POLYBROMINATED DIPHENYL ETHERS (PBDEs) IN CANADIAN HUMAN FETAL LIVER AND PLACENTAL TISSUES

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Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants used in a wide variety of products including electronics, upholstery, textiles and building materials[1]. These flame retardants persist in the environment and accumulate in biological tissues, leading to the addition of several PBDEs to Annex A of the Stockholm Convention on persistent organic pollutants (POPs), which recommended elimination of these products[2]. PBDEs have been detected in environmental compartments, wildlife, food and human samples worldwide[3]. The PBDE concentrations observed in North America are consistently higher than those observed in Europe, reflecting greater usage in North America[1]. Although diet is the major pathway of human exposure to most POPs, such as PCBs and dioxins, indoor air and dust also have been implicated as major pathways for exposure to PBDEs[4]. Due to the use pattern of these products and to the amount of time spent indoors, humans may be subject to greater exposure to PBDEs than other organisms.

Neurodevelopmental impacts resulting from PBDE exposure have been observed in rodent studies with reported impacts on the thyroid hormones which are essential for neonatal development, particularly the brain [1,2,5]. There are limited studies which have focussed on the relationship between PBDE exposure and thyroid hormone levels in children, and among these, there are conflicting results related to this association[5]. Attention deficit and poor social competence symptoms, however, have been associated with post-natal exposure to PBDE 47[5]. PBDE concentrations also have been associated with effects on reproductive hormones in infants[6]. Elevated PBDE concentrations in human milk have been positively correlated with cryptorchidism in infant males[1,7].

Numerous studies have reported detectable levels of PBDEs in human liver, adipose tissues, human milk and blood. The median level of PBDEs (Σ of 7 congeners), for example, in cord blood samples (n=210) collected from mothers in the New York City area in 2001 was 18.4 ng/g lipid (maximum 947 ng/g lipid)[8]. Additionally, PBDEs have been detected in placenta samples collected from North America and Europe. The placenta, formed during pregnancy, serves to exchange nutrients and waste between the mother and fetus during pregnancy and may provide a route of exposure for PBDEs to the fetus[9]. Total PBDE concentrations in term placenta from Denmark ranged from 0.51 – 17.1 ng/g lipid (Σ of 13 congeners)[9] and similar concentrations were measured in placental tissue from Spain (0.19 to 9.7 ng/g lipid; Σ of 13 congeners)[10]. Lower concentrations were reported in earlier work from Finland (0.88 – 4.4 ng/g lipid) although only four congeners were measured in these samples[11]. Mean PBDE levels in Canadian early to mid-gestation fetal liver samples collected in 1998 (284 ng/g lipid) and 2006 (1610 ng/g lipid) (Σ of 8 congeners)[12], were much higher than observed in mid-gestation to term liver samples from the US in 2004 (23.1 ng/g lipid) (Σ of 13 congeners)[13].

As a follow up to the earlier work, a large series of early to mid-gestation placenta and fetal liver samples were collected in Montreal, Canada and analysed for the presence of 23 PBDE congeners (15, 17, 28, 37, 47, 66, 71, 75, 77, 85, 99, 100, 119, 126, 138, 153, 154, 160, 181, 183, 190, 205, 209) to establish temporal trends (1998 – 2010) in PBDE concentrations in fetal tissues and to allow for a spatial comparison with other centres across Canada as samples become available.

Materials and methods

Human fetal tissue samples (placenta n = 150 and liver n = 48) were collected following elective pregnancy terminations between 1998 and 2010 from Montreal, Canada. Informed written consent for the sample

collection was received prior to procedures and ethics board approval was obtained from both Health Canada and McGill University for the collection and use of the human fetal tissues. Upon collection, tissue samples were flash frozen in a dry ice/acetone bath and stored at -80ºC until transport to the laboratory for analysis. Fetal ages were estimated using foot length and ranged from 6.5 to 19.5 weeks.

Two reagent blank samples, a spiked vegetable oil sample and two fish reference materials with known PBDE concentrations were included with each set of samples analysed. Prior to analysis, samples (~0.5 g) were thawed and weighed into 50 mL centrifuge tubes and homogenized with 2:1 acetone: hexane following addition of surrogate standards containing 9 ^{13}C PBDE analogues. Traces of water were removed using sodium sulphate and extracts were cleaned up using Florisil (6 g). Samples were evaporated and taken to final volume (100 µL) using performance standard prior to injection. Lipid determination was performed gravimetrically.

Analysis was performed using gas chromatography-mass spectrometry with an Agilent 6890 coupled to a Micromass Autospec Ultima. The column used was a 15 m J&W DB-5MS, 0.25 mm i.d. and 0.1 µm film thickness. Cool on-column injection was used with the injector tracking the oven temperature. The temperature program ranged from 80ºC to 300ºC. The head pressure was ramped from 28 kPa to 173 kPa. Helium was the carrier gas used and the mass spectrometer was operated in the EI positive mode (50 eV), the trap current was 650 µA and the source temperature was set to 250ºC. The resolution for these analyses was approximately 8,000 for all analytes.

Results and discussion:

The average surrogate recovery ranged from 63% (PBDE 15) to 101% (PBDE 209). PBDE concentrations in the reference samples and oil spike were within acceptable levels, based on certified ranges. Reagent blanks were consistently found to contain trace levels of PBDEs, with PBDE 47, 99 and 209 found at higher levels (1 – 10 ng). The concentrations detected in the blanks were used to correct sample concentrations for procedural background levels. The average limits of quantification (LOQ) for those PBDEs without significant background

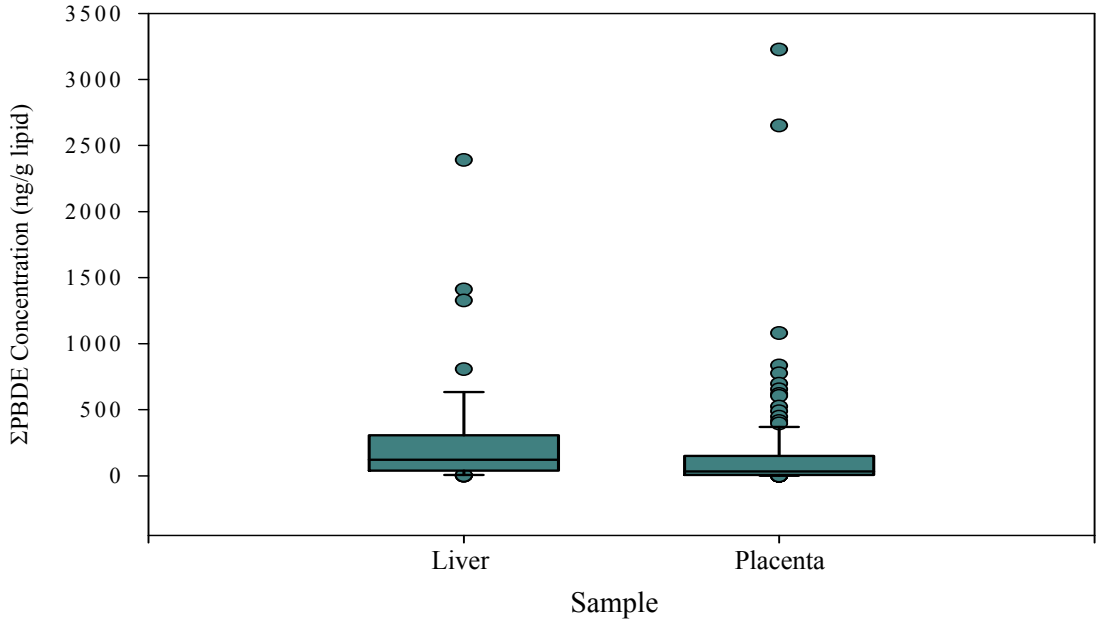

Figure 1: ΣPBDE concentrations in fetal tissue samples. Boxes indicate 25th, 50th and 75th percentiles. Points indicate data outside of 10th (⊥) or 90th (⊤) percentiles.

levels were determined using a signal to baseline noise ratio of 10:1. The average LOQ for all congeners ranged from 0.005 ng to 0.176 ng (PBDE 15 and 160, respectively), excluding PBDE 47, 99 and 209. The LOQ for PBDE 47, 99 and 209, estimated using 2 x the standard deviation in the procedural blanks were 22, 8 and 4 ng, respectively.

All 48 liver samples analysed were found to have detectable levels of PBDEs, although PBDE concentrations were below detectable levels in five of the placenta samples tested. Total PBDE concentrations in fetal liver samples ranged from <LOQ – 2390 ng/g lipid (<LOQ – 29.8 ng/g whole wt) and concentrations in placenta samples with detectable levels ranged from <LOQ to 3230 ng/g lipid (<LOQ – 36.8 ng/g whole wt) (Figure 1; Table 1). Mean and median PBDE concentrations were higher in liver (336 and 144 ng/g lipid, respectively) relative to placenta samples (157 and 34 ng/g lipid, respectively). The residue concentrations observed in placental tissues from Denmark, Finland and Spain are within the range observed in the present study, despite the median concentrations being much higher in the early gestation Canadian samples.

Table 1: ΣPBDE Concentrations (ng/g lipid) in fetal tissues 1998 -2010 (mean lipid content 1.9%, 1.2%; liver, placenta, respectively)

Year	**Liver**			**Placenta**		
	N	Median	Range	N	Median	Range
1998	7	64.7	35.1 – 250	0	–	–
1999	5	73.3	44.7 – 406	3	0.998	0.538 – 76.1
2000	17	153	6.23 – 807	41	23.6	ND[1] - 605
2001	3	162	106 – 272	49	65.9	ND – 3230
2002	8	362	0.701 – 2390	31	28.7	<LOQ[2] – 2650
2004	0	–	–	5	17.8	2.81 – 618
2005	0	–	–	1	175	175
2006	0	–	–	1	10.5	10.5
2008	3	48.8	31.4 – 358	7	16.4	3.44 – 212
2009	2	186	<LOQ – 373	8	33.6	6.73 – 1080
2010	2	14.7	10.7 – 18.6	3	98.0	23.7 - 134

[1]not detected
[2]below the limit of quantification

PBDE 47 was the predominant contributor to total PBDE levels (49%), similar to the results reported for mid-gestation to term livers in the US[13], where PBDE 47 contributed 47% to total levels. PBDE 153 was the predominant congener (33%) in adult livers collected in Belgium, although PBDE 47 was the second highest contributor to ΣPBDE concentrations[14]. PBDE 153 contributed 13% to ΣPBDE concentrations in fetal liver tissues in the present study, more closely related to the US results[13]. In contrast to observations in liver tissue, PBDE 209 had a greater contribution to ΣPBDE in the placenta samples (>50%) relative to PBDE 47 (38%), in the present study. A similarly high contribution of PBDE 209 in term placenta was reported from Denmark (50%)[9] and Spain (>50%)[10].

Thirty-three paired liver and placental samples were obtained during the study, although most pairs were collected in 2000 (Figure 2). No correlation between liver and placenta ΣPBDE concentrations was observed in the samples analysed (r = 0.037), similar to previous Canadian data[12]. The concentrations tended to increase in the pairs until the maximum concentration was observed in 2002 (Figure 2). Total PBDE concentrations were not correlated with fetal ages (r = 0.153 and r = 0.029, liver and placenta, respectively), which is consistent with the lack of correlation between postnatal ages and adult liver PBDE levels observed in Belgium[14].

Maximum PBDE concentrations were observed in samples collected during 2001/02, with an apparent subsequent decline in observed levels in later years, possibly related to changes in market use of PBDE commercial products; although fewer samples were available beyond this period.

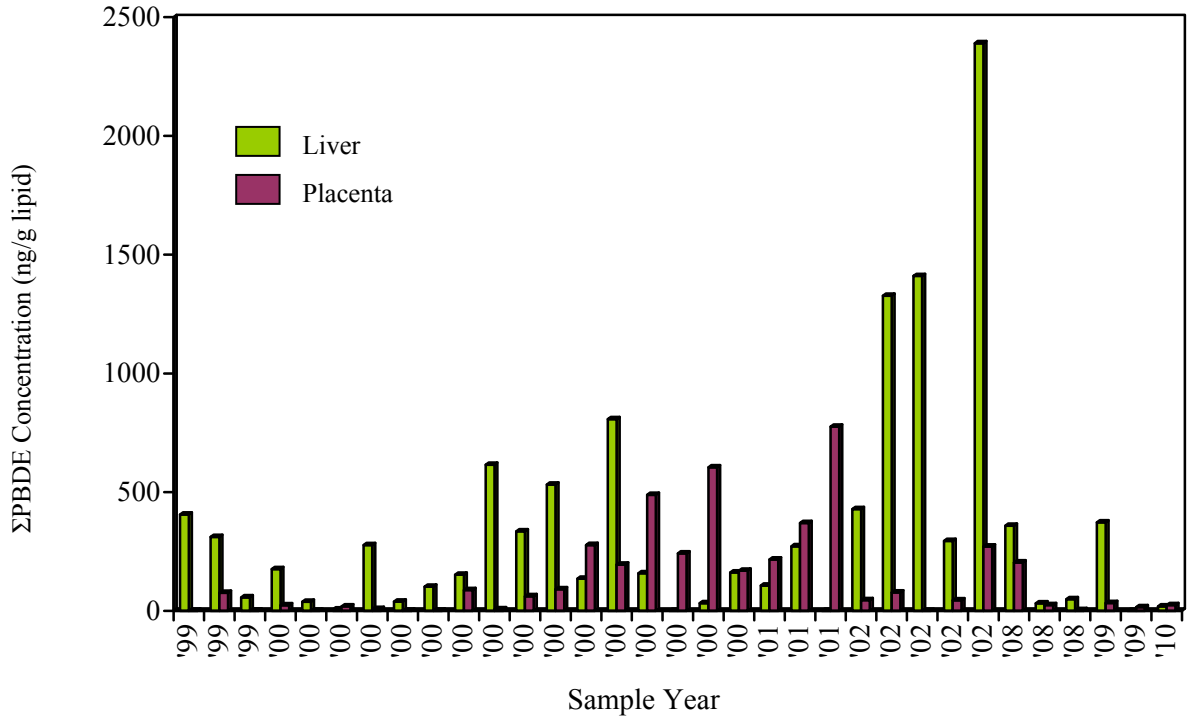

Figure 2: ΣPBDE concentrations in paired fetal liver and placenta samples collected between 1999 and 2010.

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