CONGENERS AND ENANTIOMER RATIOS OF TOXAPHENES IN EGGS FROM NORWEGIAN BIRDS OF PREY: APPLICATION OF A COMBINED APPROACH BY QUADRUPOLE NICI-MS AND ION TRAP EI-MS/MS

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Introduction

Only limited information is available about the toxaphene congener pattern and enantiomer ratio in birds. Compared to marine mammals and fish, some studies indicated the presence of more and/or different congeners in eggs from marine birds[1,2]. However, levels of other persistent polychlorinated compounds are often much higher in eggs than for toxaphenes. This requires a high selectivity of the mass spectrometric (MS) determination to avoid false positive results due to interference. Furthermore, so far no information was available about enantiomer ratios (ER) of selected toxaphenes in eggs from birds of prey.

Recently, a method for quantification of toxaphenes has been developed based on electron ionisation (EI) MS/MS in an ion trap showing a very high selectivity and low detection limits comparable to high resolution MS [3]. The aims of this work were to identify the toxaphene congener pattern in eggs of birds of prey from Norway, to determine the ER of selected toxaphenes and to compare the achieved selectivity of frequently used negative ion chemical ionisation (NICI)-MS with that of ion trap EI-MS/MS.

Methods and Materials

Selected eggs and sample clean-up

Seven not hatched (addled and deserted) eggs from birds of prey with a high toxaphene contamination were selected from an ensemble of thirty-nine eggs collected by the Norwegian Institute for Nature Research (NINA) from different regions of Norway under the license of the Norwegian Directorate for Nature Management between 1995 and 1997. Three were from white-tailed sea eagle (*Haliaeetus albicilla*), one from osprey (*Pandion haliaetus*), two from golden eagle (*Aquila chrysaetos*, same clutch) and one from goshawk (*Accipiter gentilis*). These species represent different trophic levels and migration habits. Osprey is a migrating bird feeding exclusively on freshwater fish. Golden eagle, goshawk and white-tailed sea eagle are examples of sedentary species. The eggs were stored at -20 °C in sealed polyethylene containers. Sample clean-up was carried out at the Norwegian Institute for Air Research (NILU) according to the method of Herzke et al.[4]. $^{13}C_{12}$-PCB 118 was used as internal standard added prior to extraction and octachloronaphtalene as recovery standard. More details about purity and origin of reference compounds (Parlar no. #11, #13, #15, #21, #25, #26, #31, #32, #38, #39, #40, #41, #42a/b, #44, #50, #51, #56, #58, #59, #62, #63, #69, Andrews and Vetter nomenclature B7-1453, B8-1412) are given in [5].

LEVELS IN BIOTIC COMPARTMENTS

Separation and quantification

Details of the EI-MS/MS technique are given in[3]. In brief, a Saturn 2000 ion trap MS (Varian, USA) combined with a 3400CX GC was used. The capillary column was 30 m long x 0.2 mm i.d. and coated with 0.1 µm of DB5-MS (J&W Scientific, USA) He was carrier gas (flow 1.1 mL/min). The temperature program was: 100 °C for 2 min, 15 °C/min to 160 °C, 3 °C/min to 210 °C, 15 °C/min to 260 °C, isothermal 4 min. EI-MS spectra were recorded at 70 eV (emission current 10 µA; mass range 100-400 u; scan time 1 s/scan; trap target 20000 counts). EI-MS/MS fragment ions were isolated in the resonant waveform mode (maximum ionisation time 65 ms; emission current 100 µA). For MS/MS, m/z 305 was selected as parent ion. The full scan daughter ion spectrum was obtained by collision induced dissociation (CID, excitation time 20 ms; excitation amplitude 0.5 V; excitation storage level m/z 150). Main fragment ions were m/z 269 (base ion), m/z 233, m/z 196 and m/z 159 (subsequent losses of HCl). All reference toxaphene congeners showed abundance ratios between m/z 269/233 of 1-1.5 (Cl_7), 1.1-1.6 (Cl_8) or 1.6-2.3 (Cl_9). Other polychlorinated compounds did either not show this fragmentation or gave a very different ratio (e.g. chlordanes, 9.5-13).

A 5989B mass spectrometer/5890II gas chromatograph (Agilent, USA) was employed in the NICI-mode (reagent gas, CH_4, 1.0-1.2 mbar, ion source, 200 °C, quadrupole, 100 °C). Compounds were detected in the single ion monitoring (SIM) mode (dwell time, 90 ms per ion). $[M\text{-}Cl]^-$ isotope signals were monitored (Cl_7-isomers, m/z 342.9/344.9; Cl_8-isomers, m/z 374.9/376.9/378.9; Cl_9-isomers, m/z 410.9/412.9. Separations were performed as follows: Capillary A (25 m x 0.2 mm i.d. 0.11 µm film of Ultra 2); capillary B (25 m x 0.25 mm i.d., 0.25 µm film of CP-Sil 2); temperature program for A (100 °C for 2 min, 10 °C/min to 240 °C, isothermal 10 min); for B (100 °C for 2 min, 15 °C/min to 160 °C, 2 °C/min to 255 °C, 15 °C/min to 260 °C, isothermal 5 min).

Enantiomer selective separations were carried out on two different stationary phases (0.2 mm film of 10 % heptakis(2,3,6-*O*-*tert*-butyldimethylsilyl)-b-cyclodextrin (CD) in OV-1701 or 0.15 mm of 25 % of octakis(2,3,6-tri-*O*-ethyl)-g-CD in PS086, for further details, see ref. 6).

Results and Discussion

Selectivity of NICI-MS versus EI-MS/MS

Figure 1 demonstrates that the EI-MS/MS chromatogram of an osprey egg extract contains less disturbances and interferences than those recorded by EI-MS or NICI-MS. For example, #38 is more abundant in the NICI and EI-MS mode due to a disturbing PCB, while EI-MS/MS shows that this congeners is a minor constituent. Table 1 summarises all compounds which fulfilled the identification criteria for toxaphenes by NICI-MS (isotope ratio within 10 % of reference (Cl_7 (m/z 343/345): 1.1-1.2, Cl_8 (m/z 377/379): 1.0-1.1, Cl_9 (411-413): 0.87-0.90) and by EI-MS/MS (abundance ratio m/z 269/239, see above). In addition, retention times of known congeners had to be within 2-3 s compared to reference compounds. Figure 1 shows some toxaphenes identified in this way. Signals marked with an asterisk met only the NICI criterion but were non-detectable by EI-MS/MS. Consequently, they are not toxaphenes.

Identified congeners, levels and ER

Thirteen known toxaphenes (B7-1453, B8-1412, #26, #32, #38, #40, #41, #42, #44, #50, #51, #58 and #63) were found in the eggs and fulfilled the identification criteria of both detection techniques. Six more compounds met them as well and are probably unknowns toxaphenes, among them B7-1000 (tx-1) a very early eluting Cl_7-isomer.

Compared to fish, the congener pattern of toxaphenes in eggs of birds of prey were dominated by #26, B8-1412, #40/41 and #50. Great interspecies variations were present. Additionally, minor components such as B7-1453, #32, #38, #42, #44, #51, #58 and #63 were found.

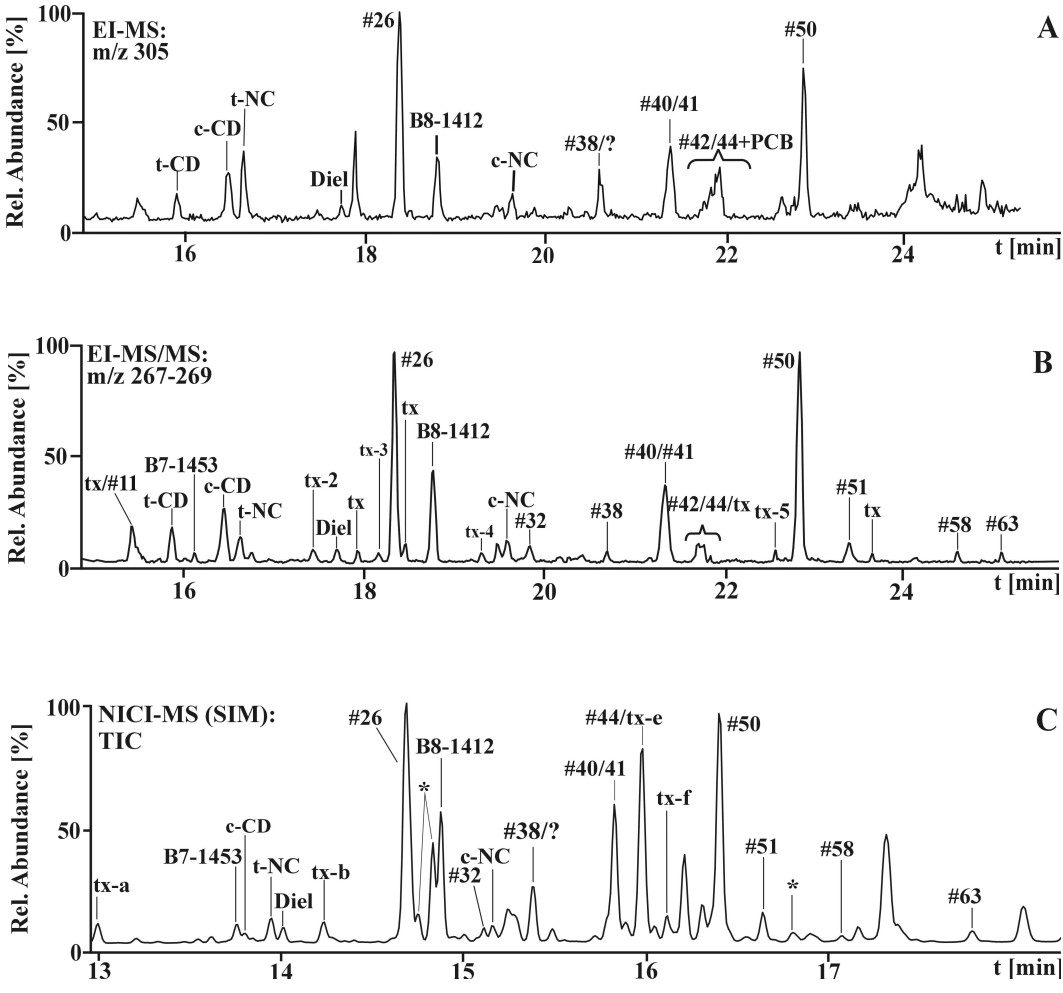

Figure 1. Mass chromatograms recorded by EI-MS (A), EI-MS/MS (B) and NICI-MS in the SIM mode (C) of a sample extract from an osprey egg. Dashed line: exceeds abundance scale; t-CD, trans-chlordane; c-CD, cis-chlordane; t-NC, trans-nonachlor; c-NC, cis-nonachlor; Diel, Dieldrin; unknown toxaphenes, tx-1 to tx-6, tx-a to tx-g or tx; asterix (*), no toxaphene (see text).

#26, B8-1412, #40/41 and #50 contributed about 80-90% to the total amount of quantified toxaphene congeners (80-740 ng/g wet weight (ww)). The sum of chlordanes (170–1600 ng/g ww) was about a factor of 2-20 higher. Up to 80 % of all quantified chlorinated compounds (2900-8000 ng/g ww,) were PCB congeners[4]. The highest toxaphene contamination was found in the osprey egg followed by golden eagle, white-tailed sea eagle and goshawk.

ER could be determined in 4-6 of the eggs and for 3 congeners. They were close to racemic for #50 except for two eggs from white-tailed sea eagle (1.33/1.34) and one from golden eagle (1.47). #26

showed a significant depletion of the first eluting enantiomer (ER 0.42-0.60) The ER of B7-1453 varied between 0.71-1.15. So far, ER in birds were only determined in two Penguin samples (*Pygoscelis adelis*, 1.34 for #50, 0.91 for #40 (TC-6) or 41, 0.74 for B7-1000 (TC-1), 0.38-0.43 for B8-1412)[7,8].

Table 1. Relative retention, isotope ratios (NICI-MS) and daughter ion ratios (EI-MS/MS) with standards deviations (n = 3-5) for selected known and unknown toxaphenes present in the eggs.

RT[1)]	Identified: by NICI-MS[2)]	by EI-MS/MS	Remarks
unknowns			
0.884 (0.795)	tx-a	tx-1	B7-1000, TC-1
0.967 (0.944)	tx-b	tx-2	Cl_7- congener
0.992 (0.953)	tx-c	tx-3	Cl_7- congener
i[3)] (1.043)	tx-d	tx-4	Cl_7- congener
1.088 (1.176)	tx-e	tx	Cl_8- congener
1.098 (1.169)	tx-f	tx-5	Cl_8- congener
knowns			
0.935 (0.899)	1.24 ± 0.11 (1.20 ± 0.07)	0.99 ± 0.22	B7-1453
1.000 (1.000)	1.05 ± 0.05 (1.02 ± 0.06)	1.35 ± 0.24	#26
1.013 (1.026)	1.02 ± 0.10 (1.08 ± 0.09)	1.21 ± 0.13	B8-1412
1.026 (1.050)	1.22 ± 0.15 (1.17 ± 0.11)	1.01 ± 0.19	#32
1.078 (1.146)	1.05 ± 0.04 (#40: 1.04 ± 0.04)	1.39 ± 0.28	#40/41
1.087 (1.183)	1.07 ± 0.09 (1.04 ± 0.04)	co-elution	#44
1.116 (1.221)	0.89 ± 0.02 (0.89 ± 0.01)	1.82 ± 0.18	#50
1.131 (1.231)	1.10 ± 0.07 (1.10 ± 0.06)	1.07 ± 0.18	#51
1.162 (1.310)	0.91 ± 0.03 (0.92 ± 0.08)	1.88 ± 0.27	#58
1.210 (1.384)	0.84 ± 0.13 (0.90 ± 0.03)	2.33 ± 0.20	#63

1) Retention relative to #26 on Ultra 2(CP-Sil 2); 2) Ultra 2 (CP-Sil 2); 3) interference

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