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Characterization of technical toxaphene using combined high-performance liquid chromatography–gas chromatography–electron capture negative ionization mass spectrometry techniques

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Abstract

More than 300 congeners in technical toxaphene were distinguished by combined HPLC–GC/electron capture negative ionization mass spectrometry (ECNIMS) techniques. Good separation of most congeners on a 60-m DB-5 GC column was achieved when the mixture was pre-separated into five fractions by silica gel HPLC. ECNI spectra of high chlorinated congeners were dominated by M − Cl while both M and M − Cl ions were important in low chlorinated ones. Other ions such as M − 3Cl, M − Cl − HCl and M − Cl − 2HCl can be used to distinguish congeners. Peaks referred to as T2 and T12 in the environment by many authors coelute with several congeners in toxaphene. The 2,5-exo-3,6-endo tetrachloro partial structure existing in both T2 and T12 may be responsible for their persistency in living systems. Results from EI-SIM mode indicated many other low chlorinated congeners not detectable by ECNI in the mixture.

1. Introduction

The attention to the presence of toxaphene residues, also known as polychlorocamphenes (PCCs), in the environment has been intensified in the recent years due to the introduction of electron capture negative ionization (ECNI) technique to the analysis of PCCs. Toxaphene residues have been found in the environment and in many species ranging from fish to wildlife [1,2]. It has been recognized as one of the major halogenated environmental pollutants along with PCBs, PCDD/PCDFs, chlordanes, DDTs and HCHs. Although several quantitation methods have been applied, most of the efforts to determine toxaphene residues are done lacking reliable standards because the individual congeners are not commercially available [3–5]. Quite a few laboratories are trying to isolate or synthesize individual PCC congeners. But before the congeners become commercially available, which probably will not happen in the near future, most laboratories have to rely on using technical toxaphene as the standard to quantify toxaphene residues in environmental samples.

Although the individual chemicals making up PCB (Aroclors and Clophens) and chlordane have been determined [6,7], toxaphene is still

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not well characterized due to its complexity. Technical toxaphene contains probably more than 600 compounds with mainly bornane and bornene skeletons [8,9]. Chlorinated compounds with a camphene skeleton were also found in the mixture [10]. Compounds in toxaphene are largely unresolved on either DB-5 or DB-1 (and their equivalent) columns which can compromise their use as quantitation standards. A few attempts have been made to study the toxaphene mixture using different gas chromatography–mass spectrometric (GC–MS) methods [8,11], but congener-specific characterization was not reported in these studies.

Because selective metabolism and bioaccumulation of toxaphene congeners have been observed [12], it is important to characterize the individual components of toxaphene in order to understand the behavior of toxaphene residues in the environment. The present paper reports the results of an investigation to separate technical toxaphene and characterize its components by their ECNI mass spectra, and how these components relate to congeners in environmental samples.

2. Experimental

2.1. Chemicals

Technical toxaphene was purchased from Supelco (USA) (Cat No. 4-9080, Lot No. LA 28615).

2.2. HPLC pre-separation

Toxaphene was pre-separated into five fractions on a Supelcosil LC-Si column (25 cm × 4.6 mm) using a Varian 9010 solvent delivery system with a Varian 9050 UV–Vis detector. Toxaphene was diluted with hexane to a concentration of 2.5 mg/ml before injection. Mobile phase was set at a flow rate of 0.5 ml/min. using hexane (0 to 15 min), methylene chloride (15 to 25 min) and returned to initial status for re-injection with hexane (25 to 30 min). UV wavelength was set at 210 nm for the first 20 min and at 254 nm from 20 to 30 min. Ten 40 μl injections were collected and the final volume of the five fractions was adjusted to 2 ml in hexane for GC–MS analysis.

2.3. GC–MS analysis

Conditions were similar to our previous description [2]. Briefly, analyses were performed on a Hewlett-Packard (HP) 5985 mass spectrometer upgraded with a HP 5890 series II gas chromatograph coupled with a HP 5988 GC–MS direct interface. Conditions were set as follows: column: DB-5 (60 m × 0.25 mm × 0.25 μm); carrier gas: helium; oven temperature: 60°C (2 min), 20°C/min to 200°C, 3°C/min to 270°C (26 min); injector: splitless (1.5 min), 250°C; transfer line temperature: 260°C; ion source temperature: 200°C (EI), 130°C (ECNI); source pressure (ECNI): 0.5 bar; reagent gas (ECNI): methane; mass scan range (ECNI): 150–500 amu; SIM (EI): 83, 159, 161, dwell time 150 ms each.

3. Results and discussion

The total ion chromatogram (TIC) of technical toxaphene using ECNI-MS (scan range 150–500 amu) is illustrated in Fig. 1. Because of the complexity of the mixture, most peaks were not fully resolved, especially in the range of 30 to 40 min where most of the hepta-, octa- and nona-

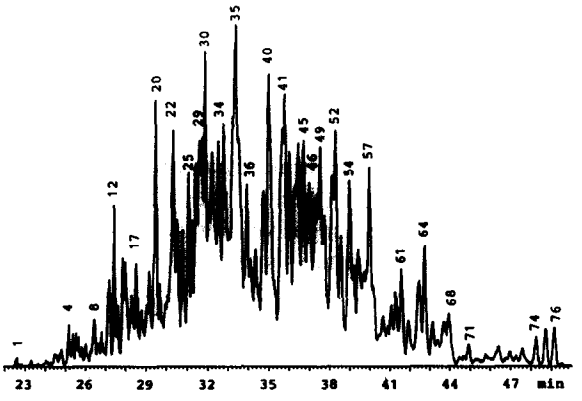

Fig. 1. Total ion chromatogram (150–500 amu) of technical toxaphene on a DB-5 column using ECNI-MS detector. The peak numbers refer to Table 2.

chlorinated congeners elute. Seventy six partially resolved peaks could be observed in the chromatogram (Fig. 1) with possible coelution within each peak. In order to achieve a better separation it is necessary to pre-separate the mixture by liquid chromatography. Several stationary phases including Florisil, silica gel and aluminum oxide have been used in the sample preparation for toxaphene residue analysis [3,13]. Reversed-phase (C_8 and C_{18}) columns were used in the isolation of two dominant congeners, T2 (a Cl_8-congener) and T12 (a Cl_9-congener), in environmental samples [14,15]. We chose silica gel HPLC for the pre-separation because the mixture was spread over quite a wide range on the HPLC chromatogram as illustrated in Fig. 2. Five fractions were collected according to the peak domains on the HPLC chromatogram.

Even though some congeners could elute in two HPLC fractions, pre-separation allowed us to achieve good resolution of congeners on the DB-5 GC column. Because the chromatogram is much simpler and the coelution problem was significantly minimized, we were able to distinguish more than 300 compounds in the five HPLC fractions. Each compound was characterized by M, M − Cl and other fragments where they were appreciable (for the mass of major fragments see Table 1). The results are summarized in Table 2. The first column of Table 2 shows the peak number in Fig. 1. Alphabetic

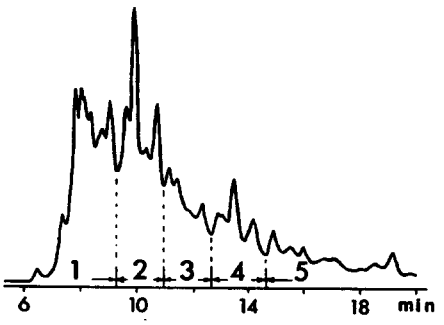

Fig. 2. HPLC chromatogram of toxaphene on Supelcosil LC-Si column using hexane as mobile phase. Five fractions were collected as indicated by the dashed lines.

characters in the second column indicate various components within each peak. Two characters in the same row imply a possibility of two components because the peak was present in more than two fractions. The chance for one compound to be present in three or more HPLC fractions is very slim, but due to the similarity of the spectra we could not confirm that more than one component was present. The third column gives the retention time of each compound. Elution overlaps were observed between different chlorine level congeners, especially for the low chlorinated ones (footnote, Table 2). The fourth and fifth columns present a brief description of the ECNI spectra. In general, toxaphene congeners produce molecular, M − Cl, M − 2Cl

Table 1
Major ions (m/z) in the ECNI spectra of chlorinated bornanes and bornenes

No. of chlorine	Mol. ion		M − Cl		M − HCl		M − Cl_2H		M − Cl_3	
	-ane	-ene	-ane	-ene	-ane	-ene	-ane	-ene	-ane	-ene
10	478	476	443	441	442	440	407	405	373	371
9	444	442	409	407	408	406	373	371	339	337
8	410	408	375	373	374	372	339	337	305	303
7	376	374	341	339	340	338	305	303	271	269
6	342	340	307	305	306	304	271	269	237	235
5	308	306	273	271	272	270	237	235	203	201
4	274	272	239	237	238	236	203	201	169	167
3	240	238	205	203	204	202	169	167		
2	206	204	171	169	170	168				
1	172	170								

Table 2
Polychlorocamphene congeners in technical toxaphene

GC peak		RT (min) [a]	m/z (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
1	a	22.63		306	5-ene	3000				
2	a	24.50	307 (70)	342, 308 (80)	6-ane	1000				
	b	24.59	305	340 (70)	6-ene	1000				
3	a	24.76		306	5-ene				1200	
4	a	25.20		340	6-ene	3000				
	b	25.20		306	5-ene				1100	
	c	25.20	307	342 (50), 306 (30)	6-ane	1200	3500			
5	a,b	25.37	273	272 (50)	5-ane		3000	2000	1600	
6	a	25.56	273	272 (10)	5-ane				2500	8000
7	a,b	25.67	273	272 (40)	5-ane		1600	800	3500	
8	a	26.34	339	374 (25)	7-ene	2500				
	b	26.43	273	272 (10)	5-ane				3000	10 000
	c	26.48	273		5-ane		1200	2000		
	d	26.50	305	340 (50)	6-ene	1500				
9	a	26.68		306	5-ene				1000	
10	a	26.82		306	5-ene				1000	
11	a	27.26	341	376 (40), 340 (10)	7-ane	12 000				
12	a	27.47	307	342 (10), 306 (40)	6-ane		30 000			
	b	27.49	307	306 (50)	6-ane	12 000				
	c	27.50	273	308 (25)	5-ane					1600
13	a	27.64	305(20)	340, 304 (20)	6-ene				3500	
	b	27.67	339		7-ene	1400				
14	a	27.86	273	308 (50)	5-ane					1200
	b	27.90	307	340 (15), 306 (20)	6-ane		17 000			
	c	27.91	307		6-ane	7000				
15	a	28.01	339		7-ene	2500				
	b	28.02	305 (50)	340	6-ene		7000			
	c	28.03	305 (60)	340	6-ene				2400	
	d	28.10	307		6-ane		7000			
16	a	28.32	305	340 (70)	6-ene					2000
	b	28.34	307		6-ane	3000	14 000			
	c	28.39	307	342 (25), 306 (10)	6-ane					4000

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
17	a	28.49	341	306 (30)	7-ane	4000				
	b	28.54		340, 304 (20)	6-ene				4000	
	c	28.54		340	6-ene					5500
	d	28.57	339	374 (40)	7-ene	6000	2000			
	e	28.57	305	340 (95)	6-ene		1200			
	f	28.57	307		6-ane	2000				
18	a	28.81	339	374 (20)	7-ene	4000	4000			
19	a	29.04	307	306 (60)	6-ane		3500	4000		
	b	29.11	339		7-ene	2000				
	c	29.21	341	376 (30), 340 (25)	7-ane	12 000				
	d	29.27	339		7-ene		5000			
20	a	29.37	307	306 (50)	6-ane		6000			
	b	29.40	307		6-ane			12 000		
	c	29.47	341		7-ane			4000		
	d,e	29.50	307	306 140)	6-ane		25 000	20 000	30 000	
	f	29.56	341	306 (15)	7-ane	3000				
	g	29.57	307	342 (40), 306 (30)	6-ane					6000
21	a	29.68	339	374 (50)	7-ene		2500			
	b	29.71	339 (40)	374	7-ene	4000				
	c	29.73	307 (50)	306	6-ane		5000	4000		
	d	29.84	307	306 (30)	6-ane				1600	
	e	29.86	307	342 (30), 306 (20)	6-ane					4000
	f	29.86	373		8-ene	1000				
22	a	29.95	339	374 (50), 304 (80), 276 (50)	7-ene		2000			
	b	29.99	373		8-ene	1000				
	c	30.06	307	306 (50)	6-ane					8000
	d	30.08	339	374 (20)	7-ene		1000	1600		
	e	30.10	341	376 (20)	7-ane		2000			
	f	30.20	339 (80)	374	7-ene		3000			
	g	30.24	339 (70)	374	7-ene				5000	
	h	30.33	341		7-ane		20 000	20 000		
	i	30.37	339	374 (70)	7-ene				4500	
	j	30.38	339	374 (100), 304 (30)	7-ene		12 000	12 000		
	k	30.40	307	306 (10)	6-ane					5000
23	a	30.48	339	374 (5)	7-ene	3000				
	b	30.52	341	376 (15)	7-ane		8000			
	c	30.52	307	306 (10)	6-ane				5000	10 000
	d	30.54	339	374 (20), 304 (90)	7-ene			3000		
	e	30.59	339 (30)	374 (30), 304	7-ene		12 000			
24	a	30.72		374	7-ene		1600			
	b	30.78	307	306 (30)	6-ane				1200	4000
	c	30.81	341	340 (30)	7-ane				4000	
	d	30.82	341		7-ane		4000			

(*Continued on p. 108*)

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
	e	30.82		374	7-ene		1000			
	f	30.90	307	306 (25)	6-ane					4000
25	a	31.00	307	306 (50)	6-ane				3000	
	b	31.03	307	306 (25)	6-ane					4000
	c	31.08	341		7-ane		6000	7000		
	d	31.08		374	7-ene			8000		
	e	31.11	339 (20)	374	7-ene				10 000	8000
	f	31.15	341		7-ane					3000
26	a	31.25	339	374 (60)	7-ene		6000	4000		
	b	31.26	339	374 (80)	7-ene				10 000	
27	a	31.39	373		8-ene	1000				
	b	31.40	341	376 (25)	7-ane					5000
	c	31.40	307		6-ane					2000
	d	31.42	341		7-ane	6000	30 000			
	e	31.48	341	376 (10)	7-ane			8000		
28	a	31.61	341		7-ane				2000	
	b	31.61	307	306 (10)	6-ane				4000	40 000
	c	31.64	341	376 (30)	7-ane					2500
	d	31.67	373		8-ene	6000				
29	a	31.69	341	340 (20)	7-ane		5000	5000		
	b	31.71	373	408 (40)	8-ene	7000	7000			
(T2)	c	31.74	375	374 (35), 303 (6)	8-ane	20 000				
	d	31.82	307	306 (20)	6-ane					3500
30	a	31.87	373		8-ene	2000				
	b	31.89	341	340 (20)	7-ane		60 000	13 000		
	c	32.00	373		8-ene	4000	2000			
31	a	32.03	341		7-ane		14 000	10 000		
	b	32.07	373		8-ene	5000				
	c	32.10	339	374 (10)	7-ene				6000	16 000
	d	32.14	341		7-ane		16 000			
32	a	32.22	375		8-ane	16 500	6000			
	b	32.23	341	340 (25)	7-ane	4000	16 000			
	c	32.27	341		7-ane			16 000	2500	
	d	32.36	373 (50)	408 (30), 325	8-ene	12 000	2500			
	e	32.43	341	340 (25)	7-ane		9000			
	f	32.45	341		7-ane			12 000		
33	a	32.55	341	376 (20)	7-ane		18 000	8000		
	b	32.55	341	376 (20), 340 (20)	7-ane				20 000	
	c	32.55	375		8-ane	6000				
	d	32.66	341		7-ane					16 000

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
34	a	32.76	341		7-ane	2000	16 000			
	b	32.82	373	408 (50)	8-ene	9000	25 000			
	c,d	32.90	341	340 (25)	7-ane		20 000	12 000	3000	
	e	33.02	373		8-ene	1000	2000			
	f	33.07	341		7-ane		10 000	9000		
	g	33.07	341		7-ane		8000			
	h	33.12	373		8-ene			2000		
35	a	33.22	339	268 (20)	7-ene					12 000
	b,c	33.26	341	340 (10)	7-ane		16 000	12 000	30 000	
	d	33.29	373		8-ene		3000			
	e,f	33.40	341	340 (10)	7-ane		30 000	45 000	50 000	16 000
	g	33.47	373	408 (30), 312 (60)	8-ene	6000				
	h	33.48	373	408 (5)	8-ene		4000	3000		
	i,j	33.56	341		7-ane			6000	8000	20 000
	k	33.57	375	410 (15)	8-ane		8000	11 000		
	l	33.62	375	305 (95), 340 (50)	8-ane	6000				
	m	33.63	373		8-ene				3000	
	n	33.65	341		7-ane				1500	
	o	33.65	373	408 (90)	8-ene		7000			
	p	33.67	373		8-ene					1200
	q	33.71	373		8-ene	2500				
	r	33.74	341		7-ane					5000
36	a	33.84	373		8-ene					1600
	b	33.85	375	340/341 (30)	8-ane				4000	
	c	33.86	375	410 (30)	8-ane		3500			
	d,e	33.94	341	340 (70)	7-ane			6000	4000	12 000
	f	33.96	373		8-ene			1800		
	g	33.97	375		8-ane	16 000				
	h	33.97	341		7-ane	2500				
	i	34.00	373	408 (40)	8-ene		3000			
37	a	34.10	341	340 (20)	7-ane					6000
	b	34.11	373		8-ene					2000
	c	34.19	341		7-ane		3500	5000		
	d	34.28	373	408 (30)	8-ene		2400			
38	a	34.33	341		7-ane					6000
	b,c	34.36	375	410 (20), 340/341 (80)	8-ane		3000	3000	3200	
	d	34.41	341		7-ane				3000	
	e	34.46	341		7-ane					5000
39	a	34.70	341		7-ane			3000	6000	
	b	34.71	373	408 (20)	8-ene		10 000			
	c	34.73	375	410 (40), 305 (5)	8-ane			7000		
	d	34.74	341		7-ane		3000			
	e	34.76	375	410 (15), 305 (10)	8-ane		12 000			
	f	34.80	341		7-ane					20 000

(*Continued on p. 110*)

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
40	a	34.96	373		8-ene					8000
	b	35.00	375	305 (60)	8-ane	5000				
	c	35.00	341	340 (30)	7-ane					4000
	d	35.01	375	305 (25)	8-ane		26 000			
	e	35.02	375	338/339 (10)	8-ane				27 000	
	f	35.09	373		8-ene					2500
	g	35.11	375		8-ane		17 000	16 000		
	h	35.15	375		8-ane	8000				
	i	35.22	341		7-ane					2500
	j	35.30	375	305 (20)	8-ane	3000	6000			
	k	35.30	375	410 (20)	8-ane		6000			
41	a	35.47	373		8-ene					2500
	b	35.55	341		7-ane					5000
	c	35.58	375		8-ane	12 000				
	d	35.63	373	302 (15)	8-ene					6000
	e	35.67	375	305 (5)	8-ane			13 000		
	f	35.81	375		8-ane		45 000	30 000		
	g	35.83	375		8-ane	6000				
	h	35.87	375		8-ane					1200
42	a	36.02	375	339 (15)	8-ane	5000	15 000			
	b	36.04	341		7-ane				2500	25 000
	c	36.07	375	305 (20)	8-ane			2500	1000	
	d	36.14	375		8-ane					8000
43	a	36.26	373		8-ene					12 000
	b	36.30	375		8-ane			5000		
	c	36.35	375		8-ane				3500	
	d	36.35	375		8-ane	6000	13 000			
44	a	36.46	407		9-ene	1500				
	b,c	36.48	375		8-ane	5000	50 000	6000		
45	a	36.63	341	304 (20)	7-ane					2500
	b	36.69	375	408 (8)	8-ane	8000	28 000			
	c	36.77	341	304 (10)	7-ane					16 000
	d	36.77	375		8-ane				2000	3000
	e	36.87	375		8-ane	2500				
46	a	36.93	375		8-ane		16 000	12 000		
	b	36.98	407		9-ene		6000	6000		
	c	37.00	375		8-ane				5000	
(T12)	d	37.07	409	337 (20)	9-ane	16 000	7000			
47	a	37.25	375		8-ane					2500
	b	37.28	375	305 (12)	8-ane			8000	16 000	
	c	37.32	375	305 (15)	8-ane		12 000			
48	a	37.34	409		9-ane		2000			
	b	37.37	375		8-ane	2000				

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
	c	37.40	375	305 (20)	8-ane					5000
	d	37.42	375	410 (10), 305 (15)	8-ane			6000	10 000	
	e	37.48	409	339 (15)	9-ane	7000				
49	a	37.53	409		9-ane		5000			
	b	37.54	409		9-ane					2000
	c,d	37.58	375		8-ane		7000	5500	12 000	10 000
50	a	37.75	375	305 (10)	8-ane		11 000	16 000		
	b	37.77	409		9-ane					2500
	c	37.79	375		8-ane				10 000	
	d	37.82	375		8-ane					2000
51	a	38.02	375		8-ane					5000
	b	38.05	409		9-ane					2000
	c	38.12	375		8-ane				5000	
	d	38.14	375		8-ane		16 000	20 000		
	e	38.15	409		9-ane		3000			
	f	38.18	407	442 (40)	9-ene		3000	4000		
52	a,b	38.28	375	340 (15), 305 (20)	8-ane		9000	15 000	22 000	12 000
	c,d	38.32	409	339 (8)	9-ane	6000	4000	6000		
53	a,b	38.56	375	305 (15)	8-ane		6500	8000	10 000	8000
	c	38.70	375	305 (5)	8-ane					3000
54	a	38.91	409	339 (30)	9-ane		2000			
	b	38.92	409		9-ane					1000
	c	38.92	407		9-ene					1000
	d	38.93	375	305 (60)	8-ane		2500	3000		
	e	39.01	407		9-ene			2500	3000	1500
	f	39.03	409		9-ane			2500		
	g	39.03	409		9-ane					1500
	h	39.04	409	339 (25)	9-ane	25 000				
55	a	39.11	375		8-ane					2000
	b	39.17	409	339 (20)	9-ane		3000			
	c	39.22	409	339 (80)	9-ane	10 000				
56	a	39.42	375	305 (15)	8-ane				2500	12 000
	b	39.46	409	444 (30), 339 (30)	9-ane	5000				
	c	39.50	409	339 (160)	9-ane		3000			
	d	39.58	375	305 (5)	8-ane					10 000
	e	39.69	409		9-ane	4000				
	f	39.69	409		9-ane					1200
57	a,b	39.82	375		8-ane			1700	6000	20 000
	c	40.01	409	373 (7)	9-ane	40 000				
	d	40.04	409	339 (20)	9-ane		2500			
	e	40.04	375		8-ane					3000

(*Continued on p. 112*)

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
	f	40.09	409		9-ane					3000
	g	40.23	409	444 (2), 339 (5)	9-ane	3000	8000			
58	a	40.48	375	305 (30)	8-ane					1200
	b	40.67	409		9-ane		3200			
	c	40.75	443		10-ane					1600
	d	40.80	409		9-ane		1100			
	e	40.92	409	339 (25)	9-ane	2000				
59	a	41.04	375		8-ane				1000	3000
	b	41.06	443		10-ane					1000
	c	41.09	411	341 (20)	9-ane + H_2					4000
60	a	41.26	375		8-ane					3000
	b	41.29	409	339 (60)	9-ane	3000				
	c	41.30	409		9-ane		10 000	3000		
	d	41.34	409	339 (30)	9-ane	2500				
61	a	41.57	409	339 (10)	9-ane			4500		
	b	41.57	409		9-ane					1200
	c	41.60	409	339 (15)	9-ane		5000			
	d	41.61	373		8-ene	1600	1000			
	e	41.61	409	339 (10)	9-ane				6000	
62	a	41.90	409	339 (80), 373 (50)	9-ane		2000			
	b	42.25	409	339 (40)	9-ane		1000			
63	a	42.41	409	339 (30), 373 (10)	9-ane		6000			
	b	42.49	411		9-ane + H_2					4000
	c	42.49	409		9-ane			1200	2500	
	d	42.52	409	339 (30)	9-ane	6000				
64	a,b	42.76	409	339 (25)	9-ane	2500	11 000	9000	3000	
	c	42.84	443		10-ane					800
	d	42.86	411		9-ane + H_2					2000
65	a	43.16	409	373 (5), 339 (5)	9-ane		7000	2500		
	b	43.33	409		9-ane		3000			
66	a	43.44	409		9-ane			1600		
67	a	43.70	409	339 (20)	9-ane					2000
	b	43.70	409	339 (20)	9-ane		4500			
	c	43.76	409		9-ane				1200	
68	a	43.93	409		9-ane		2500			
	b	43.95	409	373 (20)	9-ane			3500		
	c	44.00	443	373 (70)	10-ane	2500				
	d	44.10	409		9-ane					1200

Table 2 (*continued*)

GC peak		RT (min) [a]	*m/z* (rel. abund.) [b]		Structure	Base peak abundance, Fraction				
			M − Cl ion	Other ions		1	2	3	4	5
69	a	44.57	409		9-ane					700
	b,c	44.94	409		9-ane			1200	1200	2000
70	a	46.47	443	373 (25)	10-ane	3000				
71	a	46.99	445	375 (45)	10-ane + H_2					2500
72	a	47.30	443	373 (60)	10-ane	800				
73	a	47.60	445	375 (30)	10-ane + H_2					2000
74	a	48.27	443	373 (15)	10-ane	6000				
75	a	48.73	445	375 (45)	10-ane + H_2				1200	5000
76	a	49.14	445	375 (30)	10-ane + H_2				1000	3000

[a] Retention time range for Cl_5 isomers (22–28 min), Cl_6 (25–32 min), Cl_7 (26–37 min), Cl_8 (29–45 min), Cl_9 (36–45 min) and Cl_{10} (40–50 min).
[b] Number without ( ) is base peak.

and M − 3Cl clusters in their ECNI spectra. These four clusters were examined and those with a relative abundance greater than 5% were recorded in the table (expressed as *m/z*), except for molecular ions. The next column gives composition and structure information. The first numeral is the chlorine number of the component followed by characters which show a bornane (ane), bornene (ene) or dihydrobornane (ane + H_2) skeleton. The last five columns show the presence of components in each HPLC fraction with the base peak abundance from their ECNI spectra. Base peak height provides some idea about the relative quantity of each component.

Although the ECNI spectra of toxaphene congeners are dominated by M − Cl for high chlorinated congeners (deca- nona- and octa-) and by both M and M − Cl for low chlorinated ones (hexa- and penta-), relative abundance of these two ions varies from congener to congener (Fig. 3). Other ions such as M − 3Cl, M − Cl − HCl, M − Cl − 2HCl and M − HCl are also important and could add valuable information in distinguishing structures. Polychlorobornenes have two masses less than bornanes. The fragmentation of bornenes is very similar to bornanes, but the M − 3Cl ion is usually not observed. Polychlorobornadienes were as reported present in toxaphene [1], but we did not detect any dienes, probably due to their low concentration in the mixture.

3.1. Decachloro derivatives

Besides the M − Cl fragments, further loss of additional two chlorines (M − Cl − 2Cl) was observed. Ion 445 (M − Cl), which appears quite frequently in some peaks, implies a dihydro bornane structure (M = 480, two amu higher than that of bornane). Fig. 3A and 3B give examples of a bornane and a dihydrobornane spectra.

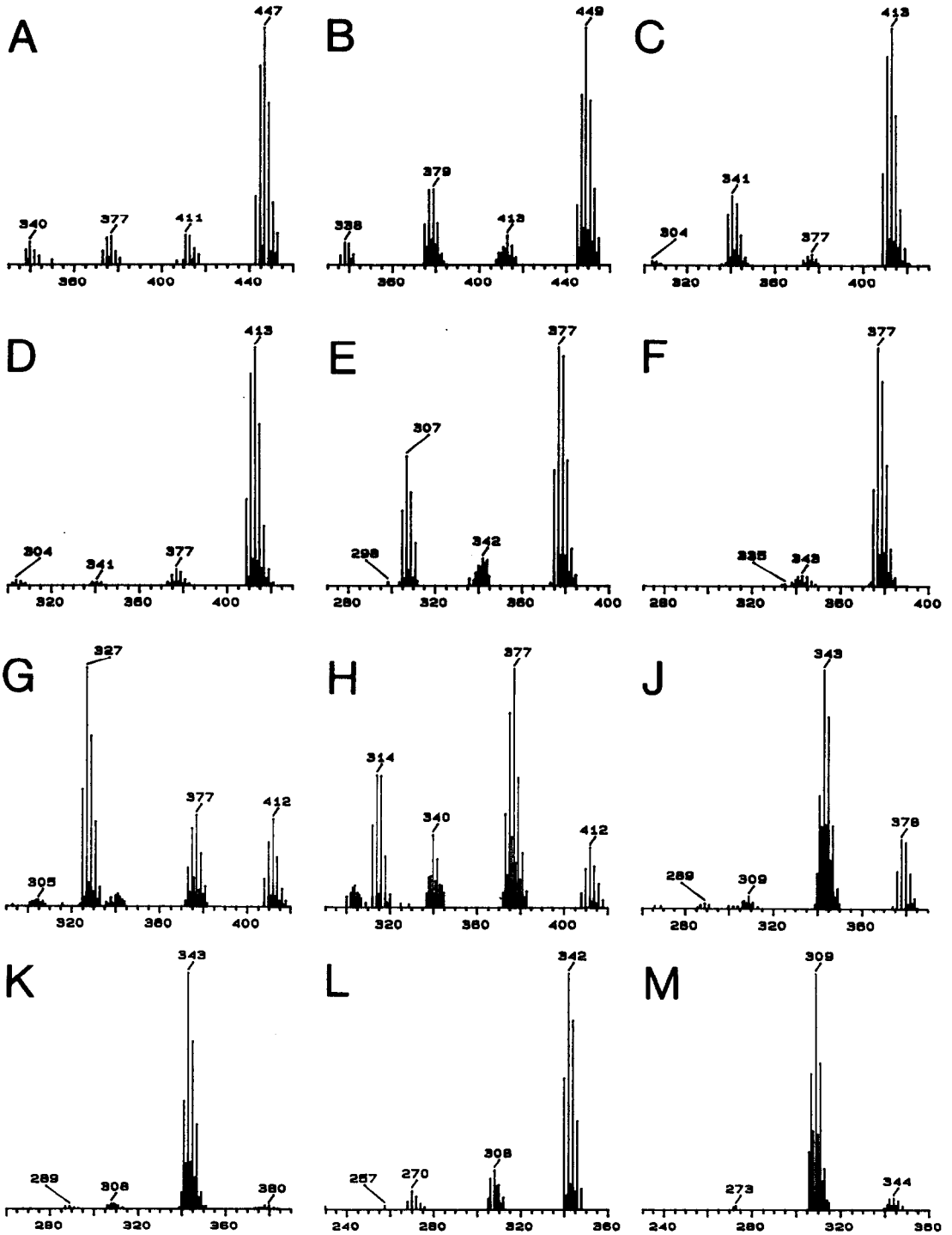

Fig. 3. ECNI spectra of selected peaks from five HPLC fractions of toxaphene. (A) 74a, Fr. 1; (B) 75a, Fr. 5; (C) 54h, Fr. 1; (D) 57c, Fr. 1; (E) 40b, Fr. 1; (F) 41c, Fr. 1; (G) 32d, Fr. 1; (H) 35g, Fr. 1; (J) 19c, Fr. 1; (K) 30b, Fr. 2; (L) 17c, Fr. 5; (M) 12b, Fr. 1. X-axis is m/z.

3.2. Nonachloro derivatives

M − Cl is the most abundant ion in all cases. In addition to M − Cl, other ions such as M, M − 3Cl (*m/z* 339 for bornane), M − Cl − HCl (*m/z* 373 for bornane) are the characteristic fragments (Fig. 3C). The relative intensity of these ions varies. In some cases M − Cl becomes the only countable peak as demonstrated in Fig. 3D. Two congeners with dihydrobornane structure (M − Cl = 411) were also detected (spectra are not presented in this paper).

3.3. Octachloro derivatives

The ECNI fragmentation patterns of octachloro derivatives are similar to those of nonachloro ones, as shown in Fig. 3E and 3F. M − 2Cl (*m/z* 340 for bornane) and M − 2Cl + H (*m/z* 341 for bornane) ions also exist in some cases besides M − Cl − HCl. One octachloro derivative at 32.36 min (peak 32d, Table 2) has a very intense peak at *m/z* 325 in addition to *m/z* 408 (M) and *m/z* 373 (M − Cl) (Fig. 3G), a loss of $CHCl_2$, mass of 83, is dominant in the fragmentation. Another one at 33.47 min (peak 47g, Table 2) has a relatively abundant peak at *m/z* 312 in addition to *m/z* 408 and *m/z* 373, which is a result of loss of $C_2H_2Cl_2$ (Fig. 3H). These two compounds have such a different fragmentation route that skeletons other than bornene are possible.

3.4. Heptachloro derivatives

Besides M and M − Cl ions, which are the two most abundant peaks, M − HCl (*m/z* 340) ions appear also in some cases in various relative intensity to M − Cl ions (Fig. 3J and 3K).

3.5. Hexa- and pentachloro derivatives

These two are dominated by molecular ion and M − Cl. In some cases the molecular ion is the only dominant peak (Fig. 3L) while in other cases the molecular ion has very low intensity compared to M − Cl ion (Fig. 3M). For these compounds both M and M − Cl ions should be monitored in a selected-ion monitoring (SIM) mode.

Fragments from a higher chlorinated congener may interfere with the quantitation of lower ones. This phenomenon is referred to as mass leakage as presented in the literature [2,12,15]. Ions of M − Cl, M − 2Cl − H and M − 2Cl have the same mass and isotope ratio as the lower congeners. For example, *m/z* 339 is the result of losing 2Cl from *m/z* 409 (M − Cl ion of a nonachlorobornane), but also is the M − Cl ion of a heptachlorobornene. *m/z* 373 is the result of losing HCl from *m/z* 409 but is also the M − Cl ion of an octachlorobornene. Masses of these ions were shown in Table 1.

Congeners T2 (2-exo,3-endo,5 - exo,6 - endo,-8,8,10,10-octachlorobornane) and T12 (2-exo, 3-endo,5-exo,6-endo,8,8,9,10,10-nonachlorobornane) appear to be the ones readily bioaccumulated in biota [13,15]. Individual congeners have been isolated from marine mammals [14,15]. With exception of a few laboratories, concentration of T2 and T12 in environment samples has been quantified with corresponding peaks in the toxaphene standard. The peaks considered as T2 and T12 in toxaphene (peak 29 and 46 in Fig. 1) are in fact a mixture of several congeners. An extended chromatogram of T2 and T12 range is presented in Figs. 4 and 5, respectively, with the ECNI spectra. The peak in toxaphene centered at 31.75 min (peak 29, Table 2) contains T2 (peak 29c, *m/z* 377). Single ion extraction revealed that this peak also contains hexa- (*m/z* 309) and hepta- (*m/z* 343) isomers (Fig. 4a). Coelution of several congeners under this peak is also demonstrated by the spectrum in Fig. 4b. By applying HPLC pre-separation the congeners were eluted into different fractions. T2 was almost quantitatively present in fraction 1 (peak 29c, Table 2) with a small amount of an octachlorobornene (peak 29b, Table 2) which was present in fraction 2 as well (Fig. 4c). The ECNI spectrum (Fig. 4d) shows a good purity of the peak with a small amount of bornene (M = 408, M − Cl = 373) present. One coeluting heptachlorobornane (peak 29a, Table 2) was found in fractions 2 and 3 and one hexachlorobornane (peak 29d, Table 2) was located in fraction 5.

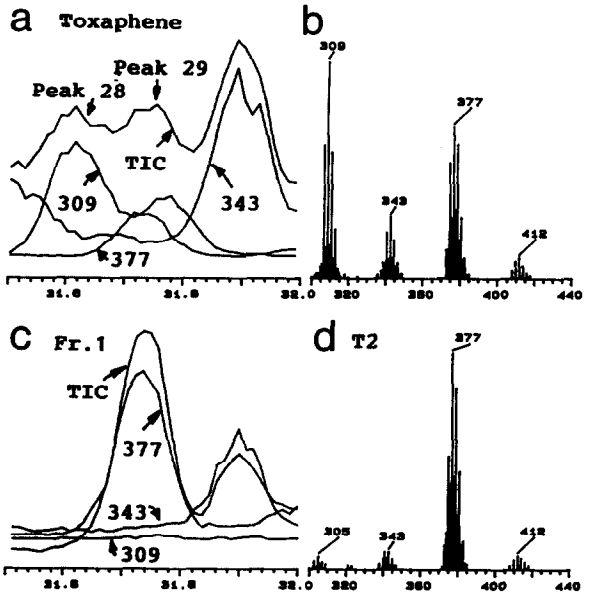

Fig. 4. Retention time range on a DB-5 column of congener T2 in toxaphene (a) and in fraction 1 (c) with their respective ECNI spectra (b) and (d). The highest ions in M − Cl clusters m/z 309 (for Cl_6), 343 (Cl_7) and 377 (Cl_8) were plotted along with the TIC curve. X-axes are retention time in min (a, c) and m/z (b, d). Note the presence of m/z 303 (M − Cl − 2HCl) in (d).

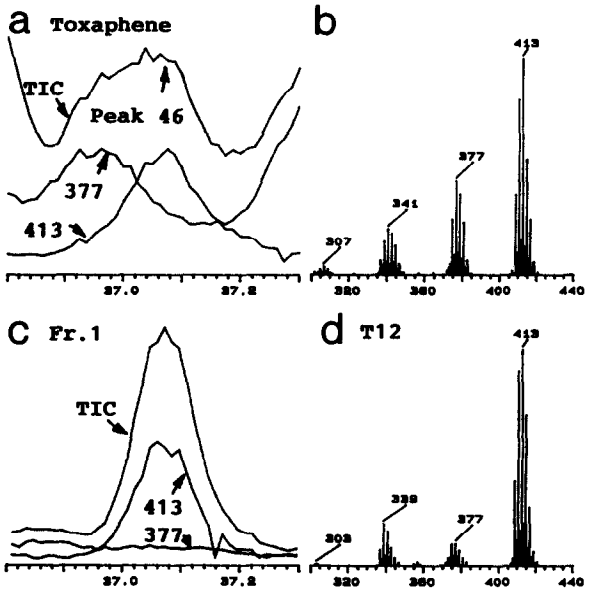

Fig. 5. Retention time range on a DB-5 column of congener T12 in toxaphene (a) and in fraction 1 (c) with their respective ECNI spectra (b) and (d). The highest ions in M − Cl clusters m/z 377 (for Cl_8) and 413 (Cl_9) were plotted along with the TIC curve. X-axes are retention time in min (a, c) and m/z (b, d). Note the presence of m/z 337 (M − Cl − 2HCl) in (d).

Thus the complex peak pattern of the T2 range is improved by HPLC separation resulting in good isolation of T2 congener from interferences. The broad peak centered at 37.05 min contains octa- (m/z 377) isomers in addition to T12 (Fig. 5a). Through HPLC pre-separation T12 (peak 46d, Table 2) was distributed two thirds in fraction 1 and the rest in fraction 2. A coeluting nonachlorobornene (peak 46b, Table 2) was found in fractions 2 and 3. Two octachlorobornanes (peaks 46a and 46c, Table 2) were eluted in fractions 2 and 3, and fraction 4 respectively. The presence of T2 and T12 in toxaphene was also confirmed using different GC columns and the ratio of m/z 408 to 312 and m/z 374 to 278 under electron impact (EI) conditions [16].

The ECNI spectra of T2 and T12 (Figs. 4d and 5d) in fraction 1 after HPLC pre-separation were in good agreement with those of individual congeners [2,15]. Both T2 and T12 produce M − Cl − 2HCl ions (m/z 303 and 337 respectively), which was not observed in other octa- and nonachlorobornanes which mainly produce M − Cl − 2Cl (m/z 305 or 339). The fragmentation by loss of two HCl could be expected through syn-elimination of the departing group (2H/3Cl and 5H/6Cl) in the coplanar position on the same side of the molecule, resulting in a bornadiene structure. The 2-exo,3-endo,5-exo,6-endo tetrachloro- structure could be partially responsible for the persistency of these two compounds in the environment and in biota.

Ions m/z 159 and 83 under Electron Impact (EI) mode have been used as an alternative to quantify toxaphene residues in the environment [3,8]. It is known that ECNI has a similar sensitivity pattern to the electron capture detector (ECD) and is very insensitive to the low chlorinated compounds. A comparison of gas chromatograms of EI-SIM mode and ECNI in the lower chlorinated congener range (15–27 min) is presented in Fig. 6. At least another 25

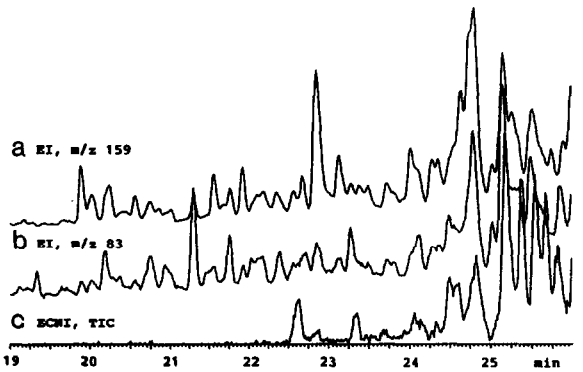

Fig. 6. Comparison of positive EI and ECNI chromatograms in the early retention time range on a DB-5 column: (a) m/z 159 from EI mode, (b) m/z 83 from EI mode and (c) TIC from ECNI mode. For conditions see Experimental.

peaks were counted in the EI mode in addition to the number detected by ECNI in this early eluting range. ECNI and ECD techniques are widely used today to determine toxaphene residue levels in many environmental samples including air, water and sediments, but low chlorinated congeners may not be detected in some of the samples due to the insensitivity of these methods. The EI mode should not be forgotten when low chlorinated congeners are concerned.

In our previous study on toxaphene residues in polar bear [2], PCC components in environmental samples were correlated to toxaphene standards by their retention time. With the help of pre-separation, PCC peaks in the toxaphene mixture can be much better recognized in the polar bear by comparing their retention times and ECNI spectra. The comparison confirmed that three nonachlorobornenes in polar bear are not present in the toxaphene standard. T2, T12 and most of the congeners in the polar bear and ringed seal were found in fraction 1 with exception of four later eluting peaks, which were found in fractions 2 through 4. If the congeners in polar bear and ringed seal represent the situation for most species, comparison with peaks in the toxaphene can be facilitated by HPLC separation. Further separation of fraction 1 using reversed-phase HPLC is in progress to concentrate the environmentally persistent toxaphene congeners. It is hoped that a toxaphene fraction which represents the residue pattern in the environment can be produced to serve as a quantitation standard.

4. Acknowledgements

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