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Environmental analysis of polychlorinated terphenyls: distribution in shellfish from the Ebro Delta (Mediterranean)

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ABSTRACT

Polychlorinated terphenyls (PCTs) have characteristics almost identical with those of polychlorinated biphenyls (PCBs) and have been used for analogous applications, but only sporadic reports of the occurrence of PCTs in the environment have been published. High-resolution gas chromatography with electron-capture detection (HRGC–ECD) and mass spectrometric detection in the selected ion monitoring mode was used to analyse samples for PCTs. The homologue distribution of Aroclor 5432, 5460, Leromoll 141 and the PCTs in samples of shellfish from the Ebro Delta (Catalonia, Spain) was established, taking into account the contribution of the $[M - Cl_2]^+$ fragments. Quantification was achieved by HRGC–ECD. Concentrations were between 790 and 3 ng/g (dry mass).

INTRODUCTION

In the last two decades, there has been considerable public concern about the presence of halogenated anthropogenic compounds in the environment, because of their persistence, bioaccumulation potential and health risks [1–4]. Many studies have been conducted to determine the extent and significance of polychlorinated biphenyl (PCB) residues, and the occurrence of these compounds in the environment has been extensively documented [5–10]. In contrast, little attention has been paid to the environmental distribution of polychlorinated terphenyls (PCTs), which are similar to PCBs in chemical characteristics and uses.

PCBs and PCTs have been used in hydraulic fluids, electrical equipment, sealants, plasticizers, paints, adhesives and casting agents because of their electrical and flame-retardant properties [1]. From an environmental protection point of view, these applications have been divided into open (from which PCBs cannot be recovered) and closed systems [1, 11], the latter having traditionally been the most important applications of PCBs. Indeed, closed systems are the only applications of PCBs to be covered by legislation since 1973 in the USA and 1976 in EEC countries. Similar regulatory attention has not been paid to PCTs but it has been argued that their usage is controlled by regulations restricting PCBs.

Commercial formulations of PCTs could be expected to be much more complex than those of PCBs. There are not only more positions and combinations of positions available for chlorination, but the phenyl rings could also have three possible arrangements. Hence the number of isomers and homologues should be higher than the theoretical

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209 PCB congeners, although the exact number has never been calculated.

Analysis for PCTs has proved to be difficult because of the complexity of the mixtures, the high boiling points of the heavily chlorinated congeners, the coincidence of the gas chromatographic retentions of the lower chlorinated PCTs with those of some PCBs and the presence of interferences in the analysis of molecular ions by mass spectrometry.

Little information is available about the distribution, fate and effects of PCTs in the environment, but as highly chlorinated aromatic compounds, PCTs might be expected to have a high resistance to biodegradation and photodegradation and to be capable of being accumulated in living organisms and through food chains. PCTs are not so widely and heavily dispersed in the environment as PCBs, but they have nevertheless been detected in river water [12], soil and sediment samples [13–16], a variety of biological samples, including shellfish, eels, seals, birds [15,17–19] and bovine milk [20], food packaging materials [21,22] and human tissue [17,23–25].

In this work, the distribution of the different chlorinated congeners in two Aroclors, 5432 and 5460, and Leromoll 141, was studied and the presence of PCTs in samples of shellfish from the Ebro Delta (Catalonia, Spain) was investigated.

EXPERIMENTAL

Chemicals

Aroclor 1254 and 1260 PCB standard mixtures were purchased from Promochem (Wesel, Germany) and Aroclor 5432 and 5460 PCT mixtures from Chem Service (West Chester, PA, USA); the commercial product Leromoll 141 was a gift from Dr. U. A. Th. Brinkman (Amsterdam, Netherlands). The internal standards 2,4-dichlorobenzyl hexyl ether (DCBE-6) and 2,4-dichlorobenzyl hexadecyl ether (DCBE-16) were a gift from Dr. D. E. Wells (Aberdeen, UK). Florisil for residue analysis (60–100 mesh), from Merck (Darmstadt, Germany) was used as a chromatographic adsorbent. It was activated at 675°C for 2 h and then stored at 130°C before use. Granular anhydrous sodium sulphate for residue analysis from Merck was dried at 450°C for 2 h and kept at 130°C. The solvents *n*-hexane and dichloromethane were redistilled in glass to obtain residue analysis grade materials. The purity of the solvents was determined by concentration of 150 ml to 0.5 ml and injection of the extract into the gas chromatographic–electron capture detection (GC–ECD) system. All glass materials were cleaned with AP-13 Extran alkaline soap (Merck) for 24 h and dried overnight at 180°C. The chromatographic clean-up column (30 cm × 1 cm I.D.) was packed with 7 *g* of activated Florisil.

Standard solutions of PCB and PCT mixtures containing 10 mg/l of individual Aroclors 1254, 1260, 5432, 5460 and Leromoll 141 were prepared in isooctane for residue analysis (Carlo Erba, Milan, Italy).

Apparatus

Gas chromatography was carried out on a Carlo Erba (Milan, Italy) Model 5300 Mega Series gas chromatograph equipped with a ^{63}Ni electron-capture detector using nitrogen as make-up gas. A DB-5 fused-silica (J&W Scientific, Folsom, CA, USA) capillary column (60 m × 0.25 mm I.D.) with a 0.25-μm film thickness, was used with helium as carrier gas at a linear velocity of 30 cm/s. The temperature was held isothermally at 90°C for 3 min, programmed to 150°C at 25°C/min and maintained at 150°C for 1 min, then programmed to 310°C at 2.5°C/min, and mantained at this temperature for 30 min. The injector and detector temperatures were 270 and 330°C, respectively. The chromatographic data were analysed using an Merck–Hitachi Model D-2000 integrator.

For HRGC–MS a Konik (Barcelona, Spain) Model 3000 gas chromatograph with a VG TS-250 (VG Instruments, Manchester, UK) mass spectrometer and a VG 11-250 data system was used. A DB-5 fused-silica (J&W Scientific) capillary column (30 m × 0.25 mm I.D.), with a 0.25-μm film thickness, was used with helium as carrier gas at a linear velocity of 25 cm/s. The temperature programme for GC–MS (full scan) was from 70°C (held for 1 min) to 200°C (held for 1 min) at 2°C/min and then to 300°C (held for 30 min) at 4°C/min. For HRGC–MS–[selected ion monitoring (SIM) mode] the temperature programme was from 70°C (held for 0.7 min) to 150°C (held for 1 min) at 20°C/min and then from 150 to 280°C (held for 15 min) at 3°C/min. The injector was kept at 250°C. The MS operating conditions were as follows: ion source and interface temperatures, 225 and 290°C, respectively; ioniza-

tion energy, 70 eV (electron impact mode); resolving power, 500; and mass range, 40–625 a.m.u. at 2 s per decade when the full-scan mode was used. In the HRGC–MS–SIM mode the ions at m/z 298, 300, 332, 334, 366, 368, 402, 404, 434, 436, 470, 472, 504, 506, 538, 540, 574, 576, 608 and 610 were monitored and m/z 207 was used as lock mass. Other conditions were dwell time 50 ms and trap current 700 μA.

Sampling and analysis

A field sampling programme was conducted over 2 years for the determination of PCBs and chlorinated pesticides in mussels and oysters from the Fangar and Alfacs bays in the Ebro Delta. Sample sites were selected to give overall information about the quality of the cultivated bivalves. The samples were collected at the breeding site and frozen at −18°C upon collection.

Whole fish were ground, homogenized and dried with a vacuum glass desiccator at 60°C for 24 h. Samples (*ca.* 8 g, dry tissue) were mixed with 200 *g* of anhydrous sodium sulphate and then Soxhlet extracted with 300 ml of hexane–dichloromethane (1:1) for 3 h. The extract was reduced in volume by rotary evaporation and solvent-exchanged to *n*-hexane (5 ml). Sample clean-up was carried out by elution of 2 ml of the extract through a Florisil column using 50 ml of *n*-hexane and 50 ml of hexane–dichloromethane (1:1). PCBs, PCTs and *p,p'*-DDE were eluted in the first fraction, whereas the second fraction contained the rest of the chlorinated pesticides. Internal standards were added and the final volume of the extract was adjusted to 0.5 ml. Samples were analysed by gas chromatography GC–ECD and GC–MS.

For the determination of PCBs in the shelfish samples, the congener specific method with Aroclor 1254 as a standard was used. The chromatographic peaks from the sample were compared with those of the Aroclor using DCBE-6 and DCBE-16 as internal standards. Total PCBs are expressed as the sum of the single congeners present. For the determination of PCTs the standard Aroclor 5460 was used; in this case the internal standards were compared with the total area of the PCT peaks because single congeners could not be identified; results were expressed on a dry mass basis. The recoveries of the PCBs and PCTs were calculated by spiking the samples with careful mixing of standard solutions. Differents amounts of PCB congeners and Aroclor 5460 standard were used in order to obtain concentrations around 50, 100, 150 and 200% ($n = 3$) of the actual concentration in the real samples. The efficiency was evaluated by studying the recoveries of PCBs and PCTs from spiked samples. The means of the analytical recoveries observed for PCBs and PCTs were 98.8% and 91.2% with relative standard deviations of 9.7 and 11.6%, respectively.

RESULTS AND DISCUSSION

Chromatography of PCT mixtures

The PCT mixtures Aroclor 5460 and 5432 and Leromoll 141 were analysed using capillary GC with ECD and MS detection. The non-polar DB-5 stationary phase was especially suitable because of its stability, allowing temperatures of up to 310°C to be used, at which the highly chlorinated PCTs are eluted.

Fig. 1 shows the TIC profiles of HRGC–MS for Leromoll 141 and Aroclor 5460. The retention of PCT congeners tends to increase with increasing degree of chlorination, although co-elution between congeners containing fewer or more chlorine atoms than a given isomer group is observed. The TIC profiles show that Aroclor 5460 is mainly composed of PCT having six to eleven chlorine substituents, whereas Leromoll 141 has five to nine. These values agree with literature data, which indicate that Aroclor 5460 is mainly constituted of terphenyls substituted with seven to ten or eleven chlorine atoms [26–28] and Leromoll from six to nine [28]. Our results show a contribution from the five substituted terphenyls in the latter, which has not been reported previously.

The mass ranges covered in the TIC profiles in Fig. 1 include the major ions in the isotope clusters for molecular ions of the terphenyls. Co-elution of the PCT congeners containing fewer chlorines did not interfere with the detection and measurement of the $M^{+\cdot}$ ions produced by a member of a given isomer group, although there may be interference from the $[M-Cl_2]^+$ fragment ions of homologues containing two additional chlorine atoms [24]. This effect is seen in Fig. 2, in which the selected ion $M^{+\cdot}$ monitoring register for the homologues of Aroclor 5460 is given. Comparison of Figs. 2b, d and/or a, c

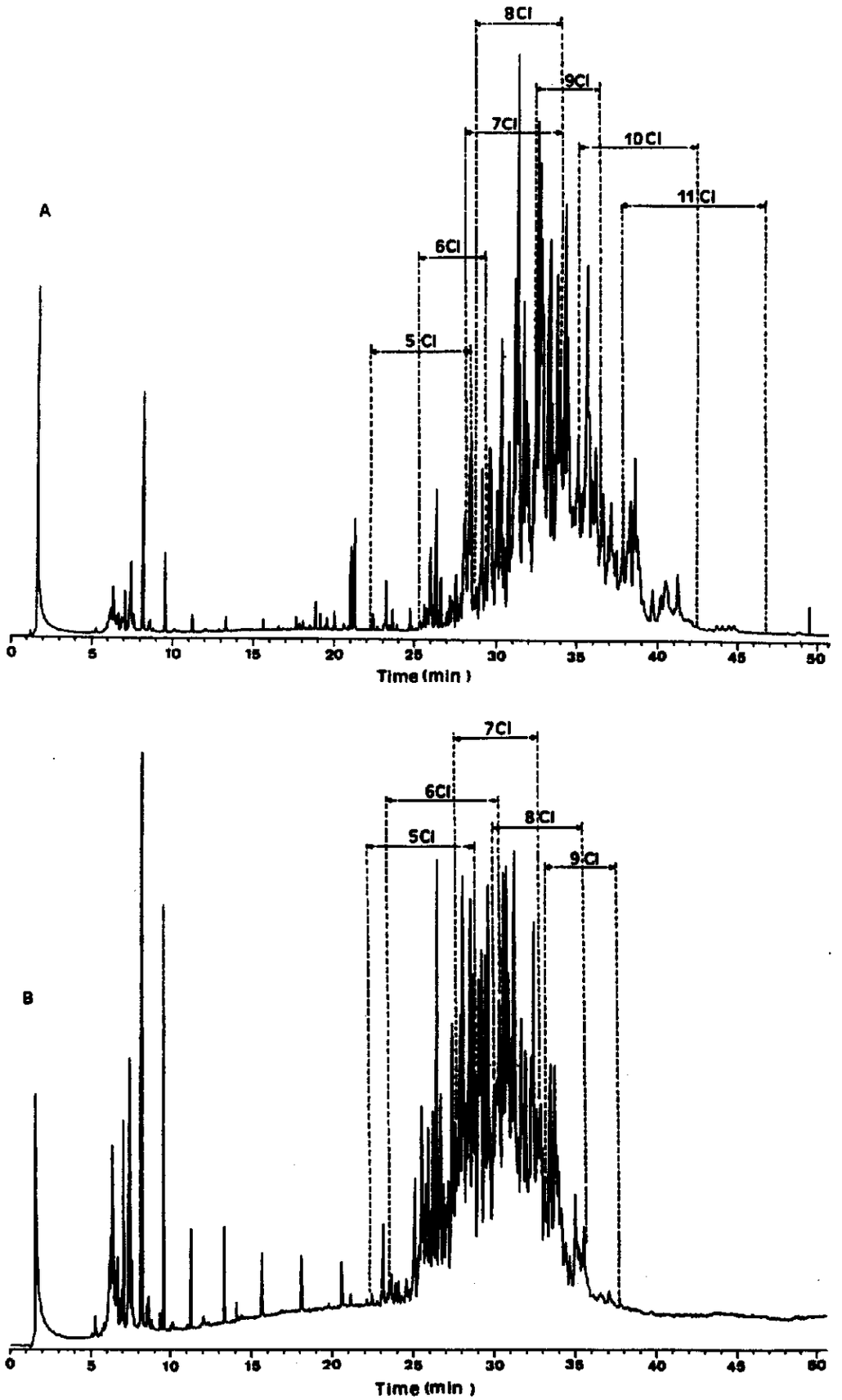


Fig. 1. Total ion current register for (A) Aroclor 5460 and (B) Leromoll 141. Numbers above the peaks designate the number of chlorine sustituents.

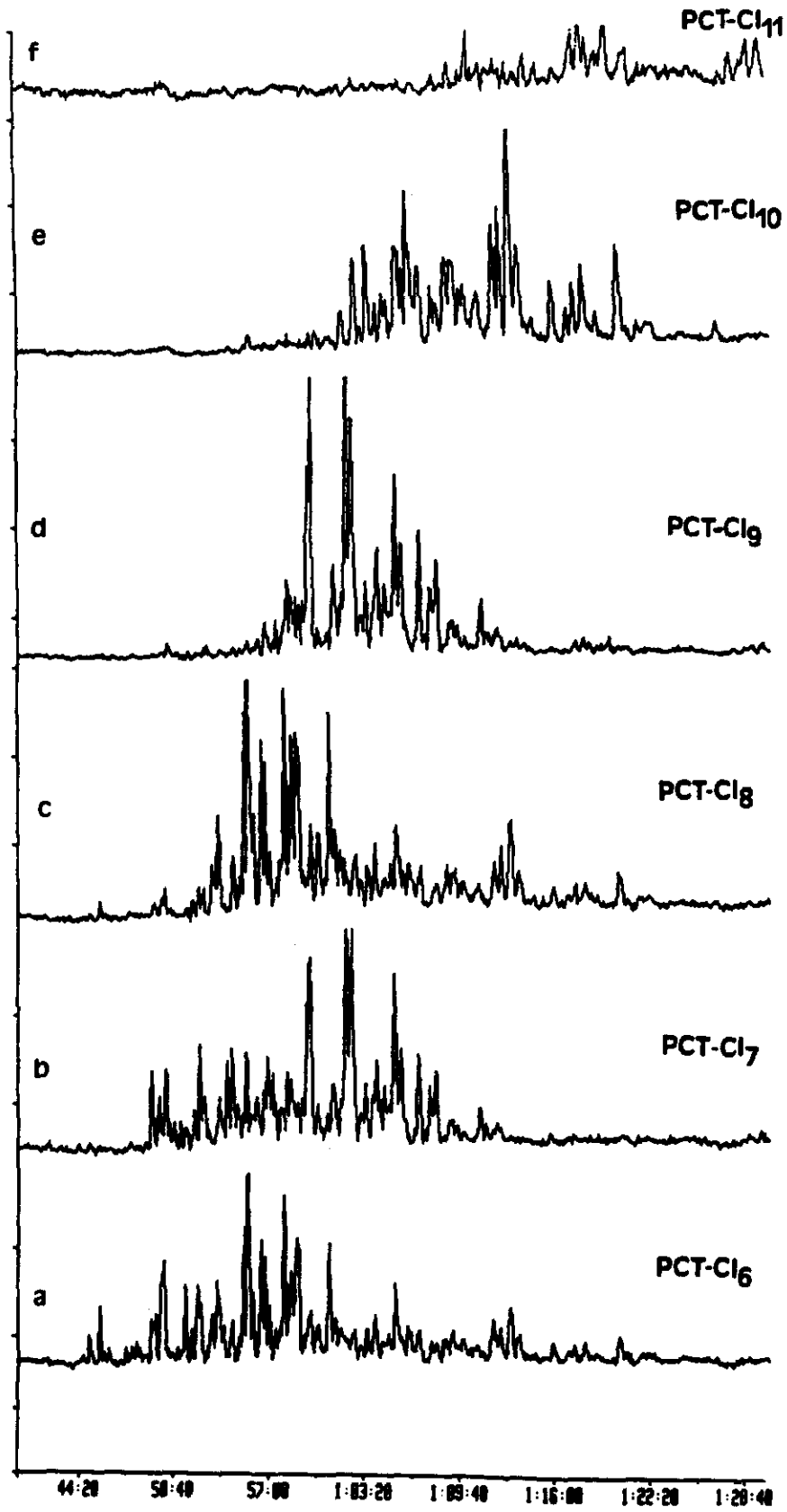


Fig. 2. Selected ion monitoring for the homologues of Aroclor 5460. (a) 6-Chloro-PCT; (b) 7-chloro-PCT; (c) 8-chloro-PCT; (d) 9-chloro-PCT; (e) 10-chloro-PCT; (f) 11-chloro-PCT. *x*-axis: time.

and e shows that the $[M-Cl_2]^+$ fragments of the nonachloroterphenyls are major contributors to the heptachloroterphenyls and fragments of the decachloroterphenyls contribute to the octachloroterphenyls, as occurs with the fragments of the latter with the hexachloroterphenyls. Another potential interference may be produced by the $[M-Cl]^+$ fragment ions, although this does not seem to be important in our case because the signal is weak as can be seen in Fig. 2.

The assigment of the level of chlorination of the homologues was calculated taking the retention times and the effect of the contribution of the $[M-Cl_2]^+$ fragments into account. Hence the contribution of the $[M-Cl_2]^+$ ions was eliminated from the selected ion register for each homologue and the approximate distribution in the original compound was calculated assuming that virtually no co-elution between homologues containing two additional chlorine atoms, as can be seen in Figs. 1 and 2. To discriminate between these two species, high-resolution mass spectrometry would be necessary (higher than 20 000). The values obtained are given in Table I, showing that Aroclor 5432 was mainly composed of PCTs having two, three and four chlorine sustituents, with minor amounts of one and five, and Aroclor 5460 had terphenyls with eight, nine and ten chlorine substituents with minor amounts of six, seven and eleven. On the other hand, Leromoll 141 was mainly composed of PCTs having five, six, seven and eight chlorine substituents with minor amounts of nine.

TABLE I

PERCENTAGE HOMOLOGUE DISTRIBUTIONS FOR AROCLOR 5432, 5460, LEROMOLL 141 AND SAMPLE 1

Compounds	Percentage of homologues[a]			
	Aroclor 5432	Aroclor 5460	Leromoll 141	Sample 1
Mono-CTs	<1	–	–	–
Di-CTs	31	–	–	–
Tri-CTs	42	–	–	–
Tetra-CTs	23	–	–	–
Penta-CTs	3	–	14	9
Hexa-CTs	–	<1	28	22
Hepta-CTs	–	6	30	40
Octa-CTs	–	17	27	27
Nona-CTs	–	42	1	2
Deca-CTs	–	28	–	<1
Undeca-CTs	–	6	–	<1

[a] Dashes indicate not detected.

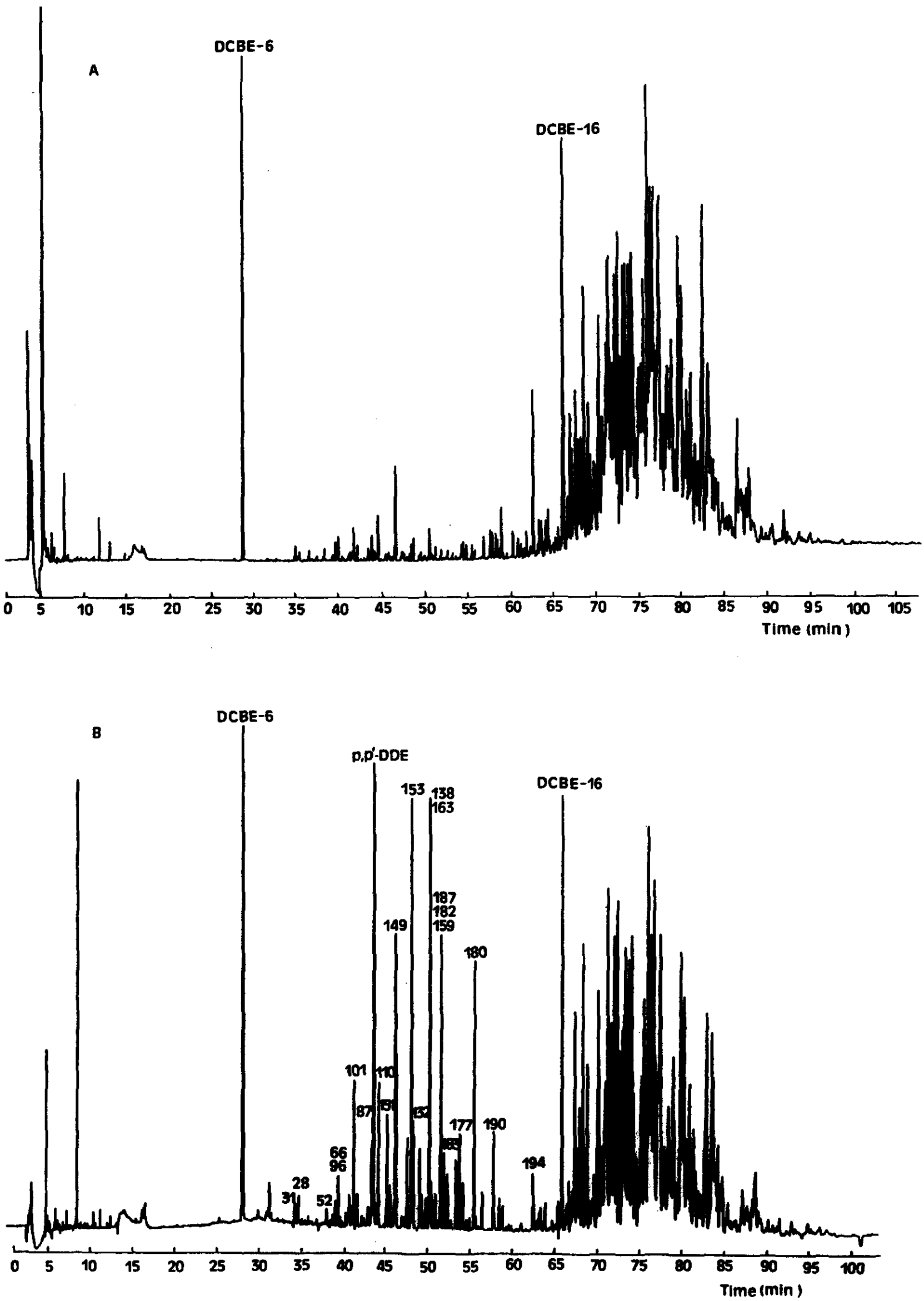
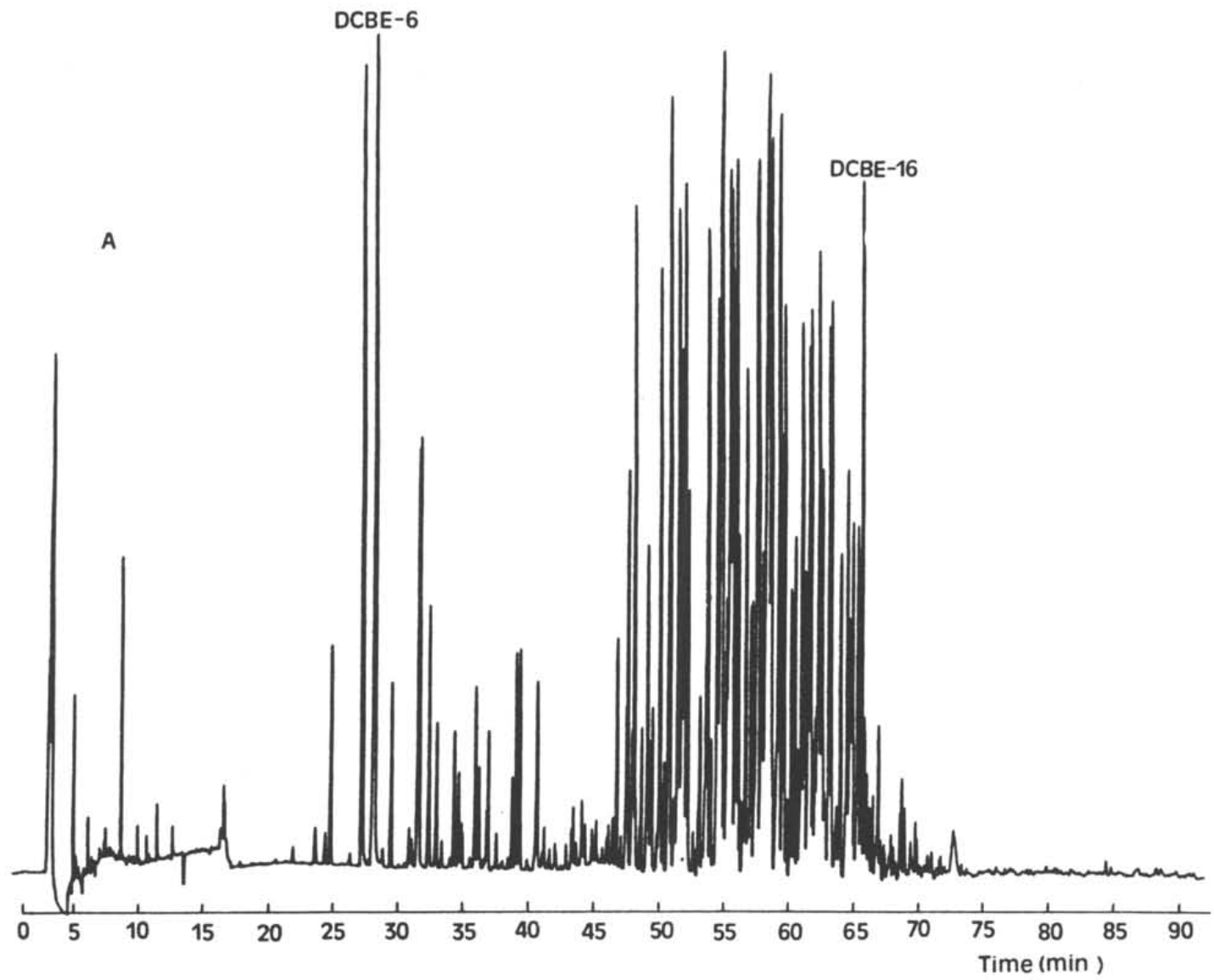


Fig. 3. HRGC–ECD (60-m DB-5 column) of (A) Leromoll 141 and (B) sample 1. PCB congener numbers and internal standards are indicated.



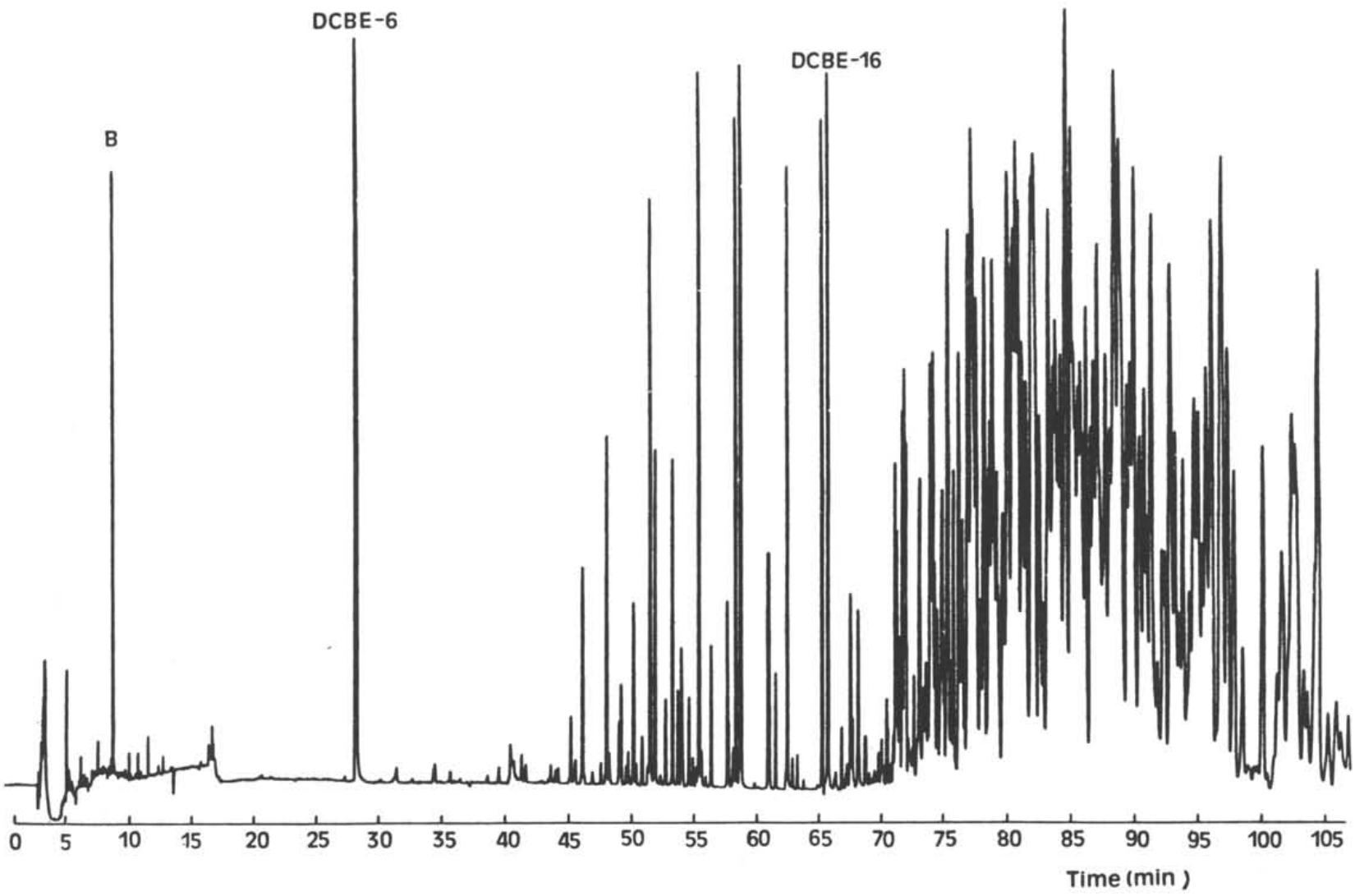


Fig. 4. HRGC–ECD (60-m DB-5 column) of (A) Aroclor 5432 and (B) Aroclor 5460.

PCT in shellfish samples

A monitoring programme of anthropogenic halogenated compounds in shellfish from the Ebro Delta was carried out in 1989 and 1990. The PCB content in these samples from mussels (*Mitylus galloprovincialis*), clams (*Tapes decussata* and *Tapes semidecussata*) and oysters (*Ostrea edulis* and *Crasostrea gigas*) lay between 2950 and 4 ng/g dry mass (600 and 1 ng/g wet mass).

The chromatograms obtained from the extracts of the samples collected in February 1989 showed a series of compounds with retention times higher than the PCBs, which were identified by HRGC–MS as PCTs. Figs. 3 and 4 show, as an example, a comparison of an HRGC–ECD trace of the extract of one sample and those of the Leromoll 141 and Aroclor 5460 and 5432 standards. It is apparent from these profiles that the PCTs in the sample are very similar to those of Leromoll 141.The peaks eluted between 34 and 65 min were identified as PCBs by HRGC–MS.

Fig. 5 shows the HRGC–MS–SIM profiles for the sample extract. PCTs in the sample are mainly isomers of between five and ten chlorine atoms. The homologue distribution of the sample is shown in Table I. Hexa-, hepta- and octachloroterphenyls are the major contributors to the PCT content in the sample, and the distribution obtained is very similar to that of Leromoll 141, so the presence in the sample of the terphenyls from this formulation or a similar one was suggested.

The amount of PCTs in the sample was calculated from the HRGC–ECD data using Aroclor 5460 as standard and DCBE-16 as internal standard. Leromoll 141 was not used because it has a relatively low content of PCTs, as can be seen in Fig. 6, where the full-scan chromatogram of this compound is shown. PCBs were not detected in this mixture. Levels of PCT in Leromoll 141 calculated from this chromatogram gave a value of about 17%, in agreement with the data reported by De Kok *et al.* [28] calculated from the chlorine content of the commercial mixture. Purification procedures and certification analysis of the PCT content in Leromoll 141 are not available. The concentrations of PCTs in the samples are given in Table II. PCTs were detected at high levels in samples collected in February 1989 (790 ng/g dry mass) but the concentrations were much lower in April (198 ng/g). All the samples collected during 1989 contained PCTs, but at low levels, whereas in 1990 PCTs were not observed.

Although the exact use of PCTs in industrial formulations is unknown, especially as the production of PCBs has been banned, and little information about their fate in the environment is available, we must point out that in all samples collected in 1989 we found PCTs in decreasing amounts from February to November. Further, we found PCBs in all the samples collected over the 2 years, but no correlation between PCBs and PCTs was observed, so it does not seem that the PCT content could be related

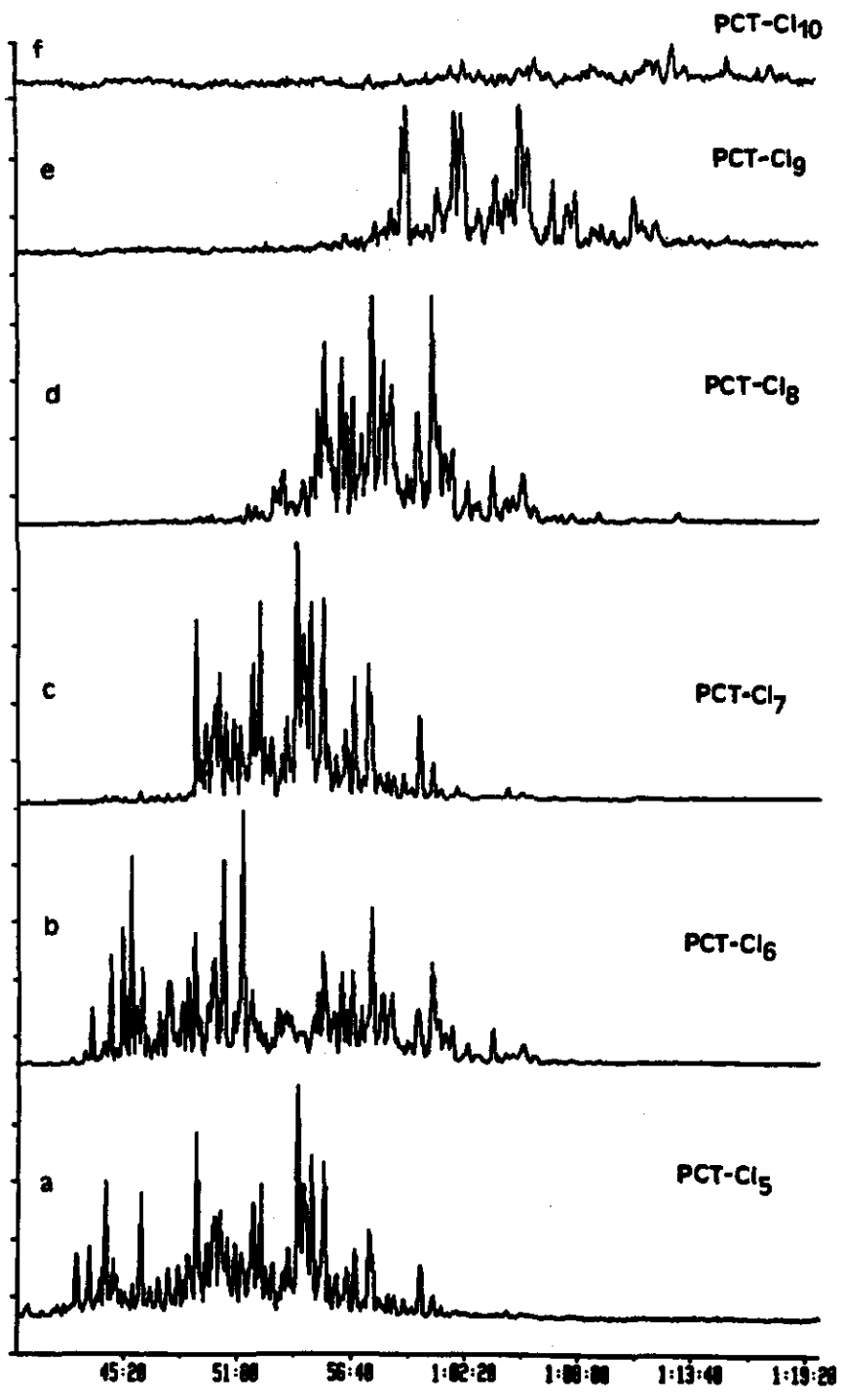


Fig. 5. Selected ion monitoring for the sample 1. (a) 5-Chloro-PCT; (b) 6-chloro-PCT; (c) 7-chloro-PCT; (d) 8-chloro-PCT; (e) 9-chloro-PCT; (f) 10-chloro-PCT. *x*-axis: time.

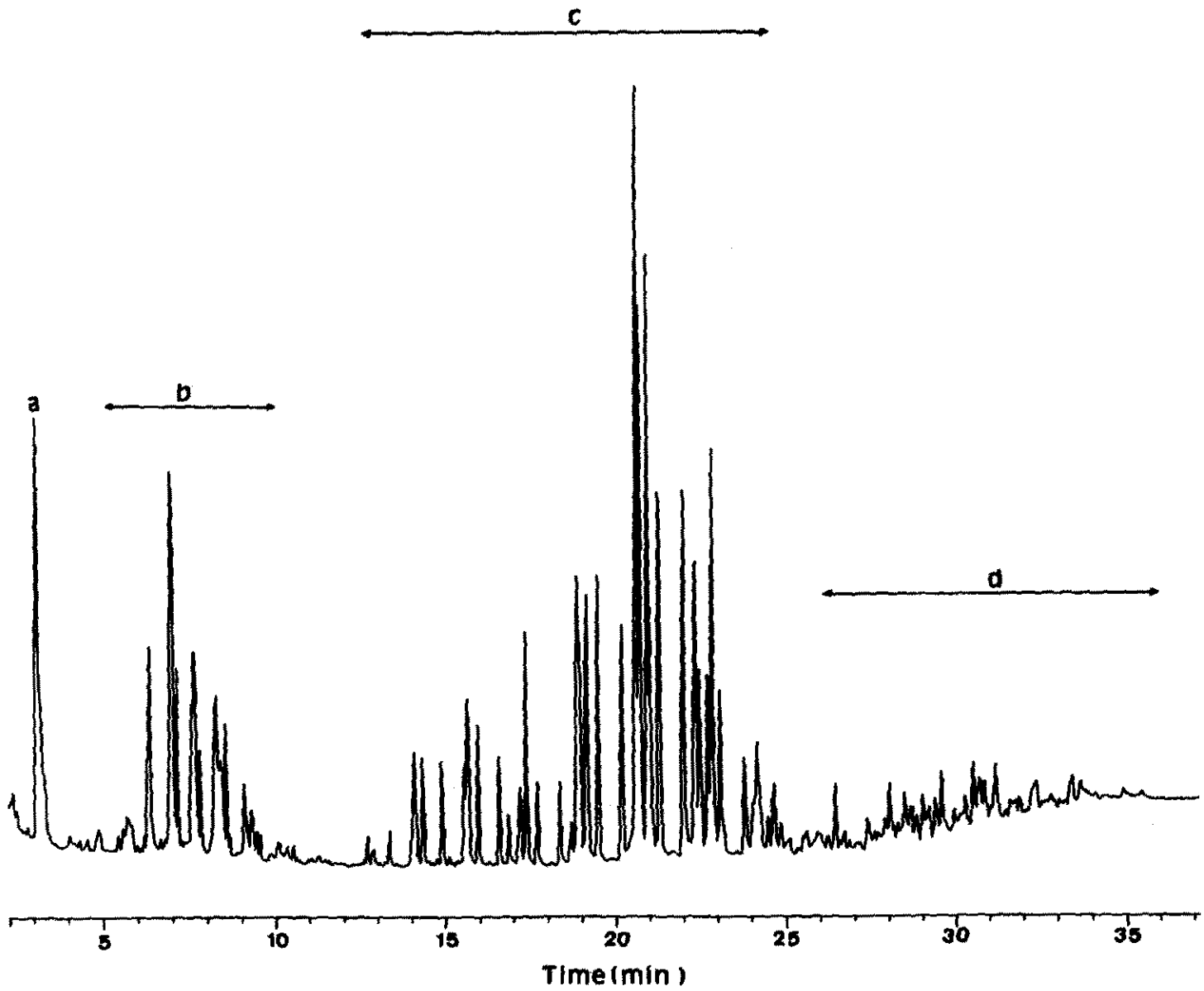


Fig. 6. Total ion current register for Leromoll 141. (a) Phenol; (b) aliphatic hydrocarbons; (c) tentative assigment phenolic resins, base peak mass 94; (d) terphenyls.

TABLE II

CONCENTRATION OF PCB AND PCT COMPOUNDS IN SHELLFISH

Sample No.	Shellfish	Date (1989)	Concentration (ng/g dry mass)	
			PCBs[a]	PCTs[b]
1	Mussels	February	131	790
2	Mussels	April	435	198
3	Mussels	July	690	10
4	Clams	July	128	7
5	Mussels	July	2000	21
6	Clams	July	730	3
7	Mussels	September	2950	115
8	Clams	September	745	118
9	Clams	October	528	97
10	Mussels	November	1797	100
11	Clams	November	215	105

[a] Whole mass between 597 and 18 ng/g.
[b] Whole mass between 180 and 1 ng/g.

to the PCBs. The fact that the Ebro River crosses an industrial zone *ca.* 60 km upstream from the delta, where there is an important chemical complex, appears to suggest a sporadic release into the river water of Leromoll 141 or other industrial formulations of similar terphenyl composition.

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