Comparison of retention time overlaps of toxaphene congeners on three different stationary phases in cod liver samples and consequences for quantification

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1. Introduction

New maximum levels of toxaphenes in food have been proposed in Germany and will probably be valid from 1 January 1996. It is assumed that they will be based on lipid weight and the quantification of 3 specific toxaphene congeners which are dominant in marine biota. Methods for the determination by high resolution gas chromatography (HRGC) combined with electron capture negative ion chemical ionization mass spectrometry (EC-NICI-MS) or electron capture detection (ECD) have been developed and compared[1,2]. The three congeners no. 26, 50 and 62 (numbering according to Parlar[3]) used for quantification were selected due to their high abundance in biota. Several correction factors have been proposed to calculated the total toxaphene amount from the sum concentrations of these three toxaphenes[1,3]

Despite many years of analysis of polychlorinated biphenyls (PCB), only a few years ago it could be shown that PCB 138 co-elute with PCB 163 and 164 on the most applied stationary phases[4]. Since a larger number of minor toxaphene congeners are present in fish samples, the same situation cannot be excluded for the selected toxaphenes. When using the ECD, toxaphenes with a different degree of chlorination might interfere. Congener no. 62 has a very low response factor when EC-NICI-MS is applied and, therefore, partly co-eluting minor congeners with a much better response can disturb. To control possible interferences, the use of two different stationary phases has been a standard procedure for PCB analysis by ECD. Furthermore, the determination of the elution order of PCB on a large number of stationary phases has been carried out. This has not been done yet for toxaphene quantification. However, at the moment only 20 to 30 pure toxaphene congeners are available for such studies. In addition, the structure of a lot of major congeners in fish is still unknown. This restricts severely the determination of the elution order and detection of possible interferences. The presented paper tries to overcome this problem for some selected toxaphene congeners by a comparison of the levels in cod livers obtained by separation on three quite different stationary phases. Similar results on all phases would indicate that there is very little risk for false results due to interferences. Toxaphene congeners were selected which were available as standards of reasonable purity and/or showed a relative intensive signal in the gas chromatograms of cod liver extracts.

2. Experimental

Samples: Pooled samples of 25 cod livers from the Barents Sea were analyzed. They belonged to a sample set described in detail elsewhere[3].

Standards: Single toxaphene congeners were obtained from Ehrensdorfer (Augsburg, Germany) or the group of Nikiforov and co-workers[5] via Promochem (Wesel, Germany). The origin of the standards is marked in Table 1.

Sample clean-up: About 1 g of cod liver was homogenized with a 4-8 times larger amount of sodium sulfate. The homogenate was filled into a 40 cm x 3 cm i.d. glass column. Both 1,4,5-exo,7,8,9,10,10-octachlorotricyclo[5,2,1,$0^{2,6}$]dec-3,8-diene (4,5-DCCD)[6] and ε-hexachlorcyclohexane (ε-HCH) were added as internal standards. Afterwards, the lipids were extracted by a slow flow of 100 ml cyclohexane (ca. 0,5 ml/min). Further clean-up of the extract was carried out by gelpermeation chromatography (GPC) on a column of 60 cm x 2,5 cm i.d. filled with 50 g Biobeads SX-3 using cyclohexane/ethylacetate (1+1) as mobile phase. The eluate was concentrated to 500 µl and fractionated further on the deactivated alumina column described before using the following solvents: 0-50 ml, 100% n-hexane; 50-100 ml, n-hexane/methyl-t-butyl ether(MTBE) 50+50; 100-150 ml, n-hexane/MTBE 20+80. For mass spectrometric quantification toxaphenes and chlordanes were collected without further separation in a single fraction from 8-130 ml. After reduction of the volume to 250 µl, 1,2,3,4-tetrachloronaphthalene was added as recovery standard.

Quantification: The obtained fraction was analyzed by HRGC combined with negative ion chemical ionization (NICI) mass spectrometry on a HP 5989 GC/MS using CH_4 at a pressure of 0,45 torr and an ion source temperature of 200°C. For quantification the following ions were employed: toxaphenes and ε-HCH, $(M\text{-}Cl)^-$ and $(M\text{-}Cl+2)^-$; 4,5-DCCD, m/z 302 or 334.

Separation conditions: The following fused silica capillaries were used: (A) 30 m length x 0,2 mm i.d. coated with a 0,11 µm film of Ultra-2 (5% diphenyl-, 95% dimethyl polysiloxane, Hewlett-Packard); (B) 30 m length x 0,25 mm i.d. coated with a 0,10 µm film of RT_x1701 (14% phenyl cyanopropyl-, 86% dimethyl polysiloxane, Restek); (C) 30 m length x 0,25 mm i.d. coated with 0,10 µm RT_x2330 (90% biscyanopropyl-, 10% phenyl cyanopropyl polysiloxane, Restek). The separation conditions were as follows: Injector temperature, 220°C; transfer line temperature, 260°C; splitless injection of 1 µl at 90°C, 2 min splitless time; for (A): 90-160°C at 30°C/min, 160-260°C at 4°/min, 2 min isothermal; (B): 90-160°C at 30°C/min, 160-180°C at 0,5°C/min, 180-260°C at 4°/min, 2 min isothermal, for (C) 90-160°C at 30°C/min, 160-180°C at 2°C/min, 5 min isothermal, 180-250°C at 4°/min, 5 min isothermal.

3. Results and discussion

The reproducibility of the quantification was determined as a first step of the comparison of the quantitative results of the toxaphene congeners on the three different stationary phases. Toxaphenes have a reduced thermal stability, and dehydrochlorination on hot and/or active surfaces might occur. Therefore, the reproducibility of the quantification was tested on the least polar stationary phase Ultra-2. 1,4,5-exo,7,8,9,10,10-octachlorotricyclo[5,2,1,$0^{2,6}$]dec-3,8-diene (4,5-DCCD) was used as internal standard since this compound elutes within the retention time range of the toxaphenes of interest. As can be seen from Table 1, the relative standard deviation of 5 injections was below 5% for most of the

congeners. Tox 58 was only partly separated from another congener resulting in a much poorer reproducibility.

Table 1. Reproducibility of the quantification of selected toxaphene congeners in a cod liver extract on Ultra-2 obtained by 5 consecutive injections and EC-NICI-MS. For structure identification, see ref. 5. P: Obtained from Promochem; E: obtained from Ehrensdorfer.

Compound	Rel. standard dev. (%) Quantified by 4,5-DCCD
Tox 26[a] (P)	2.1
Tox 31 (P)	2.5
Tox 39 (E)	3.5
Tox 40/41 (E)	4.0
Tox 42 (E)	10.7
Tox 42B/44 (E)	3.2
Tox 50[a] (P)	3.9
Tox 58 (E)	23.3
Tox 62[a] (P)	8.4
Tox 63 (E)	8.1

[a] Indicator toxaphenes proposed for quantification

Both RT_x1701 and RT_x2330 contain a different amount of cyanopropyl groups (see experimental) which can undergo dipole interactions with the Cl-C bonds of the toxaphene structure. These stationary phases are also able to separate PCB and polychlorinated dibenzo-p-dioxin and dibenzofuran isomers which co-elute on more non-polar phases. Therefore, they were selected as alternatives to the polymethyl and polymethyl phenyl polysiloxanes normally used in toxaphene analysis.

Quantifications on more polar stationary phases are normally less reproducible. Relative standard deviations of up to 10% were for example observed on RT_x2330. Therefore, a deviation of up to 15-20% between the results from the three columns was considered as acceptable. Table 2 summarizes the concentrations obtained on all three phases. Since some toxaphene congeners co-eluted on one or two of the selected stationary phases, a direct comparison of the results on all three phases was only possible for a limited number of the chosen congeners. Figure 1 shows the elution order of the octachloro bornanes on the three stationary phases.

The results can be summarized as follows:

- From all toxaphene congeners only the results of the three selected indicator compounds Tox 26, 50 and 62 give a reasonable agreement on all three phases. Some deviations might additionally be caused by the low response factor of Tox 62 or possible losses on the most polar phase RT_x2330.
- The lowest concentrations were obtained for Tox 31, 39 and 58 on Ultra-2 indicating co-elution on the other phases.
- Tox 63 is present in quite low concentrations. Therefore, the agreement between Ultra-2 and RT_x2330 is considered as acceptable.

Table 2. Quantitative results of selected toxaphene congeners in 3 cod liver extracts on the phases Ultra-2, RT_x1701 and RT_x2330. The deviation relative to Ultra-2 is given in parentheses. Averages of three injections are shown. i: Interference due to an insufficient separated signal.

Compound	Concentrations found (ng/g wet weight)		
	Ultra-2	RT_x1701	RT_x2330
Tox 26	29.4	34.2 (16)	27.2 (-7)
	20.5	24.8 (21)	21.7 (6)
	108.2	128.3 (18)	79.8 (-26)
Tox 31	5.7	7.2 (26)	5.0 (-12)
	2.8	4.7 (70)	7.2 (160)
	2.0	20.0 (900)	17.6 (780)
Tox 39	1.76	7.8 (343)	17.2 (877)
	1.62	5.8 (256)	18.9 (1070)
	4.5	30.4 (580)	56.8 (1170)
Tox 50	44.3	49.5 (12)	51.5 (16)
	28.4	29.9 (5.5)	24.7 (-13)
	140	158 (13)	154 (10)
Tox 58	1.9	3.1 (66)	i
	1.1	0.8 (-25)	i
	9.3	31.8 (240)	i
Tox 62	26.6	22.3 (-16)	17.3 (-34)
	10.5	12.6 (20)	9.2 (-7))
	53.0	46.7 (-11)	43.1 (-18)
Tox 63	0.92	1.37 (49)	0.54 (-41)
	0.45	0.73 (63)	0.58 (30)
	2.10	3.6 (72)	2.0 (-3.2)
Tox 40/41/42A			6.87
			12.9
			22.0
Tox 42B/44	47.4		
	39.5		
	87.4		
Tox 40/41	9.7		
	8.7		
	20.2		
Tox 42A	8.3		
	6.4		
	25.0		
Tox 44		20.8	i
		13.5	i
		52.0	i
Tox 41/42A/42B		10 7	
		11 4	
		27 1	
Tox 40		10 1	
		11 0	
		32.0	

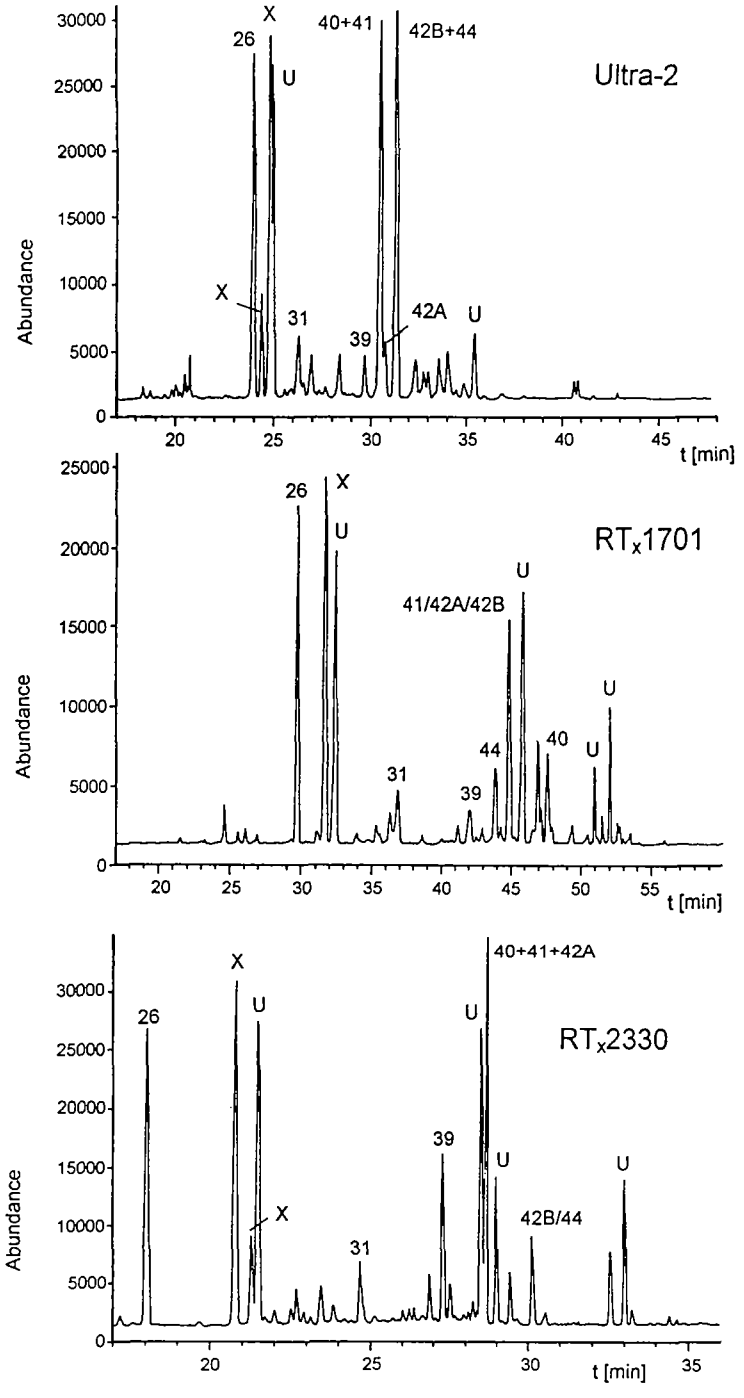

Figure 1. Elution order of selected octachlorobornanes in an cod liver extract from the Barents Sea on the three stationary phases. X: Interfering compound not being an octachloro bornane. U: Unknown octachloro bornane. Tox 31 is a octachlorobornene

- Numerous co-elutions occur with Tox 40-44. Though some of the available congeners do not overlap on RT_x1701 (Tox 40 and 44) or Ultra-2 (42A), the sums and differences of the found concentrations indicate further co-elutions with unknown congeners.
- A substantial number of major congeners marked with an "U" in Figure 1 are present which are not identified yet. Compared to samples from more temperate regions, it seems that a larger number of toxaphene congeners are present in Arctic cod liver.

The presented results indicate that it will be very difficult to find a suitable stationary phase which is able to separate most of the major toxaphene congeners in fish samples. The three indicator congeners Tox 26, 50 and 62 can be quantified without any interference on all columns by EC-NICI-MS. A combination of two columns coated with 5% diphenyl-, 95% dimethyl polysiloxane or 14% phenyl cyanopropyl 86% dimethyl polysiloxane is also used for the quantification of PCB. It can also be applied to check possible interferences of the three toxaphene congeners.

5. References

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