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Determination of toxaphene in fish samples - a congener specific approach using high resolution mass spectrometry

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Introduction

Toxaphene is a chlorinated pesticide which, owing to its persistence, is distributed globally and accumulates in the environment far from the original point of usage¹. Like other persistent organochlorine compounds, such as DDT and PCB, high concentrations are found in biological fatty tissues. Human exposure to toxaphene residues is mainly through food consumption and especially from intake of fish with high fat content².

Technical toxaphene is a complex mixture consisting primarily of polychlorinated bornanes. Analytical methods have developed from determination of total toxaphene concentration using a technical mixture as a standard to congener specific analysis^{3,4,5}. Methods for fat extraction and clean-up are usually adaptations of procedures known from determination of chlorinated pesticides and polychlorinated biphenyls in biological samples. Chromatographic separation and quantification of toxaphene congeners have been performed by capillary gas chromatography with electron capture detection⁵ or with more specific detection by different mass spectrometric techniques⁶. Recently multidimensional gas chromatography has been applied as well⁷. No single analytical procedure has been accepted to produce the most reliable results, on the contrary questions are raised concerning the great variability when comparing different methods^{7,8}. In this study an analytical method were developed based on GC/MS with high resolution mass spectrometry.

Experimental method

Standards: A quantitative mixture of three toxaphene congeners were obtained from Promochem, Germany. The standard contained:

1. 2-exo,3-endo,5-exo,6-endo,8b,8c,10a,10b-octachlorobornane (Parlar No. 26)
2. 2-exo,3-endo,5-exo,6-endo,8b,8c,9c,10a,10b-nonachlorobornane (Parlar No. 50)
3. 2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane (Parlar No. 62)

Samples: Fish of herring and salmon were filleted and homogenised in a household type blender. The samples were stored at -18°C as either fillet or homogenised sample until analysis. Figure 1 shows a flowchart of the extraction and clean-up procedure. About 10-12 g of the homogenised sample was mixed with 50 g sodium sulphate and 150 ml petroleum ether. The mixed sample was blended with an Ultra-Turrax blender, centrifuged and the solvent was decanted. This procedure was repeated two more times, with addition of 100 ml of solvent. Extractable lipids were determined gravimetricly on a subsample of the extract. Samples were evaporated to 1 ml and cleaned-up on a multi-layer column eluted with 175 ml cyclohexane-pentane (1:2). The cleaned

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sample was evaporated, the solvent changed into isooctane and adjusted to 1 ml containing ^{13}C -PCB 105 as internal standard.

The toxaphene congeners #26, #50 and #62 were measured by high resolution capillary gas chromatography with a Micromass AutoSpec Ultima high resolution mass spectrometer.

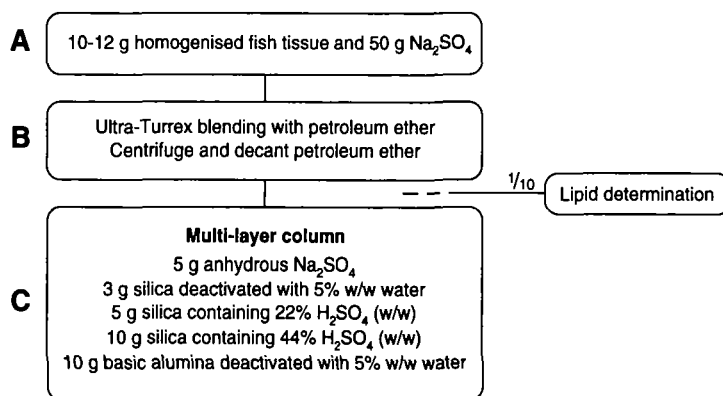


Figure 1. Clean-up scheme for toxaphene in fish samples. A. Sample pre-treatment. B. Lipid extraction. C. Lipid matrix removal.

GC parameters: Column: 25 m BPX5 (SGE), 0.22 mm I.D., 0.25 μm film thickness. Carrier gas helium at 12 psi head pressure. 1 μl injected splitless, splitless time 1 min. Injector held at 240°C. Temperature program: 100°C in 2 min., 20°C/min. to 180°C, 3°C/min. to 265°C, 20°C to 290°C, isothermal in 30 min.

MS parameters: Ionisation by electron impact, resolution 10,000, ion energy 37 eV, trap 650 μA . Source temperature 260°C, transfer lines at 270°C and 280°C. Selected ion monitoring of mass fragments at m/z 340.8806 and 342.8776 (toxaphene #26), m/z 338.8649 and 340.8620 (toxaphene #50 and #62) & m/z 335.9236 and 337.9206 (^{13}C -PCB 105). PFK was used as lock mass.

GC-ECD parameters: HP6890 gas chromatograph. Column: 50 m CP Sil 5CB, 0.25 mm I.D., 0.25 μm film thickness. Carrier gas hydrogen, constant flow controlled at 1.5 ml/min. 2 μl injected splitless, splitless time 1 min. Injector held at 220°C. Temperature program: 90°C in 2 min., 30°C/min. to 180°C, 2°C/min. to 240°C, 5°C to 280°C.

Results and discussion

The lipid extraction and clean-up procedures were chosen in order to facilitate a quick sample throughput. Blender extraction efficiency and analytical results were compared with soxhlet extraction and the two methods gave comparable results. The possibility of degradation of toxaphene congeners by the exposure to the sulphuric acid coated on silica were checked. For the three congeners measured no loss were observed. The overall recoveries for lipid extraction and cleanup were approximately 90%.

Quantification were performed by determination of relative response factors towards the internal standard ^{13}C -PCB 105. Quality assurance criteria for peak detection included retention time window and correct isotope ratio for the two mass fragments monitored for each chlorine homologue series. Detection limits were determined to 0.2 ng/g wet weight for each congener.

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Figure 2 shows an example of the mass chromatograms obtained from the analysis of a herring sample. Traces A and B are one of the two masses monitored for octachloro- and nonachlorobornanes respectively. C is a trace of the dichlorotropylium ion at m/z 158.9768, which is characteristic for toxaphene and has been used for total toxaphene determinations^{9,10}. For comparison purposes quantification using the dichlorotropylium ion were performed. Toxaphene #50 and #62 gave the same result but #26 was 12% higher indicating co-elution of another toxaphene congener or a compound generating ion fragments at the same mass. The higher mass fragments used in this study appears to be a better choice for the determination of individual toxaphene congeners.

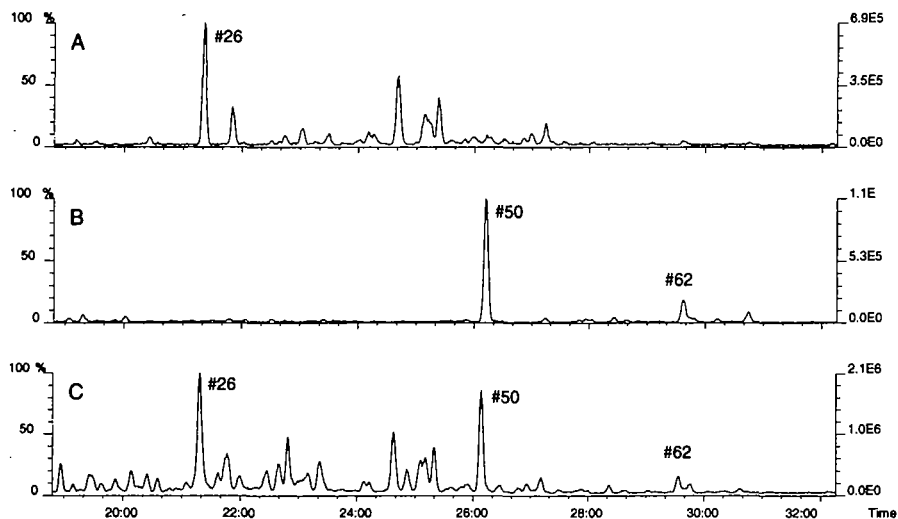


Figure 2. Mass chromatograms of herring sample. MS resolution 10,000.
A. Octachlorobornanes m/z 340.8806. B. Nonachlorobornanes m/z 338.8649.
C. Characteristic ion fragment for all chlorobornanes m/z 158.9768.

The advantage of the high resolution mass spectrometric detection is the minimisation of interferences from other e.g. chlorinated compounds eluting together with toxaphene on the multi-layer column⁹.

In order to verify this an aliquot of some samples were further subjected to fractionation on a silica column. Figure 3 illustrates the interval of elution for a standard mixture of selected PCBs, chlorinated pesticides and toxaphene congeners. The fractions containing the toxaphene congeners were accumulated and analysed by both GC/MS and GC-ECD. The GC/MS concentrations for a herring sample for #26, #50 and #62 were determined to 1.5, 1.5 and 0.4 $\mu\text{g}/\text{kg}$ wet weight. This is the same result as before the sample were subjected to fractionation on silica. The GC-ECD results were all higher: 44%, 27% and 58%, respectively.

Optimisation of the choice of GC column stationary phase might improve the results obtained by ECD¹¹. Nevertheless the ECD method is inherently more prone to interferences because a clean separation of toxaphene is difficult.

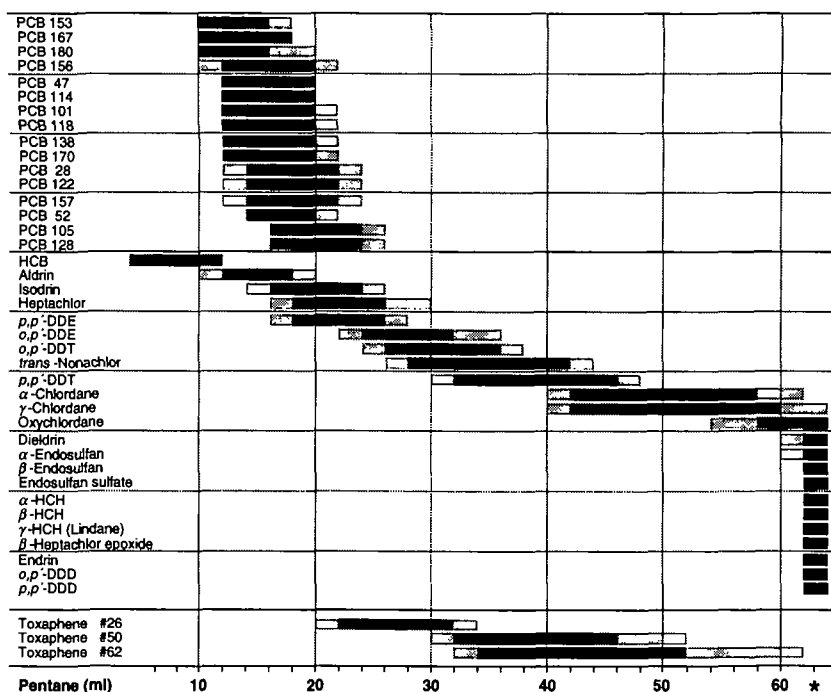


Figure 3. Elution of selected PCBs and chlorinated pesticides on 4 g silica, 1.5% deactivated. Fractions of 2 ml pentane were collected except for the last one (*): 6 ml diethyl ether. Grayscale indicates concentration profile.

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