

# PBDEs in liver and adipose tissue samples from Belgium

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## Introduction

The massive use of polybrominated diphenyl ethers (PBDEs) as flame retardants in thermoplastics (e.g. computer and TV housing), textiles, foams, interiors of cars, buses and airplanes is related to the strict fire regulations set forward by official bodies. Therefore, these products are extensively present in our daily life. Concurrent with the increasing use, environmental levels of PBDEs have risen since their first application. Spillage and emission during production and use, release from the consumer products in which they are used and also disposal at the end-of-life of the consumer products, account for this phenomenon. These compounds are chemically and biologically persistent and furthermore lipophilic, which results in their bioaccumulation in fatty tissues and enrichment throughout food chains. Although there is some data on the human exposure pathways to these chemicals through food and dust (Voorspoels et al., *in press*; Harrad and Diamond 2006; Schechter et al., 2005b), levels in human tissues are still quite limited. This study aims at expanding the limited database concerning human PBDE exposure in Belgium and discusses the extent of contamination and distribution of PBDEs in liver and adipose tissue.

## Materials and methods

*Sampling.* Human liver and adipose tissue samples were acquired from the Department of Pathology of the University Hospital of Antwerp. In total 25 paired liver and adipose tissue samples, originating from deceased individuals (18 males and 7 females), were examined. The mean age of the individuals was 37 years, range 9 to 70 years, while the mean weight was 75 kg, range 30 to 100 kg. Based on their abundance in the samples, the following contaminants (IUPAC numbering), were targeted for analysis: PBDE congeners (no. 28, 47, 99, 100, 153, 154 (co-eluting with BB 153), and 183) and PCB congeners (no. 28/31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138/163, 149, 153, 156, 170, 180, 183, 187, 194, 196, and 196).

*Analysis.* The sample preparation and analysis method was previously validated and reported elsewhere in detail (Covaci et al., 2002; Voorspoels et al., 2003). Liver (3 g) and adipose samples (0.2-0.3 g) were mixed with anhydrous Na<sub>2</sub>SO<sub>4</sub>, spiked with internal standards (BDEs 77 and 128, CBs 46 and 143) and Soxhlet-extracted with hexane:acetone (3:1, v/v). After gravimetric lipid determination (on an aliquot), the extract was cleaned on acidified silica and the analytes were eluted with 15 ml *n*-hexane and 10 ml dichloromethane. The eluate was concentrated to near dryness and reconstituted in 100 µl *iso*-octane. The analyses were performed on an Agilent 6890 GC coupled with an Agilent 5973 MS. For PCBs, electron ionisation (EI) mode was used in combination with a 30 m x 0.25 mm x 0.25 µm DB-1 capillary column. For PBDEs, electron capture negative ionisation (ECNI) mode was used in combination with a 12 m x 0.18 mm x 0.20 µm AT-5 column.

*Quality assurance.* Quantification was based on the sum of *m/z* 79 and 81 for PBDEs and on the most intense ion from the molecular cluster for PCBs. Five-point calibration curves were created for the quantification. Our laboratory participated successfully to the NIST interlaboratory exercise for PBDEs and PCBs in marine mammal tissues (Kucklick et al., *in press*). For PBDE congeners that are consistently measured in the procedural blanks, the mean blank value was subtracted and the limit of

quantification (LOQ) was calculated as  $3 \times \text{SD}$  of the blank values (Voorspoels et al., 2003). Expressed on a lipid weight (lw) basis, LOQs ranged between 0.03 and 0.2 ng/g lw for tri- to hepta-BDEs and between 1 and 4 ng/g lw for tri- to octa-CBs. The value of each PBDE congener in the procedural blank was subtracted from the corresponding value in the sample and the resulting value was compared to the LOQ calculated for each congener.

*Data treatment.* Values below the LOQ were assigned a value of  $p \times \text{LOQ}$ , with ‘p’ the proportion of measurements with levels above the LOQ (Voorspoels et al., 2002). All statistical analyses were performed using Statistica for Windows and GraphPad InStat version 3.06 for Windows. A paired t-test was used to test differences between PBDE concentrations in liver and adipose tissue.

### Results and discussion

For the liver samples, PBDE concentrations ranged from  $3.6 \pm 2.1$  ng/g lw for a lipid content of  $7.0 \pm 3.8$  %. BDE 153 and BDE 47 were the most abundant PBDE congeners, attributing for 33 % and 26 %, respectively, to the total PBDE content. In the adipose tissue, PBDE concentration ranged from  $5.3 \pm 3.0$  ng/g lw for a lipid content of  $86 \pm 6$  %. Similar to liver, BDE 153 and BDE 47 were also the most abundant PBDE congeners, contributing to 37 % and 22 % to the total PBDE content.

**Table 1.** Mean concentrations (ng/g lw), standard deviations and detection frequency of PBDE congeners in paired liver and adipose tissue samples from 25 Belgian individuals.

	Det. frequency (%)	Adipose tissue (ng/g lw)	Liver (ng/g lw)
Lipid		$86 \pm 6.1$	$7.0 \pm 3.8$
BDE 28	84	$0.08 \pm 0.06^a$	$0.06 \pm 0.04^a$
BDE 47	96	$1.2 \pm 1.1^a$	$0.95 \pm 0.83^a$
BDE 99	86	$0.55 \pm 0.47^a$	$0.38 \pm 0.36^a$
BDE 100	94	$0.34 \pm 0.33^b$	$0.17 \pm 0.20^b$
BDE 153	100	$2.0 \pm 1.8^b$	$1.2 \pm 1.4^b$
BDE 154/BB 153	100	$0.91 \pm 0.59^b$	$0.66 \pm 0.43^b$
BDE 183	96	$0.31 \pm 0.12^b$	$0.21 \pm 0.12^b$
<b>Sum PBDEs</b>		<b><math>5.3 \pm 3.0^b</math></b>	<b><math>3.6 \pm 2.1^b</math></b>

<sup>a</sup> – not significantly different; <sup>b</sup> – significantly different

Concentrations of the more lipophilic BDE congeners (BDEs 100, 153, 154 and 183) and of sum PBDEs were higher in adipose tissue compared to liver samples. This can be explained by the role of each tissue: adipose tissue displays a relatively low metabolic activity and is primarily used for energy storage, while liver is an active tissue where contaminants are (eventually) more readily metabolized. However, there were no differences in the PBDE profiles between the two tissues, contradicting this hypothesis.

The concentrations of individual PBDE congeners and sum PBDEs in liver and adipose tissue were significantly correlated (BDE 153:  $r = 0.93$ ,  $p < 0.01$ ,  $C_A = 1.21C_L + 0.52$ ; BDE 47:  $r = 0.80$ ,  $p < 0.01$ ,  $C_A = 1.04C_L + 0.20$ ; sum PBDEs:  $r = 0.74$ ,  $p < 0.01$ ,  $C_A = 1.07C_L + 1.46$ ). The lower correlation coefficients obtained for sum PBDEs may be explained by the higher number of not detects and lower values for other PBDE congeners than BDE 47 and 153 contributing to the sum.

Additionally we have summarized all available data on PBDE concentrations in Belgium (Table 2). We have compared the tissue distribution and, whenever possible, investigated relationships between age and PBDE concentrations. In general, PBDE concentrations were higher in adipose tissue samples than in other matrices. All data showed a similar PBDE distribution trend with BDE 153 being in most

of the cases the most important congener. Further, BDE 47 and 153 were the most important contributors to the sum PBDEs, independent of the sample type.

Concentrations in adipose tissue in Belgium were on the lower end compared to studies in other countries like Finland (Strandman et al., 2002) and USA (Johnson-Restrepo et al., 2005, She et al., 2002). Studies investigating serum concentrations reported lower levels in Norway (Thomson et al., 2002), but much higher in the USA (Sjödin et al., 2004, Schecter et al., 2005a). Differences between PBDE concentrations measured in various countries can be partly explained by the PBDE concentrations in the diet and especially in food items with high contribution to the total PBDE intake (e.g. fish and meat products). However, other exposure pathways (e.g. indoor air or dust) have been identified for humans and their contribution to the total PBDE intake may be higher than expected, at least for some groups of populations (Harrad and Diamond 2006; Schecter et al., 2005b).

Table 2: Summary of PBDE concentrations in Belgian human samples.

Sampling year	Sample type	Number of samples	Age	Sum PBDEs <sup>a</sup> (ng/g lw) mean ± SD	% BDE 47 and BDE 153 to sum PBDEs <sup>a</sup>	Reference
1999-2004	serum	11	n.a.	4.0 ± 1.6	31 and 43	Covaci and Voorspoels 2005
2000-2001	milk	14	26-38	2.9	59 and 15	Pirard et al., 2003
2001	adipose tissue	20	19-77	4.7 ± 2.3	31 and 52	Covaci et al., 2002
2002-2003	pooled cord serum	4	0	2.6 ± 0.4	49 and 25	Covaci and Voorspoels 2005
2002 and 2006	liver	25	9-70	3.6 ± 2.1	26 and 33	This study
2002 and 2006	adipose tissue	25	9-70	5.3 ± 3.0	22 and 37	This study
2002-2003	pooled cord serum	7	0	2.2 ± 0.5	19 and 40	Roosens et al., 2007
2003	serum	20	19-63	3.4 ± 3.4	36 and 35	Van Wouwe et al., 2004
2003-2004	pooled serum	8	14-15	4.0 ± 0.4	32 and 36	Roosens et al., 2007
2004-2005	pooled serum	8	50-65	4.6 ± 0.8	24 and 30	Roosens et al., 2007
2006	adipose tissue	53		7.6 ± 8.9	22 and 49	Naert et al., 2006

a - for each study, sum PBDEs = BDE 28 + 47 + 99 + 100 + 153 + 154 + 183.

To identify a correlation between PBDE concentration and age, a linear regression analysis was performed. No correlation ( $r = 0.004$  for sum PBDEs;  $r = 0.04$  for BDE 153) could be observed between age and PBDE concentrations in human tissues (data from Covaci et al 2002, Van Wouwe et al., 2004, Naert et al., 2006, Roosens et al., 2007). In contrast, PCBs correlated significantly with the age ( $r = 0.62$ ,  $p < 0.01$ , for the sum PCBs;  $r = 0.64$ ,  $p < 0.01$  for PCB 153) (Figure 1).

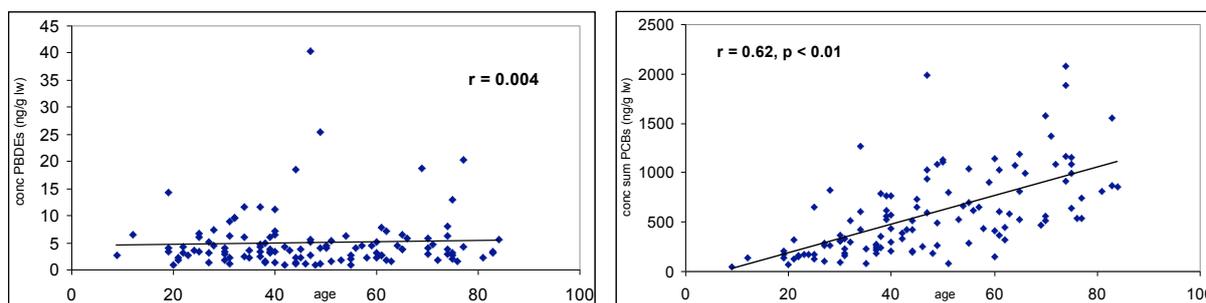


Figure 1: Correlations between age and sum PBDEs or sum PCBs in the Belgian population.

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