Determination and comparison of toxaphene congener patterns in different eggs of birds of the German wadden sea

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Introduction

The determination of organochlorine residues in biological samples often reveals a characteristic pattern. These patterns are typical and can be used like fingerprints. In a previous work (1) Denker showed distinct differences in PCB-patterns depending on the species and its diet. Using sea birds as top predators for monitoring of environmental chemicals, spatial and temporal trends, seasonal and intersite variations and interspecific differences in metabolism are visible. Detecting toxaphenes with an isomer specific analysis (2, 3) in biologically well distinguished material (4, 5) of common tern (Sterna hirundo), herring-gull (Larus argentatus) and oystercatcher (Haematopus ostralegus), we found distinct differences in pattern. By using the commercial „Parlar 22 component standard" (6) we identified 11 by their Parlar number and lots of unknown penta-, hexa-, octa- and nonachlorobornanes and -bornenes (or -camphenes).

Materials and Methods

Samples:

The eggs of the common tern were collected in may (early clutches) and july (lately clutches) 1989 at the inner German Bight at Elbe estuary (Hullen) from the same birds; those from the oystercatcher and herring-gull were layed in may 1981 on the island of Mellum, German Bight, Weser estuary.

Sample clean-up:

All samples were homogenized and stored at –21 °C. About four [g] of each material was cleaned up identically to the method described by Xu (7). Soxhlet extraction was followed by clean up with sulfuric acid and further separation with defined silica gel. 1,2,3,4-tetrabrombenzene (IS 1) and 1,4-exo,7,8,9,10,10-heptachloro-5-methoxytricyclo[5,2,1,$0^{2,6}$]dec-3,8-diene (IS 2) were added as internal standards at the beginning of the clean-up procedure. The resulting two fractions (I, II) were evaporated nearly to dryness and resolved with 50 µL toluene.

Chromatographic and detection conditions:

I:GC/ECD, Fisons Instruments, GC 8000, Ni-63 ECD, nitrogen as carrier gas, 25 m HT-5, 0.22 mm i.d., film thickness 0.1 µm, SGE, Weiterstadt. Injector temperature 230 °C, detector

temperature 300 °C, splitless injection of 1 µL at 100 °C, 1 min splitless time; 100-140 °C at 25 °C/min, 140-250 °C at 3 °C/min, held for 30 min. **II:GC/MS**, Finnigan MAT, SSQ 710, NCI ionisation-mode, using methane as reagent gas, transferline 250 °C, source 120 °C, coupled with an A200S autosampler and a Varian 3400 GC; helium as carrier gas, 50 m HT-8, 0.22 mm i.d., film thickness 0.25 µm, SGE, Weiterstadt. Injector temperature 250 °C, splitless injection of 1 µL at 105 °C, 1 min splitless time; 105-170 °C at 23 °C/min, 7.5 min isotherm, 170-275 °C at 3 °C/min, held for 25 min. The MS-analysis was running in 2 different modes: full scan 100-600 m/z, SIM with isotope cluster of $[M]^-$, $[M\text{-}1Cl]^-$, $[M\text{-}HCl]^-$, $[M\text{-}2Cl]^-$ and typical m/z for the IS. To guarantee similar sensitivity for each measurement the conditions were checked by injection of 1 µL γ-HCH, 400 ng/ml. Three time-windows were established according to different retention times of the toxaphenes resulting in a higher sensitiveness.

Window 1: (Time 10.00-24.00 min)
m/z 304.9-314.0, 336.0-348.0, 369.8-383.9, 389.0-400.0, 406.0-417.8
Window 2: (Time 24.00-31.25 min)
m/z 304.9-314.0, 329.0-348.0, 369.8-383.9, 399.0-417.8, 437.8-453.8
Window 3: (Time 31.25-65.00 min)
m/z 304.9-314.0, 336.0-348.0, 369.8-383.9, 405.0-417.8, 437.8-453.8, 471.0-488.0

Results and Discussion

The two fractions were analyzed under the conditions described before. In fraction I we fixed penta- and hexachlorobornanes and -bornenes and IS 1. Figures 1, 2 and 3 show the typical GC-MS total ion-chromatograms (RIC=Σ SIM) of the toxaphene fraction II of higher chlorinated bornanes and bornenes (hepta-, octa- and nona-), octa-chlordanes, the nona-chlordane trans-nonachlor, octachlorstyrene (OCS) and IS 2 (29.82 min). It's obvious that besides the intensity the number of detected substances varies significantly between the three bird-species. In analogy to the determination of PCBs we found nearly the same pattern of toxaphenes in different eggs of the same species as well as in different eggs of early or lately nested common terns. All of them have relatively high signals for OCS (31.70 min), trans-nonachlor (35.19 min) and an unknown nonachloro-bornadiene (40.22 min). Common terns (fig.1) show a remarkably wider pattern of detected toxaphenes comparable to the PCB congeners (5). The reason for the difference to the other two species is caused by the selective diet in fish, the spatial behavior and maybe a specific way of metabolizing selected organochlorine compounds.

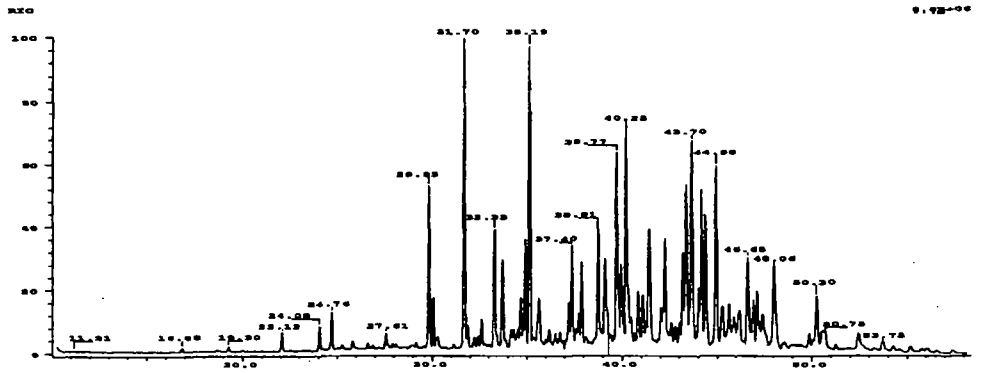

Figure 1: GC-NCI-MS chromatogram of common tern (Sterna hirundo) [min]

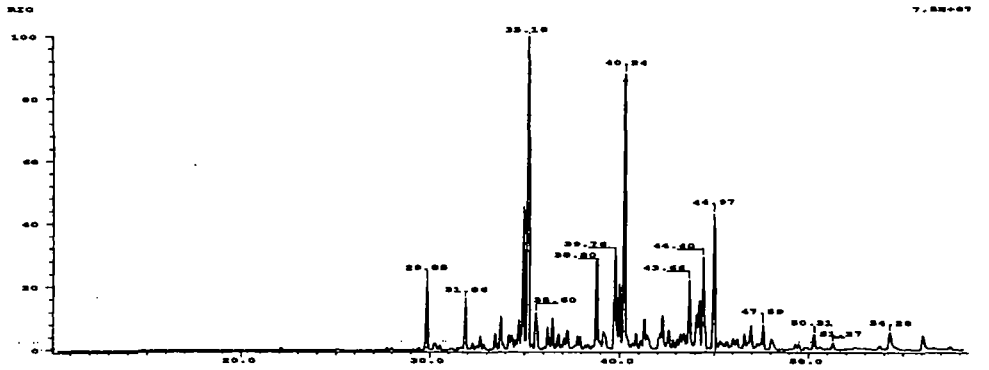

Figure 2: GC-NCI-MS chromatogram of herring-gull (Larus argentatus) [min]

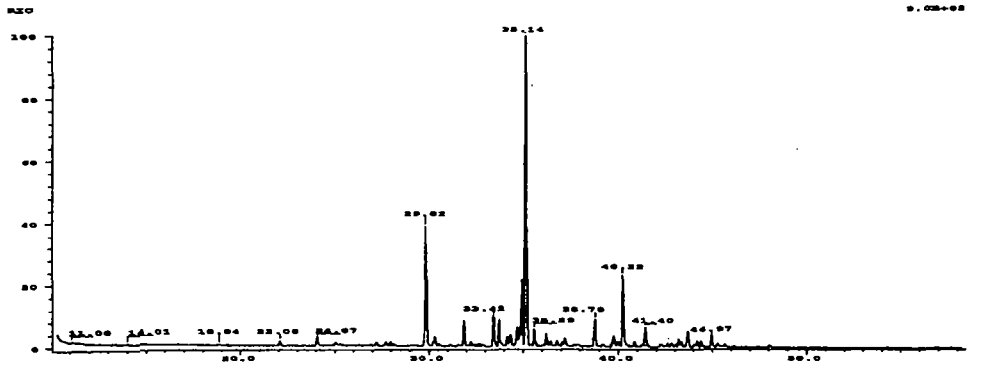

Figure 3: GC-NCI-MS chromatogram of oystercatcher (Haematopus ostralegus) [min]

Parlar No.	*Ret. Time (min)*
26	38.81/38.76
31	40.43
40	43.70
41	44.10
50	44.98/44.97
51	46.00
58	47.60
63	50.30/50.29

Table 1: Fixed toxaphenes with Parlar No.

Ret. Time (min)	*Degree of Cl*	*Bornane*	*Bornene*[+]	*Others*
36.20				x
37.22*	6	x		
37.38	7			-dien
37.93	7	x		
39.14	8		x	
39.75	8	x		
41.07	8	x#		
43.40	8	x#		
45.90	8	x#		
47.15	8		x#	

Table 2: Peaks with significant deviations

*result of coeluation; # main fragments =[M-HCl]; + (or camphenes)
(ret.times belong to fig.5, they may vary in the last digit in fig.4)

In the main part of the additionally analyzed fraction II of early (fig.4) and lately (fig.5) layed eggs of common terns 8 known toxaphenes (Parlar 26, 31, 40, 41, 50, 51, 58, 63) were fixed (tab.1), with no remarkable variations in their relative amount. Otherwise significantly higher peaks of 10 unknown toxaphenes were found in lately clutches (tab.2).

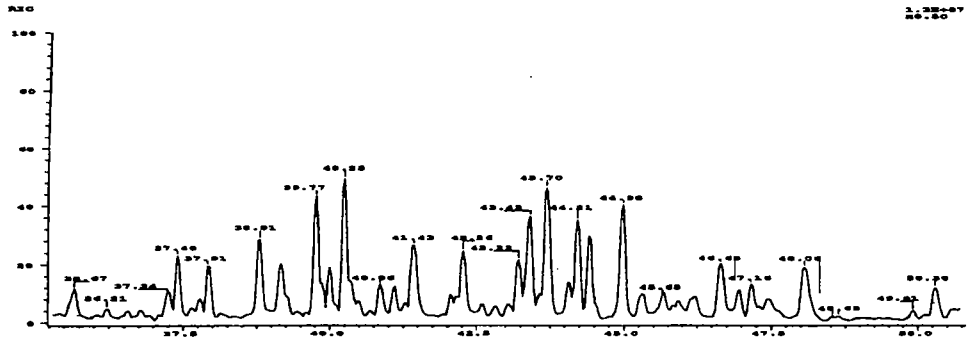

Figure 4: Essential part of GC-NCI-MS chromatogram of common tern (Sterna hirundo), early clutch [min]

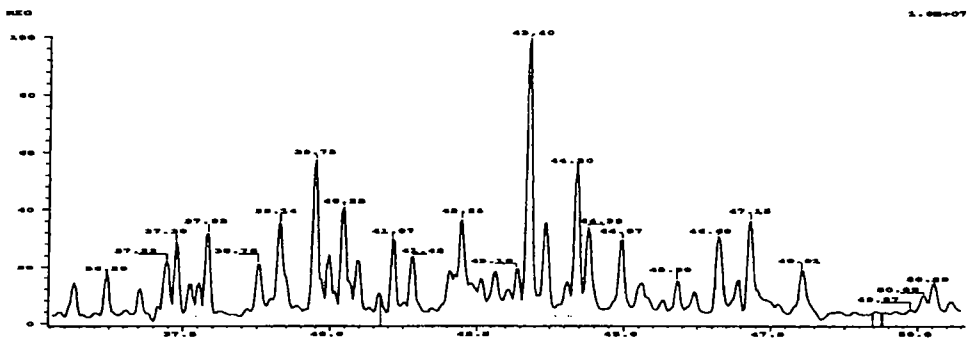

Figure 5: Essential part of GC-NCI-MS chromatogram of common tern (Sterna hirundo), lately clutch [min]

An explaination for the different amounts of the substances (table 2) is influenced by the bird migration eg. south-west africa and antarctic region (early clutch) to the northern part of Germany (lately clutch). In all samples no deca-chlorobornanes were detected. Further investigations in congener specific toxaphene analysis of different species are necessary to understand the metabolism and the toxicological relevance.

References

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