A CONTRIBUTION TO THE HRGC-MS and HRGC-ECD DETERMINATION OF TOXAPHENE RESIDUES IN FISH

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Introduction

Toxaphene, a halogenated hydrocarbon insecticide produced by chlorination of camphene, consists of a poorly defined mixture of at least 180 substances most of which are chlorinated bornanes[1-5]. The quantification of toxaphene in environmental samples has been complicated by the fact that, due to the different accumulation behaviour and degradation rates of the individual components, the composition of toxaphene residues differs significantly from that of the technical standard[6]. On the other hand, it has been possible successfully to quantify toxaphene residues in some environmental compartments, especially in fish and fish products, by using an analytical standard (CB-standard) obtained from the technical standard by UV-irradiation which led to a HRGC-peak pattern similar to that of fish samples[7,8]. For other samples, this standard seems to be less suitable for either ECD or MS/SIM quantification on account of the lower superimposability of the peak pattern. Therefore, the preparation of several environmental relevant single chlorinated bornanes as standard substances has been an important improvement. For the five most important of these substances (Parlar No. 26, 32, 50, 62, and 69)[9-11] the MS and ECD response behaviour has been investigated under different experimental conditions.

Tab. 1. Single chlorobornanes used for the determination of toxaphene residues in fish

Parlar No. 26	2-*exo*,3-*endo*,5-*exo*,6-*endo*,8b,8c,10a,10b-octachlorobornane
Parlar No. 32	2,2,5-*endo*,6-*exo*,8b,9c,10a-heptachlorobornane
Parlar No. 50	2-*exo*,3-*endo*,5-*exo*,6-*endo*,8b,8c,9c,10a,10b-nonachlorobornane
Parlar No. 62	2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane
Parlar No. 69	2,2,5,5,6-*exo*,8b,8c,9c,10a,10b-decachlorobornane

Material and Methods

Toxaphene (Camphechlor), PCB's, chlordane, HCH, HCB, DDT's, aldrin, and reference chlorobornane standards (Parlar No. 26, 32, 50, 62, and 69) were obtained from Ehrenstorfer, Germany. Organic solvents used were n-hexane, cyclohexane, dichloromethane, and carbontetrachloride of purity grade for residue analysis. Na_2SO_4 and H_2SO_4 (95-97%) were from Merck, Germany. Samples and standards 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 (ca. 2 ml/min). The chromatographic time and temperature conditions were as follows: splitless injection; initial temperature 140 °C - hold for 1 min - to 250 °C with 4 °C/min. Injection port and transfer lines were maintained at 280 °C. CH_4 was used as reactant gas. The emission current was approximately 200 µA.

HRGC-ECD routine measurements were performed with a Varian 3300 system, equipped with a 25 m x 0.2 mm capillary column (HP-5, film thickness = 0.5 µm) with nitrogen as carrier gas (ca. 2 ml/min); for better resolution helium as carrier gas is recommended. The chromatographic conditions were as follows: splitless injection; initial temperature 150 °C - to 250 °C with 5 °C/min. The injection port was maintained at 250 °C. The detector temperature was 280 °C.

HRGC-ECD/Ni^{63} on-column measurements were done with a Varian 3400 system. Column: DB-5, 60 m x 0.25 mm (film thickness = 0.25 µm); carrier gas: 1.5 ml/min; oven initial temperature: 120 °C - to 180 °C with 10 °C/min - hold for 1 min - to 280 °C with 5 °C/min - hold 13 min; injector initial temperature: 60 °C - to 250 °C with 190 °C/min - hold for 39 min; detector temperature: 320 °C.

Results and discussion

HRGC-ECD-experiments

Relative retention times (t_{RR}) and relative response factors (F_{RR}) obtained from HRGC-ECD experiments with splitless/split and on-column injection are summarized in Table 2. From the results it can be seen that the chlorobornanes are thermally unstable in the splitless/split mode and react under the high temperature in the injection port. There is a correlation between the number of the chlorine atoms and the degradation rate though it has not been investigated in detail. Especially the decachlorobornane No. 69 is very reactive. Only 4 % of the peak area of No. 69 relative to No. 50 (Toxicant Ac) could be registrated in the splitless system compared to the ratio after on-column injection. So the problem of degradation can easily be overcome by using on-column systems because under these conditions the chlorobornanes remain stable. When using on-column ECD detection, the relative response factors of the chlorobornanes are sufficiently comparable with each other, varying between 1.00 and 1.46 relative to compound No. 50. Therefore, with some restrictions, on-column ECD can be used for the quantification of unknown chlorobornanes when only one of the single chlorobornanes is used for a standard.

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 can be expected. Furthermore, the response factor differences of chlorobornanes are significantly higher than those observed with ECD detectors. Particularly the ECNI-SIM method causes remarkable deviations. The relative peak areas of the fragments used for the quantification of different chlorinated bornanes with similar concentrations vary between 20 and 100 % relative to compound No. 50. Considering these results, it is not possible to quantify unknown toxaphene components with the help of the ECNI-SIM method.

Tab. 2. Relative retention times (t_{RR}) and response factors (F_{RR}) of the five single chlorobornanes used for the determination of toxaphene residues in fish. Reference substances: **Ac** = Toxicant Ac (Parlar No. 50) and **Al** = aldrin; **PCI** = positively charged chemical ionization; **ECNI** = negatively charged chemical ionization; **SIM** = selected ion monitoring; **EC** = electron capture Ni^{63}; **OCI** = on-column

No.	t_{RR}		F_{RR}							
	Ac*	Al*	EI Ac	PCI Ac	ECNI Ac	ECNI/ SIM Ac	EC/ OCI Ac	EC/ OCI Al	EC/ splitl. Ac	EC/ splitl. 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

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