PBDEs in the terrestrial top-predator red fox (Vulpes vulpes)

Stefan Voorspoels[1], Adrian Covaci, Sophie Escutenaire, Paul Schepens

[1]University Of Antwerp - Toxicological Centre

Introduction

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

Data about PBDE concentrations in terrestrial biota are scarce. Most data are available on terrestrial birds of prey[4,5], while limited data are available on rabbits (*Oryctolagus cuniculus*), moose (*Alces alces*) and reindeer (*Rangifer tarandus*).[6] According to the author's knowledge, other data on PBDEs on terrestrial mammalian (wildlife) top-predators are virtually inexistent.

Foxes have already been proposed as bioindicator for organochlorine pollution.[7,8] They are globally present and they adapt easily to local food sources, which makes them ideal to monitor local exposure. Foxes are high on the evolutionary scale and possess well developed metabolization processes.

In the present study, fox tissues were analysed for their PBDE content. Tissue distribution and congener profiles were calculated. Special emphasis is placed on the deca-brominated congener BDE 209.

Materials and Methods

Red foxes (*Vulpes vulpes*) were collected by the staff of the Pasteur Institute (PI) (Brussels, Belgium). All foxes included in this study were found dead from traffic accident trauma's or were killed during hunt. The PI collected 33 foxes from all over Flanders (Belgium) between October 2003 and March 2004. Liver, muscle and abdominal adipose tissue were collected. Due to food deprivation related to trauma, adipose tissue could be collected only from 27 individuals. Some traffic victims did not allow dissection of the liver due to the sustained injuries (30 livers collected). All samples were stored at –20 °C until further treatment.

Based on reported abundance[1,2] and toxicity[3], the following PBDE congeners (IUPAC numbering) were targeted for analysis: 28, 47, 99, 100, 153, 154, 183, and 209. 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. ^{13}C-labelled BDE 209 was used as IS for BDE 209.

The analysis method has been previously described and is briefly presented below.[9] Between 0.3 and 3 g of homogenised sample, depending on tissue type, was dried using anhydrous Na_2SO_4, spiked with IS and was hot Soxhlet extracted for 2 h with a hexane/acetone mixture (3:1, *v/v*). The extract was cleaned-up using a cartridge containing approximately 10g of silica impregnated with concentrated sulphuric acid (48 %; *w/w*) and elution of PBDEs was realised with n-hexane and dichloromethane.

All PBDEs were analysed using GC-MS operated in electron capture negative ionisation (ECNI) in the selected ion-monitoring (SIM) mode. BDEs 28 to 183 were separated on 25 m ´ 0.22 mm ´ 0.25 µm HT-8 capillary column (SGE) and *m/z* = 79 and 81 were monitored. For analysis of BDE 209, a 5 m ´ 0.18 mm ´ 0.18 µm DB-1 capillary column (J&W) was used. Ions *m/z* = 484.7 and 486.7 and *m/z* = 494.7 and 496.7 were monitored for BDE 209 and ^{13}C-labelled BDE 209, respectively.

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 exercises (Quasimeme). Procedural blanks were consistent and therefore the mean 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 for all congeners except BDE 209, for which 3 ´ SD was used.

Results and discussion

Lipids. The lipid percentage of the different tissues (primarily muscle and liver) can be susceptible to great variance caused by nourishment and health status of the animals, what can result in a large variation in lipid percentages found in these matrices (Table 1).

The lipid content of the adipose tissue was very consistent, but the amount of adipose tissue changes when lipids are mobilised and metabolised. In the sample set of the present study, adipose tissue was readily available in 27 out of 33 foxes, which highlights that these animals were probably not deprived from food before dying.

Levels. PBDE levels found in red fox can be considered low compared to other top-predators, such as birds of prey from the same geographical region.[5] Levels were below LOQ in numerous cases (Table 1). In order to facilitate statistical data treatment, measurements below LOQ were replaced by the following formula: p ´ LOQ, where "p" is the fraction of measurement above LOQ.

Sum of PBDEs (BDE 28 to 183) ranged from 0.73 to 82 ng/g lw (0.53 to 34 ng/g ww) in adipose tissue, from 0.61 to 115 ng/g lw (0.04 to 4.3 ng/g ww) in liver and from 1.0 to 44 ng/g lw (0.05 to 1.8 ng/g ww) in muscle (Table 1).

Much higher tissue levels were expected seeing these animals' position in the food chain. Other (avian) terrestrial top-predators, such as sparrowhawks, showed tissue levels which were around 500 times higher than in fox (median in liver = 1,300 ng/g lw). These relatively low PBDE-levels in fox may however be related to it's increased metabolic capacities. It has been shown that foxes, like other canidae species, possess a very high metabolic capacity to transform PCBs.[10,11] The structural similarity between PCBs and PBDEs may suggest a high metabolic capacity for the latter compounds as well.

Foxes don't have a strict dietary pattern, they are very dependent on their territory. In highly urbanized countries, such as Belgium, have been reported even in city centres eating out of garbage bins. This makes exposure assessment extremely difficult.

BB 153. Although PBBs have never been extensively used in Europe, BB 153 could be found in nearly all individuals, although the detection frequency was tissue dependent (lower levels in muscle) (Table 1).[2] The highest concentration were seen in liver (38 ng/g lw – 2.8 ng/g ww) and can be considered as relatively low. BB 153 levels observed in liver of sparrowhawk from the same geographical region were as high as 200 ng/g lw (4.6 ng/g ww).[5]

Table 1. Statistical parameters of all PBDEs in all tissues (ng/g lw)

		BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	Sum PBDEs	BDE 209	BB 153
Fat	Median	< 0.03	0.23	0.05	0.33	0.04	0.76	0.37	2.2	< 3.7	0.18
	min	< 0.03	< 0.68	< 0.18	< 0.90	< 0.07	< 0.08	< 0.03	0.73	< 3.7	< 0.03
	max	< 0.03	23	9.5	31	3.7	33	4.9	82	120	20
	% > LOQ	0	33	26	37	48	96	67		15	96
	Lipid % (SD)	72 (22)									
Liver	Median	< 0.04	0.33	0.05	0.11	0.02	1.2	0.61	2.4	< 9.1	0.57
	Min	< 0.04	< 0.81	< 0.21	< 1.1	< 0.09	< 0.09	< 0.04	0.61	< 9.1	< 0.04
	Max	< 0.04	62	35	6.9	1.0	25	7.4	115	754	38
	% > LOQ	0	40	23	10	23	93	67		40	90
	Lipid % (SD)	5.8 (3.5)									
Muscle	Median	< 0.05	1.4	0.02	0.74	0.02	0.83	0.14	3.4	< 3.9	0.19
	Min	< 0.05	0.54	< 0.05	< 0.28	< 0.07	< 0.16	< 0.01	1.0	< 3.9	< 0.01
	Max	< 0.05	11	8.4	12	2.9	20	4.8	44	291	9.3
	% > LOQ	0	100	39	97	27	100	55		21	52
	Lipid % (SD)	3.4 (1.7)									

BDE 209.BDE 209 could be detected in 40 % of the liver samples, in 21 % of the muscle samples and in 15 % of the fat samples (Table 1). This is the first report of BDE 209 in terrestrial mammalian wildlife.

BDE 209 was previously found in humans and some avian wildlife species or in terrestrial ecosystems.[9,12] In those wildlife studies however, the highest BDE 209 levels were around 190 ng/g lw in liver.[9,12] In the foxes of the present study, however the median BDE 209 level (of the samples in which it could be determined) was around 27 ng/g lw (maximum around 755 ng/g lw). These high levels of BDE 209 are not in accordance with the expected high metabolic activity of fox. Neither are they in accordance with the expected short half-life, which is estimated at around 7 days in humans and around 2.5 days in rats.[13-14] One possible explanation for these observations are a high exposure of the foxes to BDE 209. However, even when this is the case, then the formed debromination products which results from the metabolism of BDE 209 are swiftly eliminated, because levels of BDE 28 to 183 are very low.

Highest levels of BDE 209 were found in liver, followed by muscle and adipose tissue. Further, this is the first reported finding of BDE 209 in adipose tissue of terrestrial wildlife. In a study by Mörck et al. (2003), the highest BDE 209 levels in orally exposed rats were seen in liver and serum, while the lowest concentrations were seen in adipose tissue.[14] These relative burdens have also been observed in the present study for liver and adipose tissue.

No correlation could be observed between levels of BDE 209 and the sum of the other PBDEs (BDE 28 to BDE 183), which suggests that exposure to the corresponding technical mixtures is not related for these compounds.

Tissue distribution. Total lipid-normalized PBDE burden was significantly higher in muscle (37 ± 4 %) compared to liver (29 ± 5 %). This is similar to what was seen in sparrowhawk and buzzards.[5] The total PBDE-levels in the different tissues were significantly correlated (spearman R = 0.9 between fat and muscle).

PBDE patterns.BDE 209 dominated the PBDE-burden in the fox samples whenever it was present above LOQ.

The PBDE profile was calculated using only those individuals which had measurable levels of both BDE 209 and lower-brominated BDEs. PBDE profiles were not significantly different in the various tissues. Therefore a general profile was calculated and is presented in Figure 1.

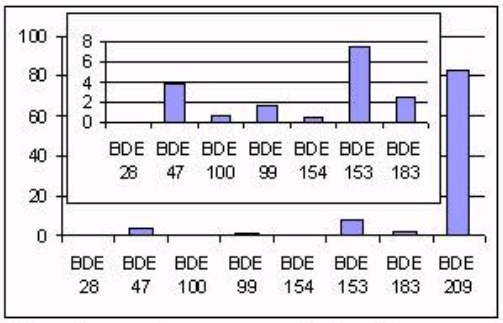

Figure 1. Overall congener profile in red fox

BDE 209 was the major congener (» 80 %) in all tissues (when it was present above the LOQ), followed by BDE 153 (» 8 %), 47 (» 4 %) and 183 (» 2 %) (Figure 1). Tribrominated BDE 28 could never be measured above LOQ, while BDE 183 could be found in the majority of the samples but at rather low levels (median = 0.61 ng/g lw in liver).

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