



## Brominated flame retardants and other organochlorine pollutants in human adipose tissue samples from the Czech Republic

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### ABSTRACT

Brominated flame retardants (BFRs) represented by polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) together with major persistent organochlorine pollutants, polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs), were determined in adipose tissue samples ( $n=98$ ) obtained by liposuction of Czech subjects. Compared to other organochlorine pollutants (mostly PCBs and DDTs), levels of PBDE were lower by 2 orders of magnitude ranging from 0.2 to 54.3 ng/g lipid weight. PBDE congeners No. 47, 99, 153 and 183 were the most abundant constituting up to 90% of these pollutants in adipose tissue. The PBDEs content measured in this study was comparable with data reported in similar samples collected in Spain, Sweden, Belgium and Japan, whilst slightly lower than in the United States. Regarding PCBs, the dominating congeners were No. 138, 153 and 180 representing up to 90% of indicator congeners. The levels of PCBs were similar to those found in other European countries.

While no age dependency was found for PBDEs, an increase of PCB and OCP levels with age was observed. Different exposure routes of donors were documented by the absence of the relationship between PCBs and OCPs.

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### 1. Introduction

Since the middle of the 20th century, numerous surveys on the levels, distribution and time trends of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDDs/Fs) and organochlorine pesticides (OCPs) occurring in various environmental compartments were initiated in many countries. In the last two decades, increasing attention has also been focused on brominated flame retardants (BFRs), a group of “emerging” halogenated POPs that similarly to chlorinated POPs tend to accumulate in lipid rich tissue found across the food chain. The human population is exposed to these man-made chemicals primarily via the diet; with fatty fish, meat and dairy products being the main sources (Sjödín et al., 2003; Stapleton et al., 2005). Inhalation is another route of human exposure, because BFRs may occur in ambient air and/or the indoor environment and is typically associated with solid dust particles (de Wit, 2002; Sjödín et al., 2003; Stapleton et al., 2005). Since infants can be exposed via contaminated breast milk containing biomagnified persistent chemicals, studies have been carried out to quantify the amount of BFRs in nursing mother’s milk. Retrospective studies on this data indicate that there is a growing body burden of BFRs in humans

(Meironyté et al., 1999). Generally; BFRs are a diverse group of chemicals which can be divided in 2 groups, reactive and additive. Since in the latter case, additive BFRs are only mixed with a polymer to be fire protected, they may leach out of goods and products relatively easily, resulting in pollution of the ambient environment. In this respect, polybrominated diphenyl ethers (PBDEs) are the most abundant representatives of this group (mainly in the EU) due to their wide use in commercial technical mixtures available in three degrees of bromination: penta-, octa- and deca-BDE. With regard to the growing concern on the potential health risk associated with the exposure of living organisms as well as humans to these BFR categories (Darnerud, 2003; Gill et al., 2004), the use of BDE technical mixtures, with the exception of deca-BDE, was banned within the EU in August 15, 2004 [Council Directive 2003/11/EC (2003) WEEE (Waste from Electrical and Electronics Equipment) and RoHS (Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment)]. Currently deca-BDE, hexabromocyclododecane (HBCD) together with tetrabromobisphenol A (TBBPA) are the most commonly used BFRs worldwide (BSEF, 2007).

The main aim of the current study was to investigate levels and profiles of (i) BFRs (PBDEs and HBCD) and (ii) “classical” organochlorine contaminants occurring in the adipose tissue of people living in the Czech Republic. To our knowledge, this is the first study comparing PBDE levels in adipose tissue of the general population of the Czech Republic with similar studies conducted elsewhere in the world.

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## 2. Materials and methods

### 2.1. Samples

Adipose fat samples for the determination of selected POPs were collected from patients (female;  $n=93$ ; male;  $n=5$ ) who underwent a tumescent liposuction for aesthetic reasons. The mean age of the group was 35.5 years with a range of 17–60 years (Table 1). Only 27 patients from the sample size of 98 were overweight or obese according to the body mass index (BMI) values ( $>26$  kg/m<sup>2</sup>) estimated from reported height and weight. To get information that could be relevant to the explanation of contamination pathways, patients were asked to provide: (i) some personal data (age, weight, height) and (ii) their eating habits, conditions in their working/household environment (e.g. regarding potential emission sources, such as electronics, textiles etc.). Samples were stored in pre-cleaned glass bottles at  $-20$  °C.

### 2.2. Materials and reagents

Individual standards of PBDE congeners and  $\alpha$ -HBCD (all with a declared purity  $\geq 98\%$ ) were obtained from Cambridge Isotope Laboratories (CIL, USA). Working standard solutions in isooctane containing the following congeners: 2,4,4'-triBDE (BDE 28), 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,5'-tetraBDE (BDE 49), 2,3',4,4'-tetraBDE (BDE 66), 2,2',3,4,4'-pentaBDE (BDE 85), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',6-pentaBDE (BDE 100), 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2,4',4,5,6'-hexaBDE (BDE 154), and 2,2',3,4,4',5,6'-hexaBDE (BDE 183) were stored at 5 °C.

The mixture of indicator PCBs (congeners No. 28, 52, 101, 118, 138, 153 and 180) in isooctane and standards (solids) of organochlorine pesticides (hexachlorobenzene (HCB),  $\alpha$ -,  $\beta$ -,  $\gamma$ -isomers of hexachlorocyclohexane (HCH),  $o,p'$ - and  $p,p'$ -DDT isomers and its degradation products DDE and DDD) used in this study were obtained from Dr. Ehrenstorfer GmbH (Germany). The purity of individual standards was at least 97%. Working standard solutions were prepared in isooctane and stored at 5 °C. Recovery standard (PCB 112) was purchased from Dr. Ehrenstorfer GmbH (Germany).

Hexane, cyclohexane, and isooctane were supplied by Merck (Germany). Ethyl acetate and dichloromethane were supplied by Scharlau (Spain). All solvents were of "organic trace analysis" grade. Anhydrous sodium sulphate obtained from Penta Chrudim (Czech Republic). Bio Beads S-X3 (styrene-divinylbenzene gel, 200–400 mesh) was purchased from Bio Rad (USA). Sulphuric acid (98%) was obtained from Merck (Germany).

### 2.3. Analytical method

#### 2.3.1. Extraction and clean up

The analytical procedure used for the analysis of adipose tissue samples has been described in detail in an earlier study (Hajšlová et al., 2007). In brief approximately 5 g of adipose fat tissue sample was homogenized with anhydrous sodium sulphate (20 g) and extracted in a Soxhlet apparatus for 8 h using a hexane:dichloromethane mixture (1:1, v/v, 150 ml). The extract was rotary evaporated at 40 °C and residues were weighted for lipid determination. An aliquot of isolated fat (cca 750 mg) was dissolved in 10 ml of an internal standard (PCB 112) solution (cyclohexane:ethylacetate, 1:1, v/v). Sample extracts were then purified on a Bio Beads S-X3 column using cyclohexane:ethylacetate (1:1, v/v) as a mobile phase and a fraction corresponding to an elution volume of 14–30 ml was collected.

#### 2.3.2. GC analysis

BFRs. An Agilent 6890 gas chromatograph coupled to a mass spectrometer 5975 XLD Inert (Agilent Technologies, USA) operated in a negative chemical ionization (NCI) was used for the GC/MS analysis of PBDEs and HBCD. A concentrated extract (1  $\mu$ l) was injected onto a DB-XLB (30 m  $\times$  250  $\mu$ m i.d.  $\times$  0.1  $\mu$ m film thickness) capillary column. DecaBDE was determined using the same GC instrument equipped with a shorter DB-XLB column (15 m  $\times$  0.25 mm i.d.  $\times$  0.1  $\mu$ m film thickness). Both GC/MSD-NCI methods have been described in an earlier study (Hajšlová et al., 2007).

PCBs and OCP. An HP 5890 gas chromatograph equipped with 2 electron capture detectors from Agilent Technologies (USA) was used for the analysis of PCBs and OCPs. The GC conditions were as follows: DB-5 (5% phenyl-methylpolysiloxane) and DB-17 (50% phenyl-methylpolysiloxane) capillary columns (60 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness); column temperature program: 60 °C (2 min) to 220 °C at 30 °C/min, to 240 °C at 0.5 °C/min and to 280 °C at 2.5 °C/min (10 min); carrier gas helium with constant flow 1.7 ml/min, injector temperature: 280 °C; injection volume: 1  $\mu$ l using the splitless injection mode (splitless time). Quantification of both target groups was performed by a multi-level calibration.

**Table 1**

Details of the Czech human adipose samples analysed in the study ( $n=98$ )

Parameter	Mean	RSD (%)	Median	(5%–95% percentile)
Age (years)	35.5	27	36	(20.9–51.2)
Weight (kg)	69.6	17	66.5	(53.9–92.6)
BMI (kg/m <sup>2</sup> )	24.9	15	23.9	(20.2–33.3)
Extractable lipid (%)	83.0	10	84.3	(69.1–94.3)

**Table 2**

Contents of brominated flame retardant and other organochlorine pollutants in human adipose tissue samples (ng/g lipid)

Analyte	Mean	RSD (%)	Median	(5%–95% percentile)
BDE 28	0.1	196	0.05	(<0.05–0.4)
BDE 47	1.1	157	0.7	(0.2–2.2)
BDE 49	0.05	193	0.05	(<0.05–0.2)
BDE 66	0.1	162	0.05	(<0.05–0.4)
BDE 85	0.05	185	0.05	(<0.05–0.2)
BDE 99	0.5	182	0.2	(<0.1–1.0)
BDE 100	0.5	166	0.3	(<0.1–1.1)
BDE 153	1.3	158	1.0	(0.3–2.3)
BDE 154	0.2	181	0.1	(0.1–0.8)
BDE 183	0.7	116	0.4	(0.1–1.9)
BDE 209	5.4	168	<2	(<2–24.9)
$\Sigma$ 10 PBDEs	4.4	139	3.1	(0.9–9.1)
HBCD	1.2	150	<0.5	(<0.5–7.5)
CB 28	2.0	49	1.8	(0.9–4.0)
CB 52	1.6	59	1.3	(0.6–3.6)
CB 101	4.2	110	1.9	(0.8–14.0)
CB 118	17.7	58	14.3	(7.1–35.2)
CB 138	121.6	57	110.1	(46.7–240.8)
CB 153	233.6	64	219.8	(82.8–492.4)
CB 180	245.0	64	230.7	(61.6–483.8)
$\Sigma$ PCBs	625.5	60	595.0	(214.6–1284.6)
HCB	120.4	111	72.1	(24.2–469.4)
$\beta$ -HCH	23.9	81	17.5	(5.9–68.0)
$p,p'$ -DDE	582.5	73	478.8	(121.8–1616.8)
$p,p'$ -DDD	1.7	90	1.3	(0.6–4.1)
$p,p'$ -DDT	24.9	72	20.4	(10.0–55.3)
$\Sigma$ DDTs	615.6	71	509.7	(137.7–1668.4)

### 2.4. Quality assurance/quality control

Analysis was carried out in an accredited testing laboratory (No. 1316.2) in the Czech Republic (current standard: EN ISO/IEC 17025). The scope of accreditation included the analyses of PCBs, OCP and BFRs in environmental, human and food samples. To document the trueness of generated data, the laboratory participates every year in several inter-laboratory studies organized by the Institute for Reference Measurements and Materials (IRRM, Geel, Belgium) and/or in FAPAS® (Food Analysis Performance Assessment Scheme) coordinated by the Central Science Laboratory (CSL, York, United Kingdom).

The trueness of generated data was controlled by the simultaneous analysis of certified reference materials CRM 350 (PCBs in mackerel oil) and CRM 430 (OCPs in pork fat) in each batch ( $n=10$ ) of analysed adipose tissue. Limits of quantification (LOQs) were: for PBDEs 28–183 (0.05–0.1 ng/g lipid), BDE 209 (2 ng/g lipid), HBCD (0.5 ng/g lipid), PCBs (0.2–0.7 ng/g lipid) and OCPs (0.3–1.5 ng/g lipid).

## 3. Results and discussion

The first data on the occurrence of BFRs in the Czech Republic, in an aquatic ecosystem and humans, were reported in 2004 (Hajšlová et al., 2007; Pulkrabová et al., 2007; Kazda et al., 2004). In the latter case, human breast milk was used as a bioindicator matrix documenting the lactating women's exposure to these "emerging" POPs. However, until now, no information on the exposure of the general Czech population has been available. Table 2 shows aggregated data illustrating 98 healthy (free from disease) body burden of BFRs in these Czech subjects. Adipose tissue obtained during a tumescent liposuction that these individuals underwent for aesthetic reasons, was employed for the purpose of this study. To our knowledge, the analysis of adipose fat obtained from people undergoing a liposuction procedure has been performed only once before in a study conducted in the United States, in which the authors analysed 52 samples (Johnson-Restrepo et al., 2005). In other comparable studies analysing BFRs and other halogenated POPs in adipose tissues, samples were taken from post mortem autopsies (Covaci et al., 2002; Naert et al., 2006; Kunisue et al., 2007). In a recent Spanish study, adipose tissue samples were obtained from women undergoing a surgery for malignant and/or benign tumors (Fernandez et al., 2007a).

An overview of the most abundant PBDE and PCB congeners together with OCP levels in Czech human adipose tissues determined in this study is presented in Table 2 and Fig. 1. The total PBDE concentrations (sum of BDEs 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183) ranged between 0.2 and 54.3 ng/g lipid weight (mean 4.4; median 3.1 ng/g lipid). For BDE congeners, 47, 153 and 183 were predominant and accounted for 28, 37 and 16% of the total PBDEs, respectively. Other relatively abundant representatives of this group were congeners BDE 99 and 100 and BDEs 28, 49, 66, 85 and 154 were detected only in several samples, mostly close to the limit of quantification.

As shown in Fig. 2, PBDE profiles in samples examined in our study were very similar to those reported in Spanish and Japanese studies (Fernandez et al., 2007a; Kunisue et al., 2007) but different to those observed in samples from Belgium. In any case, in all European countries hexabrominated congener BDE 153 was dominant,

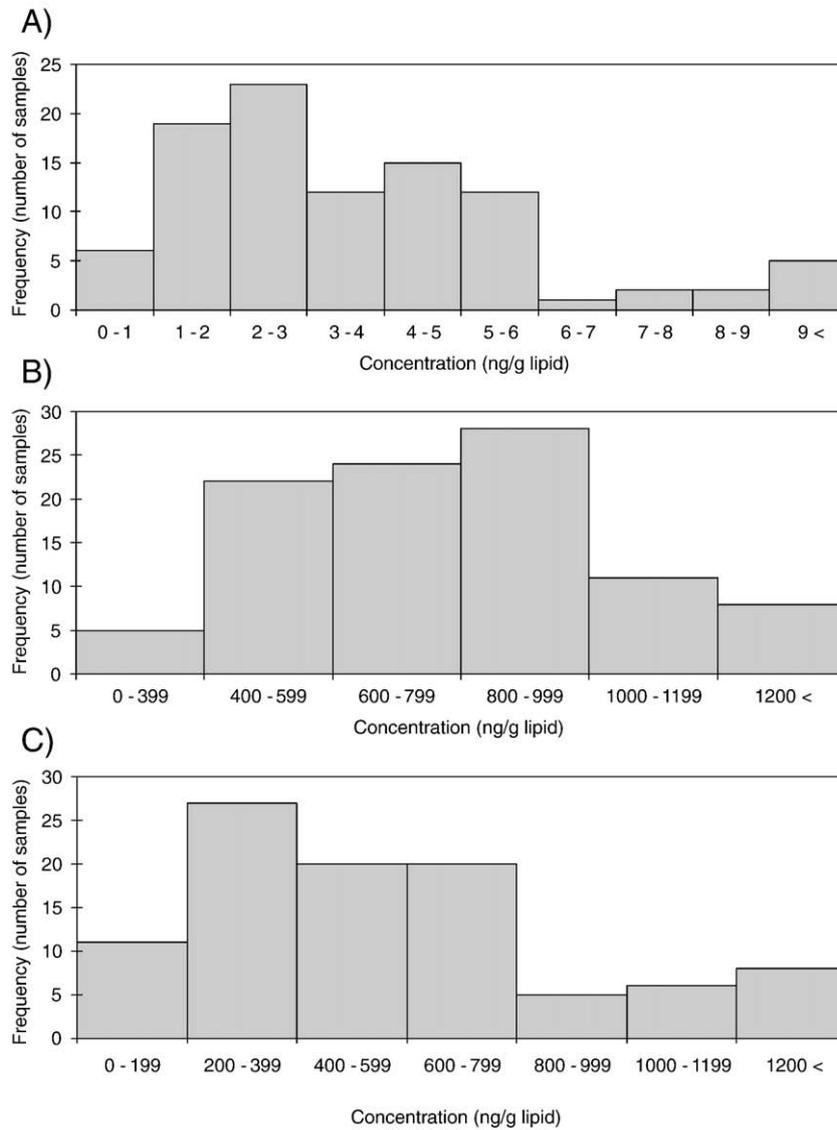


Fig. 1. Frequency distributions of the concentration of PBDEs, PCBs and DDT in human adipose tissue samples from the Czech Republic (n=98); A) PBDEs, B) PCBs, C) DDTs.

except in Sweden where the most abundant congener was tetrabrominated BDE 47. The difference in Sweden could be attributed to different uses of these products or a difference in the diet of individuals.

Interestingly, PBDE profiles in human adipose tissue found in the present study were not identical to those observed in human milk samples analysed in a recent study

by the current authors (unpublished data). While in human milk BDE 47 was clearly the most abundant brominated pollutant, the more hydrophobic BDE 153, recognized as one the most persistent congeners (Geyer et al., 2004), was the dominating congener in adipose tissue. Biotransformation and accumulation kinetic properties of individual PBDE congeners after human exposure may contribute to those differences. As shown in

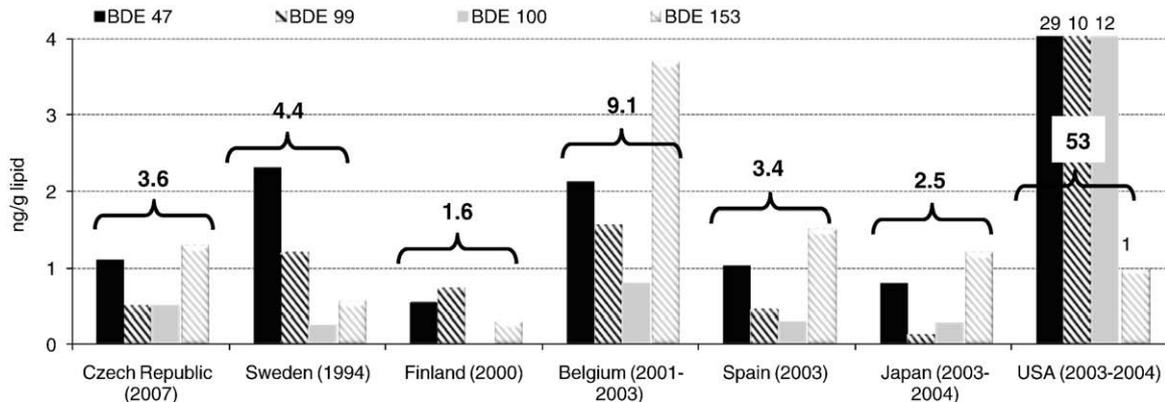


Fig. 2. Mean concentration and congener profile of the major PBDEs (ng/g lipid) in human adipose tissue samples from various countries. The numbers above bars are the mean sum of PBDE congeners.

**Table 3**

Comparison of the relationship between mean values of BDE 47 and BDE 153 in human milk and adipose tissue analysed in different countries

		Year of sampling	Number of samples	BDE 47 ng/g lipid	BDE 153 ng/g lipid	BDE 47/BDE 153	Ref.
Adipose tissue	Czech Republic	2007	98	1.1	1.3	0.85	This study
Milk	Czech Republic	2007	56	1.7	0.6	2.83	Unpublished data
Adipose tissue	Japan	2003–2004	28	0.79	1.2	0.66	Kunisue et al., 2007
Milk	Japan	2004	1000	0.68	0.27	2.52	Eslami et al., 2006
Adipose tissue	Sweden	1994	5	2.3	0.57	4.04	Guvenius et al., 2001
Milk	Sweden	2000–2001	97	2.35	0.6	3.92	Lind et al., 2003
Milk	Sweden	1997	40	2.28	0.46	4.96	Meironyté et al., 1999

Table 3, a similar trend was observed in a recent Japanese study (Kunisue et al., 2007). However, this was not reported in a study conducted in Sweden which documented, in both human milk and adipose tissue, the levels of BDE 47 to be approximately 4 times higher than BDE 153. Penta-BDE technical mixture with the majority consisting of BDE 47 was probably predominantly used in this country (Guvenius et al., 2001).

Generally, based on available studies, PBDE levels in adipose tissue of the European population were considerably lower (by almost one order of magnitude) than those in samples from the United States (Johnson-Restrepo et al., 2005), probably due to a less extensive use of this group of BFRs in common goods and products.

With regard to the deca-BDE congener representing currently the most widely used BDE-technical mixture product, little data on the presence of this congener in human tissues has been published until now. The reason is mostly due to analytical difficulties, as it is a poorly volatile, heavy congener and therefore difficult to determine together with other lower brominated PBDEs in a single run. In this study, levels exceeding the LOQ were found only in 10% of adipose tissue samples and the maximum concentration found was 28 ng/g lipid. This low incidence does not necessarily indicate low exposure to this congener. According to some studies (Kunisue et al., 2007; Johnson-Restrepo et al., 2005), the distribution of deca-BDE in adipose tissues is relatively low in humans, because of its rapid biotransformation or binding to proteins. The results reported by Thuresson et al. (2006) showed that under physiological conditions the half life of higher brominated congeners (hepta-deca PBDE) decreases with increasing numbers of bromine substituent. In other words, deca-BDE can also indirectly contribute to the pollution of the food chain.

Regarding HBCD, another widely used BFR, its fate has been investigated in several studies, mostly concerned with aquatic biota (Covaci et al., 2006); however, little is known about its presence in human tissues. In the current study, this compound (sum of  $\alpha$ -,  $\beta$ - and  $\gamma$ -isomers) was found in only 15% of the analysed samples. Its occurrence is comparable to that reported in human milk obtained from Czech women (Kazda et al., 2004). Unfortunately, under conditions of the commonly used GC/MS operated in NCI mode (this approach provides low detection limits needed in BFRs analysis) which was also employed in the current study, quantification of the individual HBCD isomers was not possible. This is because the diastereomers are not separated and moreover they interconvert at a temperature above 160 °C (Baroniti et al., 2001). The knowledge on the contamination pattern may provide some information on the extent of biotransformation of HBCD across the food chain. While in the technical mixture, the  $\gamma$ -isomer is the dominating component (up to 80%),  $\alpha$ -HBCD may become the most abundant isomer in biota. Therefore, for the determination of all individual HBCD diastereomers, LC/MS

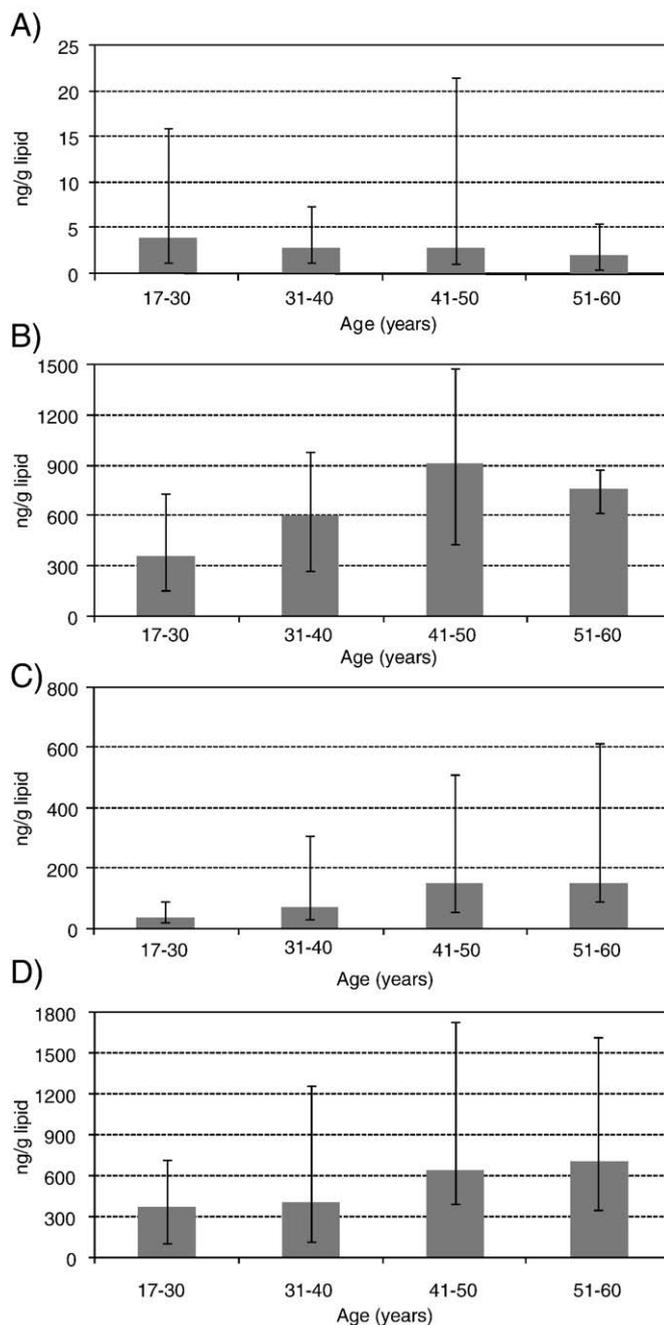


Fig. 4. Mean concentration of halogenated POPs in different age groups of donors (ng/g lipid); A) PBDEs, B) PCBs, C) HCB, D) p,p'-DDE.

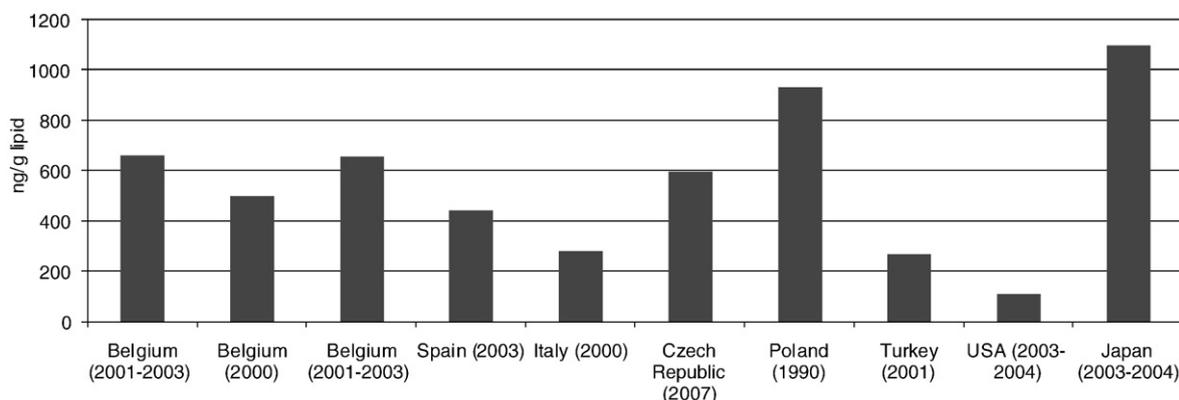
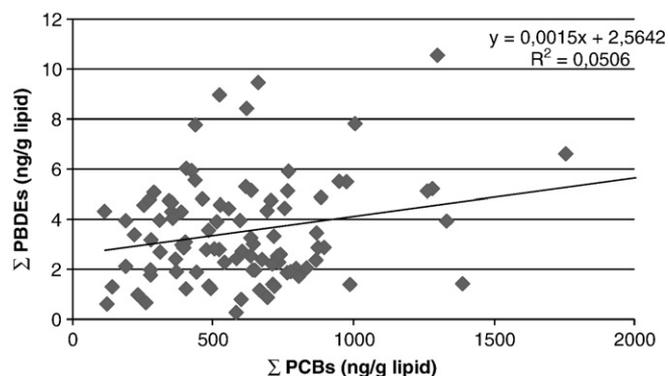


Fig. 3. Mean concentration of  $\Sigma$  PCBs (ng/g lipid) in human adipose tissue samples from various countries.



**Fig. 5.** Relationship between total PCB and total PBDE concentrations in human adipose tissue from the Czech Republic (3 PBDE and 1 PCB outliers excluded).

employing chiral separation is the preferred and most sensitive method of analysis (Morris et al. 2004).

To compare the halogenated POPs pattern and their levels vs. age dependence, also “classical” organochlorine contaminants were also analysed in adipose tissue. As expected, the levels of PCBs and DDTs were 2 orders of magnitude higher, than those determined for PBDEs. The mean value for the sum of seven indicator PCB congeners (No. 28, 52, 101, 118, 138, 153 and 180) was 625 ng/g lipid and ranged from 112.9 to 2931.3 ng/g lipid. In accordance with other European studies, the latter 3 congeners accounted for an average of more than 90% of the  $\Sigma$  PCBs concentration. This extensive pollution was a result of food being the major contamination source in the former Czechoslovakia as reported in late 1980s (NIPH, 2006).

The PCB levels in human adipose tissue from the Czech Republic do not significantly exceed those from other European countries (see Fig. 3) (Çok et al., 2004; Covaci et al., 2002; De Saeger et al., 2005; Falandyz et al., 1994; Fernandez et al., 2007a,b; Johnson-Restrepo et al., 2005; Kunisue et al., 2007; Mariottini et al., 2000; Naert et al., 2006; Smeds and Saukko, 2001).

The most abundant OCP, present in all samples, was p,p'-DDE [1,1-bis(4-chlorophenyl)-2,2-dichloroethene], but levels of its precursor, p,p'-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] were lower than this OCP. Other chlorinated POPs, like HCB (hexachlorobenzene) and  $\beta$ -HCH (hexachlorocyclohexane), were also detected in all samples in levels above the LOQs (see Table 2).

Statistically significant ( $p < 0.05$ ) age-dependent differences were not observed in the current study, when all the age categories were considered. Nevertheless, when donors were grouped according to their age (see Fig. 4A), the highest mean PBDE levels were found in individuals belonging to the youngest group (<30 years) and with the lowest BMI (23.7). On the other hand, the lowest mean values of PBDEs were found for the oldest donors with the highest BMI (26.9). As the BMI is proportional to the amount of particular individual's adipose tissue and assuming that everyday exposure is comparable, then a lower BFR content per fat unit in the older donor group may be due to some dilution effect. The continuing exposure of all population groups to PBDEs from various sources (including the diet as well as inhalation either from indoor air or house dust as potential sources: Sjödin et al., 2003; Stapleton et al., 2005) was probably responsible for the absence of a correlation between the age and levels in other similar studies conducted in the United States, Spain, Japan and Belgium (Johnson-Restrepo et al., 2005; Kunisue et al., 2007; Fernandez et al., 2007a; Covaci et al., 2002).

Contrary to BFRs and in the agreement with many other studies performed outside the Czech Republic, a significant clear correlation between age and the concentration of OCs was observed in the present study up to the age group 41–50 years (see Fig. 4B–D). For these contaminants, diet, particularly fish and other animal products, are the main exposure sources. Some decrease in the mean PCBs content that occurred in the age group 51–60 was observed. This may be due to either a statistical error (only 8 samples were available) or to earlier liposuctions which might have removed some of the body burden during these previous procedures.

Based on the differences in the exposure pathways and with regard to different pharmacokinetics, it was not surprising that there was no relationship between the concentrations of PBDEs and PCBs (Fig. 5) in human adipose tissue samples. Following the ban of PCBs in many countries, a slow successive decrease of PCBs may have occurred in the human food chain. This is the major difference of PCBs compared to PBDEs.

#### 4. Conclusions

This study reports for the first time the residue levels of PBDEs and HBCD together with “classical” OCs in human adipose tissue obtained from 98 patients who underwent liposuction in the Czech Republic. The results clearly show the ubiquitous occurrence of BFRs in the general Czech population, with BDE 153 being the most abundant

congener in examined biotic samples, followed by BDE 47. The contamination pattern was rather different from that found previously in a set of 103 human milk samples collected from Czech mothers in 2003 (Kazda et al., 2004). In this pilot study, BDE 47 dominated in respective bioindicator matrix. The observed differences in PBDE congener distribution among body fluids and tissues (due to diversity of their kinetics) is in accordance with other studies (Kunisue et al., 2007).

Despite its broad use, BDE 209 was detected in only a few adipose tissue samples. The mean values of PBDEs in human fat from Czech donors did not largely differ from those recorded from other European countries.

Also PCB and OCP levels were comparable to those reported in similar studies conducted outside of the Czech Republic. It should be noted that the production and use of PCBs has been banned for three decades, whereas the use and production of PBDEs still continues. In spite of the fact, that penta- and octa-BDE technical mixtures were banned in the EU 4 years ago, humans are still exposed in their daily lives from food and emissions from various products into their environment.

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