Brominated flame retardants in birds of prey from Flanders, Belgium

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Introduction

Since their introduction on the market, environmental levels of polybrominated diphenyl ethers (PBDEs) are continuously increasing[1]. This is caused by spillage and emission during production and use, but also by improper disposal at the end-of-life of the products in which they are used. These chemicals are highly persistent and lipophilic[2] which results in bioaccumulation in fatty tissues of biota and biomagnification throughout the food chain[3]. Because PBDEs have a high toxicological potential[3-6], this biomagnification can have serious health consequences for top-predators, such as birds of prey.

Data about PBDE concentrations in terrestrial biota, especially in birds of prey, is scarce. A rapid increase of PBDE concentrations has been seen in pooled guillemot (*Uria algae*) eggs from the Baltic proper[7] during the late 1970's and early 1980's, followed by a decrease during the 1990's[8]. In herring gull eggs from the Great Lakes[9], the PBDE concentrations increased exponentially from 1981 to 2000. Most of the studies look at concentrations in eggs[8-10], while less is known about tissue levels and distribution of these pollutants in birds of prey.

Materials and Methods

Birds of prey were collected by the staff of the Wildlife Rescue Centre (WRC) at Opglabbeek (Belgium). All birds included in this study were found dead or dying from traffic accident trauma's. No bird was killed for the purpose of this study. The WRC collected 44 birds from all over Flanders (Belgium) between

Table 1. Sample inventory

Common Name	Scientific Name	Individuals
Buzzard	*Buteo buteo*	29
Sparrowhawk	*Accipiter*	7
Long-eared	*Asio otus*	4
Barn Owl	*Tyto alba*	2
Tawny Owl	*Strix aluco*	2

November 2001 and September 2003. Liver, brain, muscle and adipose tissue were collected. Due to food deprivation related to trauma, adipose tissue could not be collected from all individuals. All samples were stored at –20 °C until further treatment.

Most birds sampled were Buzzard (*Buteo buteo* – 29 individuals) and Sparrowhawk (*Accipiter nisus* – 7 individuals) (Table 1). The 3 different owl species are further discussed as one group and are referred to as “Owl species”.

Based on reported abundance[5,7,11] and toxicity[1], the following PBDE congeners (IUPAC numbering) were targeted for analysis: 28, 47, 99, 100, 153, 154, and 183. BDE 77 was used as internal standard (IS) for BDE 28, 47, 99, 100, 153, and 154, and BDE 128 was used as IS for BDE 183.

The analysis method has been previously described[11] and is briefly presented below. Between 0.2 and 2 g of homogenised sample, depending on tissue type and availability, was chemically dried using anhydrous Na_2SO_4, transferred into a hexane-rinsed extraction thimble and spiked with IS. The thimbles were then hot Soxhlet extracted for 2.5 h with a hexane/acetone mixture (3:1, *v/v*). The extract was cleaned-up using a cartridge containing approximately 5g of silica impregnated with concentrated sulphuric acid (44%) and elution of PBDEs was realised with n-hexane and dichloromethane.

All PBDEs were analysed[11] using GC-MS operated in electron capture negative ionisation (ECNI) in the selected ion-monitoring (SIM) mode at the m/z = 79 and 81. A 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE) was used.

The quality control was done by regular analyses of procedural blanks, blind duplicate samples, and random injection of solvent blanks and standards. The quality of the methods used was verified by regular participation in interlaboratory excercises (Quasismeme). Procedural blanks were consistent and therefore the median blank values for these compounds were used for subtraction. Limit of quantification (LOQ) for PBDEs, based on GC/MS performance and procedural blanks, was dependent of the sample intake and was set at 2 times the standard deviation of the procedural blanks. Sample intake was adapted to the expected pollution load of the sample when possible.

Results and discussion

Lipids. Concerning the investigated tissues, no significant differences in the lipid content could be observed between the different species. However, the lipid percentage of the different tissues can be susceptible to great variance caused by nourishment and health status of the animals. The lipid content of brain tissue was the most consistent troughout all individuals. This can be explained by the importance of this tissue: even under extreme starvation, the brain is kept in good condition and the lipids are not used for any other purpose such as energy production. Muscle and liver are much more susceptible for lipid content changes, which results in a much wider variation of lipid percentages found in these matrices (Table 2). The authors therefore preferred expressing all results on fresh weight basis.

The lipid content of the adipose tissue was very consistent, as expected. The lipid content of adipose tissue does not alter, but the amount of adipose tissue changes when lipids are mobilised and metabolised. When fat from the adipose tissue is used by the bird, the lipophilic pollutants that were stored in the adipose tissue are remobilised since they lose their substrate and pollutants may redistribute throughout the body until a new steady-state is reached. This “remobilisation effect” can theoretically result in higher pollutant levels in other tissues than before the remobilisation of the adipose tissue. However, this could not be observed in this sample set. Birds for which no adipose tissue was available did not display significantly higher levels in the other tissues.

Levels. When looking at the sum of PBDEs in the individual birds in the present study, levels span a concentration range of almost 4 orders of magnitude. The lowest levels were generally found in buzzard brain, while the highest levels originated from sparrowhawk liver (up to 600 ng/g ww). Adipose tissue even had higher levels, but seen the nature of this tissue, consisting for almost 90% of pure fat, it was not included in this comparison.

It is well known that difference in diet constitution may explain most of the variation in pollution levels between different bird species as well as differences between individuals of the same species. However, different metabolic abilities can also influence levels and abundance of the congeners that can be found, which makes data interpretation very complex. In the present study levels of total PBDEs in buzzard and owls were comparable, while those in sparrowhawk were around 1 order of magnitude higher (Table 2 and Figure 1). This also explains why BDE 28 could be measured in most sparrowhawk samples.

In the present study, the diet of sparrowhawk seems the most plausible explanation for the inter-species difference. Sparrowhawks primarily feed on small birds, such as passerines, and small mammals, such as field mice. Buzzards primarily feed on small mammals, such as field mice, but they also eat worms and insects. Owls have a wider dietary spectrum; their diet ranges from insects, over amphibia, to small mammals and small birds. Based on the diets of these raptor species, one would expect the sparrowhawks to have the highest pollutant loads, because his preys are positioned on a higher scale in the food web when compared to buzzards and owls.

Body weight is also a factor that can influence the PBDE levels. Body weight in these raptors is dependant on sex and of course health status. The normal mean body weight of a sparrowhawk ranges from 150 to 290 g. Buzzards can weigh up to 1200 g. The owls in this study can weigh between 300 and 500g. It is clear that smaller animals have less tissue to distribute the pollutants, while heavier birds, such as buzzards, can distribute the pollutants over their relatively big bodymass, resulting in lower concentrations. Seen the diets of these birds, it is highly unlikely that the lower PBDE levels found in buzzard were only related to their their higher body mass.

Buzzards prefer living in forests and far from human activity, while sparrowhawks live in closer contact with humans and can even be found at industrial sites. Sparrowhawks are therefore likely to accumumate higher levels of pollution.

In general, PBDE related studies on terrestrial bird tissues are scarce. Most studies that have been performed were based on eggs. Median sum of PBDEs was reported to be of 72 ng/g lw in liver of black guillemots (*Cepphus grylle*)[12], while the median in the present study was between 70 (buzzard) and 1300 ng/g lw (sparrowhawk).

The use of birds of prey as geographical indicators for local pollution is very complex. All bird species analysed in this study are endemic throughout the year in Belgium, although they can migrate to other parts of Europe and Africa. It is virtually impossible to preccisely predict were they picked up the pollution they are bearing, although we can assume they represent local pollution.

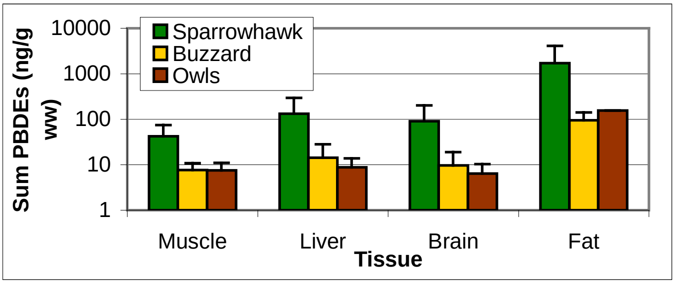

Figure 1. Mean PBDE levels in different tissues/organs of the different species.

BB 153. Although PBBs have never been extensively used in Europe[3], BB 153 could be found in most raptor samples analysed (Table 2). Similar as for PBDEs, the lowest BB 153 levels were seen in muscle and brain. The highest concentration was seen in sparrowhawk liver (4.6 ng/g ww - 200 ng/g lw) and can be considered as relatively low.

The presence of this compound in terrestrial wildlife is in clear contrast with marine biota, where this compound could not be measured[11]. Whether this is related to exposure or metabolisation cannot be concluded from the present data. Another study also report BB153 in eggs of peregrine falcon (*Falco peregrinus*) at concentrations up to 370 ng/g lw[10], which is comparable to the levels found in sparrowhawk liver in the present study.

PBDE patterns. Significant congener profile differences were observed between buzzard, sparrowhawk and owls (Fig. 2). Because no significant profile differences were observed between the different organs/tissues, the profiles are presented as a mean of the different tissues.

BDEs 47, 99, and 153 were the most abundant congeners and contributed each between 15 and 35 % to the total sum of PBDEs. BDEs 100, 154, and 183 represented between 3 and 13 % of total PBDEs (Fig. 2). Tribrominated BDE 28 could only be determined in some liver and fat samples with only 30 and 15% of buzzard and owl muscle samples, having measurable amounts of BDE 28. Slightly higher abundancies were seen in buzzard and owl brain (55 and 25 %, respectively). Sparrowhawk muscle and brain was more contaminated with BDE 28, which could be determined in 75 % of all sparrowhawk samples.

Except some brain samples, BDE 183 could be found in nearly all samples. The presence of this congener is often taken as indicative of the presence of the octa-mix[13]. However, BDE 209 was shown to be broken down into lower brominated compounds (but not into BDE 183) in marine organisms. It cannot be ruled out that BDE 183 in the raptor samples of the present study originated from BDE 209 after metabolic breakdown.

The contribution of BDE 47 and 183 to the total PBDE load was similar for the 3 species, but for the other congeners, species specific differences could be observed. BDE 28 was significantly more abundant in buzzard samples than in the other raptors. For BDE 99, 100, 153, and 154, differences seen in Fig. 2 are statistically significant.

The differences observed in the congener profiles might indicate that these 3 raptor species have a different metabolic capacity. It is also possible that the preys of these raptors bear different BPDE pollution patterns, but this is quite unlikely since all 3 raptor species feed on terrestrial preys, which suggests that the PBDE pattern in the preys will be quite similar. However, the influence of habitat and food specialisation cannot adequately explain the pattern differences of the PBDE contamination[13].

Not much is known about metabolisation of PBDEs in terrestrial birds of prey. In a study performed in fish[14], metabolisation of higher brominated congeners to form lower brominated ones was observed. The differences observed in the present study between buzzard and sparrowhawk PBDE profiles could also be related to debromination processes. BDE 153 and 154 can both be theoretically debrominated to form BDE 99 and BDE 100, respectively. More research is needed to verify such hypothesis however, but is is clear that congener profiles vary between species.

PBDE profile differences between peregrine falcon eggs and other birds have been previously reported[13]. In other study on eggs from sparrowhawk[15], a predominance of BDE 47 was noticed, while in the tissues of sparrowhawk in the present study the dominating congener is BDE 99.

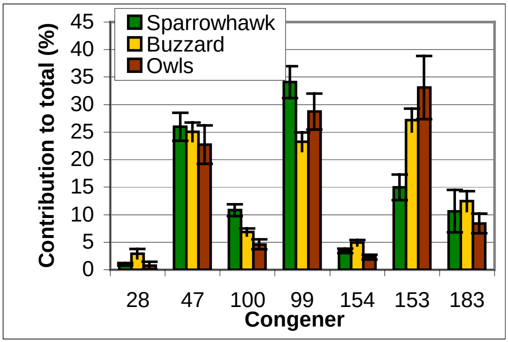

Fig. 2. Mean PBDE patterns in the 3 raptor species. Note that BDE 28 contribution is magnified 10 times to highlight the difference between buzzard and the other species. Pattern differences for BDE 99, 100, 153, and 154 were statistically significant.

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References

1. de Wit C.A. (2002) Chemosphere 46, 583-624.
2. Gustafsson K., Björk M., Burreau S., Gilek M. (1999) Environ. Toxicol. Chem. 18, 1218-1224.
3. de Boer J., de Boer K., Boon J.P. (2000) In The Handbook of Environmental Chemistry, New Types of Persistent Halogenated Compounds; Paasivirta, J., Ed.; Springer-Verlag; pp 61-95, ISBN 3-540-6583-6.
4. Darnerud P.O., Eriksen G.S., Jóhannesson T., Larsen P.B., Viluksela M. (2001) Environ. Health Perspect. 109, 49-68.
5. Bergman Å. (2000) Organohalogen Compd. 47, 36-40.
6. McDonald T.A. (2002) Chemosphere 46, 745-755.
7. Sellström U., Jansson B., Kierkegaard A., de Wit C., Odsjö T., Olsson M. (1993) Chemosphere 26, 1703-1718.
8. Sellström U., Bignert A., Kierkegaard A., Häggberg L., de Wit C., Olsson M., Jansson B. (2003) Environ. Sci. Technol. 37, 5496-5501.
9. Norstrom R.J., Simon M., Moisley J., Wakeford B., Weseloh D.V.C. (2000) Environ. Sci. Technol. 36, 4783-4789.
10. Lindberg P., Sellström U., Häggberg L., de Wit C. (2004) Environ. Sci. Technol. 38, 93-96.
11. Voorspoels S., Covaci A., Schepens P. (2003). Environ. Sci. Technol. 37, 4348-4357.
12. Vorkamp K., Christensen J.H., Glasius M., Riget F.F. (2004) Mar. Pollut. Bull. 48, 111-121.
13. Law R.J., Alaee M., Allchin C.R., Boon J.P., Lebeuf M., Lepom P., Stern G.A. (2003) Environ. Int. 29, 757-770.
14. Stapleton H.M, Letcher R.J., Baker J.E. (2004) Environ. Sci. Technol. 38, 1054-1061.
15. Hertzke D., Kallenborn R., Nygård T., Sandanger T. (2001) The Second International Workshop on Brominated Flame Retardants, Stockholm, Sweden, 321-324.

Table 2. Statistical descriptors of total PBDEs and BB 153 in the different tissues of the different species. Concentrations are given in ng/g ww. Lipid content is expressed in %. n.a. = not available.

		Muscle		Liver		Brain		Adipose tissue	
Species		**Sum PBDEs**	**BB 153**	**Sum PBDEs**	**BB 153**	**Sum PBDEs**	**BB 153**	**Sum PBDEs**	**BB 153**
Buzzard	Mean (SD)	7.7 (8.5)	0.16 (0.21)	14 (37)	0.10 (0.14)	9.6 (25)	0.17 (0.54)	95 (93)	0.95 (0.89)
	Median	5.6	0.09	2.7	0.04	1.8	0.03	55	0.71
	N	29	29	29	29	29	29	16	16
	Lipid content (SD)	4.4 (2.3)		3.6 (1.3)		7.4 (0.8)		89 (4.4)	
Sparrowhawk	Mean (SD)	42 (42)	0.71 (0.80)	133 (215)	1.3 (1.9)	91 (150)	0.73 (1.0)	1700 (1700)	2.7 (2.5)
	Median	28	0.36	40	0.40	30	0.19	1700	2.7
	N	7	7	7	7	7	7	2	2
	Lipid content (SD)	2.7 (2.4)		3.6 (1.1)		7.7 (0.6)		91 (3.8)	
Owls	Mean (SD)	7.5 (5.0)	0.20 (0.17)	8.8 (7.2)	0.18 (0.21)	6.4 (5.5)	0.15 (0.17)	160 (n.a.)	2.4 (n.a.)
	Median	7.8	0.15	8.8	0.07	6.3	0.06	160	2.4
	N	8	8	8	8	8	8	1	1
	Lipid content	3.9 (2.2)		3.8 (0.5)		7.0 (0.7)		80 (n.a.)	