Journal of Chromatography, 634 (1993) 79-86
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 783

Determination of polychlorinated naphthalenes in polychlorinated biphenyl products via capillary gas chromatography-mass spectrometry after separation by gel permeation chromatography

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(First received August 18th, 1992; revised manuscript received November 25th, 1992)

ABSTRACT

A gel permeation chromatographic method was developed for the isolation and determination of small amounts of polychlorinated naphthalenes (PCNs) present in polychlorinated biphenyl (PCB) products. The method was applied to nine commercial PCB products: Aroclor 1016, 1232, 1242, 1248, 1254 and 1260 and Clophen A30, A40 and A50. All products were found to contain total PCN concentrations between 1.8 and 870 μg/g. High concentrations of PCNs were detected in Clophen A30 and A40 whereas a low concentration was found in Clophen A50. The PCN congeners were dominated by compounds substituted with two to six chlorine atoms.

INTRODUCTION

Polychlorinated naphthalenes (PCNs) have been produced commercially since World War I [1]. PCNs have mainly been used in applications where high thermal stability was necessary, e.g., in dielectric fluids in transformers and capacitors, but have also been used as pesticides [2,3], plasticizers, and oil additives [1]. The production of PCNs was restricted soon after their introduction owing to serious occupational health effects, such as chloracne and liver damages [4]. Recently some PCN congeners have been shown to induce enzymes such as EROD (7-ethoxyresorufin 0-deethylase) and AHH (aryl hydrocarbon hydroxylase) in a similar way to dioxins [5].

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0021-9673/93/$06.00

PCNs are environmental contaminants detected in wildlife worldwide [6–8] and are also found in human adipose tissue and mothers' milk at the ng/g lipid level [9,10]. The presence of PCNs in biota, including humans, and their potential effect on human health are of public concern. It is therefore important to investigate potential sources for PCNs in the environment.

PCNs were reported in the early 1970s to be present as contaminants in commercial polychlorinated biphenyl (PCB) products [11,121. However, these investigations were focused on the measurements of polychlorinated dibenzofurans, and no quantitative data for PCNs were reported.

Technical PCN products and samples highly contaminated with PCNs can be analysed, without extensive clean-up, by either gas or liquid chromatography [13,14]. The determination of PCNs in matrices with a high content of PCBs has so far mainly been achieved with either perchlorination [15,161 or dechlorination [17–19] of the chlorinated compounds prior to analysis. These methods do not permit the determination of any of the individual PCN congeners. An alternative method, based on separation of PCBs and PCNs on activated charcoal, has been used to determine tetra- to octachloronaphthalene isomers [20].

The aim of this study was to develop a high-resolution gel permeation chromatographic (HR-GPC) method for the separation of PCNs and PCBs to make it possible to identify and determine individual PCN congeners, present as by-products in commercial PCBs.

EXPERIMENTAL

Chemicals

The PCB products analysed were Aroclor 1016 (batch no. 1151 77029), 1232 (batch unknown), 1242 (batch no. 399 71696), 1248 (batch no. 398 71691), 1254 (batch no. 370 71698) and 1260 (batch no. 371 71699) [US Federal Drug Administration (FDA)] and Clophen A30 (batch no. M639 18324), A40 (batch unknown) and A50 (batch no. 17012(9479D)) (Bayer, Leverkusen, Germany). The PCN products were Halowax 1001, 1013, 1014 and 1051 (Koppers, Pittsburgh, PA,USA). 1,3-Di-,1,3,6-tri-,1,3,7-tri-,1,4,6-tri-, 2,3,7-tri-, 1,3,5,7-tetra- 1,2,4,6-tetra-, 2,3,6,9-tetra- and 1,2,3,5,7-pentachloronaphthalene were generous gifts from Professor U. A. Th. Brinkman (Free University, Amsterdam, Netherlands). 1,2,3,5,6,7-, 1,2,3,4,6,7-, 1,2,4,5,6,8-, 1,2,4,5,7,8-Hexa-, 1,2,3,4,5,6,7-hepta and octachloronaphthalene were synthesized as described elsewhere [21,22]. Hexachlorobenzene, l-mono- and 1,5-dichloronaphthalene were purchased from Promochem (Wesel, Germany). 1,2,3,4,5,6-Hexachloronaphthalene was a gift from Dr. R. Williams (Environmental Health Directorate, Ottawa, Canada). 2,3,3′,4,4′,5,5′-Heptachlorobiphenyl was prepared according to Sundstrdm [23]. 1 ,1,1-Trimethylpentane was distilled prior to use. Dichloromethane (DCM) and unstabilized tetrahydrofuran (THF) were of HPLC grade (Lab-scan, Dublin, Ireland) and used as received.

Instruments

The HR-GPC equipment consisted of a Merck-Hitachi L-6200 liquid chromatograph, a Rheodyne Model 7125 injector with a 100-μl loop, a variable-wavelength UV detector set at 260 nm and a Merck-Hitachi D-2000 integrator. The HR-GPC column (30 cm x 7.8 mm I.D., 5-μm particles, polystyrene-divinylbenzene copolymer, 50 Å pore size) was obtained from Polymer Labs. (Church Stretton, UK).

Gas chromatography (GC) was performed on a Varian Model 3700 gas chromatograph equipped with an electron capture detector and a DB-5 fused-silica capillary column (30 m × 0.25 mm ID, 0.25-μm film thickness) (J&W Scientific, Folsom, CA, USA). Injections of 2 μl were made in the splitless mode at an injector temperature of 260°C. Helium was used as the carrier gas with a head pressure of 12 p.s.i. (1 p.s.i. = 6894.76 Pa). The GC temperature programme was initially 90°C (2.5 min, splitless), increased at 10°C/min to 150°C, then at 2.5°C/min to 280°C (held for 1 min).

Gas chromatography-mass spectrometry (GC-MS) was performed on a Varian 3400 gas chromatograph coupled with a Finnigan ITS40 ion-trap mass spectrometer. All GC parameters were as described above for GC with electron-capture detection (ECD). The mass spectrometer was programmed to scan for appropriate

intervals of 15 u in seven sequences altering during the acquisition, giving 21 μscans/scan to improve the sensitivity of the mass spectrometric determination.

Methodological development

A test mixture containing equal concentrations of Clophen A50 and Halowax 1014 *(ca. 20* ng/μl) was used to optimize the separation between PCBs and PCNs. Aliquots of 50 μl of this solution were injected on to the HR-GPC column and chromatograms were recorded with UV detection. The optimum separation between PCNs and PCBs was selected from a series of mobile phase-flow-rate combinations, *viz.*, THF or DCM at 0.25, 0.5, 0.7 and 1.0 ml/min.

Using the optimum mobile phase-flow-rate combination, *i.e.*, THF at 0.5 ml/min (see Results and Discussion), the collection window for PCNs was established by injecting two test solutions: (I) Clophen A50 (110 mg/ml) and a mixture (110 μg/ml) of Halowax 1001, 1013, 1014 and 1051 (2 : 2 : 5 : 1); and (II) Clophen A50 (110 mg/ml). The samples were injected on to the HR-GPC column using an over-filled 100-μl loop. The HR-GPC elution windows for PCBs and PCNs were 12-17 and 16-19 min, respectively. The column was severely overloaded in this experiment and it was therefore decided that replicate injections/fractionations were necessary to obtain sufficient purity of the PCN fraction.

The injections were repeated, as described above, and PCNs were recovered by collecting the column effluent from 16 to 21 min after injection. The PCN fractions were then reinjected until complete separation of PCBs and PCNs was achieved. In the final fractionation step, PCNs were collected from 16.5 to 21 min after injection to maintain the purity of the PCN fraction (Fig. 1). The GC calibration internal standard, 2,3,3′,4,4′,5,5′-heptachlorobiphenyl (75 ng), was added to the PCN fractions and the volume was reduced to 100 μl with a gentle stream of nitrogen prior to analysis by GC–ECD.

Determination of PCN in PCB products

Nine PCB products, Aroclor 1016, 1232, 1242, 1248, 1254 and 1260 and Clophen A30, A40 and A50, were fractionated according to the method described above. The PCB products analysed were selected to cover products with different degrees of chlorination. Each PCB product (20 mg) was dissolved in THF and hexachlorobenzene (1.0 μg) was added as an internal standard. The volume was adjusted to 200 μl and a 100-μl aliquot was passed through the HR-GPC column, as described above, and subsequently analysed by GC-MS.

The PCN components were identified from the isotopic patterns of the molecular ion clusters and the retention times relative to octachloronaphthalene. All PCN congeners determined in Halowax 1014 were given numbers according to the number of chlorine atoms and a suffix relating to the GC elution order (see Fig. 2 and Table II).

The analytes were determined against multi-level calibration graphs for each congener level using the internal standard method. The Data Master II data program was used to create the calibration graphs. One PCN congener was used for calibrating each congener level. This will naturally introduce an error into the determination as not all congeners have the same response, but normally the response factors for polychlorinated aromatic compounds, such as PCNs and PCBs, differ by only ±20–40% (95% confidence level) within a congener level [24]. Also, the uncertainties in the determinations will affect only the absolute levels and not the relative levels between different technical PCBs.

Mass chromatograms of the sum of the ions M^+, $(M + 2)^+$ and $(M + 4)^+$ were used for quantification. Peak areas were used for calculating PCN concentrations of >100 pg and peak heights for PCN concentrations of <100 pg.

RESULTS AND DISCUSSION

The optimum separation between PCNs and PCBs was obtained when THF was used as the mobile phase at 0.5 ml/min. At this flow-rate the efficiency was *ca.* 70 000 plates/m for individual isomers of PCB or PCN. The retention times were longer and the resolution between PCNs and PCBs deteriorated if DCM was used as mobile phase instead of THF. The retention times of PCB and PCN were 16.2 and 18.0 min,

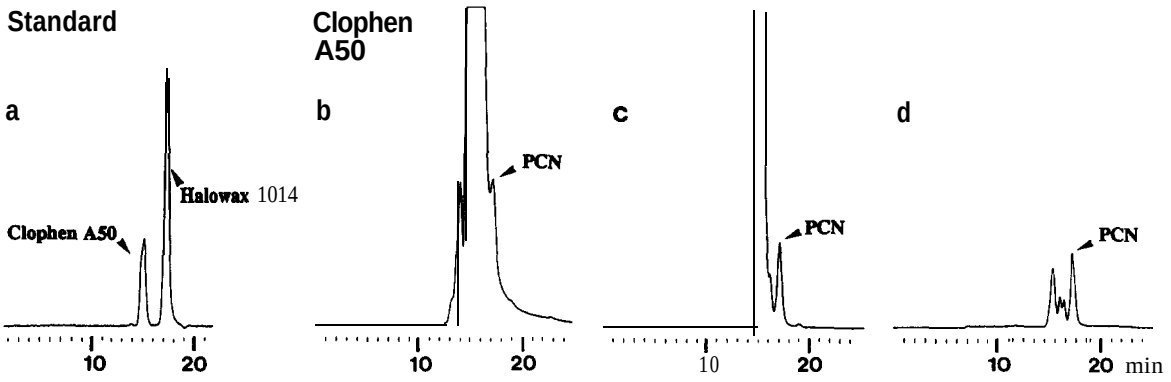

Fig. 1. Chromatograms obtained with UV detection of (a) a mixture of Clophen A50 and Halowax 1014, (b) Clophen A50, first fractionation, (c) Clophen A50, second fractionation, and (d) Clophen A50, third fractionation. For conditions, see Experimental.

respectively, with DCM as mobile phase, and 15.1 and 17.3 min, respectively, with THF. Toluene is an unadsorbed compound that totally permeates the pores of the stationary phase and had a retention time of 16.7 min with both DCM and THF as mobile phase. This suggests that both size-exclusion and adsorption processes are involved in the separation, and that the adsorptive effect is less pronounced when a polar solvent, such as THF, is used. Both PCBs and PCNs interact strongly with the π-electrons of aromatic compounds [25], and the interaction is due to electron donor-acceptor (EDA) complexation. Consequently, the adsorptive effect observed when DCM is used as mobile phase is probably due to EDA interactions between the solutes (electron acceptors) and the stationary phase, which is a polystyrene-divinylbenzene copolymer (r-electron donor).

PCBs (Clophen A50) and PCNs (Halowax 1001, 1013, 1014 and 1051) were completely separated after three separation cycles (Fig. 1). Analysis of the final PCN fraction by GC-ECD showed the recovery of the PCN congeners to be >90% (after correction for the intrinsic PCN content of the PCB). No discrimination of either higher or lower chlorinated naphthalenes (CNs) was observed.

The concentrations of CN homologues and total concentrations of PCNs in the nine PCB products are given in Table I. The total concentrations range from 1.8 to 870 μg/g. The highest

TABLE I

CONCENTRATIONS (μg/g) OF PCN HOMOLOGUES IN AROCLOR AND CLOPHEN PCB PRODUCTS

$C_{10}H_{8-n}Cl_n$	Aroclor[a]						Clophen[a]		
	1016 (38%)	1232 (32-33%)	1242 (40–42%)	1248 (48%)	1254 (52–54%)	1260 (60%)	A30 (40–42%)	A40 (48%)	A50 (52–54%)
?1=1		–	–	–	–	–	–	–	–
$n=2$		96	5.1	–	–	–	–	–	–
$n=3$	1.5	73	77	58	–	–	850	440	–
$n=4$	4.8	6.3	6.7	5.5	–	–	21	74	0.16
$n=5$	0.08	0.58	14	3.2	2.5	–	2.0	290	1.4
$n=6$	0.16	–	0.69	–	0.99	0.53	–	20	0.25
$n=7$		–	0.03	–	–	1.4	–	0.17	–
$n=8$		–	0.008	–	–	0.86	–	–	–
Total PCNs	6.5	170	100	67	3.5	2.7	870	810	1.8

[a] Chlorine contents (%) are given in parentheses in the column headings.

TABLE II

CONCENTRATIONS (μg/g) OF PCN COMPONENTS IN AROCOLOR AND CLOPHEN PCB PRODUCTS

No. of chlorine atoms	Relative retention vs. octaCN	Aroclor"						Clophen"		
		1016 (38%)	1232 (32-33%)	1242 (40–42%)	1248 (48%)	1254 (52–54%)	1260 (60%)	A30 (40–42%)	A40 (48%)	A50 (52–54%)
DiCN										
2-l	0.260			3.3						
2-2	0.262		34							
2-3	0.266		37	1.8						
2-4	0.272		16							
2-5	0.307		9							
TriCN										
3-1	0.341	0.05	6.7	7.7	8.4			67	62	
3-2	0.347	0.95	51	36	50			750	240	
3-3	0.358		6.2	6.0				34	25	
3-4	0.361		3.9	4.8					30	
3-5	0.367		5.7	7.4					62	
3-6	0.386	0.46		11					19	
3-7	0.410			4.0						
TetraCN										
4-l	0.422	0.02	0.33	0.37	0.2				7.5	
4-2	0.444	1.3	2.5	2.1	2.6			19	36	0.16
4-3	0.458	0.64	0.91	0.71	1.3			1.6	11	
4-4	0.468	0.11	0.45	0.06	0.21			0.66	1.7	
4-5	0.471	0.05		0.055					0.7	
4-6	0.473	0.73		0.58					2.8	
4-7	0.481	0.15		0.05					1.1	
4-8	0.484	0.17		0.05					1.5	
4-9	0.489	0.08		0.02					0.36	
4-10	0.494	0.42	0.66	0.52					2.6	
4-11	0.500									
4-12	0.503	0.91	1.5	1.9	1.2				8.5	
4-13	0.523	0.04		0.27						
4-14	0.539			0.05					0.64	
PentaCN										
5-l	0.563	0.08	0.25	2.2	1.7	1.6		2.0	100	1.4
5-2	0.576			0.21					6.6	
5-3	0.582			2.2					47	
5-4	0.586			0.57	0.18				15	
5-5	0.589			0.91	0.15				26	
5-6	0.607			0.07					0.72	
5-7	0.611		0.14	1.6	0.55	0.45			28	
5-8	0.618		0.19	2.2	0.64	0.41			51	
5-9	0.623			1.6					4.6	
5-10	0.634			1.8					1.3	
5-11	0.640			0.21					0.56	
5-12	0.663			0.22					0.73	

(Continued on p. 84)

TABLE II *(continued)*

No. of chlorine atoms	Relative retention vs. octaCN	Aroclor[a]				Clophen[a]				
		1016 (38%)	1232 (32-33%)	1242 (40–42%)	1248 (48%)	1254 (52-54%)	1260 (60%)	A30 (40–42%)	A40 (48%)	A50 (52–54%)
HexaCN										
6-l	0.706			0.10		0.66	0.34		9.6	0.25
6-2	0.725			0.13			0.09		3.4	
6-3	0.733	0.08		0.18			0.05		3.5	
6-4	0.740	0.08		0.13					0.80	
6-5	0.761			0.10		0.33	0.05		2.3	
6-6	0.775			0.05						
HeptaCN										
7-l	0.869			0.02			1.3		0.17	
7-2	0.871			0.01			0.06			
OctaCN										
8	1.00			0.008			0.86			

[a] Chlorine contents (%) are given in parentheses in the column headings.

concentrations of PCNs were found in products containing 30–40% of chlorine. The levels of PCN in Clophen A30 and A40 exceeded those found in Aroclor 1232 and 1242 by factors of 5 and 8, respectively.

The average degree of chlorination of the CNs was higher than the corresponding chlorination degree of the PCB products, which is to be expected as naphthalene has a greater mean electron localization energy than biphenyl, and consequently a higher reactivity [26–28].

The concentrations of PCN homologues follow a general trend in most of the PCB products. Normally, the percentage of the individual CN homologues increases as the degree of chlorination increases up to the most abundant homologue. The percentage of each homologue then decreases with a further increase in the degree of chlorination. However, in Clophen A40 and Aroclor 1242, a different pattern occurs between the different PCN homologues. The total concentration of tetraCN isomers in these two products is lower than the level in both tri- and pentaCNs. These two PCB products also contain the widest range of PCN homologues.

The concentrations of the individual CNs measured are given in Table II. Relative retention times with respect to octachloronaphthalene (retention time 53.5 min) are also given in Table II. Seventeen of the PCN congeners present in the PCB products and Halowax 1014 have been tentatively identified as follows: 1,3- and/or 1,5-diCN (2-l); 1,3,6- and/or 1,3,7- and/or 1,4,6-triCN (3-2); 2,3,7-triCN (3-5); 1,3,5,7-tetraCN (4-l); 1,2,4,6-tetraCN (4-2); 1,4,6,7-tetraCN (4-3); 1,2,3,5,7-pentaCN (5-l); 1,2,3,4,6,7- and/or 1,2,3,5,6,7-hexaCN (6-l); 1,2,4,5,6,8- and/or 1,2,4,5,7,8-hexaCN (6-4); 1,2,3,4,5,6-hexaCN (6-5); 1,2,3,4,5,6,7-heptaCN (7-l); 1,2,3,4,5,6,8-heptaCN (7-2); octaCN (8).

The peak patterns of the PCB products studied, except Aroclor 1242, are all similar. The peak-patterns of Aroclor 1242 are different not only from those of the other PCB products, but also from those of commercial PCN products (Halowax 1001, 1013, 1014 and 1051) (*cf.*, Fig. 2).

Minor amounts of naphthalene have been identified in technical biphenyl used for PCB production [29], and the chlorination of naphthalene would most likely occur simultaneously with the chlorination of biphenyl. The differences in peak patterns betwen the PCNs in the PCB products and the technical PCN products, which are produced by a similar chlorination process to PCBs, are difficult to explain, however.

Even though the levels of PCNs in the PCB

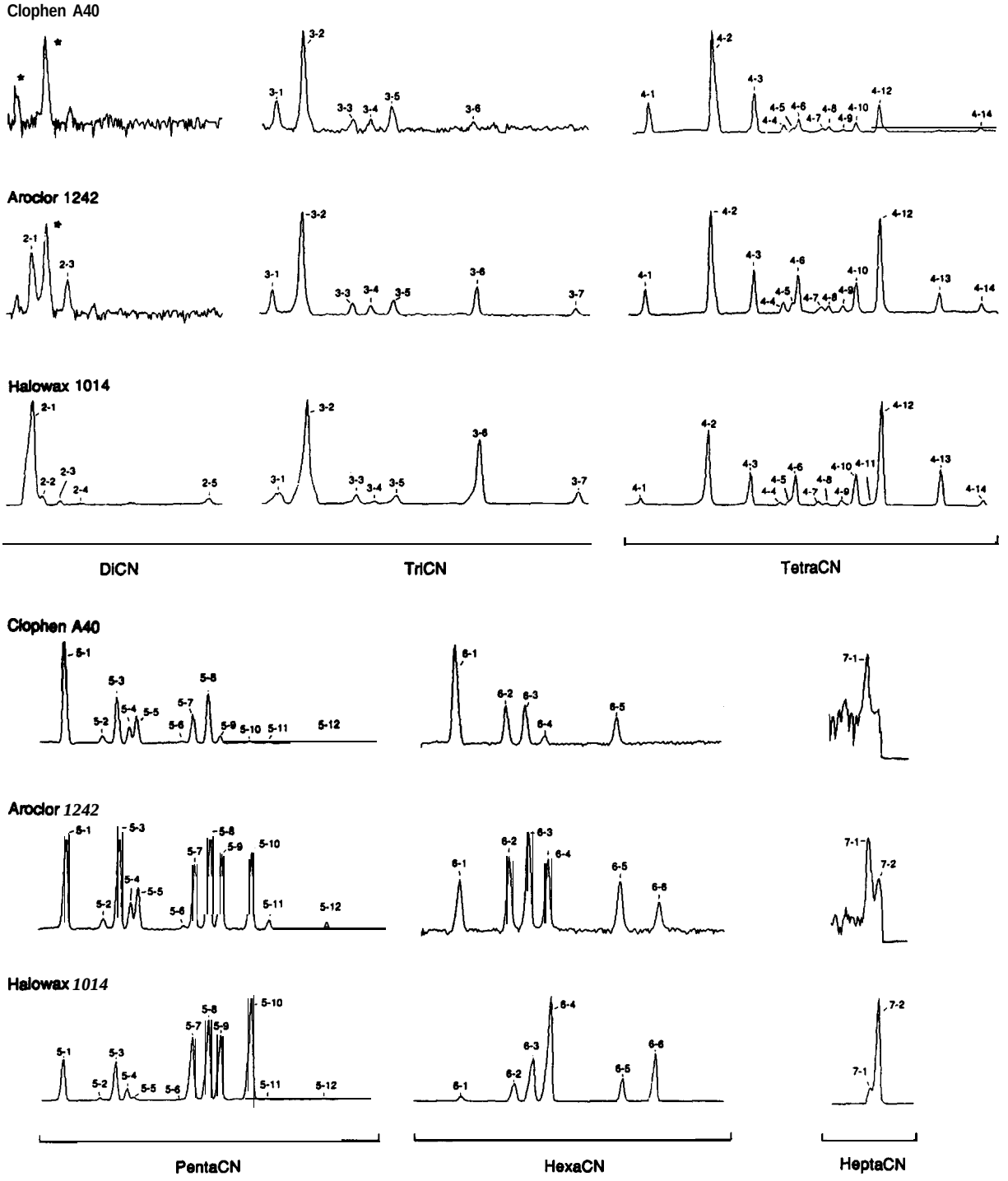

Fig. 2. Mass chromatograms of **diCN** to **heptaCN** in Clophen A40 (top), Aroclor 1242 (middle) and **Halowax** 1014 (bottom). The isomers are numbered according to the number of chlorine substituents and GC elution order of the isomers in **Halowax** 1014. The peaks marked with asterisks are not PCN peaks. For conditions, see Experimental.

products may seem high, the total amount of PCNs formed in this way must be smaller than the total world production of technical PCNs. If the batches studied are representative of the world production, and the relative amounts Clophen A30, A40 and A50 produced are the same as those for Aroclor 1242, 1248 and 1254, the total amount of PCNs formed can be calculated from PCB production figures found in the literature [24,30]. Using these assumptions, the total amount of PCN present as by-products in the PCB products investigated was calculated to be cu. 120 metric tons. Total national or world production figures for PCNs are difficult to obtain but it is known that the annual production from the largest PCN manufacture, Koppers, was 3200 metric tons in 1956, and that the production had declined to ca. 2300 metric tons in 1972 [31]. Hence the amount of PCNs present as by-products in PCBs is most likely <1% of the total production of PCNs.

ACKNOWLEDGEMENT

This study was given financial support from the Swedish Environmental Protection Agency, project programme PETOS.

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