HRGC AND HRGC-ECNI DETERMINATION OF TOXAPHENE RESIDUES IN FISH WITH A NEW 22 COMPONENTS STANDARD

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SUMMARY

The HRGC and HRGC-ECNI determination of toxaphene residues in the presence of other chlorinated hydrocarbons with similar retention times is often difficult. This problem can satisfactorily be overcome by using 22 purely isolated representative highly chlorinated bornane derivatives as a standard. This method is highly selective for the quantification of toxaphene in complex environmental matrices, especially fish and fish products.

Introduction

Toxaphene is one of the chlorinated hydrocarbon insecticides with the world's highest production volume [1-8]. In the 1980s, use and production of this preparation were banned, first in the USA and then in other countries [9, 10]. But even in recent years some cotton growing countries have continued to manufacture or to apply toxaphene [10]. Although some research groups [11-19] have investigated the contamination of various environmental samples the trace analysis of toxaphene has not been paid the attention due to it in view of its distribution behaviour and persistence in the biosphere [20-25]. The cause of this were problems in the separation of the individual components by capillary gas chromatography [8, 9, 26-29] as well as difficulties in quantification resulting from the differing accumulation behaviour and the decomposition rates of the individual toxaphene compounds which led to environmental samples varying greatly in their composition from that of the industrial mixture [30-32]. These difficulties have now been overcome by the possibility of using a new 22 component standard. These environmentally representative compounds were isolated from a photochemically modified toxaphene by column chromatography [33] (s. Table 1 and Fig. 1).

Experimental

Toxaphene (Camphechlor) and reference standards (incl. a new standard with 22 congeners) were obtained from Ehrenstorfer, Germany, and diluted with n-hexane to known concentrations. Organic solvents used were n-hexane, cyclohexane, dichloromethane, and carbon tetrachloride of purity grade for residue analysis. Na_2SO_4 and H_2SO_4 (95-97 %) were from Merck, Germany. Standards and sampleds were analyzed on a Hewlett-Packard 5890/5988A GC-MS system equipped with a 25 m

x 0.2 mm capillary column (HP-5, film thickness = 0.33 µm) with helium as carrier gas (~ 1 ml/min). The chromatographic time – temperature conditions were as follows: splitless injection; initial temperature 140 °C – hold for 1 min – to 250 °C at 4 °C/min. The injection port and transfer lines were maintained at 280 °C. The ion source temperature for the ECNI mode was 100 °C. CH_4 was used as reactant gas. The emission current was approximately 200 µA.

All routine analyses were carried out on a Varian 3400 gas chromatograph (Injection: on column, 120 to 230 °C with 200 °C/min; column: 60 m DB-5, fused silica, i.d. 0.25 mm; film thickness 0.32 µm; EC detector 320 °C; carrier gas: 1.5 ml/min; temperature program (column): 120 °C (0 min) – 10 °C/min – 180 °C (1 min) – 5 °C/min – 250 °C). Comparative analyses with determination of the response factors (Table 1) were carried out on a Varian 3300 with splitless/split injector (230 °C, 30 sec split open, column: HP-5, 25 m, i.d. 0.2 mm, film thickness 0.5 µm, carrier gas: 2.5 ml/min; EC detection 320 °C, the same column temperature program as described above).

The analyzed fish and fish products were obtained from Iceland, Greenland, Japan, Norway, and Germany. The samples were extracted as reported before [26].

Table 1. Data of all 22 isolated congeners

Parlar No.	Name	Purity[a] [%]	t_r[b]	F_r[c] [%]	F_r[d] [%]
11	2,2,3-exo,8b,9c,10α-Hexachlorocamphene	80	0.714	68	46
12	2-exo,3-endo,8b,8c,9c,10α-Hexachlorocamphene	99	0.719	135	84
15	2-exo,3-endo,7a,8b,9c,10α-Hexachlorocamphene	99	0.741	127	85
21	2,2,5,5,8c,10a,10b-Heptachlorobornane	87	0.780	113	73
25	2,2,3-exo,8b,8c,9c,10α-Heptachlorocamphene	99	0.808	104	83
26	2-exo,3-endo,5-exo,6-endo,8b,8c,10a,10b-Octachlorobornane	98	0.820	145	120
31	2,2,3-exo,8a,8b,9b,9c,10α-Octachlorocamphene	82	0.857	54	57
32	2,2,5-endo,6-exo,8b,9c,10a-Heptachlorobornane	95	0.861	131	121
38	2,2,5,5,8b,8c,10a,10b-Octachlorobornane	83	0.903	113	104
39	2,2,3-exo,5-endo,6-exo,8b,9c,10a-Octachlorobornane	96	0.924	142	131
40	2-exo,3-endo,5-exo,6-endo,8b,9c,10a,10b-Octachlorobornane	93	0.930	89	99
41	2-exo,3-endo,5-exo,8b,9c,10a,10b-Octachlorobornane	91	0.933	131	114
42a+b	2,2,5-endo,6-exo,8b,8c,9c,10a- and 2,2,5-endo,6-exo,8c,-9b,9c,10a-Octachlorobornane	90	0.943	97	75
44	2-exo,5,5,8b,8c,9c,10a,10b-Octachlorobornane	91	0.956	92	87
50	2-exo,3-endo,5-exo,6-endo,8b,8c,9c,10a,10b-Nonachlorobornane	98	1.000	100	100
51	2,2,5,5,8b,9c,10a,10b-Octachlorobornane	99	1.021	116	114
56	2,2,5-endo,6-exo,8c,9b,9c,10a,10c-Nonachlorobornane	82	1.074	101	131
58	2,2,3-exo,5,5,8b,9c,10a,10b-Nonachlorobornane	96	1.094	119	151
59	2,2,5-endo,6-exo,8b,8c,9c,10a,10b-Nonachlorobornane	99	1.105	92	117
62	2,2,5,5,8b,8c,9c,10a,10b-Nonachlorobornane	97	1.151	104	134
69	2,2,5,5,6-exo,8b,8c,9c,10a,10b-Decachlorobornane	98	1.400	43	97

[a] Established with GC-ECD, 60 m DB-5 column, i.d. 0.25 m, film 0.32 µm

[b] Retention times relative to toxicant Ac (50), same column ass under [a]

[c] Response factors relative to compound 50 (= 100); split injection; injector temperature 230 °C; 25 m HP-5 column, i.d. 0.2 mm, film 0.5 µm

[d] Response factors relative to compound 50 (= 100); on column injection, injector temperature program: 120 °C (0 min) – 200 °C/min – 230 °C (hold); same column as under [a]

RESULTS AND DISCUSSION

HRGC-ECD experiments

Figure 1 shows a typical HRGC-ECD splitless chromatogram of the extract of a cod liver oil sample from the German market, the photochemically modified toxaphene mixture, and the chlorobornane standard. It can be seen that the compounds studied are separated by capillary gaschromatography with some restrictions. The first exception is Parlar No. 32 (Toxicant B), which co-eluates with cis-chlordane. Although it is possible to pre-separate both compounds on a silica gel column interferences cannot be excluded under practical conditions because of the high relative response factor of cis-chlordane. The second exception are the congeners 31/32 and 40/41 which can be separated only by the 60 m column, but not by the 30 m column. The relative retention times (t_{RR}) and the relative response factors (F_{rr}), obtained from HRGC-ECD with splitless/split and on-column injection experiments are summarized in Table 2. From the results it can easily be seen, that the chlorobornanes are thermally unstable in the splitless/split mode and react at the high temperatures in the injection port. This problem can easily be overcome by using on-column systems, because under these conditions the chlorobornanes remain stable (s. Tab. 1). The relative response factors of chlorobornanes are nearly comparable to each other, if on-column ECD detection is used. Under these conditions they vary between 1.00 and 1.46 relatively to compound No. 50 (Toxicant Ac).

HRGC-MS/SIM experiments

HRGC-MS systems are usually equipped with splitless/split injection systems. Therefore the same disturbing effects as in the case of HRGC-ECD are to be expected. Furthermore, the response factor differences of chlorobornanes are significantly higher than the differences observed by ECD detectors. (Tab. 2).

Table 2. Relative retention times (t_{RR}) and response factors (F_{rr}) of some environmentally relevant chlorinated bornane derivatives
[Ac = Toxicant Ac (Parlar No. 50); Al = Aldrin as reference substance (F_{rr}Aldrin = 1.00; PCI = positively charged chemical ionisation; ECNI = negatively charged chemical ionisation; SIM = selected ion monitoring, EC = electron capture Ni^{63}; OCl = on column]

Compound	t_{RR} Ac*	t_{RR} Al*	F_{rr} EI Ac	F_{rr} PCI Ac	F_{rr} ECNI Ac	F_{rr} ECNI/SIM Ac	F_{rr} EC/OCl Ac	F_{rr} EC/OCl Al	F_{rr} EC/splitless Ac	F_{rr} EC/splitless Al
26	0.84	1.18	1.42	-	-	1.11	-	-	-	-
32	0.91	1.28	0.99	1.55	2.54	1.99	1.29	0.86	1.86	0.30
50	1.00	1.41	1.00	1.00	1.00	1.00	1.00	0.69	1.00	0.16
62	1.09	1.54	0.52	0.26	0.22	0.39	1.38	0.95	0.71	0.11
69	1.22	1.72	0.18	0.10	0.17	0.45	1.46	1.00	0.19	0.03

Residue analysis

In this work the silica gel separation has been used to eliminate the interfering substances such as PCBs or cyclodiene insecticides [13, 15, 33]. The ions 340, 341, 342, 343, 375, 377, 411, 413, 447, and 449, which are representative for both compound families, chlorobornanes and bornenes, are monitored by ECNI method for the quantification. The compounds No. 26, 50 and 62 are present at concentrations similar to important PCB isomers and cyclodiene insecticides in most of the aquatic biota samples, especially from the North Atlantic (Tab. 2). These three chlorobornanes constitute the major proportion of the total toxaphene residues observed by GC-MS/ECNI-SIM and GC–ECD analysis of aquatic biota extracts. The amount of 26, 50, and 62 constitute 25 - 30 % of the toxaphene residues in cod liver oils determined by using the 22 component standard. In fresh fish and in caviar these substances amount to approximately 18 - 23 % of the chlorobornanes. The so-called Toxicant B (No. 32), which was the first product isolated and identified from technical toxaphene, could be found in all samples investigated. In contrast to the major contaminants No. 26, 50, and 62, this compound was detected only in very low concentration levels. The decachloro compound (No. 69) could not be detected in fish extracts but it is detectable in toxaphene mixtures at amounts between 50 and 500 mg/kg. The data of the toxaphene residues quantified by using the new standards with the ECD or ECNI-SIM technique show that especially cod liver and salmon oil are highly contaminated by chlorobornanes. In some of the samples the levels of toxaphene residues exceed those of the PCB levels. The results of these residue analyses confirm the general opinion that toxaphene is present in high quantities in tissue and fish products. Therefore, it is necessary to determine the residue levels of frequently occurring toxaphenes in other biological samples to be able to compare the contamination of different ecosystems.

Table 3. Concentrations (µg/kg) of the chlorobornane standards (No. 26, 50, and 62) and toxaphene (determined by using the CB-standard[7]) in samples of aquatic biota [I = HRGC/ECD splitless; II = HRGC/MS - ECNI-SIM

Sample/Origin		C_{26}	C_{50}	C_{62}	$C_{\Sigma 26,50,62}$	$C_{22\ standard}$
Caviar-substitute Iceland	I	58 ± 21	64 ± 28	28 ± 12	150	750 ± 230
	II	32 ± 14	56 ± 25	21 ± 7	109	555 ± 170
Caviar-substitute Germany	I	33 ± 14	36 ± 15	14 ± 4	83	410 ± 150
	II	18 ± 8	57 ± 26	16 ± 5	91	490 ± 180
Fish oil 1 Germany	I	10 ± 4	11 ± 4	2 ± 1	23	75 ± 30
	II	10 ± 4	19 ± 8	6 ± 2	35	90 ± 35
Fish oil 2 Japan	I	4 ± 2	6 ± 2	2 ± 1	12	38 ± 16
	II	3 ± 1	7 ± 2	2 ± 1	12	40 ± 18
Salmon oil 1	I	33 ± 13	60 ± 28	9 ± 3	102	350 ± 115
	II	21 ± 7	75 ± 29	14 ± 5	110	400 ± 150

Table 3 cont.

Salmon oil 2 Norway	I	11 ± 4	40 ± 16	1 ± 1	52	154 ± 74
	II	14 ± 5	35 ± 15	5 ± 2	54	158 ± 75
Cod liver oil 1 Germany	I	220 ± 75	343 ± 98	171 ± 63	734	2100 ± 730
	II	245 ± 90	390 ± 110	120 ± 55	755	2300 ± 880
Cod liver oil 2 Germany	I	210 ± 70	405 ± 240	80 ± 30	695	2120 ± 725
	II	220 ± 75	420 ± 250	110 ± 60	750	2210 ± 910
Cod fish Greenland	I	50 ± 21	82 ± 33	50 ± 24	182	620 ± 230
	II	40 ± 16	86 ± 36	54 ± 29	180	610 ± 210
Red fish Greenland	I	7 ± 2	15 ± 8	8 ± 4	30	150 ± 60
	II	9 ± 3	27 ± 15	17 ± 5	53	250 ± 90
Halibut Germany	I	4 ± 2	12 ± 5	3 ± 1	19	120 ± 35
	II	5 ± 3	14 ± 5	2 ± 1	21	138 ± 39

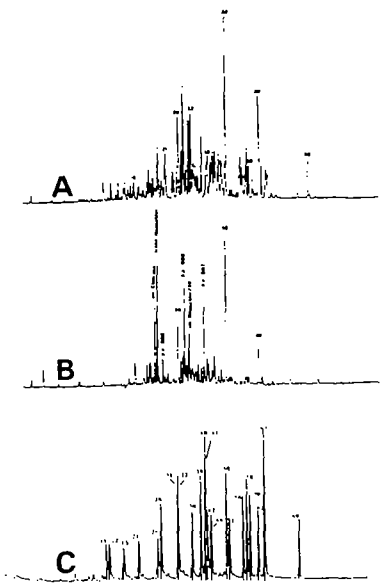

Fig. 1 HRGC-ECD splitless chromatograms of (A) the CB standard, (B) a cod liver oil extract, and (C) a standard mixture of the chlorobornanes 26, 32, 50, 62, and 69

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