LEGACY AND CURRENT-USE BROMINATED FLAME RETARDANTS IN THE BARN OWL (*TYTO ALBA*)

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Introduction

Predatory bird species exhibit specific characteristics that make them suitable biomonitoring species for environmental pollution, especially as they represent the endpoints of food chains[1]. As such, predatory birds are interesting to biomonitor for persistent organic pollutants (POPs), because especially these xenobiotics bioaccumulate in tissues and biomagnify through food chains[2]. At present, biomonitoring brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A) is of high importance: they are, or were recently, consumed in high volumes[3], and are additionally suspected to have endocrine-disrupting properties[4].

Since limited information is available for pollution of terrestrial environments, we will report accumulation levels and profiles of legacy and current-use BFRs in barn owls (*Tyto alba*) and evaluate if this species is suitable for biomonitoring terrestrial pollution with BFRs. We discuss here tissue distribution, spatial variability and metabolism. Doing so, we focus on the use of feathers as a non-destructive sampling strategy.

Materials and methods

In 2010, we collected carcasses of barn owls from Flanders (Belgium; N = 9) and Normandy (France; N = 5). The studied individuals were the subject of road-kills and were thus not sacrificed for this study. From each individual, we sampled feathers (outermost primary and tail feather) and tissues (muscle, liver, preen gland and adipose tissue).

Feathers were thoroughly washed with distilled water, dried overnight at ambient room temperature and cut in ~1 mm pieces. Feather samples were incubated overnight at 45 °C with HCl (4 M) and hexane:dichloromethane (4:1, v/v). Organic layers were liquid-liquid extracted and cleaned up on columns containing acidified silica (44% sulphuric acid) topped with anhydrous Na_2SO_4.

Tissues were homogenised with anhydrous Na_2SO_4 and organic layers were extracted with hexane:acetone (3:1 v/v) in an automated hot Soxhlet extractor for 2h. Lipid content was gravimetrically determined on an aliquot of the extract, dried for 1h at 100 °C.

Resulting extracts from feathers and tissues were further fractionated on silica SPE cartridges: a first fraction (fraction A), containing PBDEs, was eluted with hexane, while a second fraction (fraction B), containing HBCDs and TBBP-A, was eluted with dichloromethane. Both fractions were concentrated under a gentle nitrogen flow until dry and re-dissolved in iso-octane and methanol, respectively. Fraction A was analysed by GC/ECNI-MS, while fraction B was analysed by LC/MS-MS. All feathers and tissues were analysed for ten PBDE congeners (IUPAC: BDE 28, 47, 49, 66, 85, 99, 100, 153, 154, 183), TBBP-A and three HBCD stereoisomers (α, β and γ). We refer to Dauwe et al.[5] for further methodological details.

All statistical analyses were performed using R 2.13.0 (The R Foundation for Statistical Computing) and PASW Statistics 18.0 (SPSS Inc., 2009). For each compound, individual data below limit of quantification (LOQ) were assigned a value f x LOQ, where f is the proportion of samples quantified ≥ LOQ[6]. Compounds with $f < 0.50$ were not taken into account for further statistical analysis. All data were transformed according to $Y = \log_{10}(X + 1)$: Shapiro-Wilk normality tests and visual judgment of QQ plots showed that normality was met. Levels of significance were set to $\alpha = 0.05$ and $\alpha = 0.01$. Additionally, $0.05 \leq P < 0.10$ was considered a trend since the total sample size of this study is quite low (N = 14). Correlations between concentrations in feathers (primary and tail) and those in tissues (liver, muscle and preen gland) were tested using Pearson correlation tests on LOG-transformed data of feathers (dw) and tissues (ww). Correlation results were not Bonferroni post hoc corrected, given the low sample size[7].

Results and discussion

Accumulation levels.

We could quantify all targeted PBDEs in both feather (Figure 1) and tissue (Table 1) samples. With the exception of liver, concentrations of HBCDs seem lower in tissues of French barn owls, compared to these of Belgian individuals, but variation is too high to be significant (P = 0.169). HBCD accumulation can be spatially variable due to point sources[8]. Lower HBCD levels in French individuals could explain why HBCDs were present in only very low levels in their tail feathers, and absent in their primary feathers (Figure 1). As such, these results suggest that feathers can reflect a geographical pattern. Although not significant, levels of PBDEs seem to be higher in muscle and liver from French individuals (P = 0.516), but not in feathers (P = 0.811). Levels of PBDEs are consistently higher in tail feathers than in primary feathers (P = 0.039): this is not due to variations in sample weight, but could be due to extensive external contamination of preen oil on tail feathers[9]. TBBP-A was not detected in a single feather or tissue sample. This suggests that the chemical properties of BFRs should be taken into account for their risk assessment: although in Europe TBBP-A is the BFR with the highest production volume[3], it does not seem to pose an environmental threat, probably since TBBP-A is a reactive BFR and therefore leaches much less easily into environmental media[3].

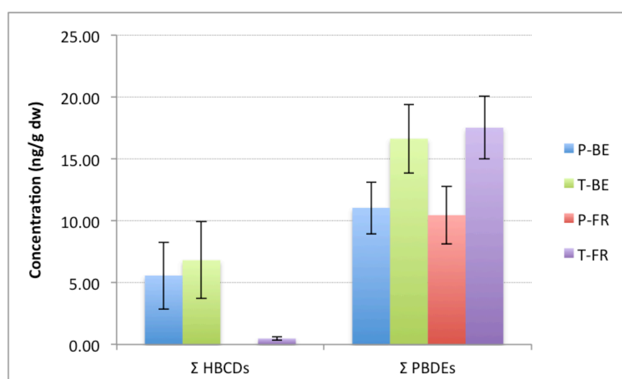

Figure 1: Mean (± SE) concentrations (ng g^{-1} dw) of BFRs in primary (P) and tail (T) feathers of Belgian (BE) and French (FR) barn owls.

Table 1: Mean (± SE) concentrations (ng g^{-1} lw) of BFRs in tissues of barn owls from Belgium (BE) and France (FR). Compounds that could not be quantified above limit of quantification are marked '< LOQ'.

	muscle		liver		adipose tissue		preen gland	
	BE (N = 9)	FR (N = 5)	BE (N = 9)	FR (N = 5)	BE (N = 9)	FR (N = 3)	BE (N = 9)	FR (N = 5)
α-HBCD	32 ± 11	10.4 ± 4.5	14.9 ± 6.2	12.8 ± 5.8	63 ± 28	17 ± 11	114 ± 85	6.1 ± 2.5
β-HBCD	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
γ-HBCD	< LOQ	< LOQ	1.97 ± 0.27	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Σ HBCDs	32 ± 11	10.4 ± 4.5	16.9 ± 6.4	12.8 ± 5.8	63 ± 28	17 ± 11	114 ± 85	6.1 ± 2.5
TBBP-A	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
BDE 28	0.14 ± 0.02	0.20 ± 0.07	0.09 ± 0.01	0.19 ± 0.08	0.18 ± 0.05	0.38 ± 0.29	0.15 ± 0.03	0.16 ± 0.05
BDE 47	37 ± 15	42 ± 21	32 ± 17	58 ± 30	77 ± 43	43 ± 24	40 ± 22	18.7 ± 7.4
BDE 49	0.20 ± 0.07	0.44 ± 0.21	0.20 ± 0.08	0.89 ± 0.48	0.49 ± 0.34	0.69 ± 0.55	0.16 ± 0.07	0.16 ± 0.08
BDE 66	0.35 ± 0.12	0.79 ± 0.34	0.37 ± 0.18	1.03 ± 0.50	0.50 ± 0.26	0.91 ± 0.58	0.38 ± 0.17	0.34 ± 0.11
BDE 85	1.00 ± 0.38	3.3 ± 1.5	0.70 ± 0.36	3.4 ± 1.5	2.6 ± 1.7	2.7 ± 1.3	1.05 ± 0.62	0.76 ± 0.30
BDE 99	46 ± 23	82 ± 34	42 ± 25	95 ± 43	96 ± 65	95 ± 45	41 ± 25	24 ± 11
BDE 100	9.2 ± 4.4	13.6 ± 5.8	9.3 ± 5.3	13.7 ± 6.2	19 ± 13	14.9 ± 6.8	8.5 ± 5.1	4.9 ± 2.3
BDE 153	42 ± 20	110 ± 51	33 ± 20	130 ± 61	84 ± 61	79 ± 37	23.5 ± 13.6	15.5 ± 6.6
BDE 154	7.6 ± 3.9	21.4 ± 9.9	6.2 ± 3.8	21 ± 12	15 ± 12	14.1 ± 6.9	4.5 ± 2.7	3.1 ± 1.2
BDE 183	5.3 ± 2.1	7.3 ± 3.4	4.16 ± 2.00	6.6 ± 3.3	8.6 ± 6.2	3.8 ± 1.8	1.99 ± 0.94	1.8 ± 1.0
Σ PBDEs	149 ± 68	280 ± 120	130 ± 74	330 ± 150	300 ± 200	250 ± 120	120 ± 70	70 ± 27
Σ BFRs	182 ± 77	290 ± 120	140 ± 80	350 ± 160	370 ± 230	270 ± 120	240 ± 110	76 ± 29

Our reported HBCD concentrations in liver and muscle are much lower than those reported for liver of cormorants (*Phalocrocorax carbo*; mean: 796 ng g^{-1} lw)[10] and for muscle of sparrowhawks (*Accipiter nisus*; mean: 993.5 ng g^{-1} lw), respectively[11] from England. Additionally, low TBBP-A levels (mean: 7.1 ng g^{-1} lw) were reported in liver of these English cormorants[10]. Earlier results for Belgian barn owls indicated much higher PBDE levels in liver (mean: 1600 ng g^{-1} lw) and muscle (mean: 1400 ng g^{-1} lw)[12] compared to the current study. In contrast, our reported PBDE concentrations in tail feathers of Belgian Barn owl are twice to three times higher than those reported earlier[13].

Accumulation profiles.

We could quantify all targeted HBCD stereoisomers in Belgian barn owl feathers. Their accumulation profile (Figure 2) shows that proportions ranked according to α > γ > β, with respectively 75%, 15% and 10%. This deviates from their ratios in the technical HBCD mixtures[8], but is in agreement with earlier reported data for predatory birds[8]. Furthermore, the presence of α- and γ-HBCD in tissues may suggest that HBCD stereoisomers can be subject to metabolization and that the accumulation profile is not subject to variable uptake or partitioning behaviour[8].

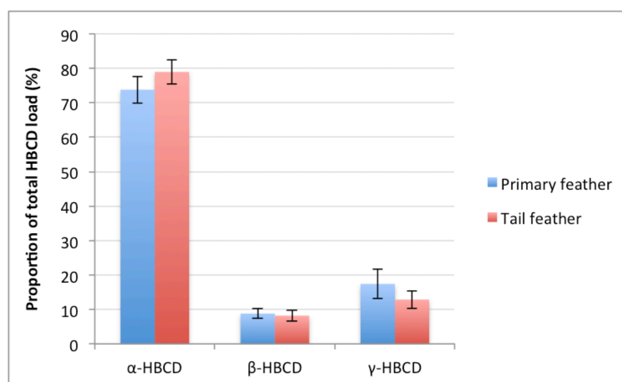

Figure 2: Accumulation profile (mean % ± SE) of HBCD stereoisomers in feathers of Belgian barn owls.

The PBDE accumulation profile (Figure 3) shows that accumulation of BDE congeners is similar between primary and tail feathers of individuals from both countries. In agreement with previous studies[12,13], proportions of BDE 47 and BDE 99 are elevated. The dominance of BDE 99 and the presence of BDE 153 and 154 indicate that the barn owl is indeed a terrestrial feeding top predator[12]. The PBDE accumulation profile for feathers is generally in agreement with the one for tissues, suggesting that feathers can reflect the internal state of contamination[13]. However, proportions of BDE 47, 99 and 153 are equally dominant in tissues, compared to the absolute dominance of BDE 99 proportion in feathers[13]. The accumulation profiles of both feathers and tissues show that error bars for metabolised congeners (BDE 47, 99 and 153) are larger than those for other congeners, possibly reflecting individual metabolic capacity.

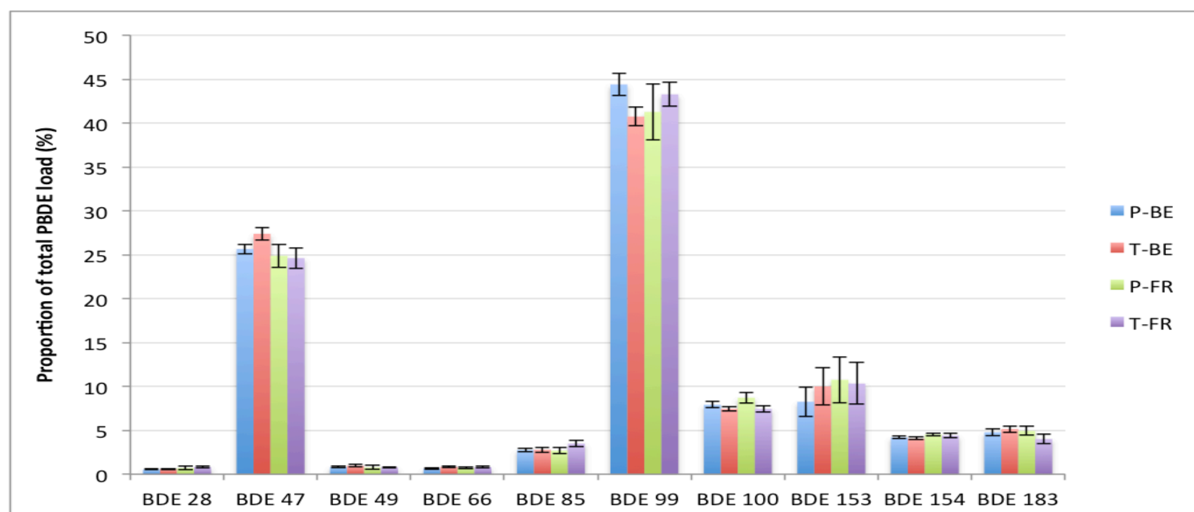

Figure 3: Accumulation profile (mean % ± SE) of PBDE congeners in primary (P) and tail (T) feathers of Belgian (BE) and French (FR) barn owls.

Correlations between feathers and tissues.

Correlations between HBCD levels in feathers and those in tissues (liver, muscle and preen gland) are not significant ($0.030 \leq R \leq 0.531$; $0.943 \geq P \geq 0.062$; $N = 8$). Only correlations between tail feather and muscle sample concentrations exhibit a trend ($0.479 \leq R \leq 0.531$; $0.098 \geq P \geq 0.062$; $N = 13$). PBDE concentrations in tail feathers and tissues correlate significantly for all congeners, with the exception of BDE 66 and 85 (Table 2). This confirms and expands previous results on Belgian barn owls[13]. In contrast, results on PBDE correlations for primary feathers are only significant for BDE 153. This unexpected discrepancy between feather types could possibly be due to higher external contamination[9] of tail feathers with preen oil compared to primary feathers, as suggested by significance levels of correlations between tail, primary feathers and preen gland concentrations.

Table 2: Correlation coefficients and their significances for correlations between feather (primary and tail) and tissue (liver, muscle and preen gland) concentrations in barn owls from Belgium and France. Wet weight BDE 66 preen gland data were not available (n.a.).

	Primary feather (N = 13)						Tail feather (N = 14)					
	Liver		Muscle		Preen gland		Liver		Muscle		Preen gland	
	R	***P***	***R***	***P***	***R***	***P***	***R***	***P***	***R***	***P***	***R***	***P***
BDE 47	0.34	0.26	0.59^{*}	0.03	0.54^{T}	0.06	0.67^{**}	< 0.01	0.69^{**}	< 0.01	0.66^{*}	0.01
BDE 66	-0.11	0.72	-0.27	0.38	n.a.	n.a.	0.35	0.22	0.43	0.12	n.a.	n.a.
BDE 85	-0.29	0.34	-0.24	0.44	0.02	0.95	0.39	0.16	0.17	0.57	-0.07	0.81
BDE 99	0.15	0.63	0.32	0.29	0.36	0.22	0.59*	0.03	0.58^{*}	0.03	0.52^{T}	0.06
BDE 100	0.41	0.17	0.40	0.18	0.47	0.11	0.62^{*}	0.02	0.66^{*}	0.01	0.56^{*}	0.04
BDE 153	0.61^{*}	0.03	0.73^{**}	< 0.01	0.83^{**}	< 0.01	0.82^{**}	< 0.01	0.92^{**}	< 0.01	0.86^{**}	< 0.01
BDE 154	0.27	0.37	0.37	0.21	0.61^{*}	0.03	0.85^{**}	< 0.01	0.89^{**}	< 0.01	0.74^{**}	< 0.01
BDE 183	0.56^{*}	0.05	0.57^{*}	0.04	0.54^{T}	0.06	0.73^{**}	< 0.01	0.79^{**}	< 0.01	0.57^{*}	< 0.01
Σ PBDEs	0.24	0.43	0.42	0.15	0.49^{T}	0.09	0.70^{**}	< 0.01	0.72^{**}	< 0.01	0.66^{**}	0.01

* Correlation is significant at the 0.05 level - ** Correlation is significant at the 0.01 level - ^{T} Correlation shows a trend towards significance

Conclusion

A wide range of PBDEs and HBCD stereoisomers could be detected in feathers and tissues from barn owls. Generally, accumulation levels and profiles show accumulation differences between Belgian and French populations, suggesting that Flanders is more polluted with HBCD, while Normandy more with PBDEs. The recognition of these spatial patterns positively argues for the use of barn owls to monitor terrestrial ecosystems. Furthermore, accumulation profiles and correlations between tissues and tail feathers indicate that tail feathers are suitable biomonitor matrices for PBDEs, although maybe not for HBCDs. Results for primary feathers do not support this conclusion, suggesting that external contamination should be systematically investigated for different feather types.

Acknowledgements

We would like to express our gratitude to the Kerkuilwerkgroep Vlaanderen and the Association du CHENE d'Allouville-Bellefosse for their cooperation in providing barn owl carcasses and their assistance in dissections. Furthermore, Igor Eulaers, Rianne Pinxten and Marcel Eens acknowledge the University of Antwerp for funding (NOI-BOF UA). Veerle Jaspers and Adrian Covaci wish to thank the Funds for Scientific Research in Flanders (FWO) for funding their post-doctoral research.

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