Multidimensional GC analysis of toxaphene

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Abstract

A technical toxaphene mixture and a number of biological samples were analysed by multidimensional GC. The technical mixture at least consists of 246 congeners, whilst only 107 peaks were found in the first dimension-chromatogram. Furthermore it is shown that the major toxaphene peaks present in the technical mixture and biological samples mostly consist of 2-10 different toxaphene congeners, and only occasionally can be determined as a pure peak in the first dimension-chromatogram.

1. Introduction

Toxaphene is a complex mixture primarily consisting of chlorinated bornanes, and has widely been used as a pesticide[1]. Different chlorine substitution can theoretically lead to 32,768 possible congeners[2], of which a number also show chiral activity[3,4]. Technical toxaphene mainly consists of Cl_7 to Cl_9 congeners and therefore could contain 6,840 congeners.
However, a number of these CHBs are unlikely to be present because of unfavourable substitution positions on ring and bridge carbons[5]. Jansson and Wideqvist claim to have separated 670 individual congeners in a technical toxaphene mixture[6]. In environmental samples the total number of congeners will be smaller due to degradation and metabolism. However, the separation power of one-dimensional gas chromatography (GC) is insufficient for separating these numbers of congeners. The use of mass spectrometry (MS) adds extra selectivity but with the sensitive negative chemical ionisation (NCI) technique, needed for the

determination of low concentrations[7], mainly the molecular ion is formed which makes single congener identification more difficult.
The use of multidimensional (MD) GC has shown to be very beneficial for other complex mixtures such as PCBs[8,9]. By making sequential heart-cuts it is possible to make a better estimation of the minimum number of toxaphene congeners present in a sample. Furthermore, the quantification of toxaphene congeners can be checked on purity and false positive results can be avoided.
In this study the number of congeners present in a technical toxaphene sample was estimated by making sequential heart-cuts. Heart-cuts at retention times of some specific CHBs were made in the technical toxaphene mixture and in some fish and dolphin extracts to check the composition of these peaks, and to determine their concentration .

2. Experimental

A technical toxaphene mixture was obtained from Polyscience (Warrington, PA, USA). Five individual toxaphene congeners were obtained from Promochem (Wesel, Germany). Their codes and structures (Fig. 1) are:
Parlar No. 26 (T_2) 2-exo,3-endo,5-exo,6-endo,8b,8c,10a,10b-octachlorbornane
Parlar No. 32 (Tox B) 2,2,5-endo,6-exo,8b,9c,10a-heptachlorbornane
Parlar No. 50 (T_{12}, Tox Ac) 2-exo,3-endo,5-exo-6-endo,8b,8c,9c,10a,10b-nonachlorbornane
Parlar No. 62 2,2,5,5,8b,8c,9c,10a,10b-nonachlorbornane
Parlar No. 69 2,2,5,5,6-exo,8b,8c,9c,10a,10b-decachlorbornane.
Fish muscle tissue and liver, dolphin blubber and human milk samples were extracted and cleaned-up according to de Boer and Wester[7].
All analyses were performed on a Sichromat 2-8 GC with two independently controlled ovens and two electron capture detectors (ECDs) (Siemens AG, Karlsruhe, Germany). The column dimensions and operation conditions are given in Table 1. A DB-5 (5% phenyl 95% methylsilicone) column (J&W Alltech, Deerfield, IL, USA) was connected via a "live-T-piece" to a DX-4 (15% dimethylsilicone 85% polyethyleneglycol) column (J&W, Alltech). Heart-cuts from the first column were transferred to the second column through the "live-T-piece" by pressure switching[9]. The temperature programme of the first oven was optimised to obtain a maximum resolution. The second column was used isothermally, close to its maximum allowable operating temperature to prevent unnecessary lengthening of the total run time. Heart-cuts were made of T_2 and T_{12} in the technical toxaphene mixture and the biological sample, and additionally of the CHB 32 and 62 in the toxaphene standard. It was not possible to elute CHB 69 from the second column, probably due to a strong retention on this column and a too low operating temperature. Heart-cuts of the four remaining CHB congener standards were made

and checked on purity, which appeared to be >99% for T_2 and T_{12}, >98% for CHB 62, and > 95% for CHB 32.

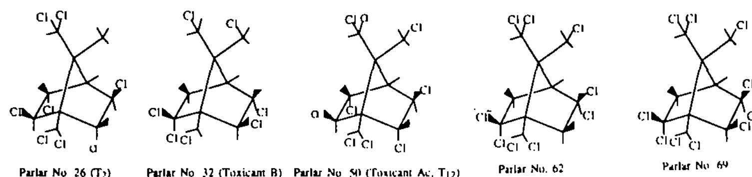

Figure 1 Structure of five CHB congeners

Table 1 MDGC/ECD conditions

Parameters	DB-5	DX-4		DB-5
Column length (m)	30	15	Initial isothermal period (min)	1
Column i.d. (mm)	0.25	0.25	Initial programming rate (°C.min^{-1})	20
Film thickness (μm)	0.25	0.25	Second isothermal temperature (°C)	220
Carrier gas (Pressure, kPa)	N_2(108)	N_2(70)	Second isothermal period (min)	1
Linear carrier gas velocity (cm.s^{-1})	10	22	Second programming rate (C.min^{-1})	5
Injection	manual		Third isothermal temperature (°C)	260
Injection volume (μl)	1.0		Third isothermal period (min)	1
Splitless time (min)	2.0		Third programming rate (°C.min^{-1})	3
Injector temperature (°C)	270		Final isothermal temperature (°C)	280
Septum purge (ml.min^{-1})	2			
Detection	^{63}Ni ECD	^{63}Ni ECD		
Detection temperature (°C)	300	300		
Make-up gas (flow, ml.min^{-1})	N_2(36)	N_2(38)		
Initial oven temperature (°C)	90	220		
Final isothermal period (min)	30	55		

3. Results and discussion

The first dimension-chromatogram of the toxaphene standard and a hake liver sample are shown in Fig. 2. The sections and peaks of these chromatograms which are heart-cutted are

indicated. The series of 40 heart-cuts throughout the toxaphene standard chromatogram resulted in 246 peaks, corrected for peaks which appeared in more than one heart-cut. This is more than twice the number of the visible peaks (107) in the first dimension chromatogram (Fig. 2). This shows that the chance on co-elution in a one-dimensional chromatogram of toxaphene is relatively high.

An accurate total number of toxaphene congeners present in the biological samples could not yet be given because with the repeated silica elution used[7)] losses of some congeners occur. Especially T_2 was only present in the final toxaphene fraction at 5% of its original concentration. Concentrations of the CHBs 32, 50 and 62 varied from 54 to 82%. An improved fractionation is currently investigated.

Second dimension-chromatograms of the heart-cuts of T_2 and T_{12} in the toxaphene standard and the hake liver on the DX-4 column are shown in Fig. 3. In Table 2 the concentrations and the peak height ratios of T_{12} and the number of peaks present in the T_2 and T_{12} heart cuts in the toxaphene standard and a number of biological samples are given. The calculations of the T_{12} concentrations are based on single point calibration.

Due to the poor fractionation characteristics of T_2, T_2 concentrations could not be calculated.

Table 2 Number of peaks, peak height ratios and concentrations of T_2 and T_{12} in heart-cuts of a toxaphene standard and biological samples[a]

Sample	Location	T_2	T_{12}			Total toxaphene[f]	Lipid[b] (g/kg)
		n[c]	R[d]	n	c[e]	(µg/kg)	
Toxaphene standard[g]		15	48	9	4.6	-	-
Hake liver *(Merluccius merluccius)*	Ireland	8	92	2	63	900	440
Cod liver *(Gadus morhua)*[h]	North Sea	8	40	5	<6	300	379
Herring fillet *(Clupea harengus)*	Skagerrak	5	5	4	3.7	40	220
Twait shad fillet *(Alosa fallax)*[h]	North Sea	4	-	0	<2	20	78
Human milk	Nicaragua	10	34	4	3.7	34	26
Whitebeaked dolphin blubber *(Lagenorynchus albirostris)*	North Sea	17	97	9	8100	19000	696

[a] Fish samples: pooled samples of 25 fishes; [b] total lipid content according to Bligh and Dyer; [c] n= number of peaks present in heart-cut; [d] R= peak height ratio (%): peak height of T_{12} divided by sum of peak heights of other peaks present in heart-cut; [e] c= concentration in µg/kg wet weight (for the toxaphene mixture in %); [f] total toxaphene concentration in µg/kg wet weight determined by GC/NCI-MS; [g] T_2: R = 29%, c=0.5 %; CHB 32: R= 56, n= 11, c= 4.2%; CHB 62: R= 80, n= 2, c= 0.4%; [h] determination around the detection limit.

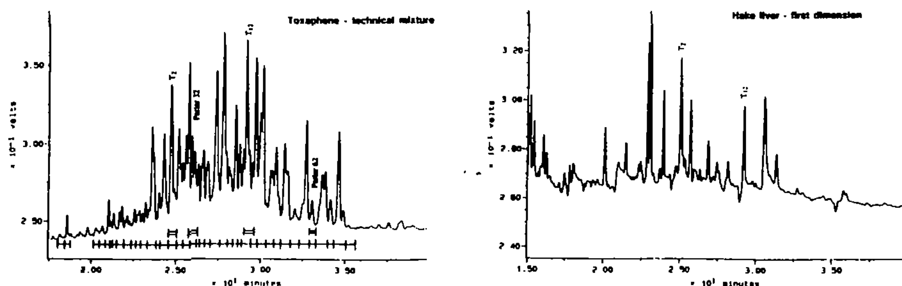

Figure 2 First dimension chromatograms of the toxaphene mixture and a hake liver sample on the DB-5 column (GC conditions in Table 1); T_2 in hake liver is only 5% of its original concentration

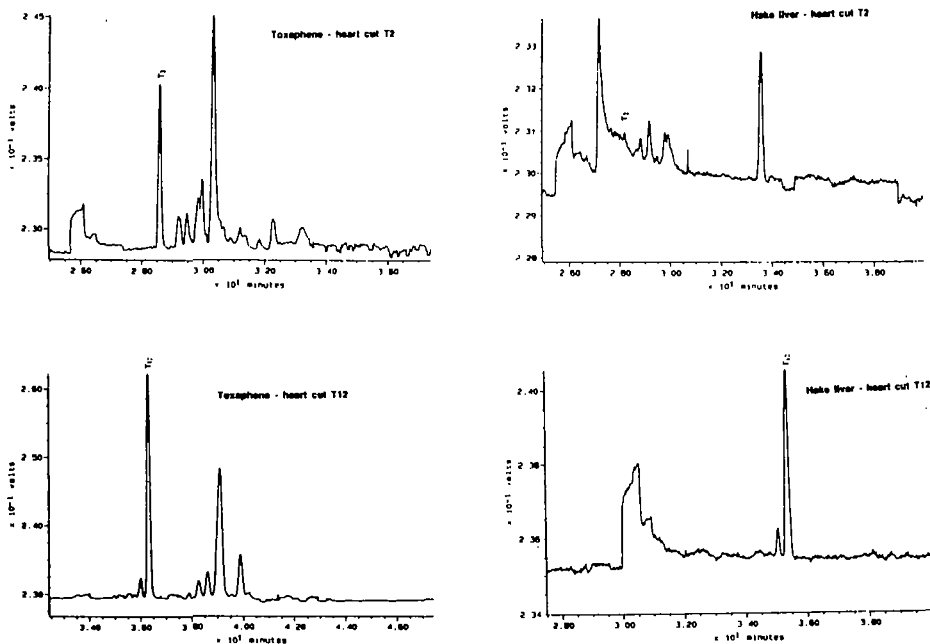

Figure 3 Heart-cuts of T_2 and T_{12} from the chromatograms shown in Fig. 2 on the DX-4 column (GC conditions in Table 1); T_2 in hake liver is only 5% of its original concentration

The heart-cuts show, however, that at the same retention time 4-17 peaks are present. Although some of these peaks may be negligible compared to T_2, this analysis shows that one should be very careful with a congener-specific analysis of T_2 with single-column GC. A different stationary phase may of course solve this problem, but it is rather likely that again other congeners will coelute then. T_{12} concentrations are <2-11% of the total in most samples except in dolphin blubber in which T_{12} is ca. 40% of the total toxaphene concentration.
Most heart-cuts of T_{12} contain 2-10 peaks. In some samples however, e.g. dolphin blubber and hake liver, the heart-cut of the T_{12} peak contains only a few other minor peaks, resulting in a purity of T_{12} of 97% and 92%, respectively. Therefore, there may be possibilities for a direct determination of T_{12} by single-column GC.

4. Conclusions

MDGC/ECD is a suitable technique for the determination of individual toxaphene congeners and a check on the purity of their peaks in single-column GC chromatograms. MDGC/ECD analysis of a technical toxaphene mixture has shown that there are at least 246 different congeners present in this mixture.
The data obtained until now with MDGC/ECD show that only T_{12} in some samples may be determined by single-column GC/ECD without significant coelution. Other stationary phases should be investigated and more samples should further be analysed by MDGC.
The use of MDGC/ECD is recommended for a reliable identification and quantification of individual toxaphene congeners.

5. References

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