DISTRIBUTION OF POPs AND PFCS IN TISSUES OF WHITE TAILED EAGLES (*HALIAEETUS ALBICILLA*) FROM GREENLAND

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Introduction

The white-tailed eagle (*Haliaeetus albicilla)*, also referred to as white-tailed sea eagle (WTSE), is a large predatory bird from the northern part of Eurasia. It is a top predator from the aquatic ecosystem and is mainly feeding on fish, waterfowl and carrion.[1] Because of its high trophic position, the WTSE is a very relevant species to monitor accumulation of organohalogen compounds (OHCs). The WTSE population in Greenland is distributed in the southwest and was estimated at 150-170 breeding pairs in 1990[2] and has been expanding to Disko Bay during the last decade (pers. comm. D. Boertmann).

In this study we investigated the distribution of different OHCs, comprising brominated flame retardants (BFRs), perfluorinated chemicals (PFCs) as well as traditional persistent organic pollutants (POPs), within the body of WTSEs from Greenland. As such, we will be able to characterize tissue-specific accumulation of OHCs with different chemical properties. The information gathered here can be valuable for further risk assessment studies.

Materials and methods

White-tailed eagles (n=17), found dead in West Greenland between March 1997 and January 2009, were analyzed in this study. Eight birds were juveniles, two birds in 2nd, one bird in 4th, one in 5th plumage and 5 birds were adults. The cause of death was either shooting, lead intoxication, trauma/shock, a broken wing or unknown (in 3 cases). According to the legislation, dead birds that are not left in nature must be delivered to the Greenland Institute of Natural Resources, where they are stored at -18 °C. More details on the sampling area and procedures can be found in Krone et al.[3] Muscle, liver and kidney were dissected and preen oil, blood and adipose tissue were collected when available in a sufficient amount for analysis. Feathers were sampled as well to investigate POPs variation among different feather types[4] and to study their suitability as biomonitoring matrices for PFCs (see abstract Herzke et al.[5]). Samples were shipped with CITES permission from Greenland to the National Environmental Research Institute (NERI, Aarhus University, Denmark) and subsequently to the Toxicological Centre (University of Antwerp, Belgium) for analysis of POPs and to NILU (FRAM, Tromsø, Norway) for analysis of PFCs.

For the analysis of POPs, approximately 1 g of muscle, liver and kidney, 2.5g of blood, 10-80 mg of preen oil, 90 mg of adipose tissue were weighed and spiked with internal standards (CB 143 and BDE 77/128). Tissues were homogenised with anhydrous Na_2SO_4 and extracted with hexane:acetone (3:1, v/v) in an automated hot Soxhlet extractor for 2h. The lipid content was determined gravimetrically on an aliquot of the extract. Further sample treatment and analysis were performed according to previously described methods.[6] Detection and quantification of compounds were performed by gas chromatography-mass spectrometry (GC-MS).

For the analysis of PFCs, we followed the method in biological matrices described by Powley.[7] Approximately one gram of homogenized tissue sample was spiked with internal standard (^{13}C-PFOA/PFOS) and thoroughly mixed with 8 mL acetonitrile. After centrifugation (2000 rpm, 5 min), the supernatant was concentrated to 4 mL in Rapidvap and cleaned up with ENVI-carb and glacial acetic acid. Samples were analyzed with liquid chromatography-mass spectrometry (LC-MS).

Statistical analyses were performed using XLSTAT (version 2011.2.02; Addinsoft™). Samples with data below LOQ were assigned a value according to f x LOQ, where f is the proportion of samples ≥ LOQ (or detection frequency). Compounds with f < 0.50 were not taken into account for statistical analysis. To meet the requirement of normality, all POP data were transformed according to Y = log10 (X + 1). Pearson correlations were performed on the log transformed data. Correlations could not be investigated between preen oil and blood, because samples from both tissues were only available for three individuals. Furthermore, only data from 3 adipose tissues were available, so these were not included in the correlation analyses either.

Results and discussion

Traditional POPs were found in high levels in all tissues (Table 1), except in adipose tissue. High variation in contamination was found among individuals with more than a 100 times difference in concentrations. Visual inspection of the data revealed that this was due to age differences of the birds and not to temporal trends of POPs. Very high levels were quantified in individual GR54, which was an adult female bird sampled in August 2007. A high age may be an important cause of the high POPs concentrations found in this bird. This individual could not be sampled for blood and adipose tissue, which could explain maximum levels in blood and adipose tissue being lower compared to the other tissues (Table 1). CB 153 was the dominating PCB congener in all tissues, followed by CB 138 and 180. Compared to the other tissues, preen oil had a higher contribution of lower chlorinated congeners (e.g. CB 118) and a lower contribution of the higher congeners (e.g. CB 180). This is consistent with findings of previous studies.[8,9]

PBDEs could be quantified in all tissues with the highest concentrations in the liver (Table 1). Levels were a 100 times lower than levels of PCBs and DDTs. BDE 47 was the dominating congener in all tissues (45-70% of the total sum PBDEs), followed by BDE 99 and BDE 100 (10-15%) and BDE 153 and BDE 154 (5-10%). BDE 28 and BDE 49 contributed less than 5% to the total sum of PBDEs.

Table 1: Median concentrations and ranges (ng/g lipid weight) of POPs in the tissues of Greenland WTSE.

	Muscle	**Preen oil**	**Liver**	**Kidney**	**Blood**	**Adipose tissue**
n	17	13*	6	6	5*	3
Lipid %	1.5-11	16-96	3.4-6.2	3.0-6.8	0.2-1.5	94-96
Sum PCBs	36000 (1500-930000)	14000 (830-170000)	11000 (620-1500000)	9100 (490-1000000)	6900 (1400-96000)	1800 (1800-1900)
Sum DDTs	18000 (700-530000)	12000 (440-160000)	7200 (330-910000)	6300 (230-630000)	5100 (600-53000)	1100 (840-1200)
Sum CHLs	7800 (360-160000)	5200 (380-77000)	3000 (200-210000)	2600 (130-190000)	2100 (330-18000)	520 (410-530)
HCB	1200 (110-10000)	1100 (140-6000)	780 (81-12000)	740 (58-13000)	880 (150-1500)	170 (120-200)
Sum HCHs	260 (19-3700)	230 (17-4300)	220 (17-8600)	160 (10-6100)	230 (28-620)	46 (26-49)
Sum PBDEs	420 (26-15000)	190 (16-3200)	180 (12-24000)	150 (10-18000)	150 (37-1700)	32 (29-35)

As shown in Figure 1, PCBs have the highest contribution to the total sum of POPs (around 50%), followed by DDTs (30%), CHLs (15%) and HCB (5%). HCHs and PBDEs contribute only less than 5% to the total POPs load.

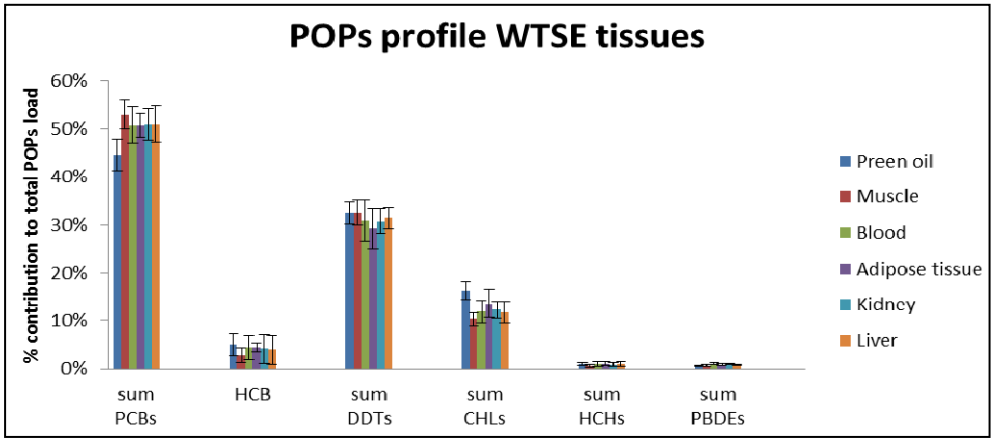

Figure 1: Profile of POPs (mean % ± 2SE) in tissues of WTSE from Greenland.

PFOS was commonly detected above LOQ in all tissues, except for the adipose tissue samples (Table 2). PFNA, PFUnA and PFTriA could also be quantified in liver and kidney. The amount of blood for analysis was very low and this may have limited the quantification of other PFCs than PFOS. PFOSA was not commonly detected in any of the tissues (Table2: < LOQ: below LOQ in more than 50% of the cases). From the six kidney samples, two had a concentration of PFOSA above LOQ and four above LOD. Further, PFOSA was above LOQ in only one preen oil and two muscle samples. One blood sample had high levels of PFOSA (121 ng/mL).

Table 2: Median concentrations and range (ng/g wet weight) of PFCs in the tissues of WTSE from Greenland.

	Muscle	**Preen oil**	**Liver**	**Kidney**	**Blood**	**Adipose tissue**
n	17	7	6	6	4	3
PFOS	40 (2.1-120)	18 (6.0-37)	19 (1.4-46)	36 (24-76)	23 (9.2-92)	< LOQ
PFNA	< LOQ	< LOQ	6.2 (3.1-14)	5.7 (3.1-16)	< LOQ	< LOQ
PFUnA	< LOQ	< LOQ	11 (3.1-54)	15 (5.6-36)	< LOQ	< LOQ
PFTriA	< LOQ	< LOQ	7.4 (4.1-22)	21 (5.1-71)	< LOQ	< LOQ
PFOSA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

We have constructed the PFCs profile for liver and kidney, being the only tissues where other PFCs than PFOS could be quantified. The profile of PFCs (Figure 2) shows that PFOS was the dominating compound, followed by PFUnA in liver and PFTriA in kidney, although error bars are overlapping. PFNA had the lowest contribution to total sum PFCs in both liver and kidney.

We have also investigated correlations between the different compounds quantified in WTSE tissues. The results of the correlation analyses showed that sum PCBs concentrations were significantly correlated between all tissues ($0.97 \leq r \leq 0.99$; $0.0001 \leq p \leq 0.013$; excluding the 3 adipose tissue samples). The same pattern was found for HCB ($0.96 \leq r \leq 0.99$; $0.0001 \leq p \leq 0.008$) and sum PBDEs ($0.98 \leq r \leq 0.99$; $0.0001 \leq p \leq 0.012$). For sum DDTs ($0.97 \leq r \leq 0.99$; $0.0001 \leq p \leq 0.033$), sum CHLs ($0.98 \leq r \leq 0.99$; $0.0001 \leq p \leq 0.006$) and sum HCHs ($0.98 \leq r \leq 0.99$; $0.0001 \leq p \leq 0.042$) significant correlations were found between all tissues, excluding the adipose tissue samples.

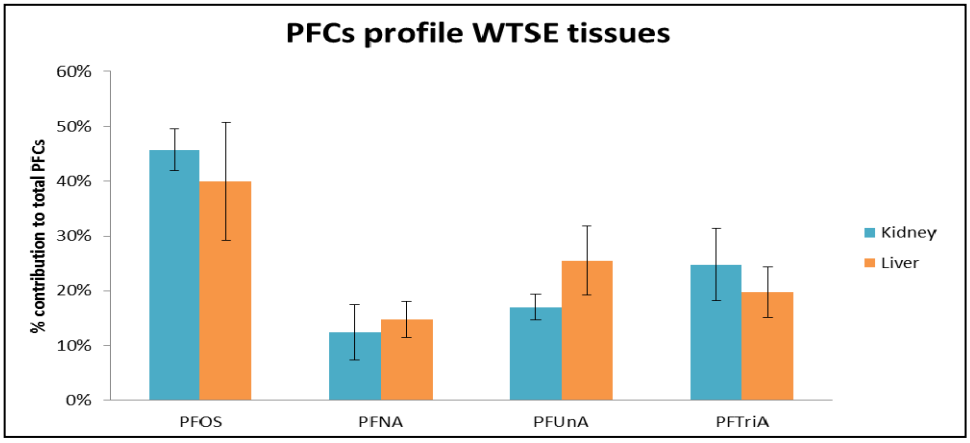

Figure 2: Profile of PFCs (mean % ± 2SE) in liver and kidney samples of WTSE from Greenland.

No significant correlations were found between concentrations of PFOS and concentrations of PCBs, HCB, DDTs, CHLs, HCHs and PBDEs. This suggests that PFCs and POPs may come from different sources. Furthermore, PFOS was not correlated with the other PFCs in liver or kidney. Only a trend was found between levels of PFOS and level of PFUnA in kidney (r = 0.81; p = 0.053). All other PFCs were significantly intercorrelated in kidney, but not in liver, where only a significant correlation was found between PFNA and PFUnA (r = 0.85; p = 0.032). This might be due to different physiological properties of liver and kidney, like blood circulation and protein content, in addition to different transport mechanisms in these two organs, potentially causing a compound specific retention.

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