A COMPREHENSIVE SURVEY OF PERSISTENT ORGANIC POLLUTANTS IN NORWEGIAN BIRDS OF PREY EGGS

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Introduction

Already in the early 1960's birds of prey served as model organisms to demonstrate the toxicological potential of environmental pollutants such as trace metals and persistent organochlorines. The high concentrations registered in those top predators due to biomagnification processes lead to physiological dysfunction like egg shell thinning caused by disturbance of the Ca^{2+} transfer across the oviduct[1,2]. Thus, this ecotoxicological effect proven for several bird of prey species played an important role as it led to the official ban of dichlorodiphenyltrichloroethane (*p,p*'-DDT) as an insecticide in western industrial countries[2,3]. The occurrence of polychlorinated biphenyls (PCB) as an environmental pollutant was proven for the first time in the 1960s for White-tailed Sea Eagles in Sweden[4]. In the past decades birds of prey have shown their vulnerability towards persistent bioaccumulative pollutants and, thus, are sensitive monitoring organisms for environmental pollutants. However, birds of preys are usually protected species in most western countries. Therefore, it is rather difficult to get sufficient tissue material from healthy birds for a comprehensive trace analytical program. At the other hand, birds of prey eggs represent an excellent opportunity to investigate the content of pollutants in the respective species without destroying the mother organism.

Material and methods

Norwegian environmental authorities have been monitoring birds of prey since 1965 continuously, and a comprehensive report is summarising the status of the contamination including time trends (1965-1994)[5]. A new initiative was started in 1994, where unhatched eggs of several species have been collected by the Norwegian Institute for Nature Research (NINA) throughout Norway under the licence of the Norwegian Directorate for Nature Management. The egg samples were biologically characterised and transferred to the laboratory of the Norwegian Institute for Air Research (NILU) for analysis of persistent organic pollutants (POPs). A total of 47 egg samples from eight Norwegian birds of prey species were analysed. Representing migratory birds preying mostly on small birds, the Sparrowhawk *(Accipiter nisus)*, Peregrine Falcon (*Falco peregrinus*) and Merlin (*Falco columbarius*) were chosen. Osprey (*Pandion haliaetus*) was chosen as a migrating bird species which exclusively feeds on freshwater fish. As examples of sedentary species, Golden Eagle (*Aquila chrysaetos*), Gyrfalcon (*Falco rusticolus*), Goshawk (*Accipiter gentilis*) and the White-tailed Sea Eagle (*Haliaeetus albicilla*) were available. The Golden Eagle and the Gyrfalcon feed mainly on terrestrial herbivores (including Ptarmigan *Lagopus spp.*), the Goshawk has a wide diet of birds, while the Sea Eagle feeds on marine fish and seabirds. All chemicals used for sample preparation and analysis were purchased form Merck (Darmstadt, Germany) in SupraSolv quality. The egg samples were homogenised with a 10-fold of pre-treated sodium sulphate (8h at 650°C). The extraction of the sodium sulphate homogenate was performed with accelerated solvent extraction (ASE® 300: Dionex Sunnyvale, CA) using 100 ml of a 50:50 (v:v), acetone/n-hexane mixture (Merck, Darmstadt, Germany). The 15 min. extraction procedure was repeated three times and the three extracts obtained were unified, dried with pre-treated sodium sulphate and concentrated to 2 x 2 ml for parallel clean-up with a Turbovap 500 (Zymark, Hutchinson, USA). Lipid removal was performed on a GPC system consisting of a dual pre-packed Waters Envirogel system (Taunton, MA, USA) and a glass column system (13 mm id, 1090 mm length) purchased from LATEK (Eppelheim, Germany) packed with 50 g Biobeads S-X3 (Biorad, Hercules, CA, USA). A rough clean-up was performed with cyclohexane/ ethyl acetate (1:1; v,v) at a flow rate of 5 ml/min. The POP fraction between 75 and 125 ml was transferred after concentration to 1 ml into the LATEK column, for final matrix removal. A flow rate of 1 ml/min was used and the POP-fraction between 60 and 105 ml was collected. An additional fractionation was carried out on a silica column (10 mm id, 80 mm length) purchased from Baker (Phillipsburg, NJ, USA), packed with 1g pre-treated silica (8 h at 600 °C, 1.5 weight-% water added). The column was conditioned with 10 ml *n*-hexane/ toluene (60:35; v,v). The eluate was added to the silica column for the final claen-up (1 fr: 10ml *n*-hexane/toluene 60:35 v:v, 2 fr.: 15 ml *n*-hexane/toluene 50:50, v:v). Both combined fractions (25 ml) were collected for subsequent POP analysis. After the final volume reduction to 200 µl (under a gentle nitrogen stream) the analysis was carried out. A CE instruments 8560 Mega II gas chromatograph (Milan, Italy) was equipped with a 30m DB5-MS (0.25 mm id and 0.25 µm, film thickness) purchased from J&W (Folsom, CA, USA). Helium (He, 5.0 quality) was used as carrier gas at a flow rate of 1 ml/min. 2µl of the extract was injected on-column with an AS800 autosampling system (CE instruments). The transfer-line was held at 280 °C. Temperature program: 60 °C, 2 min, than 15 °C/min to 180 °C and 5 °C/min to 280 °C (5 min isothermal). The quantification was carried out on a Finnigan MD800 quadrupole mass spectrometer (Finnigan, San Jose, CA, USA) in negative ion chemical ionisation (NICI). Methane (5.0 quality) was used as reactant gas for NICI at an ion source pressure of 4 x 10^{-4} kPa and a source temperature of 160 °C. The distribution and patterns of polychlorinated alkanes (Sum PCA), 5 brominated biphenyls and 2 biphenyl ethers (47, 99), 6 chlorinated bornanes (Parlar #26, #32, #50, #69, #40, #58), 5 cyclodiene pesticides (*trans-*, *cis-*chlordane: t-CD/c-CD, *trans-*, *cis-*nonachlor: t-NC/c-NC dieldrin), 3 hexachlororcyclohexanes (α-,β-,γ-HCH), 3 pesticide transformation products (heptachlorepoxide: HCE, oxychlordane: oxy-CD, *p,p'*-DDE), 18 polychlorinated biphenyl congeners (PCB #28, #52, #99, #101, #105, #118, #126, #128, #138, #149, # 153, #169, #170, #178, #180, #183, #187, #194) were investigated. The median distribution with maximum and minimum values for chlorinated pesticides and species are listed in Tables 1.

Results and discussions

In addition to assessment of the contamination status of these birds of prey in Norway, additional 'new contaminants' were added to the list of compounds analysed. The concentration levels are in general within the same order of magnitude as those published in the first status report[5]. In general, indications for a continuous reduction of the overall POP levels were found. *p,p'*-DDE, still the most abundant persistent-pesticide related compound in all egg samples, shows a clear reduction over the past two decades[3]. These findings must be considered as a clear effect of the strong national and international regulation measures, which lead to a complete ban of *p,p'-DDT* as pesticide in the western world. In addition to conventional persistent pollutants, for the first time, chlorinated bornane contamination (toxaphene congeners) was investigated in Norwegian birds of prey eggs (Table 1). The concentration levels were in the same order of magnitude as determined for HCH-isomers.

Table 1. Median (Med.), maximum (Max.) and minimum (Min.) concentration values for selected chlorinated pesticides and transformation products in egg samples from eight bird of prey species. Int.: Interference, no quantification possible; <LOQ: Levels below limit of quantification (LOQ = 5 x limit of detection). Please note: in case of three equal values for median, minimum and maximum, only one value was determined in all samples collected and analysed from this species.

COMP.	White-tailed Sea Eagle (n=8)	Golden Eagle (n=13)	Sparrow-hawk (n=2)	Goshawk (n=7)	Peregrine Falcon (n=6)	Merlin (n=3)	Osprey (n=5)	Gyrfalcon (n=3)
	Med . ng/g wet weight] (Max/ Min)							
Parlar #26	28.6 127.9/20.6	24.2 96.7/10.7	13.7 13.7/13.7	20.5 54.8/4.6	11.1 16.9/5.3	<LOQ	17.1 221.9/8.0	<LOQ
Parlar #32	36.8 57.9/7.9	3.9 4.4/3.4	<LOQ	<LOQ	2.9 2.9/2.9	<LOQ	18.8 79.8/7.7	<LOQ
Parlar #40	38.4 96.0/2.4	24.9 115.0/19.5	8.4 8.4/8.4	42.2 103.8/15.3	6.7 6.7/6.7	<LOQ	32.6 127.1/9.2	<LOQ
Parlar #50	41.8 62.1/33.7	15.6 58.5/8.1	19.2 34.6/3.8	5.0 78.1/2.2	6.0 11.7/5.6	<LOQ	21.1 315.3/3.4	<LOQ
Parlar #58	2.8 3.0/2.5	<LOQ	<LOQ	3.3 3.4/3.2	<LOQ	<LOQ	4.1 4.1/4.1	<LOQ
c-CD	8.2 24.9/1.8	4.6 11.4/0.8	3.4 4.9/1.9	4.75 26.04/0.8	3.8 4.8/0.5	0.4 0.4/0.4	1.9 77.5/0.4	0.6 0.6/0.6
t-CD	4.3 14.7/1.2	<LOQ	5.1 5.1/5.1	2.7 9.7/0.8	5.6 6.5/4.7	0.9 1.2/0.6	1.3 27.7/1.0	0.7 0.7/0.7
HCE	29.8 79.7/22.4	22.7 95.3/5.4	64.7 87.0/42.3	61.0 86.5/35.6	<LOQ	1079.6 1141.2/124.7	267.5 284.6/44.1	<LOQ
Dield-rin	49.3 142.9/23.6	12.9 264.5/2.6	24.4 42.1/6.7	83.4 247.3/16.4	65.4 617.7/5.1	73.4 91.2/55.6	59.9 209.1/6.1	8.1 8.5/7.6
t-NC	56.7 302.3/10.9	12.4 373.3/0.9	22.4 40.9/3.9	33.0 156.9/9.0	37.3 37.9/19.1	20.2 30.9/4.5	23.0 326.5/3.9	4.5 6.7/2.3
c-NC	67.9 332.1/22.0	267.2 752.5/112.1	11.6 12.6/10.6	20.9 56.9/2.1	36.3 167.1/1.3	42.2 109.3/2.9	225.3 315.7/4.8	6.4 29.8/2.9
oxy-CD	174.8 421.8/104.5	104.1 402.1/2.3	190.7 190.7/190.7	534.8 596/426.5	180.7 184.8/86.0	291.8 291.8/291.8	363.4 363.4/363.4	<LOQ
pp-DDE	546.4 4132.9/27.0	86.5 6016.6/6.2	2479.4 4911.1/2468	232.4 723.5/45.4	2058.4 5136/140.9	1711.0 2867/1520.8	431.9 4689.3/132.3	127.6 486.5/72
γ-HCH	Int	Int	24.1 24.1/24.1	29.6 60.2/24.3	22.5 27.5/17.6	18.2 30.5/6.0	12.5 15.2/9.8	Int
β-HCH	25.0 234.1/6.5	6.5 8.0/5.3	<LOQ	8.1 13.4/2.7	3.4 66.0/2.5	3.2 3.2/3.2	2.5 2.5/2.5	<LOQ
HCB	30.9 44.2/20.7	25.7 25.7/25.7	47.1 47.1/47.1	32.1 373.9/25.8	52.8 63.3/35.5	27.3 32.0/22.6	24.2 69.5/20.4	<LOQ

A general comparison of the concentration levels in the species investigated revealed species dependent differences. The highest contamination of chlorinated bornanes was found in Osprey eggs (Table 1, max. 315 ng/g ww), a species feeding mainly on freshwater fish. In this context, it should be noted that low levels of toxaphene have been found in Dipper (*Cinclus cinclus*) eggs and livers in Norway[6], a species feeding entirely on freshwater organisms. Interestingly, toxaphene was used in Canada and USA in combination with rotenone as piscicide (for regulation of fish populations in fresh water lakes)[8].

It is not known whether this compound has been used in African wintering grounds of the Norwegian Ospreys. Merlin eggs are dominated by heptachlorepoxide, the main transformation product of the pesticide heptachlor, indicating high metabolic capacity of this species. In contrast, no chlorobornanes were found in Merlin eggs. This probably reflects the low toxaphene contamination status in its main prey species (e.g. passerines). Minor contamination of chlorobornanes was found in Peregrine Falcon egg samples, a species feeding mainly on marine birds in Norway, which represent higher trophic levels. The highest overall toxaphene contamination was found in White-tailed Sea Eagle eggs. This species is a predator of marine fish known to be heavily contaminated with chlorobornanes[7]. A considerable contribution of the congeners #32 and #40 should be noted. Usually congener #50 is the most abundant chlorobornane. However, an unusual distribution pattern was determined for Goshawk, as congener #40 was the most abundant chlorobornane congener in this species. The Goshawk is a sedentary species preying on both migrating and sedentary birds of medium size, thus being exposed to contaminants from areas both from northern and southern regions. Of all hexachlorocyclohexane isomers, γ-HCH dominated in all species except for the Gyrfalcon and the Golden Eagle (unfortunately, an interference in the chromatograms made it impossible to quantify the γ-HCH isomer in some of the samples). More or less similar contamination levels of dieldrin (between 40 and 80 ng/g ww), a still used modern agrochemical, were found for all species except Gyrfalcon (8 ng/g ww) and Golden Eagle (13 ng/g ww), two species closely connected to mountainous habitats in Norway. The highest median concentration for *p,p'-DDE* was found in a Sparrowhawk egg, a bird of prey species feeding mainly on migrating passerines, which often spend time in agricultural landscapes. Oxychlordane, the major transformation product of *cis-* and *trans-*chlordane, is dominating the pesticide contamination in Goshawk with a median concentration of 535 ng/g ww. As outlined above, these documented species related differences are reflecting the sum of influences from their preferred habitat, behavioural patterns and food preferences. In general, PCB contamination was high compared to the pesticide levels. The general PCB pattern found was similar to the PCB distribution in other Norwegian top predators[5]. PCB 153 and 138 were the most abundant congeners. More information about contamination levels for chlorinated biphenyls, brominated flame retardants and polychlorinated alkanes (PCA) will be given in the presentation.

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