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Multidimensional Gas Chromatographic Analysis of Toxaphene

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The pesticide toxaphene with a total worldwide production of 1.3 Mt is found in the aquatic environment with a widespread, global distribution. Its analysis is difficult due to its complex character. A technical toxaphene mixture and a number of biological samples were analyzed by multidimensional gas chromatography (MDGC). Four different column combinations were used: DB5/DX4, DB5/FFAP, Ultra 2/DX4, and Ultra 2/Rtx 2330. The technical mixture consists of at least 246 congeners, while only 107 peaks were found in a one-dimensional GC/ECD chromatogram. The major peaks present in the technical mixture and biological samples mostly consist of 2−10 toxaphene congeners. These results show that in most cases single-column chromatography is not suitable for a congener-specific determination of the toxaphene congeners. The Rtx 2330 column is recommended as the best choice as a second column in MDGC of toxaphene, particularly because of its stability at higher temperatures and because of a slightly better separation, although the differences in results obtained with the different column combinations were relatively small. The relatively high concentrations of toxaphene in European fish, which may even lead to exceeding the tolerance levels for human consumption, and in marine mammals demand a proper monitoring of total toxaphene and individual CHBs in relevant samples from European aquatic ecosystems.

Introduction

Toxaphene is a complex mixture primarily consisting of chlorinated bornanes (CHBs) with an average elemental composition of $C_{10}H_{10}Cl_8$ (*1*−*3*). Prior to its ban in 1982 by the U.S. Environmental Protection Agency (*1*), toxaphene was the most extensively used pesticide in the United States and many parts of the world. The total global production is estimated to be 1.3 Mt (*4*), which is higher than that of polychlorinated biphenyls (PCBs). Considerable amounts may still be stored, although exact data are difficult to obtain (*5*). Toxaphene is still produced and used in several countries throughout the world. Toxaphene has been detected as a contaminant in various environmental compartments and has a widespread distribution (*6*−*10*). Due to aerial transport, toxaphene was detected even in very remote areas (*11, 12*). Condensation at low temperatures is supposed to result in elevated concentrations in polar regions (*13*).

Different chlorine substitution patterns can theoretically lead to 32 768 congeners (*14*), of which a number also show chiral activity (*15*). Technical toxaphene mainly consists of Cl_7 and Cl_8 congeners or, in principle, up to 6840 CHBs. However, a number of these CHBs are unlikely to be present because of unfavorable substitution positions on ring and bridge carbons (*16*). Actually, Jansson and Wideqvist separated 670 individual congeners in a technical toxaphene mixture by using a very long carbon column (*17*). Their method is, however, not suitable for routine analysis because it is very time-consuming. In environmental samples, the total number of congeners will be smaller due to degradation and biotransformation. A determination of the total toxaphene content has a lot of disadvantages. Due to the differences in peak patterns between the samples and the technical mixture, the accuracy of such a determination is rather low. This phenomenom was described earlier for other complex mixtures such as polychlorinated biphenyls and terphenyls (*18, 19*). A congener-specific determination offers the possibility of determining the compounds with a higher toxicological relevance. Also, the behavior of individual congeners in the environment can be studied in this way.

Recent attempts to improve the mutual comparability of analytical data on the determination of total toxaphene and individual CHBs showed that there are major separation problems when using single-column gas chromatography (GC) (*20*). Nevertheless, a single-column GC/ECD (electron capture detection) method was recently introduced as a congener-specific method for the determination of individual CHB congeners (*21*). For reasons of sensitivity, the use of electron capture negative ion mass spectrometry (ECNI−MS) is preferred, although due to the softer ionization technique as compared to electron impact (EI) MS, less structural information is available from the mass spectra. Selective ion monitoring of the $[M - Cl]^-$ ions would, however, give greater specificity than single-column GC/ECD. The use of high-resolution mass spectrometry (HRMS) would only reduce the possibility of interference of other organochlorine compounds but would not solve the problem of co-eluting CHBs from the same homologue group.

This study presents a CHB congener-specific method using multidimensional gas chromatography (MDGC). The use of MDGC has shown to be very beneficial in the analysis of other complex mixtures such as polychlorinated biphenyls (PCBs) (*22, 23*). By making sequential heart-cuts, it is possible to obtain a better estimation of the minimum number of CHBs present in a sample. Additionally, the quantification of CHBs can be checked with regard to purity, and false positive results can be avoided. In this study, the number of CHBs present in a commercial toxaphene mixture was estimated. Using various column combinations, heart-cuts at the retention times of some selected CHBs were made both for the technical mixture and several biota samples in order (i) to check the composition of those peaks and (ii) to determine the concentration of these CHBs.

Materials and Methods

Standards and Samples. A technical toxaphene mixture was obtained from Polyscience (Warrington, PA). Five individual toxaphene congeners were obtained from Promochem (dr. Ehrenstorfer, Wesel, Germany). Their codes (Parlar numbers) (*24*) and structures (Figure 1) are as follows:

CHB 26 (T_2), 2-exo,3-endo,5-exo,6-endo,8b,8c,10a,10b-octachlorobornane

CHB 32 (ToxB), 2,2,5-endo,6-exo,8b,9c,10a-heptachlorobornane

CHB 50 (T_{12},ToxAc), 2-exo,3-endo,5-exo,6-endo,8b,8c,9c,-10a,10b-nonachlorobornane

S0013-936X(96)00565-2 CCC: $14.00

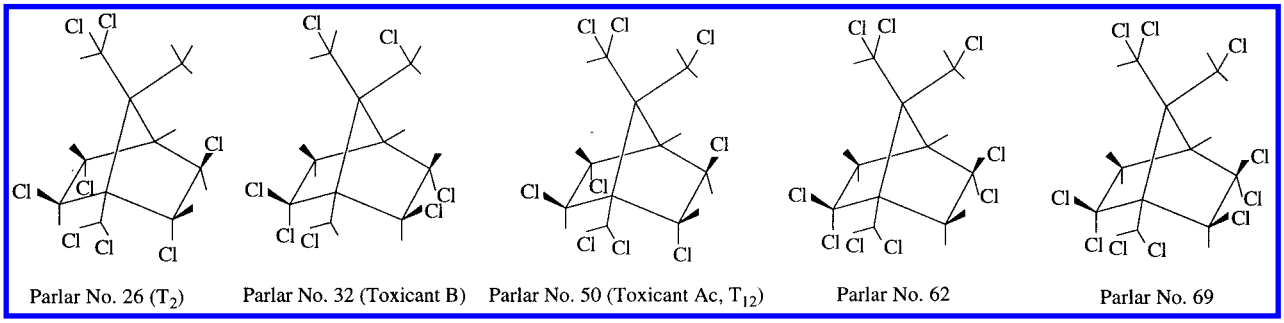

FIGURE 1. Structure of five CHB congeners.

TABLE 1. Relevant Sample Data

sample type	Latin name	year	location	*n* [a]	lipid content[b] (g/kg)
hake liver	Merluccius merluccius	1991	West of Ireland	25	465
hake liver	Merluccius merluccius	1989	South of Ireland	25	465
herring muscle	Clupea harengus	1993	Shetland Islands	80	196
white-beaked dolphin blubber	Lagenorynchus albirostris	1991	Southern North Sea	1	696
human milk	Homo sapiens	1992	Managua, Nicaragua	1	11

[a] Number of specimen in pooled sample. [b] Total lipid content according to Bligh and Dyer (*31*).

CHB 62, 2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane

CHB 69, 2,2,5,5,6-exo,8b,8c,9c,10a,10b-decachlorobornane

The CHBs were available as standard solutions, ampuled in cyclohexane, with a concentration of 1 mg/L ± 15%. The purity of four congeners was tested by heart-cuts made with a DB5/DX4 and an Ultra 2/Rtx 2330 column combination. With ECD detection, the purity appeared to be >99% for CHBs 26, 50, and 69; >98% for CHB 62; and >95% for CHB 32. The heart-cuts of the CHB standards also showed that no degradation occurred during their analysis in the MDGC system. The concentrations of CHBs 26, 32, 50, and 62 were later checked against those of another CHB mixture (Promochem, Code USL 421), stated to contain 5 mg/L of these CHBs, which appeared to be more pure. The CHB concentrations were corrected to the latter standard.

Hake liver, herring muscle tissue, and dolphin blubber were obtained from animals caught in the Atlantic Ocean southwest and west of Ireland and in the North Sea (Table 1). A human milk sample was obtained from Managua, Nicaragua (*8*).

Cleanup Methods. All samples were extracted and cleanedup according to de Boer and Wester (*8*). This method included a Soxhlet extraction in *n*-pentane/dichloromethane (50:50, v/v) for the fish and dolphin samples; a cold *n*-hexane/acetone extraction for the human milk; and, for all types of sample, alumina column chromatography with *n*-pentane and a repeated silica gel column fractionation. This silica fractionation was carried out with 11 mL of isooctane and 12 mL of diethyl ether/isooctane (25:75, v/v) to obtain a first and second fraction. Subsequently, the second frcation was concentrated and eluted again according to the same procedure, which resulted in a third (isooctane) and fourth (diethyl ether/isooctane) fraction. The CHBs were supposed to be eluted in the fourth fraction (*8*). Total toxaphene recoveries are reported to vary between 70 and 80% (*8*). All experimental results were corrected accordingly. All solvents were of nanograde quality (Promochem).

After the first series of experiments (DB5/DX4 columns), it was found that CHB 26 was only present in the toxaphene fraction at 5% of its original concentration. The recoveries of CHBs 32, 50, and 62 varied from 54 to 82%. So, despite a reported acceptable recovery percentage of total toxaphene (cf. above), recoveries of some specific congeners were clearly less acceptable. Similar difficulties were reported by Andrews (*20*) after evaluation of the results of an interlaboratory study on toxaphene. The silica gel fractionation was improved by using columns of 2.5 g of SiO_2·2% H_2O (w/w) rather than 1.8 g of SiO_2·2% H_2O (w/w) columns in all further experiments. The bulk of the toxaphene compounds, including the most relevant congeners, was now eluted in a second fraction of 12 mL of diethyl ether/isooctane (20:80, v/v) after a first fraction of 13 mL of isooctane that contained most PCBs. The idea of a repeated fractionation, which was initially carried out to avoid any possible interference by PCBs that could cause false positive toxaphene signals with ECNI−MS due to the formation of oxygen adducts, was now deleted. This was mainly done because (i) in the present experiments an ECD was used as detector and (ii) PCBs were only present at 1−2% in the toxaphene fraction, which did not seriously interfere with the toxaphene quantification using ECD (*8*). With the entire cleanup procedure including the improved fractionation, the recovery of total toxaphene was 80−96%. The recoveries of the CHBs 32, 50, and 62 with this procedure were 84−100% (Table 2). CHB 26 was divided over the two fractions (about 40% in the first fraction and 60% in the second fraction) with an overall recovery of 85−95%. This made it necessary to determine CHB 26 in both fractions. Because of the low recovery of CHB 26 in the first series of experiments, this series was repeated using the improved silica fractionation and a Ultra 2/DX4 column combination, which has essentially the same characteristics but somewhat different dimensions (cf. below).

MDGC Analysis. All MDGC analyses were performed on a Sichromat 2-8 GC with two independently controlled ovens and two ECDs (Siemens, Karlsruhe, Germany). All column dimensions and operating conditions are given in Table 2. Five different columns in four different combinations were used: DB5 (5% phenyl/95% methylsilicone) (J&W Alltech, Deerfield, IL), DX4 (15% dimethylsilicone/85% polyethylene glycol) (J&W Alltech), Ultra 2 (5% phenyl 95% methylsilicone) (Hewlett Packard, Amstelveen, The Netherlands), FFAP (polyethylene glycol terephthalic acid ester) (Hewlett Packard) and Rtx 2330 (10% cyanopropyl/90% biscyanopropylpolysiloxane) (Restek Corporation, Bellefonte, PA). DB5 and Ultra 2 were used as first columns, because these relatively nonpolar phases are frequently used to produce total toxaphene chromatograms (*23*−*26*). Because of the importance in MDGC to use a second column with a stationary phase clearly different from the first one (*27*), the other three columns used were rather polar phases, each of them with a different structure.

TABLE 2. MDGC/ECD Conditions[a]

parameter	DB5/DX4	DB5/FFAP	Ultra 2/DX4	Ultra 2/Rtx 2330
First Dimension				
column type	DB5	DB 5	Ultra 2	Ultra 2
column length (m)	30	30	25	25
internal diameter (mm)	0.25	0.25	0.20	0.20
film thickness (μm)	0.25	0.25	0.30	0.30
carrier gas pressure (kpa)	108	108	150	150
linear gas velocity (cm/s)	10	10	18	18
makeup gas flow (mL/min)	36	36	36	36
initial oven temp (°C)	90	90	90	90
initial isothermal period (min)	1	1	1	1
initial programming rate (°C/min)	20	20	30	30
second isothermal temp (°C)	220	220	220	220
second isothermal period (min)	1	1	20	20
second programming rate (°C/min)	5	5	3	3
third isothermal temp (°C)	260	260		
third isothermal period (min)	1	1		
third programming rate (°C/min)	3	3		
third isothermal temp (°C)	280	280	270	270
final isothermal period (min)	30	30	40	40
Second Dimension				
column type	DX4	FFAP	DX4	Rtx 2330
column length (m)	15	15	15	15
internal diameter (mm)	0.25	0.20	0.25	0.25
film thickness (μm)	0.25	0.30	0.25	0.20
carrier gas pressure (kpa)	70	110	70	70
linear gas velocity (cm/s)	22	10	22	26
makeup gas flow (mL/min)	38	38	38	38
isothermal oven temp (°C)	220	200	210	215

[a] For all column combinations: manual 1−2 μL splitless injection; splitless time, 1 min; injector temperature, 270 °C; ^{63}Ni electron capture detector; temperature, 300 °C; makeup and carrier gas, N_2; septum purge, 2 mL/min.

Heart-cuts from the first column were transferred to the second column by use of a valveless switching technique, in which instead of the gas flow, the pressure drop over the coupling piece—a glass capillary, i.d. 0.17 mm (live-T-piece)—is used (*28*). Through special auxiliary flow arrangements, the transfer of the heart-cut to the second column can be carried out with negligible deterioration of the peak shape. The pressure drop type of adjustment of the flow is facilitated by digital reading. The adjustments are stable over a long period of time (weeks−months). The number of heart-cuts made during a run is in principle not limited (*29*, *30*). Although the system also functions when helium or hydrogen is used, nitrogen is preferred because a better precision (0.01 min) for the heart-cuts is obtained.

The temperature of the first oven was optimized to obtain maximum resolution. The second columns were run under isothermal conditions, close to their maximum allowable operating temperatures to prevent an unnecessary increase of the total run time. Only the maximum temperature of the Rtx 2330 column was higher (275 °C) than the working temperature (215 °C). However, in this instance, a higher working temperature resulted in less resolution.

A series of 40 heart-cuts throughout the first-dimension chromatogram of the technical toxaphene mixture was made using the DB5/DX4 column combination. Although several heart-cuts can be included in one chromatogram, in this case only one heart-cut was analyzed per run to prevent any possible interference from peaks in one heart-cut with those of another heart-cut in the same run.

Finally, heart-cuts of the five CHBs were made from first-dimension chromatograms of the technical toxaphene mixture and the five biota samples (Table 1), using the three other column combinations. The number of peaks and the peak area ratios were determined, and the concentrations of the five CHBs (indicative because an internal standard was not always used—incidentally 2,3,5,6,3′-pentachlorobiphenyl was used, added before injection—and only single-point calibration was used) were calculated.

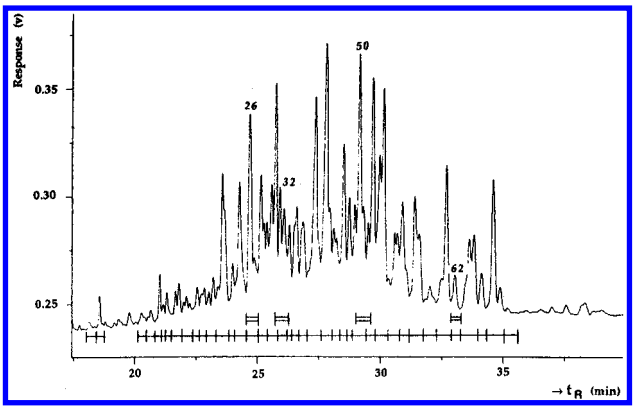

FIGURE 2. First-dimension chromatogram of the technical toxaphene mixture on the DB5 column (GC conditions in Table 3).

Results and Discussion

Sequential Heart-Cuts Technical Toxaphene. A series of 40 heart-cuts was made with the DB5/DX4 column combination throughout the first-dimension chromatogram of the technical toxaphene mixture (Figure 2). The chromatograms of the CHBs 26, 32, 50, and 62 heart-cuts on the DX4 column are shown in Figure 3. It was not possible to elute CHB 69 from the DX4 column, presumably due to strong retention in combination with a relatively low maximum allowable temperature (220 °C).

The complete series of 40 heart-cuts resulted in 246 peaks, after correction for peaks that appeared in more than one heart-cut. This is more than twice the number of peaks visible (107) in the first-dimension chromatogram (Figure 2). The peak patterns of the various heart-cuts of Figure 3 show that even here some overlap of peaks occurs. This indicates that the number of 670 CHB congeners in commercial toxaphene separated by Jansson and Wideqvist (*17*) may well be a realistic estimate. It certainly indicates that co-elution of toxaphene

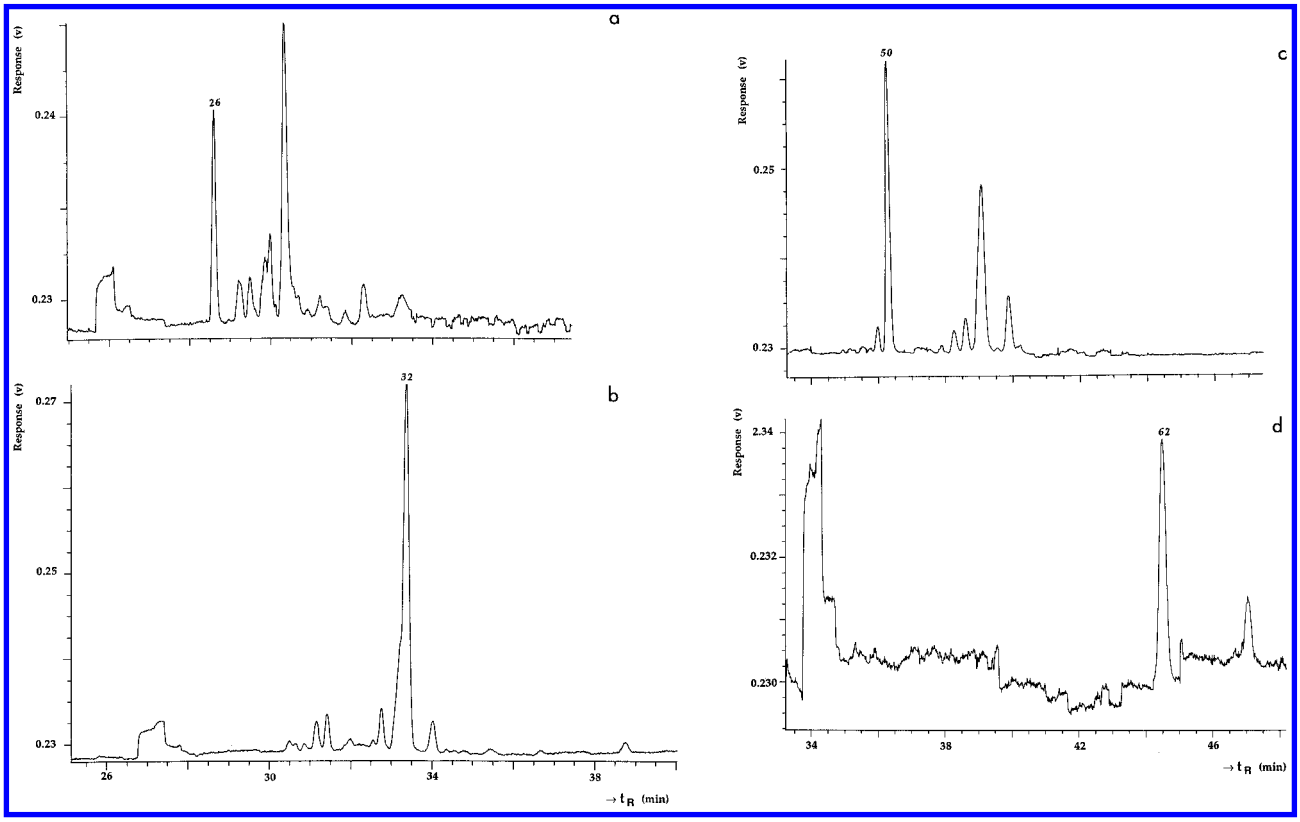

FIGURE 3. Heart-cuts from the first-dimension chromatogram of the technical toxaphene mixture (cf. Figure 2) of (a) CHB 26, (b) CHB 32, (c) CHB 50, and (d) CHB 62 analyzed on the DX4 column.

TABLE 3. Number of Peaks, Peak Area Ratios, and Estimated Concentrations of CHBs 26, 32, 50, 62 and 69 in Heart-Cuts of Technical Toxaphene Mixture Made with Three Column Combinations

CHB	column combination	*n* [a]	*R* [b]	concn (%, w/w)
26	DB5/FFAP	4	18	0.6
	Ultra 2/DX4	7	11	0.4
	Ultra 2/Rtx 2330	9	8	0.3
32	DB5/FFAP	6	55	16
	Ultra 2/DX4	9	59	3.8
	Ultra 2/Rtx 2330	7	31	1.0
50	DB5/FFAP	4	61	2.2
	Ultra 2/DX4	7	52	1.4
	Ultra 2/Rtx 2330	6	40	1.7
62	DB5/FFAP	7	47	12
	Ultra 2/DX4	12	66	3.3
	Ultra 2/Rtx 2330	5	19	1.2
69	DB5/FFAP			
	Ultra 2/DX4			
	Ultra 2/Rtx 2330	1	100	0.3

[a] *n*, number of peaks present in heart-cut. [b] *R*, peak area ratio (%), peak area of target CHB divided by sum of areas of all peaks present in the same heart-cut.

congeners will be a very serious problem in any one-dimensional chromatogram.

Table 3 gives an overview of the number of peaks in the heart-cuts of the five CHBs studied, obtained with three different column combinations, and their estimated concentrations. All heart-cuts, except that of CHB 69, consist of 4−12 peaks. The number of peaks (*n* in Tables 3 and 4) is corrected for compounds present in visible shoulder peaks in the first dimension chromatogram, which were occasionally included in the heart-cuts. This is to avoid an over-estimation of *n*. The concentrations of CHBs 32 and 62 calculated from the DB5/FFAP column combination are clearly positively biased, which shows that co-elution still occurs in the heart-cut. Considering possible co-elution in the heart-cuts and the somewhat incomplete quality control of the data, the following CHB concentrations in technical toxaphene seem to be reasonable estimates: CHB 26 0.3−0.4%, CHB 32 1%, CHB 50 1.4−1.7%, CHB 62 1.2%, and CHB 69, 0.3%. These data show that the CHBs that are most stable in biological samples, CHBs 26 and 50 (*26, 32*), are only present at low concentrations of 0.3−1.7% in the commercial toxaphene mixture. CHB 69, which could only be eluted from the Rtx 2330 column, appears to be the only pure peak isolated from a first-dimension chromatogram. The Ultra 2/Rtx 2330 column combination generally provides the lowest CHB concentrations whereas DB 5/FFAP tends to give invariably positively biased results.

Heart-Cuts of Biota. Heart-cuts of CHBs 26, 32, 50, 62, and 69 were made from first-dimension chromatograms of five biological samples (cf. Table 1), using the same three column combinations as above: DB5/FFAP, Ultra 2/DX4, and Ultra 2/Rtx 2330. The results of this study in terms of the number of peaks present in the heart-cuts and estimated CHB concentrations are given in Table 4. Two first-dimension chromatograms, one of a hake liver and one of a white-beaked dolphin are shown in Figure 4. Several relevant heart-cuts from these chromatograms are shown in Figure 5.

The determination of CHB 26 obviously is not straightforward. In the first place, the distribution of this CHB over two fractions is a clear disadvantage and results in less accurate data. A fractionation in which CHB 26 also would be eluted in the second fraction would be preferable, but no proper strategy has been devised as of yet. Secondly, the number of peaks in the two CHB 26 heart-cuts varies from 3 to 13. The percentage of CHB 26 generally is some 20−40% (Table 4) with the white-beaked dolphin (77−85%) as an exception. The differences in the calculated concentrations, such as with the 1989 hake liver are mainly caused by co-elution of peaks in the heart-cuts. There is no distinct preference for one of the column combinations used. The

TABLE 4. Number of Peaks, Peak Area Ratios, and Concentrations of CHBs 26, 32, 50, and 62 in Heart-Cuts of Biological Samples Made with Three Column Combinations[a]

		n [b]			*R* [c]			concentration (μg/kg)[d]		
CHB	sample	I	II	III	I	II	III	I	II	III
26[e]	hake liver 1991		3 (4)	13 (4)		72 (20)	40 (15)	na[f]	100	70
	hake liver 1989	3 (4)	4 (4)	10 (4)	45 (3)	39 (29)	38 (25)	50	30	90
	herring muscle		5 (2)	5 (2)		39 (37)	29 (70)	<1	2	<1
	dolphin blubber		9 (4)	8 (4)		85 (37)	77 (30)	na	3300	2600
	human milk	3 (1)	5 (2)	9 (2)	21 (100)	21 (92)	22 (89)	1	1	1
32	hake liver 1991	2	4	8				<1	<1	<1
	hake liver 1989	1	2	11				<1	<1	<1
	herring muscle		4	5		13		<1	0.4	<1
	dolphin blubber	7	9	13				<1	<1	<1
	human milk			6				<1	<1	<1
50	hake liver 1991	1	3	3	100	95	92	20	30	10
	hake liver 1989	2	4	5	92	95	85	10	30	7
	herring muscle	1	2	3	100	84	40	2	2	1
	dolphin blubber		10	6	95	95	84	4400	3500	1900
	human milk	1	3	3	100	92	95	3	6	3
62	hake liver 1991							<1	<1	<1
	hake liver 1989		2	3		83	32	<1	2	1
	herring muscle		2	2		57	51	<1	2	1
	dolphin blubber	4	6	3	75	83	28	100	190	40
	human milk		4	3		29	14	<1	0.6	0.4

[a] I, DB5/FFAP; II, Ultra 2/DX4; III, Ultra 2/Rtx 2330. [b] *n*, number of peaks present in heart-cut. [c] *R*, peak area ratio (%), peak area of target CHB divided by sum of peak areas of all peaks in same heart-cut. [d] Concentrations in μg/kg wet weight. [e] CHB 26 was present in two fractions, which were both analyzed. The concentration given is the sum of CHB 26 concentrations in both fractions. Under *n* and *R*, the number of peaks and peak area ratios in the second fraction are given, followed by those in the first fraction in parentheses. [f] na, not analyzed. Total toxaphene concentrations determined by GC/NCI−MS (8) in μg/kg wet weight: hake liver 1991, 1300; hake liver 1989, 690; herring, 60; dolphin, 19 000; human milk, not determined.

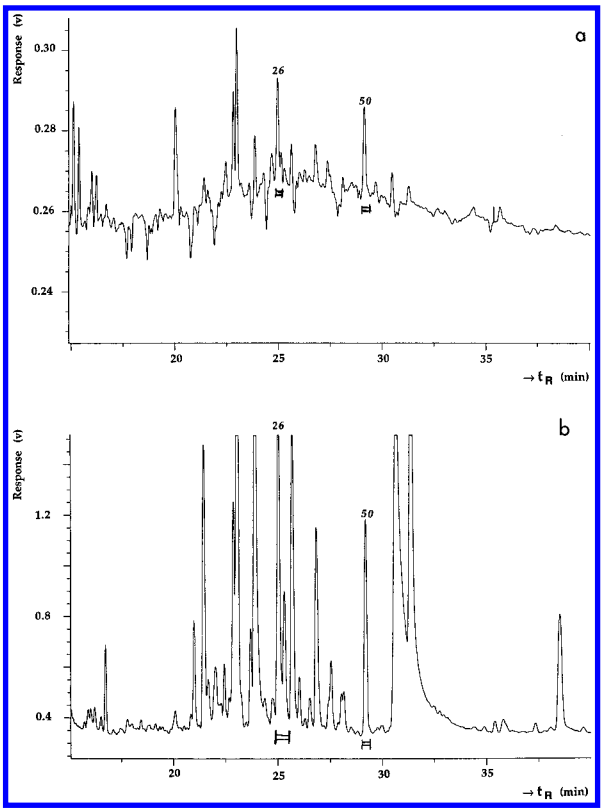

FIGURE 4. First-dimension chromatograms of (a) hake liver (west of Ireland) and (b) white-beaked dolphin blubber obtained on the DB5 column (GC conditions in Table 3).

CHB 26 percentage of the total toxaphene concentration in the biota samples analyzed varies from 3 to 14% (Table 4), which is clearly higher than its percentage in technical toxaphene (0.3−0.4%). This confirms the idea that CHB 26 is one of the more persistent CHBs (*26, 32*).

The CHB 32 data suggest that this congener is subject to biotransformation in all samples analyzed. Whereas CHB 32 percentages in the technical toxaphene are distinctly higher than those of CHB 50 (Table 3), CHB 32 contributes much less than CHB 50 in the biota samples and cannot be detected in most cases (Table 4). Despite its absence in most heart-cuts, there are several other peaks present in these heart-cuts, which shows that, if small amounts of CHB 32 would be present, it would not be possible to quantify these amounts accurately by single-column GC. The Ultra 2/Rtx2330 column combination generally results in more peaks per heart-cut for CHB 32 than the two other combinations.

The CHB 50 percentage of the total toxaphene concentration in the biota analyzed varies from 1 to 10%, comparable to the contribution of CHB 26. This confirms also that CHB 50 is one of the more persistent CHBs (*26, 32*). The peak area ratios are relatively high in most heart-cuts except in one herring muscle analysis (Ultra 2/Rtx 2330). This can be explained by the presence of a few other congeners due to biotransformation (most samples are from organisms placed high in the food chain). Based on the number of peaks observed in the heart-cuts (Table 4; Figure 5), one should prefer the Ultra 2/DX4 and Ultra 2/Rtx 2330 column combinations over the DB5/FFAP combination.

The number of peaks present in the heart-cuts of CHB 62 varies from 2 to 6, and the peak area ratios vary from 14 to 83% (Table 4). The Ultra 2/Rtx 2330 obviously is the preferred column combination in this instance. The CHB 62 percentage in the samples analyzed varies between <0.1 and 1.7%, which is less than or comparable to the 1.2% found for CHB 62 in technical toxaphene (Table 3). This indicates that CHB 62 is one of the less persistent CHBs.

CHB 69 could only be determined by the Ultra 2/Rtx 2330 column combination because of its extremely long retention with the other column combinations. It was found to be present in the samples at or below the detection limit of 1 μg/kg wet weight.

Comparison with Literature Data. There is only one report on MDGC analysis of toxaphene (*33*). However, that study did not focus on the determination of individual CHBs but only showed the presence of many low abundant peaks in the GC chromatogram of a large heart-cut (5 min), which is in agreement with our results. Data on CHBs 26, 50, and

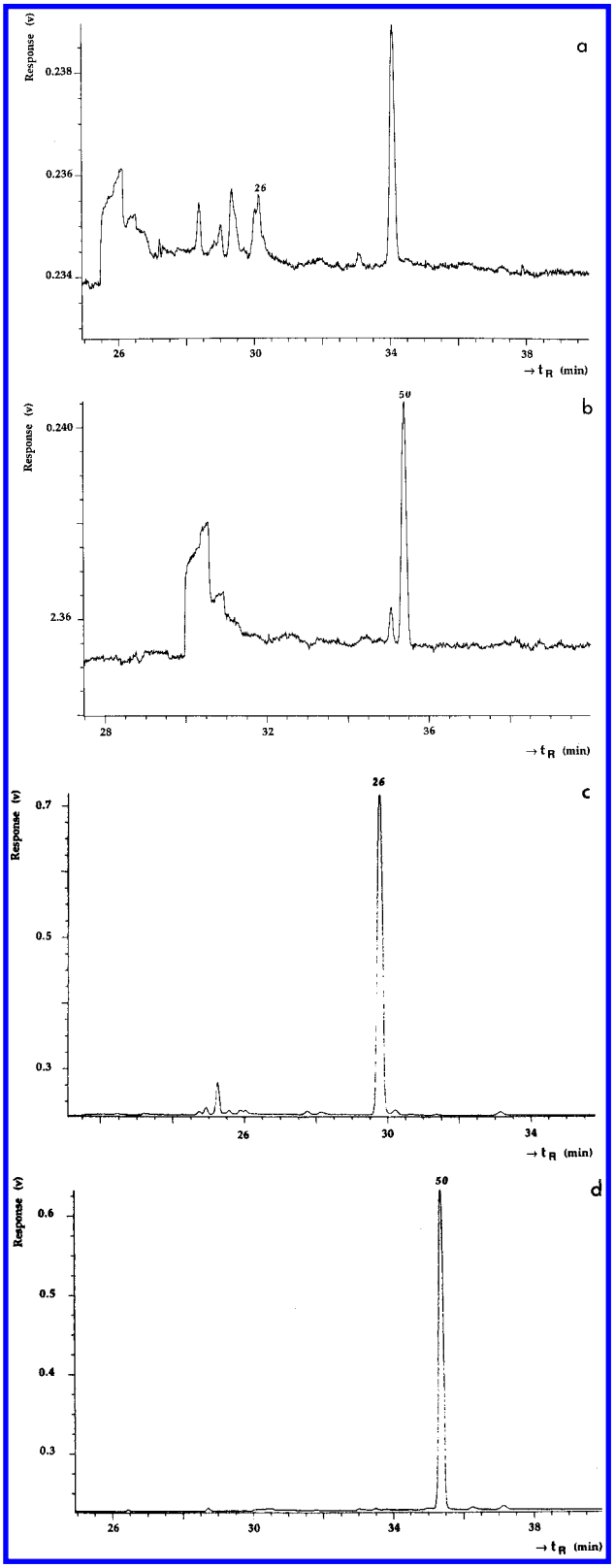

FIGURE 5. Heart-cuts from the first-dimension chromatogram of hake liver (cf. Figure 4a) of (a) CHB 26 (fraction II) and (b) CHB 50 analyzed on the DX4 column and from the first-dimension chromatogram of white-beaked dolphin (cf. Figure 4b) of (c) CHB 26 (fraction II) and (d) CHB 50 analyzed on the DX4 column (GC conditions in Table 3).

62 in fish samples obtained by single-column GC were reported by Alder et al. (*34*) and Parlar et al. (*35*). The former group reported concentrations of 6, 12, and 10 μg/kg wet weight for CHBs 26, 50, and 62, respectively, in herring from the Shetland Islands, which is ca. 5−10-fold higher than our results for a similar sample (Table 4). When expressed on a lipid weight basis, the differences are even larger. The discrepancies between the two sets of results can be explained by co-elution in the first place by other CHBs but, given the fact that the differences in concentrations are larger than expected on the basis of the peak area ratios in our heart-cuts (Table 4), presumably also by co-elution with compounds such as PCBs. Alder et al. used a single-column GC/ECD method, in which toxaphene is not even separated from most PCBs and organochlorine pesticides prior to the GC analysis. Despite the encouraging results obtained in an interlaboratory study in which the quoted method was used (*21*), the analytical results should be regarded with caution as far as concentrations of individual CHBs are concerned. The present study shows that the percentage of CHBs 26, 50, and 62 present in the first-dimension peaks generally is some 30−50%.

Parlar et al. presented data on the same congeners as above, using both GC/ECD and HR GC/ECNI−MS (*35*). Unfortunately, the nature of most samples studied by these authors is rather different from that of our samples. However, they reported that the sum of CHBs 26, 50, and 62 constitutes 25−30% of the total toxaphene residues in cod liver oil, which is ca. 3-fold higher than the present calculations indicate (Table 4). Again, co-elution probably is mainly responsible for this difference. In agreement with our results, the authors report that CHB 32 is only found in very low concentrations in fish samples and that CHB 69 cannot be detected at all. Parlar et al. (*35*) also refer to possible disturbing effects (CHB degradation) due to the use of a splitless rather than an on-column injection technique. The first-dimension chromatograms of the standards were clean, which at least indicates that formation of lower chlorinated CHBs did not take place in our experiments.

The total toxaphene levels found in the fish samples analyzed exceed the German tolerance level of 0.1 mg/kg lipid weight (*36*) 3−30-fold (Table 4). The toxaphene level in the dolphin sample (19 mg/kg in blubber) was also extremely high. Therefore, although we do not really agree with Parlar et al. (*35*) regarding the CHB concentration levels in fish samples, we do confirm their findings that toxaphene generally is present in European fish products at high concentrations and that a proper monitoring of frequently occurring and toxicological relevant CHBs in biological samples and sediments is required to compare the contamination of various ecosystems.

Obviously, further improvement may be expected from a combination of MDGC with MS. Improvements with regard to selectivity and sensitivity may also be expected from future developments in comprehensive MDGC (*30*, *37*).

Conclusions

Concentrations of CHB congeners determined by MDGC/ECD can be up to 10-fold lower than those determined by single-column GC/ECD, and heart-cuts analyzed by second-dimension GC show that peaks of CHBs in biota samples and, even more so, in technical toxaphene obtained by single-column GC may contain numerous peaks. Actually, heart-cuts made of some peaks in the technical mixture may still suffer from some co-elution. This is due to the complexity of the toxaphene mixture before biotransformation takes place. A combination of MDGC with (preferably HR)MS or application of comprehensive MDGC may be required to separate all congeners in such a complex mixture. For biota samples with their somewhat less complex CHB composition, the use of MDGC/ECD can be recommended for congener-specific routine analysis as well as in monitoring programes, especially because the resolution that can be achieved is better than with any other technique currently available.

Our results suggest that for CHB 50 a single-column GC analysis would be reliable. However, most samples studied were from organisms placed relatively high in the food chain,

and biotransformation in those samples may well have caused chromatograms to be rather clean. The presence of more peaks in the CHB 50 heart-cut (Ultra 2/Rtx 2330) in herring, which is placed lower in the food chain, confirms this idea. The real problem is that different samples, even of one species, may have different toxaphene patterns (*38*). Because MS detection does not offer the selectivity required for a reliable congener-specific analysis, MDGC separation is the only suitable method available today. This observation is important regarding to discussions on tolerance levels for toxaphene. If such discussions would result in congener-specific tolerance levels, because of the toxicity or persistence of some CHBs, one should be aware of the limitations of the use of single-column GC for this purpose.

Regarding the analytical conditions, there were only small differences between the GC column combinations tested. Based on the results for the five congeners studied until now, there is a slight preference for the Ultra 2/Rtx 2330 column combination because separations were generally better and bleeding was essentially absent because of the good thermostability.

Finally, the relatively high concentrations of toxaphene in European fish, which may even lead to exceeding the tolerance levels for human consumption, and in marine mammals demand a proper monitoring of total toxaphene and individual CHBs in relevant samples from European aquatic ecosystems.

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