin-layer chromatography (TLC) are given in the next section.

Apparatus

Packed column GC was performed on a Packard Becker (Delft, the Netherlands) Model 419 gas chromatograph equipped with a ^{63}Ni electron capture (EC) and a flame-ionisation detector. The glass column (2.5 m × 2 mm I.D.) was packed with 4% OV-101 on Chromosorb W HP (80–100 mesh). For PCT pattern analyses the oven temperature was initially held at 200°C for 5 min and then linearly programmed to 310°C at a rate of 5°C/min. Analyses of perchlorinated reaction mixtures were done isocratically at 300°C.

Capillary GC was carried out on a Packard Becker Model 427 gas chromatograph equipped with a ^{63}Ni EC detector and an all-glass solid injector (moving needle). The fused silica WCOT column (25 m × 0.25 mm I.D.) was coated with CP™Sil-5 (film thickness, 0.13 μm). For pattern analysis the oven temperature was linearly programmed from 150 to 325°C at a rate of 6°C/min, and for measurement of perchlorinated mixtures from 200 to 325°C, at a rate of 10°C/min.

For both GC instruments the injector and detector temperature was held at 300 and 320°C, respectively. Nitrogen was used as carrier gas at a flow-rate of 30 ml/min (packed column) or 1 ml/min (capillary column). The nitrogen purge-gas flow-rate was 30 ml/min in both cases. Injection volumes were 1–5 μl.

Gas chromatography/mass spectrometry (GC/MS) was done on a Finnigan (Sunnyvale, CA., U.S.A.) 4000 series quadrupole GC/MS system interfaced with an INCOS 2000-series data system with electron impact ionization at 70 eV and an ion-source temperature of 250°C. The GC conditions were described as above.

The HPLC system consisted of an Orlita (Giessen, G.F.R.) Model FE 034 sRC reciprocating pump, a Rheodyne (Cotati, CA., U.S.A.) Model 7125 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, CA., U.S.A.) silica, and a Pye–Unicam (Philips, Eindhoven, the Netherlands) Model LC 3 variable-wavelength UV detector. Hexane dried on molecular sieve 5A was used as mobile phase. All chromatograms were run at ambient temperature.

Straight-phase TLC was carried out on activated pre-coated silica gel plates (Merck) using dry ChromAR hexane as mobile phase. Development was done in a sandwich chamber, and detection was effected under UV-light. Reversed-phase TLC was carried out on Kieselguhr G impregnated with paraffin oil. Development, with solvent mixtures of varying composition, was done in a saturated chamber. Spots were revealed by spraying with an acidified solution of tolidine in ethanol–water, subsequent irradiation under UV-light. For a more detailed description of the procedure, see ref. 52.

Methods

Sample preparation of PCT-containing paper and sewage sludge samples included treatment with a 2% ethanolic NaOH solution plus extraction with hexane, or direct soxhlet extraction with hexane, and subsequent clean-up over a 5% deactivated aluminium oxide column. The procedure was identical to that for PCB-containing samples, and is extensively reported in ref. 51. For the detailed description of perchlorination with $SbCl_5$, and dechlorination with $LiAlH_4$–both carried out overnight at 170–180°C–the reader is referred to refs. 51 and 20, respectively.

RESULTS AND DISCUSSION

Chromatography of PCT mixtures

Aroclor series. As was observed in the review section of this paper (capillary) GC, with EC detection, is the preferred method of analysis for the complex commercial PCT mixtures. The highly thermostable and apolar CP^{tm}–Sil-5 turned out to be especially suitable as stationary phase, because temperature-programmed GC has to be carried out up to 325°C in order to elute the highest boiling PCT isomers.

Fig. 1 shows GC chromatograms of the Aroclor 5400 series. From the GC/MS data included in the figure it is evident that retention of the individual PCT isomers tends to increase with increasing degree of chlorination. The dependence of retention time on chlorine content is rather similar to that observed for PCBs, and is less straightforward than that found with polychlorinated naphthalenes.[53] All three chromatograms display many tens of peaks and shoulders and, from the observation that baseline separation hardly occurs even with the highly efficient capillary GC system used here, one concludes that the number of PCT constituents per mixture may well be over one hundred.

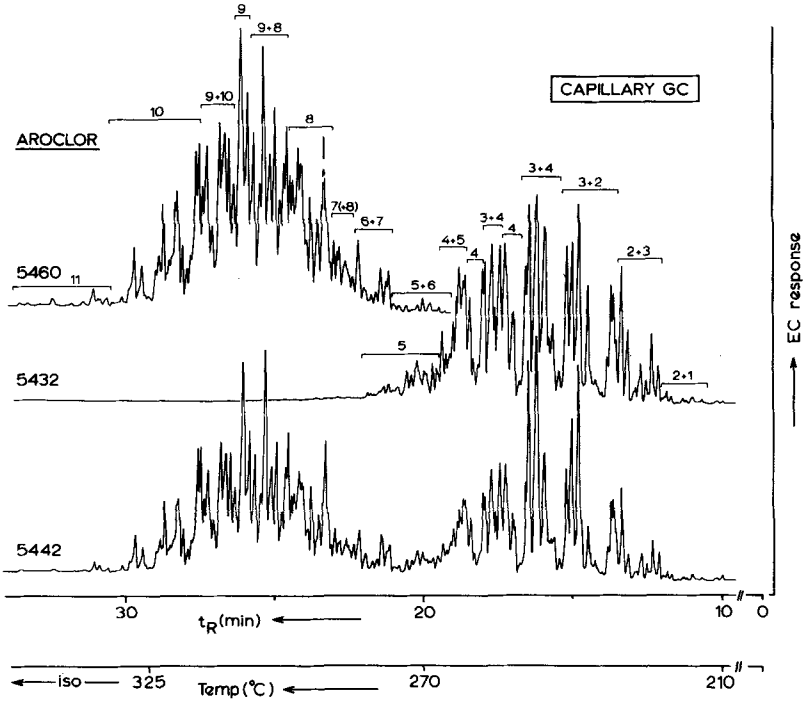

FIGURE 1 Capillary GC-EC chromatograms of the commercial PCT mixtures Aroclor 5432, 5442 and 5460. For experimental details, see text. Numbers above the peaks designate the number of chlorine substituents as derived from GC/MS, the first number indicating the dominating isomer.

Close inspection of the chromatograms reveals a striking similarity between the peak patterns of Aroclor 5432 and the early eluting (t_R, 10–20 min) peaks of Aroclor 5442, and between the late eluting (t_R, 20–30 min) peaks of the latter mixture and the peak pattern of Aroclor 5460. This

suggests that Aroclor 5442, rather than being produced by using a time of reaction intermediate between that for Aroclor 5432 and 5460, is a composite mixture of the latter two Aroclors. The mixing ratio–deduced either from the chlorine contents provided by the manufacturer or from peak area measurements–probably is Aroclor 5432:Aroclor 5460 = 1.5–2:1.† For the test, Aroclor 5432 and 5460 are seen to be mainly composed of PCTs having 2–5 and 6–10 chlorine substituents, respectively. For Aroclor 5460, this result is in agreement with literature data,[21,23] which state its main constituents to be hepta- through deca- (or undecyl-) substituted PCTs. It clearly contradicts, however, an earlier observation[22] on Aroclor 5442, said to be mainly composed of PCTs substituted with no more than 6 chlorine atoms. GC/MS data on Aroclor 5432 have not been reported in the literature.

HPLC in the system silica/dry hexane has repeatedly been shown to be a convenient method to characterize, e.g., commercially available PCB[52,54] or polychlorinated naphthalene[55] mixtures, even though the separation efficiency of such a system is considerably less than that of capillary GC. In the adsorption HPLC system used, PCTs display a distinctly higher retention than do PCBs owing to the presence of an additional phenyl ring. For the rest, retention decreases with an increasing number of chlorine substituents on the aromatic nucleus, as has also been observed with other classes of halogenated aromatic compounds. As a consequence, only the more highly chlorinated PCTs, i.e., the majority of those present in Aroclor 5460, elute in the same retention-time range as do the PCBs. As an example, the chromatogram of Aroclor 5442 is given in Fig. 2; the arrow indicates the position of the last-eluting major peak of the (relatively low-chlorinated) PCB mixture Aroclor 1242. Comparison with the chromatograms of Aroclor 5432 and 5460, which are not shown here, revealed that fractions *a* and *c* (cf. Fig. 2) comprise peaks present in Aroclor 5460 and Aroclor 5432, respectively. The peaks of fraction *b* show up in the chromatograms of both mixtures. These results were fully confirmed by analysis of the several fractions[5] via GC.

Finally, in order to demonstrate the merits which TLC has for the analysis of even complex mixtures, straight-phase TLC, on silica, and reversed-phase TLC, on paraffin oil-impregnated kieselguhr, were carried out as done previously for PCBs[52] and polychlorinated naphthalenes.[55] On activated silica, and with dry hexane as mobile phase, 4–6 bands showed up for each of the Aroclor mixtures (Fig. 3A). According to

†When comparing GC-EC chromatograms of the various PCT mixtures, one should keep in mind that the more highly substituted mixtures generally will display a higher sensitivity towards the EC detector than do the less chlorinated ones.

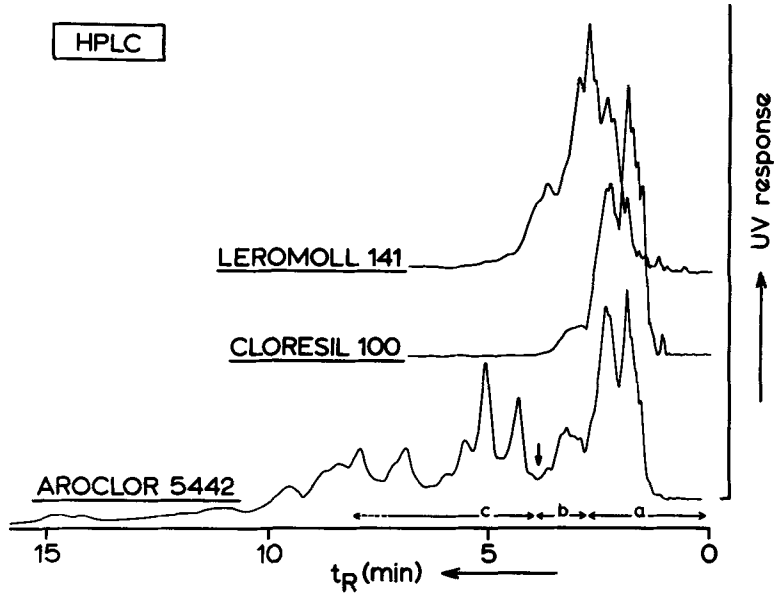

FIGURE 2 Adsorption LC chromatograms of the commercial PCT mixtures Aroclor 5442, Cloresil 100 and Leromoll 141. LC system: LiChrosorb SI 60/dry hexane; UV detection at 207 nm; flow-rate, 2.5 ml/min. Fractions used in fractionation studies for GC and TLC are denoted by *a*, *b* and *c* (also see text). The arrow designates the position the last eluting PCB isomer would have under the experimental conditions used. For details, see text.

expectation, the results are closely analogous to those obtained in adsorption HPLC. With appropriately selected mobile phases, i.e., acetonitrile–methanol–water (8:9:3) for Aroclor 5432 and 5442, and acetonitrile–methanol–acetone–water (20:20:9:1) for Aroclor 5460, more efficient separations (6–9 bands per mixture; Fig. 3B) were achieved in reversed-phase TLC. Analysis of the various zones by GC and HPLC led to the definite assignment of fractions *a*, *b* and *c* (cf. Fig. 2) although now, of course, the order of elution was reversed as compared to that obtained in the adsorption system. So far, no attempts have been made to further improve the separation of the Aroclor PCT mixtures by using either reversed-phase TLC on apolar chemically bonded stationary phases, or reversed-phase HPLC.

Other PCT mixtures. Leromoll 141 and 112-90, Clophen-Harz W, and Cloresil 100 were analyzed by capillary GC and adsorption HPLC, which yielded similar conclusions. Two representative GC chromatograms, and GC/MS data, are shown in Fig. 4. The PCTs present in both Leromolls and in Clophen-Harz W possess virtually the same degree of chlorination, which is between that of Aroclor 5442 and 5460 (cf. Fig. 1), and may be

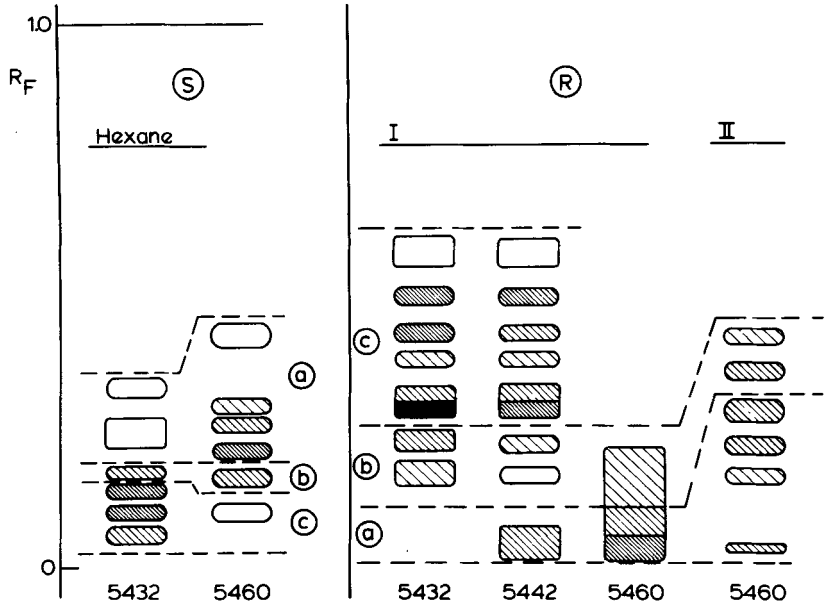

FIGURE 3 TLC of Aroclor PCT mixtures. Straight phase (S): silica/*n*-hexane; reversed phase (R): Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–acetone–water, 8:9:0:3 (I) or 20:20:9:11 (II). For details, see ref. 52. Fractions *a*, *b* and *c* correspond with those in HPLC (see Fig. 2 and text).

estimated to be about 50%. For all three mixtures, GC/MS reveals the presence of, predominantly, PCTs having 6–8 chlorine substituents. It should be added that it is known[1] that the Leromoll products were based on a mixture of substituted aromatic and aliphatic hydrocarbons, with chlorine contents of 36 and 11 wt% for Leromoll 112 and 141, respectively. No data are known on the PCT content of these mixtures. On the basis of the above results PCT levels can be concluded to have been relatively low, and cannot have been more than, e.g., about 20 wt% for Leromoll 141.

Cloresil 100 which, according to the manufacturer, has a chlorine content of 50–60 wt%, showed chromatographic peak patterns rather similar to those of Aroclor 5460, with the main constituents having 8–10 chlorine atoms. However, as is evident when comparing Figs. 1 and 4, the relative intensity of the peaks due to the more highly chlorinated PCTs is higher in the Caffaro as compared with the Monsanto product. Since elemental analysis indicated a chlorine content of 59 wt% (C, 39.60%; H, 0.91%; Cl, 58.97%) Cloresil 100 possibly contains, next to the PCTs, a few wt% of additives.

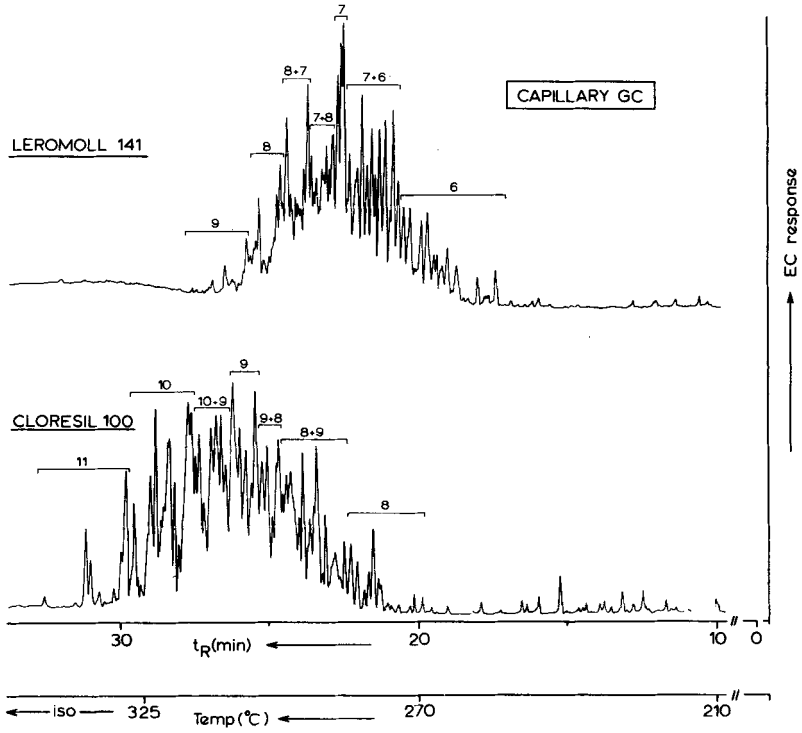

FIGURE 4 Capillary GC-EC chromatograms of the commercial PCT mixtures Leromoll 141 and Cloresil 100. For experimental details, see text. Numbers above the peaks designate the numbers of chlorine substituents as derived from GC/MS, the first number indicating the dominating isomer.

UV spectrometry of PCT mixtures

The UV absorption spectra of all seven PCT mixtures studied are rather similar, with the maximum absorption of the so-called main band invariably occurring in the region 200–210 nm. With Aroclor 5432, a distinct κ band also shows up with a maximum at 245 nm. With increasing substitution of the aromatic nucleus this κ band shifts to shorter wavelengths–a phenomenon well known from spectral data on PCBs and polybrominated biphenyls–and is completely lost in the main band in the case of highly chlorinated mixtures such as Aroclor 5460 and Cloresil 100. Pertinent data are summarized in Table III.

From the data on the Aroclors and Cloresil, it is evident that molar extinction increases with increasing chlorine content. As for the Bayer products the ε_{max} value of Clophen-Harz W suggests this product to be a

fairly "pure" mixture of only PCTs. The low extinction values recorded for the Leromolls agree with the data reported in the previous section, if it is assumed that the non-PCT constituents present in these mixtures do not display appreciable UV absorption in the 200–210 nm region and are not too highly chlorinated. To quote an example, the presence of a PCT mixture with a chlorination degree of about 50 wt% in Leromoll 112-90—which contains 36 wt% of chlorine—indicates a PCT content of at most some 70%. The ε_{max} value of 41,000 found for this Leromoll, on the other hand, is about 70% of that typical for a 50 wt% chlorinated PCT mixture (60,000–65,000).

TABLE III

UV absorption characteristics of commercial PCT mixtures

PCT mixture	λ_{max} (nm)	$\varepsilon_{max} \cdot 10^{-3}$ (1/mole·cm)	PCT mixture	λ_{max} (nm)	$\varepsilon_{max} \cdot 10^{-3}$ (1/mole·cm)
Aroclor 5432	207	46	Leromoll 141	203–207	18
5442	208	57	112-90	200–209	41
5460	208	78	Clophen-Harz W	201–209	61
Cloresil 100	208	76			

Terphenyls and tetradecachloroterphenyls

UV spectrometry, and characteristic GC and adsorption HPLC retention-time data of the isomeric parent terphenyls and the TDCTs are summarized in Table IV.

The UV spectra of the terphenyls all display a main band with a maximum at around 206 nm and a κ band in the region 225–300 nm. The position of the κ band is strongly dependent on the position of the third phenyl ring, with a considerable shift towards the visible region occurring upon going from *o*- via *m*- to *p*-terphenyl. The UV spectra of the three isomeric TDCTs are rather similar, showing a single broad band between about 200 and 270 nm, which features a maximum at 212–214 nm and several more or less well defined shoulders at higher wavelengths. The marked influence of chlorine substitution on the molar extinction coefficient of the main band is clearly manifest from the data in Table IV (terphenyls: ε_{max}, 50,000; TDCTs: ε_{max}, 120,000). As is to be expected, no separate κ band shows up in the UV spectra of the TDCTs. The UV spectra of the parent terphenyls are in good agreement with literature data. Their characteristic nature has been used[20] to advantage in the analysis of PCT-containing samples via dechlorination (cf. below). UV spectral data of TCDTs have previously been published by Hutzinger *et al.*.[13] They should be regarded with some caution because (1) the ε_{max}

TABLE IV

Characteristics or terphenyls and TDCTs. HPLC: silica/dry hexane; column I: 25 cm × 3 mm I.D., 5 μm LiChrosorb SI 60 (Merck); column II: 25 cm × 4.6 mm I.D., 5 μm LiChrosorb SI 100 (Brownlee); flow-rate: 1.4(I) and 2.5(II) ml/min. GC: CP™-Sil-5: temperature from 200 to 325°C at 10°C/min; 4% OV-101: temperature, 300°C isotherm.

		t_R(min) in			
		HPLC		GC	
	λ_{max} in nm ($\varepsilon.10^{-3}$ in l/mole.cm)	Column I	Column II	CP™-Sil-5	4% OV-101
o-Terphenyl	207(40); 232(28)	31.2	26.4	—	—
m-	205(46); 245(39)	22.9	21.2	—	—
p-	205(56); 274(32)	23.8	23.2	—	—
o-TDCT	212(112)	2.68	—	14.7	12.2
m-	214(126)	2.03	—	17.1	19.1
p-	214(123)	2.23	—	16.4	17.2

values are unrealistically high with values of between 10^6 and 10^8, and (2) the position of some of the absorption maxima is quite different from what is to be expected for highly substituted linearly condensed chloroaromatic hydrocarbons.

As for chromatography, the TDCTs can easily be separated both on packed and capillary GC columns. The detection limits in GC-EC were found to be about 0.2 pg, with relative EC responses of 1.0:0.45:0.50 for ortho:meta:para. For the rest one notes that *p*-TDCT elutes between the ortho and meta isomer, with the relative retention $\alpha_{o/p} > \alpha_{p/m}$. The reversed order of elution–with, however, again, $\alpha_{o/p} > \alpha_{p/m}$–was obtained for both the TDCTs and the parent terphenyls during HPLC in the system silica/dry hexane. Here, detection limits for the TDCTs are around 0.5 ng, while those for the terphenyls are 2–5 ng. The latter, relatively high, values are partly caused by the unfavourably high retention times of 20–30 min (which are seen to be slightly dependent upon the type of silica HPLC column used). The situation can be improved by adding a small proportion of an organic modifier to the mobile phase; this will decrease retention but, unfortunately, also resolution. Besides, such a modification would make it impossible to use a single adsorption HPLC system for the determination of the terphenyls, the (much) earlier eluting PCT (and PCB) mixtures, and the TDCTs.

Perchlorination of PCT mixtures

Perchlorination of PCB and PCT mixtures has repeatedly been propagated as a means to simplify the analysis of environmental samples and to improve sensitivity. In a recent paper,[51] the severe limitations in the use of perchlorination for the quantitative analysis of PCBs have been discussed in detail. As an extension of this work, perchlorination of PCT mixtures has now been shortly investigated. Special attention was given to the amount of DCB formed during reaction, since appreciable decomposition of PCTs to PCBs under high-temperature conditions, plus additional perchlorination of the PCBs so formed, might partly explain the grossly inaccurate, i.e., too high, results of PCB analyses carried out via perchlorination/GC.

Using standard reaction conditions (reagent, $SbCl_5$; time, 17 h; temperature, 170–180°C), the mean recovery of Aroclor 5432, 5442 and 5460 after perchlorination was 90, 85, and 95%, respectively ($n=3$; rel. S.D., $\pm 5\%$). The high yield obtained for Aroclor 5460–the PCT mixture almost invariably detected in environmental samples–means that no correction factor for incomplete recovery has to be applied when studying real samples. The relative peak heights of the three TDCTs after perchlorination were found to be closely similar for the various mixtures tested, viz. ortho:meta:para = 0.12:1.0:0.45, 0.13:1.0:0.46 and 0.09:1.0:0.44 for Aroclor 5432, 5442 and 5460, respectively. Obviously, an identical mixture of the parent terphenyls was used as feedstock in the production of all Aroclor PCT mixtures. This statement, however, does not necessarily imply that the feedstock indeed had a composition ortho:meta:para = 0.1:1.0:0.45, since preliminary experiments have revealed that perchlorination of each of the pure terphenyls caused the formation of–next to typically 85–95% of the corresponding TDCT and 0–3% of DCB–5–15% of the two isomeric TDCTs. These results pertain to analytical-scale perchlorination of the parent terphenyls and, therefore, cannot be said to yield reliable values either for perchlorination of the chlorinated terphenyls present in PCT mixtures, or for the industrial-scale production of these mixtures from the terphenyls. They merely serve as a warning for those trying to interpret the above data.

As regards the *o*-TDCT:*m*-TDCT:*p*-TDCT ratios mentioned above, it is interesting to note that in experiments on dechlorination of Aroclor 5460 with $LiAlH_4$–in which the recovery, admittedly, was only about 50%–very similar results were obtained for the parent terphenyls, viz. ortho:meta:para = 0.1–0.2:1.0:0.4–0.5. As for the literature data on the TDCTs, ratios rather similar to those recorded by us can be read from a paper by Doguchi and co-workers,[33] while Hassell and Holmes,[37] and Jan *et al.*[14,15] find much higher values for *o*-TDCT.

The percentage conversion of the Aroclor PCT mixtures into DCB was found to be strongly dependent on the temperature. For, e.g., Aroclor 5442, values of less than 0.1%, 1.2% and 7.5% were obtained at temperatures of 120, 170 and 240°C, respectively. Using the standard temperature of 170°C, the influence of the time of reaction was found to be fairly small (Table V). Results for Aroclor 5460 were similar to those for Aroclor 5442.

TABLE V

Dependence of percentage conversion of Aroclor 5442 into DCB on perchlorination reaction time[a]

Time (h):	2	6	17	66
% DCB found:	0.7–1.1	0.5–0.8	1.1–1.2	1.3–3.2

[a]Temperature, 170°C; results calculated as % of Aroclor PCTs converted into DCB; $n=4$; final analysis by GC.

A HPLC chromatogram of a perchlorinated Aroclor 5442 mixture is shown in Fig. 5. It is interesting to note that, next to the small peak of DCB, an early eluting peak due to hexachlorobenzene occurs. The formation of hexachlorobenzene may well be explained by the reaction PCTs→PCBs+chlorinated benzenes, with subsequent perchlorination of the chlorobenzenes.

When interpreting data such as the above, one should consider a possible interfering role of PCBs present as contaminants in PCT mixtures. In this study, using GC/MS we have unambiguously detected biphenyl, monochlorobiphenyls and (traces of) dichlorobiphenyls in Aroclor 5442. The total amount of these compounds, however, was estimated at distinctly less than 1 wt%. Besides, the degree of conversion of biphenyl and low-chlorinated PCBs into DCB upon perchlorination is known to be relatively low. In other words, at least a large part of the DCB formed in the above experiments can be attributed to PCTs.

Finally, we should emphasize that the amount of DCB formed during perchlorination of PCTs under standard conditions is rather small. Consequently, the presence of PCTs cannot explain the high results often obtained in the analysis of PCB-containing samples via perchlorination/GC (cf. Table I and II in ref. 51).

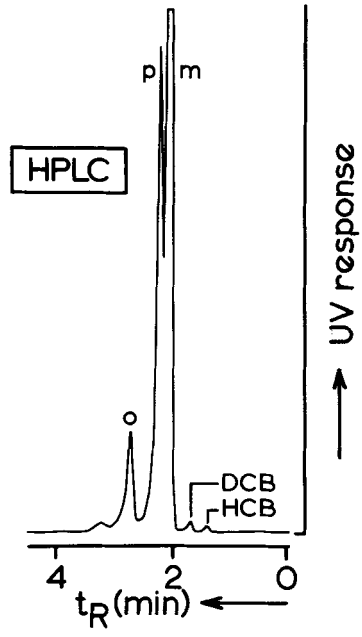

FIGURE 5 Adsorption LC chromatogram of a perchlorinated Aroclor 5442 mixture. LC system LiChrosorb SI 60/dry hexane; UV detection at 214 nm; flowrate, 1.4 ml/min.

PCTs in paper and sewage sludge samples

Large discrepancies between PCB contents calculated on the basis of perchlorination/GC as compared with the GC pattern-comparison method have primarily been noted[51] with samples of a non-biological nature, such as (recycled) paper and sewage sludge samples. A number of these was therefore re-analysed and the PCT levels were determined. In all cases Aroclor 5460 was used as standard PCT mixture for pattern-comparison purposes, peak heights of standard and sample being compared for, typically, 10–20 peaks. Part of the analyses was done by packed-column, part by capillary GC. For a restricted number of paper samples, next to the conventional alcoholic NaOH treatment the soxhlet extraction with hexane was used. The results are summarized in Table VI.

Comparison of the PCT content as μg of TDCTs per g of sample (determined via pattern comparison; conversion factor Aroclor 5460→TDCTs, 1.29) with the TDCT content determined by perchlorination shows perchlorination/pattern comparison (PE/PC) ratios

TABLE IV

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

Sample		Pattern (μg A5460 per g)	Perchlorination (μg TDCT/g)				PE/PC ratio	Excess[b] DCB/TDCT
			ortho	meta	para	total		
Toilet paper	I	—	0.04	0.27	0.18	0.49	»	0.6
	III	—	0.08	1.01	0.50	1.59	»	3.4
	IV	0.95	2.0	13.6	15.1	30.7	25.0	0.25
	IV[c]	0.83	1.5	12.8	14.0	28.3	26.4	0.23
	V	1.00	0.5	4.2	5.1	9.8	7.6	0.5
	V[c]	0.83	0.4	2.9	4.0	7.3	6.8	0.7
Recycled paper	I	—	0.20	0.65	1.36	2.2	»	3.0
	II	—	1.6	16.5	12.6	30.7	»	1.5
	III	—	0.17	0.78	0.74	1.69	»	2.7
	IV	—	2.2	18.7	14.4	35.3	»	1.2
	V	—	1.2	10.4	6.8	18.4	»	2.0
	VI	—	0.10	2.2	1.2	3.5	»	3.2
	VII	—	0.12	0.71	0.75	1.58	»	1.5
	IX	—	0.32	3.9	1.8	6.0	»	1.4
	X	—	0.21	2.5	1.01	3.7	»	2.0
	XI	—	0.2	1.4	1.8	3.4	»	2.0
	XI[c]	—	0.2	1.2	1.5	2.9	»	2.2
Paper board	II	7.85	2.1	14.0	9.4	25.5	2.5	1.3
	V	0.17	0.30	2.0	1.3	3.6	16.6	1.5
	VI	—	0.5	5.6	4.9	11.0	»	1.1
	VI[c]	—	0.3	3.5	2.6	6.4	»	1.5
	VII	0.25	0.21	0.38	0.16	0.75	2.3	2.6
	VIII	0.18	0.36	0.47	0.19	1.02	4.4	0.9
Sewage sludge[d] AW	V	0.68	2.7	2.1	1.2	6.0	6.8	3.7
	VI	0.96	4.7	4.2	1.8	10.7	8.6	3.1
	VIII	0.96	3.5	3.1	1.6	8.2	6.6	—
	IX	0.83	3.8	3.1	2.0	8.9	8.3	—

[a]Sample codes correspond with those used in Tables I and II in ref. 51. Conversion factor from PCTs (Aroclor 5460) to total TDCT: 1.29.

[b]Excess DCB (or TDCT) calculated by subtracting the amount of DCB (or TDCT) calculated from pattern comparison from that found after perchlorination.

[c]Hexane soxhlet extraction used; all other samples extracted with alcoholic NaOH.

[d]Calculated on dry-weight basis.

of between about 2 and 25 for those samples in which the presence of PCTs was demonstrated by means of direct GC analysis. More surprisingly, for samples which apparently did not contain PCTs, TDCT levels after perchlorination were of the same order of magnitude as for the

samples that did contain PCTs. Such results were obtained with both packed-column and capillary GC. The data quoted in Table VI also demonstrate that results obtained by the hexane-extraction and the alcoholic NaOH treatment satisfactorily agree. In short, for the present types of sample, perchlorination is as unreliable a method of analysis for PCTs as it is for PCBs; here, it is interesting to note that the excess DCB/excess TDCT ratios recorded in Table VI lie within a rather narrow range of 0.2–3.7 for all samples tested. In the literature, perchlorination has repeatedly been used in the analysis of PCT-containing samples (cf. Table II). Unfortunately, however, in all cases the technique was only used to confirm the presence of PCTs, which were quantitatively determined by means of pattern comparison, or perchlorination was the sole analysis technique applied. That is, no meaningful conclusions can be drawn from these data.

Further, one should note that with a large majority of the paper samples examined, the ratio *o*-TDCT:*m*-TDCT:*p*-TDCT = 0.1–0.15:1:0.5–0.9 is fairly constant and does not deviate too much from the ratio 0.1:1.0:0.45 found for perchlorinated Aroclor mixtures. The para isomer, however, shows a relative increase. The results obtained for the, admittedly, limited number of sewage sludge samples are markedly different, with large contributions for *o*-TDCT and a relatively small one from *p*-TDCT. In the literature, the relative increase of the para isomer has been observed with, e.g., bird[37] and human fat[33,36] samples; unexpectedly high proportions of *o*-TDCT have also been reported, viz. in a study on eagles and seals.[45] Obviously, no simple, generally valid conclusion on the relative ease of degradation of chlorinated *o*-, *m*- and *p*-terphenyls can be reached. (Besides, the obvious presence of interfering compounds which are also converted into TDCTs further confuses the issue.)

In Fig. 6 capillary GC chromatograms for a paper sample extract and an Aroclor 5460 standard, analysed with and without perchlorination, are shown.

Lastly, we mention that the successful application of dechlorination/HPLC to the analysis of a recycled paper sample has been reported[20] before; good use was made of the favourable spectral properties of *o*-, *m*- and *p*-terphenyl. Unfortunately, analysis of a further number of samples has revealed that frequently strongly UV-absorbing compounds (possibly hydrogenated polyphenyls; cf. next section) elute in the same retention-time range as do the terphenyls, thereby obscuring their peaks. In other words, without further sample clean-up, dechlorination with subsequent HPLC/UV analysis is not generally applicable for the determination of PCTs in real samples.

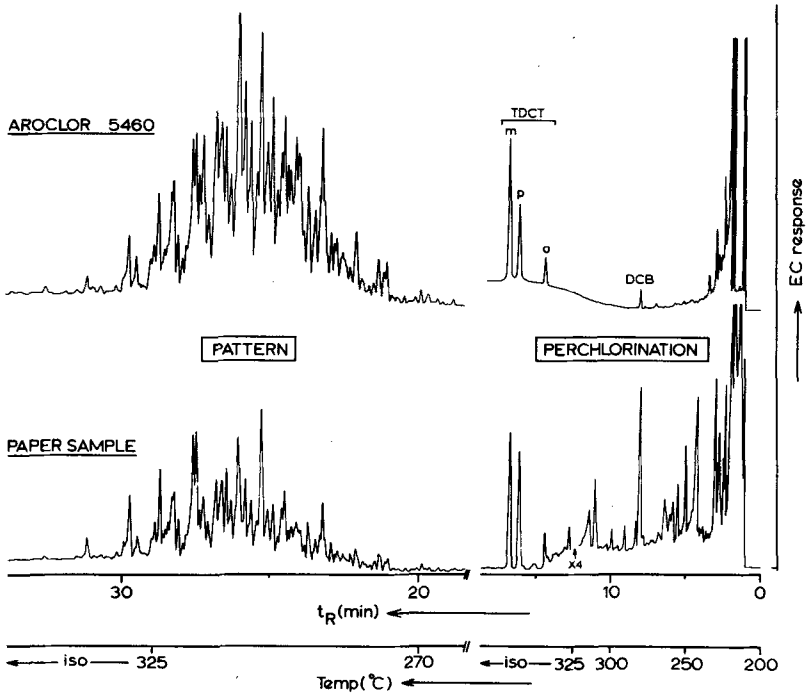

FIGURE 6 Capillary GC-EC chromatograms of a paper sample (no. V in Table VI) extract and an Aroclor 5460 standard obtained using (left) pattern comparison or (right) perchlorination. For experimental details, see text.

Interfering compounds

Recently, we have reported[51] on the identification of hydrogenated terphenyls in (recycled) paper samples, and evidence has been presented to show that these apparently are the main interfering compounds in PCB analysis via perchlorination/GC. On the basis of their structure, a partial conversion of the hydrogenated terphenyls, upon perchlorination, into TDCT can be expected. This would explain the high PE/PC ratios for PCTs as well.

A more detailed examination was made using a mixture of polyphenyls and hydrogenated polyphenyls commercially available from Monsanto under the trade name HB-40; for GC/MS, HPLC and UV spectral data of HB-40 one is referred to the paper quoted above. In the present study, attention was solely devoted to the perchlorination (for 17 h at 170°C) of HB-40. Fractionation of HB-40 by means of HPLC in the system silica/dry hexane was done as before and fraction A (t_R, 0–6 min), B (t_R, 6–11 min) and C (t_R, 18–30 min) were collected. Results of perchlorination of

these fractions and their subsequent analysis by capillary GC are shown in Table VII. Next to a considerable amount of DCB (211 ng per μg of HB-40) large amounts of the TDCTs (171 ng per μg of HB-40) are found. Even though the ratio *o*-TDCT:*m*-TDCT:*p*-TDCT varies widely from one fraction to another, the overall ratio of 0.1:1.0:0.5 is surprisingly close to that found above after the perchlorination of standard Aroclor mixtures.

The above results cannot be explained on the basis of the conversion of traces of *o*-, *m*- and/or *p*-terphenyl present in HB-40 into the fully chlorinated end-products. These parent hydrocarbons all elute in fraction C and the only isomer detected so far in this fraction (by GC/MS; cf. ref. 51) is *o*-terphenyl, which to all appearances is a rather minor component. In other words, even though care should be taken in interpreting data

TABLE VII

Amounts of DCB and TDCTs formed upon perchlorination of 97.6 μg of HB-40

HPLC fraction[a]	DCB (ng/μg)	TDCTs (ng/μg)			Rel. int. TDCTs			Excess[b] DCB/TDCT
		o-	*m*-	*p*-	*o*-	*m*-	*p*-	
A	59.4	6.1	34.8	4.5	0.18:1:0.13			1.31
B	70.7	6.7	34.8	40.0	0.19:1:1.15			0.87
C	80.9	1.3	99.4	43.0	0.01:1:0.43			0.56
Total	211.0	14.1	169.0	87.5	0.08:1:0.52			0.78

[a]For details, see text of this paper and Fig. 6 in ref. 51.
[b]Cf. Table VI.

obtained by processing a complicated mixture of closely related polyphenyls, it seems safe to state that perchlorination of partly hydrogenated polyphenyls yields, next to DCB, appreciable amounts of the isomeric TDCTs. If a mean molecular weight of 236 (terphenyl hydrogenated in one ring) is assumed for HB-40, then conversion percentages are of the order of 10% for both DCB and total TDCT.

It should be emphasized here that the above results do not provide any evidence that the high PE/PC ratios obtained with the samples mentioned in Table VI are caused by the actual presence of HB-40. The large differences observed between the excess DCB/excess TDCT ratio of about 0.8 for HB-40 and the mutually divergent values quoted in Table VI rather demontrate that solving our problem is not as simple as that.

CONCLUSION

Commercially available PCT mixtures can conveniently be characterized by either capillary GC with EC detection or adsorption HPLC with UV

detection. These chromatographic techniques have been used, for the first time, for a close comparison of seven mixtures obtained from three different manufacturers. In the paper and sewage sludge samples examined the identifiable PCT mixture always had a high degree of chlorination, with a peak pattern closely analogous to that of Aroclor 5460. This agrees with literature results; the PCT levels typically vary between 0 and 10 ppm. Quantitation via the well known pattern-comparison procedure must certainly be preferred to analysis by means of perchlorination which–for the present samples–invariably yielded inaccurate, i.e., excessively high, results. This conclusion ties in with earlier results on the limited usefulness of perchlorination in the analysis of PCB-containing samples.

During perchlorination, decomposition of PCTs into PCBs (with subsequent conversion into DCB) occurs to an insignificant extent. That is, high results of PCB analyses cannot be attributed to the presence of PCTs in the samples studied, less so since in a large majority of all samples described in the literature, the ratio PCTs/PCBs has a value of less than 0.1. Hydrogenated polyphenyls, on the other hand, which have already been shown to be interfering compounds during the determination of PCBs in paper and such-like samples via perchlorination, also are responsible for the erroneous results of PCT analyses carried out via perchlorination. Besides, the presence of hydrogenated polyphenyls probably is at least partly responsible for the fact that–contrary to what has been observed with PCBs–dechlorination/HPLC cannot be recommended as a generally useful independent check method (next to pattern comparison) for PCT analyses.

The above conclusions can be summarized very succinctly. At the present moment pattern-comparison analysis via capillary GC-EC is the only reliable routine method for the quantitation of PCTs. Fortunately, it is an excellent one, combining good separation efficiency with high sensitivity.

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