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ORGANOCHLORINES, ORGANOBROMINES AND THEIR METABOLITES IN EGGS OF NORWEGIAN BIRDS OF PREY

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Introduction

Owing to their high trophic position, birds of prey are important indicator organisms for anthropogenic pollution. The harmful effect of chlorinated hydrocarbon contamination on bird-reproductivity has been demonstrated in a large number of reports [1,2,3]. Birds of prey, feeding both on terrestrial and marine prey, are characterised by high pollutant levels. The investigation of addled eggs has been proven to be a very useful method to characterise the contamination of both the mother organism and the offspring. PCBs and pesticides in eggs of birds of prey have been monitored in Norway since 1974 [4,5]. On the other hand, very little is known about the contamination with other persistent pollutants like polychlorinated camphenes (toxaphene), brominated flame retardants [polybrominated diphenylethers (PBDEs) and biphenyls (PBBs), tetrabromobisphenol A (TBBPA)] and other brominated compounds. Additionally, information about the contribution of metabolites of the main contaminant, PCBs, to the contamination load is very sparse [6].

Methods and Materials

Within the period of 1991-2002, 78 egg samples were collected representing eight different species from both densely populated and pristine areas in Norway (Table 1).

Table 1: Species chosen for the monitoring campaign

Species (abbreviation)	*Scientific name*	*Trophic chain*	*Main food*	*Sampling period*	*Number of samples*
White-tailed Sea Eagle (WS)	*Haliaeetus albicilla*	Marine	Marine fish, seabirds	1992-2000	16
Peregrine falcon (P)	*Falco peregrinus*	Marine/ terrestrial	Sea- and terrestrial birds	1993-2000	11
Merlin (M)	*Falco columbarius*	Terrestrial	Passerines	1995-2000	10
Golden Eagle (GE)	*Aquila chrysaetos*	Terrestrial	Grouse, hare, ungulates	1992-2002	20
Osprey (O)	*Pandion haliaetus*	Limnic	Freshwater fish	1993-2000	8
Goshawk (GH)	*Accipiter gentilis*	Terrestrial	Terrestrial birds, small mammals	1991-2002	13

We analysed in all samples 8 chlorobornanes, 17 PCBs congeners, 8 PBDEs (incl. 209) and 6 PBBs (incl. 209) using the method described elsewhere [7], briefly by extracting the Na_2SO_4-homogenate, GPC for lipid removal, florisil chromatography for further clean-up prior to GC/MS. Eight selected eggs from Norwegian bird of prey species (two eggs of each of four different species) were analysed for hydroxy-PCBs, pentachlorophenol (PCP, a fungicide mainly used as preservation additive) and TBBPA. The clean up procedure consisted of the extraction of acidified Na_2SO_4-homogenate with cyclohexane/ acetone (3:1), lipid removal via GPC and further clean up by florisil chromatography. Subsequently the extracts were split in two aliquotes. One part was directly analysed by HPLC-MS while the second part was quantified via GC-MS after derivatisation with methylchloroformiate.

GC /MS investigations of bird of prey extracts:
Quantification was carried out by GC/LRMS using a Finnigan MD800 quadrupole mass spectrometer as detector in selected ion monitoring mode (SIM). Electron impact (EI) was used as ionisation method for the determination of PCBs, DDT, PBBs and PBDEs and NCI was used for the analyses of toxaphenes, chloropesticides as well as PBDE 183 and 209. For the verification of the LRMS results high resolution measurements (HRMS) with a Micromass VG Autospec were used at a resolution of $M/\Delta M$ 10,000 and 8000 V acceleration voltage using EI and SIM mode.

Results and Discussion

Due to the relatively long data series available for *p,p'*-DDE and PCBs in Norway (about 30 years of monitoring for birds of prey) our here presented data set can be put into a wider perspective for the species analysed (Table 2) [4,5,8]. The decrease of levels for *p,p'*-DDE in eggs of bird of prey is confirmed for Goshawk, White-tailed Sea Eagle and Osprey. Reduced levels for Merlin and Peregrine falcon have been found as well, but still on a relatively high level (about 2000 ng/g ww). These values are still close to the recommended NOAEL (no observed adverse effect level) of 3500 ng/g ww for other bird species [9]. A possible reason for these high concentrations may be specialized prey habits, mainly consisting of marine birds (Peregrine falcon), fish and small migrating passerines (Merlin) [4] as well as migratory habits. The *p,p'*-DDE levels in Golden Eagle stayed at approximately the same low level over the monitored period (ca. 200 ng/g ww). Comparable trends have been found for PCBs, with Peregrine falcons expressing the most dramatic level reductions of all species investigated (35 000 ng/g ww in the 1970's to 4 000 ng/g ww in the present samples). However, the concentrations found in Peregrine falcons and White-tailed Sea Eagle are still elevated and further monitoring is strongly recommended (NOAEL of 4000 ng/g ww in related species)[9].

As shown in Figure 1 the egg samples of Peregrine falcons (P), Merlin (M) and the Golden Eagle (GE) deviated from the typical PBDE pattern reported for biota. PBDE 153 was the most abundant PBDE congener in these samples. For the first time, BDE 209 (decabromodiphenyl ether) was identified in egg samples of Norwegian bird of prey. All PBDE 183 and 209 findings were verified using a second, less polar GC column.

Table 2: Median, maximum and minimum (Max./Min) concentrations (ng/g wet weight) of selected chlorinated hydrocarbons in egg samples from six bird of prey species. (nd: levels below limit of detection ; *: coelution with γ-HCH)

Compound	*White-tailed Sea Eagle*	*Golden Eagle*	*Goshawk*	*Peregrine Falcon*	*Merlin*	*Osprey*
Parlar #26 (B8-1413)	34.4 (128/1)	6.3 (97/1)	14.8 (55/nd)	5.7 (17/2)	0.4 (3/nd)	5.0 (222/1)
Parlar #50 (B9-1679)	40.3 (130/1)	3.2 (58/1)	5.0 (78/nd)	5.8 (15/2)	0.3 (2/nd)	3.4 (315/1)
cis-chlordane	21.8 (94/1)	3.7 (77/1)	2.6 (26/nd)	2.6 (8/nd)	0.8 (6/nd)	1.9 (77/1)
trans-chlordane	4.9 (15/1)	1.4 (11/nd)	2.8 (10/nd)	1.9 (6/nd)	0.3 (1/nd)	1.0 (27/nd)
trans-nonachlor	104 (302/11)	14.9 (373/1)	23.5 (157/ 2)	16.4 (38/2)	12.0 (95/2)	4.9 (326/1)
cis-nonachlor	58.3 (332/21)	35.5 (752/nd)	14.6 (57/nd)	6.3 (167/2)	2.9 (109/1)	12.7 (316/1)
oxychlordane	67.0 (741/7)	120 (402/5)	52.9 (595/3)	110 (185/40)	41.3 (292/2)	5.8 (363/3)
p,p'-DDE	740 (4130/216)	160 (1947/8)	330 (2380/32)	2550 (8000/730)	1850 (3830/680)	670(4700/330)
γ-HCH			9.9 (60/nd)	6.7 (27/2)	2.1 (30/1)	9.3 (15/1)
β-HCH	11.9 (234/1)*	8.5 (27/3)*	1.7 (13/nd)	44.5 (92/2)	19.4 (91/3)	9.5 (16/2)
α-HCH	0.5 (1/nd)	7.2 (7/nd)	0.1 (0.1/nd)	0.8 (6/nd)	0.3 (0.3/nd)	0.3 (0.3/nd)
HCB	17.3 (31/6)	8.0 (26/2)	16.2 (374/1)	44.1 (126/17)	22.6 (33/3)	22.3 (118/2
Sum PCB	3700 (25800/1100)	610 (5340/80)	875 (12000/34)	3500 (25800/1200)	1100 (2540/330)	2100 (13100/230)

Eggs from two White-tailed Sea Eagle, one Golden Eagle, one Merlin and one Peregrine falcon were selected because of their high contaminant load with both traditional POPs and brominated flame retardants. 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'bipyrrole (Q1), the $C_{10}H_{13}Br_2Cl_3$ monoterpene (MHC1) as well as 2,4,6-tribromoanisole could be identified in all samples except the one from Peregrine. Traces of 5,5´-dichloro-3,3´,4,4´-tetrabromo-1,1'-dimethyl-2,2´-bipyrrole (4-2-HDBP; BC-10) were detected in both White–tailed Sea eagle samples. All these compounds are known as biogenic halogenated compounds, mainly detected in marine biota. Semi-quantitative determinations confirmed highest concentrations in the samples of White–tailed Sea eagle (Q1: 3000 pg/g ww; MHC-1: 12600 pg/g ww).

PCP was found in all eggs analysed by HPLC-MS and GC-MS with concentrations ranging from below limit of quantification (LOQ, <100 pg/g ww) to 1350 pg/g ww. TBBPA was also detected in all egg samples, but only by GC-HRMS at very low levels (<4 pg/g ww - 13 pg/g ww). The HPLC method was not sensitive enough to detect trace amounts of this compound. The highest PCP concentration was found in a Golden Eagle egg whereas TBBPA was most abundant in an Osprey sample. However, individual differences between single eggs from the same species were substantial, thus no conclusions about species-specific accumulation of these POPs can be drawn from the limited sample set.

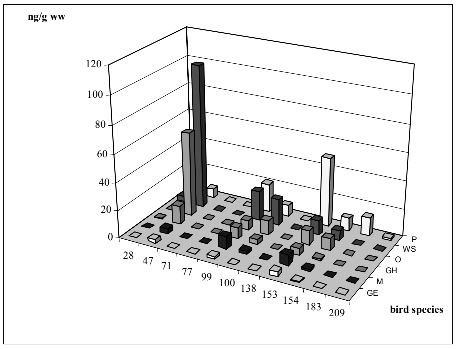

Figure 1: Median concentration of PBDEs in eggs of Norwegian bird of prey

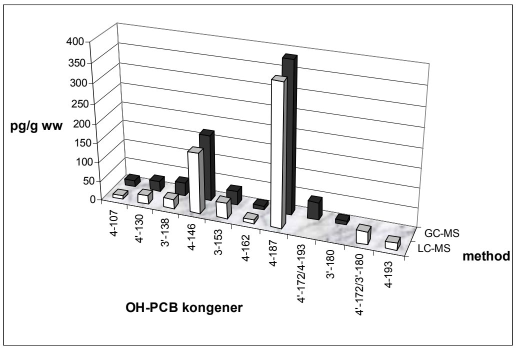

Figure 2: Comparison of results for identified hydroxy - PCBs in a Peregrine falcon egg sample obtained by GC/HRMS and HPLC/MS

The GC-MS method based on selected ion monitoring of hydroxylated penta- to heptachloro PCBs revealed a high number of different hydroxy-PCBs. However, only 13 reference standards of environmental relevance were available. These 13 congeners were all detected and two of them (4-OH-PCB 146 and 4-OH-PCB 187) were among the most abundant. A comparison of the levels of these 13 compounds found by HPLC-MS and GC-MS in a Peregrine falcon sample is shown in Figure 2. Additionally, many unknown congeners were detected at concentrations comparable or higher than those of the reference hydroxy -PCB.

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