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Polybrominated diphenyl ethers and their methoxylated and hydroxylated analogs in Brown Bullhead (*Ameiurus nebulosus*) plasma from Lake Ontario

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HIGHLIGHTS

- PBDEs, MeO-PBDEs and OH-PBDEs were detected in Brown Bullhead from three locations in Lake Ontario.
- Concentrations of OH-PBDEs in plasma were about 50 folds higher than the MeO-PBDEs.
- OH- and MeO-PBDEs were evaluated against 20 authentic standards; eight OH-PBDEs and five MeO-PBDEs were identified.
- Additional seven unidentified OH-PBDEs and three unidentified MeO-PBDEs were detected in fish plasma.

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs), methoxylated PBDEs (MeO-PBDEs) and hydroxylated PBDEs (OH-PBDEs) were detected and quantified in Brown Bullhead (*Ameiurus nebulosus*) from Lake Ontario. Samples were collected in 2006 from three different locations near the city of Toronto: Frenchman's Bay, Toronto Island, and Tommy Thompson Park. A total of 117 plasma samples were pooled into 19 samples, separating males and females by site of capture. Pooled samples were analyzed for 36 PBDEs, 20 MeO-PBDEs and 20 OH-PBDEs, but only six PBDEs, five MeO- and eight OH-compounds were confirmed against standards currently available. These peaks were quantified as "identified" peaks, while peaks matching ion ratios but not matching the retention time of the available standards were quantified as "unidentified" peaks. Both "identified" and "unidentified" concentrations were combined to obtain a total concentration. No significant variations were obtained for total PBDE concentrations, ranging from 3.33 to 9.02 ng g^{-1} wet weight. However, OH- and MeO-PBDE totals ranged over 1 order of magnitude among the samples (not detected – 3.57 ng g^{-1} wet weight for OH-PBDEs and not detected −0.10 ng/g wet weight for MeO-PBDE). The results of this study suggested that these compounds are ubiquitous in biota. Source estimation of MeO- and OH-PBDEs in freshwater fish were discussed. Considering that up to date no freshwater sources for MeO- or OH-PBDEs have been reported, concentrations found should be mainly related to bioaccumulation from anthropogenic sources, although other sources could not be dismissed.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been produced and used in large quantities as flame retardants in a wide variety of consumer products (furniture, PCs, TVs, etc.). Their persistence, potential bioaccumulation and toxic effects to humans and wildlife have caused large concern (European Communities, 2001, 2002, 2003) and as a result, tetra-, penta-, hexa- and hepta-PBDEs have been included in Annex A of the Stockholm Convention United Nations Environmental Programme (UNEP), 2001. Over the past several years significant efforts have been put forth on understanding the source and fate of PBDEs in the environment however there is a lack of knowledge regarding their methoxylated (MeO-PBDEs) and hydroxylated (OH-PBDEs) analogs.

OH- and MeO-PBDEs have been detected as natural products of marine organisms (Fu et al., 1995; Handayani et al., 1997; Cameron et al., 2000; Vetter et al., 2001; Marsh et al., 2004; Kierkegaard et al., 2004; Teuten et al., 2005; Malmvärn et al., 2005, 2008). Several studies have also identified OH-PBDEs as metabolites of PBDEs in mice and rats exposed to PBDEs (Örn and Klasson-Wehler, 1998; Malmberg et al., 2005; Marsh et al., 2006; Qiu et al., 2007). In fact,

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Qiu et al. (2007) proposed three metabolic pathways for OH-PBDEs formation: (i) direct metabolic derivation resulting from cytochrome P450 enzyme mediated metabolism of precursor PBDEs, (ii) a 1,2-shift of a bromine atom after epoxidation of the parent PBDEs, and (iii) debromination and hydroxylation of the parent PBDEs. In addition, MeO-PBDEs have been detected as products of OH-PBDEs via O-methylation in bacteria (Allard et al., 1987) and also metabolic production of OH-PBDEs from MeO-PBDEs have been reported (Wan et al., 2009). Reactions involving PBDE parent precursors including thermal heat stress, pyrolysis and incineration, transformation by free radicals such as OH and/or CH_3 (Haglund et al., 1997) and oxidative processes in sewage treatments plants (Ueno et al., 2008) could be other potential sources of these compounds.

MeO-and OH-PBDEs have been identified in abiotic environmental matrices such as surface water and precipitation in Ontario, Canada by Ueno et al. (2008). These compounds have also been detected in the biotic environment including algae, mussel (Malmvärn et al., 2005), herring, seal, salmon muscle and fish oil (Haglund et al., 1997), marine fish (Asplund et al., 1999; Marsh et al., 2004), marine mammals (van Babel et al., 2001; Vetter, 2001; Vetter et al., 2002; Pettersson et al., 2004; Sinkkonen et al., 2004; Marsh et al., 2005; Stapleton et al., 2006; Wan et al., 2009), freshwater fish (Letcher et al., 2003; Kierkegaard et al., 2004; Valters et al., 2005; Houde et al., 2009), birds (Haglund et al., 1997; McKinney et al., 2006), as well as humans (Hovander et al., 2002; Vetter and Jun, 2003; Athanasiadou et al., 2008; Qiu et al., 2009; Lacorte and Ikonomou, 2009). Presence of MeO- and OH-PBDEs in humans arouses high concern, since these compounds have some structural resemblance to the thyroid hormone thyroxine (T4), and have been shown to have up to three times stronger affinity for transthyretin (TTR) than thyroxine (Malmberg, 2004) thus providing a mechanism for potential disruption in thyroxine homeostasis (Meerts et al., 2001).

The aim of this study was to investigate the occurrence of methoxylated and hydroxylated PBDEs in fish plasma from Lake Ontario. The Brown Bullhead (*Ameiurus nebulosus*), an abundant benthic feeding and high trophic species in Lake Ontario was used in this study. This species is widely used for monitoring purposes in the Great Lakes region due to its common occurrence in polluted habitats (International Joint Commission (IJC), 1989). Concentrations and congener patterns are discussed to estimate the possible origin of these compounds in the sampled fish.

2. Materials and methods

2.1. Sample collection

A total of 117 fish plasma samples were collected in October 2006 from three different sites near the city of Toronto: Toronto Island and Tommy Thompson Park, located within the city of Toronto, and Frenchman's Bay, located 40 km North to Toronto. Complete details about sample collection are provided in the Supplementary materials (SM: Material and methods). In brief: blood was sampled and stored in heparinized vials keep in ice and stored at −80 °C until analysis. Due to the small volumes of individual samples, 19 different pools of plasma were created with samples mixed together from the three locations – male and female samples were pooled separately by site. Percent lipid in these samples ranged between 0.6% and 2.3%. Detailed biological descriptions of the pooled samples are summarized in Table 1, and complete details of for each pooled sample are listed in Table S1.

2.2. Chemicals and materials

For MeO- and methylated OH-PBDEs determination, twenty individual analytical grade solutions, ranging from mono to hexa brominated native MeO-PBDEs, one labeled $^{13}C_{12}$ OH-PBDE and two $^{13}C_{12}$ MeO-PBDEs solutions as recovery and performance standards were purchased from Accustandard Inc. (New Haven, CT, USA) and Wellington Laboratories (Guelph, ON, Canada), completed details are described in SM. For PBDE determinations five individual calibration solutions including 41 PBDEs from mono to deca brominated were purchased from Wellington Laboratories (Guelph, ON, Canada). Diazomethane was prepared from N-methyl-N-nitroso-p-toluenesulfonamide (Diazald) (Sigma Aldrich) (Fieser and Fieser, 1967).

2.3. Extraction, clean up, and quantification

The extraction procedure has been described elsewhere (Hovander et al., 2000; Athanasiadou et al., 2008) and is only summarized here. Briefly, pooled plasma samples (average wet weight of 2.72 g) were spiked with ^{13}C recovery standards before extraction ($^{13}C_{12}$-6′-MeO-BDE-100 and $^{13}C_{12}$-6′-OH-BDE-100). Hydrochloric acid (1 mL) and 2-propanol (6 mL) were added to denature the proteins and help with the emulsification, respectively. The organic phase was extracted twice with 6 and 4 mL of hexane/methyl tert-butyl ether mixture (1:1; v/v). Organic extracts were combined and washed with 4 mL of potassium chlorine (1%), reduced to dryness with nitrogen for gravimetric lipid determination, and subsequently redissolved in a hexane/methyl tert-butyl ether mixture (1:1; v/v).

Fractionation was performed using a florisil column (Berger et al., 2004), 1.5 g activated for 12 h at 450 °C and deactivated with 0.5% v/v water topped with 2 g anhydrous sodium sulfate which was washed with 10 mL hexane and dichloromethane (3:1; v/v). Fraction A, containing PBDEs and MeO-PBDEs, was obtained eluting the column with 11 mL hexane and dichloromethane (3:1; v/v) and subsequently with 2 mL of hexane and acetone (85:15; v/v).

Table 1
Summary of biological characteristics according to capturing site of the fish collected.

	Frenchman's Bay[a]		Toronto Island[a]		Tommy Thompson[a]		Female mean	Male mean
	Mean	(min–max)	Mean	(min–max)	Mean	(min–max)		
Fork length (cm)	32.3	(30.1–35.5)	33.2	(30.6–35.6)	27.6	(26.2–29.7)		
Total weight (g)	467.6	(365.7–546.0)	520.3	(430.0–645.1)	292.5	(230.7–387.2)	411.6	454.1
Gonad weight (g)	3.11	(0.73–7.03)	3.66	(0.60–0.11)	2.28	(0.36–5.01)	5.25	0.62
GSI[b]	0.69	(0.15–1.29)	0.71	(0.11–1.36)	0.83	(0.12–1.59)	1.29	0.14
Liver weight (g)	11.3	(9.2–13.6)	10.7	(8.3–14.0)	8.28	(6.4–10.6)	10.3	9.97
LSI[c]	2.42	(2.21–2.59)	2.16	(1.72–2.42)	2.82	(2.61–3.11)	2.58	2.30
Age (years)	6.4	(6.0–6.8)	6.9	(6.1–7.5)	7.6	(7.3–7.6)	6.95	6.97
Plasma lipid percentage (%)	1.48	(0.99–1.91)	0.89	(0.64–1.14)	1.46	(1.04–2.33)	1.40	1.09

[a] Pooled samples.
[b] GSI = (Gonad weight/Total weight) × 100.
[c] LSI = (Liver weight/Total weight) × 100.

Fraction B, containing OH-PBDEs was then obtained with 4 mL of hexane and acetone (85:15; v/v) and 10 mL of dichloromethane:methanol (88:12; v/v). Fraction B was evaporated to dryness and derivatized with 1 mL of diazomethane for 2 h although previous studies have demonstrated that 30 min is sufficient for quantitative methylation (Athanasiadou et al., 2008). Finally, excess diazomethane and ether were removed by the addition of 10 mL of hexane to the extract followed by evaporation to 4 mL. Fraction A and B were subjected to further cleanup. Co-extracted lipids were removed by treatment with 2 mL of concentrated sulfuric acid and washed with 4 mL of hexane, and the organic phase was eluted on acid and neutral silica columns. The final fraction was concentrated until incipient dryness and re-dissolved in the performance standard ($^{13}C_{12}$-6-MeO-BDE-47 in isooctane) prior to GC–MS.

Fraction A, containing MeO-PBDEs and PBDEs, and Fraction B, containing OH-PBDEs (as MeO-PBDEs), were analysed by high resolution mass spectrometry (MicroMass Autospec Ultima HRMS) operated in electron ionization mode at a resolution greater than 10000, details are described in the SM. Peaks which matched the retention times and isotopic ratio with authentic MeO-PBDEs standards were quantified as “identified” MeO- or OH-PBDEs, while peaks that matched only the isotopic ratio were quantified as “unidentified” using an average response factor of same homologue group.

2.4. Quality control

Three criteria were used to ensure the correct identification and quantification of analytes: (a) ±3 s retention time between the analyte and standard, (b) the ratio of quantifier and qualifier ions had to be within ±15% of the theoretical values and (c) signal to noise ratio had to be greater than 3:1. Recoveries for $^{13}C_{12}$-6′-MeO-BDE-100 and $^{13}C_{12}$-6′-OH-BDE-100 during this study averaged 72 ± 9% and 56 ± 15%, respectively. Method detection limits (MDLs) were defined as three times the standard deviation analytical mean procedural blank value ($n = 3$). MDLs ranged from 0.005 to 0.015 pg g^{-1} wet weight (w.w.) for MeO-PBDEs, from 0.009 to 0.020 pg g^{-1} w.w. for OH-PBDEs, and from 0.001 to 0.009 pg g^{-1} w.w. for PBDEs. Nonane, used as an instrumental blank was injected between samples to ensure that there was no carry over between samples. No PBDEs, MeO-PBDEs and OH-PBDEs were detected in the procedure and instrument blanks.

2.5. Statistical analysis

Statistical analysis was performed using SPSS statistical software (Version 17.0). Principal component analysis (PCA) was conduced to evaluate correlation between biological characteristics and total PBDE, OH-PBDE and MeO-PBDE concentrations.

Table 2
Summary of concentrations (ng g^{-1}) of PBDEs, OH-PBDEs and MeO-PBDEs according to capturing sites evaluated in this study. Only detected congeners are described for both identified and unidentified compounds.

Compounds		Frenchman's Bay[a]		Toronto Island[a]		Tommy Thompson[a]		Female mean	Male mean
		Mean	(min–max)	Mean	(min–max)	Mean	(min–max)		
PBDEs									
Tetra	BDE-47	2.21	(1.36–3.00)	1.53	(1.10–2.01)	2.12	(1.58–2.50)	1.85	2.02
Penta	BDE-100	0.75	(0.56–0.98)	0.74	(0.47–1.26)	0.43	(0.28–0.53)	0.62	0.67
	BDE-99	1.60	(1.15–2.05)	2.02	(1.16–3.28)	1.68	(1.39–1.90)	1.68	1.89
Hexa	BDE-153	0.36	(0.26–0.51)	0.4	(0.19–1.03)	0.21	(0.15–0.29)	0.29	0.36
	BDE-154	0.26	(0.12–0.36)	0.43	(0.16–1.30)	0.22	(0.15–0.30)	0.26	0.37
Hepta	BDE-183	0.03	(0.01–0.04)	0.06	(0.02–0.15)	0.04	(0.03–0.06)	0.03	0.05
	Total PBDEs	5.21	(3.78–6.02)	5.18	(3.33–9.02)	4.69	(3.69–5.40)	4.74	5.36
OH-PBDEs									
Tri	2′- OH-BDE-28	n.d.		0.009	(0.006–0.016)	0.013	(0.006–0.022)	0.014	0.009
	4′- OH-BDE-17	0.002	(n = 1)	0.018	(0.009–0.037)	0.033	(0.006–0.072)	0.025	0.022
	Unidentified	0.075	(n = 1)	0.041	(0.001–0.14)	0.054	(0.016–0.106)	0.053	0.033
Tetra	2′-OH-BDE-68	n.d.		0.022	(0.013–0.034)	0.023	(0.009–0.052)	0.029	0.019
	6- OH-BDE-47	0.043	(0.01–0.15)	0.83	(0.18–2.55)	1.09	(0.26 - (2.77)	1.03	0.37
	5- OH- BDE-47	0.011	(n = 1)	n.d.		0.011	(n = 1)		0.011
	4′- OH-BDE-49	0.15	(0.07–0.35)	0.24	(0.060–0.50)	0.24	(0.055–0.453)	0.28	0.14
	4- OH-BDE-42	0.012	(0.008–0.018)	0.014	(0.009–0.026)	0.005	(n = 1)	0.009	0.013
	Unidentified	0.026	(0.014–0.043)	0.04	(0.002–0.10)	0.038	(0.010–0.107)	0.040	0.030
Penta	4′- OH-BDE-101	0.044	(0.025–0.064)	0.063	(0.021–0.14)	0.029	(0.022–0.037)	0.043	0.048
	Unidentified	n.d.		0.164	(0.118–0.211)	0.04	(0.022–0.070)	0.069	0.095
Hexa	Unidentified	n.d.		0.029	(n = 1)	n.d.			0.029
Total identified OH-PBDEs		0.24	(n.d.–0.51)	1.15	(0.31–3.10)	1.42	(0.37–3.36)	1.37	0.60
Total unidentified OH-PBDEs		0.03	(n.d.–0.04)	0.13	(0.003–0.35)	0.11	(0.05–0.21)	0.09	0.10
Total OH-PBDEs		0.27	(n.d.–0.55)	1.28	(0.33–3.34)	1.53	(0.42–3.58)	1.46	0.70
Total ident/Total OH-PBDEs		0.90	(0.87–0.92)	0.89	(0.75–1.00)	0.91	(0.86–0.95)	0.93	0.87
Total OH-PBDEs/Total PBDEs		0.05	(0.02–0.13)	0.25	(0.08–0.67)	0.35	(0.09–0.97)	0.32	0.14
MeO-PBDEs									
Di	Unidentified	n.d.		0.006	(n = 1)	n.d.			
Tri	2′- MeO-BDE-28	0.003	(n = 1)	n.d.		n.d.			
	4′- MeO-BDE-17	0.001	(n = 1)	n.d.		n.d.			
Tetra	2′-MeO-BDE-68	0.003	(0.001–0.004)	0.012	(0.002–0.021)	0.004	(0.002–0.008)	0.010	0.004
	6- MeO-BDE-47	0.008	(0.005–0.014)	0.028	(0.006–0.076)	0.036	(0.006–0.089)	0.025	0.023
	Unidentified	n.d.		0.009	(n = 1)	n.d.			
Penta	4′- MeO-BDE-103	n.d.		0.002	(n = 1)	n.d.			
Total identified MeO-PBDEs		0.010	(n.d. - 0.017)	0.037	(0.008–0.097)	0.034	(0.008–0.089)	0.032	0.025
Total unidentified MeO-PBDEs		n.d.		0.016	(n = 1)	n.d.			
Total MeO-PBDEs		0.010	(n.d. - 0.017)	0.039	(0.008–0.097)	0.034	(0.008–0.089)	0.032	0.026
Total ident/Total MeO-PBDEs		1.00		0.95	(0.64–1.00)	1.00		1.00	0.96
Total MeO-PBDEs/Total PBDEs		0.002	(0.001–0.003)	0.008	(0.001–0.02)	0.007	(0.002–0.018)	0.007	0.005

[a] Pooled samples, n.d. = not detected.

Correlations between PBDE, OH-PBDE and MeO-PBDE congeners were also evaluated by Pearson's test. Concentrations of pollutants related to the different sampling locations were compared using Mann–Whitney Test. Correlation matrices are shown in supporting information (Tables S5 and S6).

3. Results

Concentrations of PBDEs, MeO-PBDEs and OH-PBDEs were summarized in Table 2. Detail results for PBDEs, MeO-PBDEs and OH-PBDEs are provided in supplemental material Tables S2-4 respectively. Plasma lipid content did not correlate with concentrations of PBDEs, MeO- and OH-PBDEs, and thus concentrations in this study were expressed on a wet weight basis (w.w.), (Table S5).

3.1. PBDEs

Average of total PBDE concentrations (sum of BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183) was 5.03 ± 1.31 ng g^{-1} w.w. (mean ± SD). Valters et al. (2005) reported levels of PBDEs and OH-PBDEs (levels of MeO-PBDEs were below LODs) in different fish species from the Detroit River. Among them, two pools of four Brown Bullhead fish were evaluated, reporting an average concentration of total PBDEs (BDE-47, -99, -100, -154, -153) for the two samples of 1.71 pg g^{-1} w.w.

Only six PBDEs were detected in all samples: BDE-47, -99, -100, -154, -153 and -183, with an average contributions to the total PBDE concentrations of: 39% for BDE-47, 37% for BDE-99, 13% for BDE-100, 6% for BDE-153, 6% for BDE-154 and 1% for BDE-183. Similar congener pattern was observed by Valters et al. (2005) and was also well correlated to those reported for two penta-BDEs commercial mixtures, DE-71 and Bromkal 70-5DE, by (La Guardia et al., 2006). Concentration of BDE-99, -100, -153, -154 and -183 were closely correlated ($p < 0.01$; $r = 0.647$ to 0.954; min–max). However no significant correlations were obtained between BDE-47 and any other BDE congeners detected in this study (see Table S6).

3.2. MeO- PBDEs

Fraction A was assessed for 20 MeO-PBDEs, but only five MeO-PBDEs were identified while three unidentified peaks were also detected. To evaluate the importance of unidentified concentrations, the identification ratios (Total identified MeO-PBDEs/Total MeO-PBDEs) were calculated. Mean value was 0.98 ± 0.08 (mean ± SD), indicating that the unidentified compounds contributed less than 10% to the total concentration.

Concentration of total MeO-PBDEs ranged between N.D. and 0.10 ng g^{-1} w.w. (0.03 ng g^{-1} w.w.; mean) for females; and between 0.01 and 0.09 ng g^{-1} w.w. (0.02 ng g^{-1} w.w.; mean) for males. These levels were low compared to those reported by Kierkegaard et al. (2004) in a temporal trend study in Pike from Swedish Lakes; where a decreasing trend for the sum of two MeO-PBDEs, 2′-MeO-BDE-68 and 6-MeO-BDE-47, from 5.4 to 0.5 ng g^{-1} w.w. between 1967 and 2000 was reported.

Similar congener patterns were observed in all locations evaluated, both for males and females. The major MeO-PBDEs in the samples were tetra-brominated diphenyl ethers congeners: 2′-MeO-BDE-68, and 6-MeO-BDE-47. This data correlated well with those obtained in Pike from Swedish lakes by Kierkegaard et al. (2004), where 2′-MeO-BDE-68, and 6-MeO-BDE-47 were detected in all fish. Both congeners have been reported previously in marine mammals (Marsh et al., 2005; Stapleton et al., 2006). To the best of our knowledge, this is the first time that 2′-MeO-BDE-28, 4′-MeO-BDE-17, and 4′-MeO-BDE-103, were identified in freshwater fish. These MeO-PBDEs were detected at much lower concentrations in three of the 19 pooled samples analyzed.

3.3. OH-PBDEs

Fraction B was assessed for 20 OH-PBDE congeners, however only eight were identified with the standards while seven were quantified as "unidentified". Mean value for Total identified OH-PBDEs/Total OH-PBDEs ratio was 0.90 ± 0.08 (mean ± SD).

Total OH-PBDEs ranged between N.D. and 3.58 ng g^{-1} w.w (1.46 ng g^{-1} w.w.; mean) for females; and between 0.16 and 1.51 ng g^{-1} w.w. for males (0.70 ng g^{-1} w.w.; mean). Concentration of OH-PBDEs in plasma were approximately 46- and 56-fold higher than the MeO-PBDEs in male and female fish plasma respectively. Concentrations presented in this study were higher than those reported by Valters et al. (2005), in two pooled samples of Brown Bullhead from Detroit River (12.8 pg g^{-1} w.w; mean of total OH-PBDEs including 2′-OH-BDE-68, 6-OH-BDE-47, 4′-OH-BDE-49, 4-OH-BDE-42 and 2-OH-BDE-123). This fact is in agreement with levels of OH-PBDEs (MeO-PBDEs were not analyzed) reported in the abiotic environment from the Great Lakes region, including the Detroit River and Lake Ontario by Ueno et al. (2008). In that study, snow samples presented the highest value in Guelph Lake located 45 km north of Lake Ontario, and 65 km from Toronto Island and the Tommy Thompson Park sampling locations of our study. In addition, higher concentrations of OH-PBDEs in surface water samples were found in Lake Ontario compared to those obtained from the Detroit River. The authors suggested that sources of OH-PBDEs were related to populated areas and they may be produced in wastewater treatment plants (WWTPs). Since urban wastewater treatment is a highly oxidative process, it is not surprising to find OH-PBDEs in these effluents (Hua et al., 2005), although a fraction of OH-PBDEs may also arrive to the WWTPs in their influent, and could be due to a multitude of sources, like human or animal excretion (Hakk et al., 2002, 2006; Ueno et al., 2008). Possible sources of MeO- and OH-PBDEs detected in this study are discussed later.

As in the case of MeO-PBDEs, for OH-PBDEs similar congener patterns were also observed in all locations evaluated, both for males and females. The major OH-PBDEs were tetra-BDEs: 6-OH-BDE-47, and 4′-OH-BDE-49, which accounted for 77 ± 14% (mean ± SD) of the total OH-PBDE concentration and were detected in 18 of 19 samples analyzed. Other congeners such as 2′-OH-BDE-28, 4′-OH-BDE-17, 2′-OH-BDE-68, 5-OH-BDE-47, 4-OH-BDE-42 and 4′-OH-BDE-101 were detected in some of the samples but at lower concentrations. 5-OH-BDE-47, a tetra OH-BDE, was only detected in two samples and at very low levels. These results are consistent with congener patterns reported by Valters et al. (2005) in plasma of fish from the Detroit River where 6-OH-BDE-47, and 4′-OH-BDE-49 accounted for 75% of the total OH-PBDE concentration without considering 2-OH-BDE123 that we did not analyze in our study. 2′-OH-BDE-68 has also been frequently detected in fish (Asplund et al., 1999; Marsh et al., 2004).

4. Discussion

Principal component analysis (PCA) was performed including biological characteristics and total PBDE, OH-PBDE and MeO-PBDE concentrations, respectively. Results showed that three principal components (PC) depicted 79.2% of the variance. The first component, included age, total weight, fork length and liver weight. Total weight, fork length and liver weight were closely positively correlated ($p < 0.01$; $r = 0.980$, 0.789 and 0.818, respectively), see Table S5. However, negative correlations were obtained between these variables and age ($p < 0.01$; $r = -0.749$, -0.760 and -0.738,

respectively). The second component described total concentrations of OH- and MeO-PBDEs, which are positively correlated ($p < 0.05$; $r = 0.454$), and the third component was only influenced by gonad weight.

Fig. 1A shows the relationship between the first and second component, sample scores were labelled according to the sampling site. It is observed that samples from Tommy Thompson were located on the negative side of the first component, while samples from Frenchman Bay and Toronto Island were situated on the positive side. This indicated that samples from Tommy Thompson were older and smaller when compared to the other two sampling sites. Tommy Thompson location was the final destination of the sediments produced during the dredging of Toronto Port Channels (TPA, 2010), so differences in growing ratio could be explained by the less availability of food in this location. In addition, samples from Frenchman's Bay were situated in the negative side of the second component, indicated that these samples had a lower concentration of MeO- and OH-PBDEs when compared to the other sampling sites. Fig. 2 shows the concentrations of total PBDEs, total MeO-PBDEs and total OH-PBDEs for males and females in the three locations studied. No significant variations could be obtained between sites of capture for PBDEs, however levels of MeO- and OH-PBDEs were lowest ($p < 0.05$) at the Frenchman's Bay sampling site compared to the Toronto Island and the Tommy Thompson

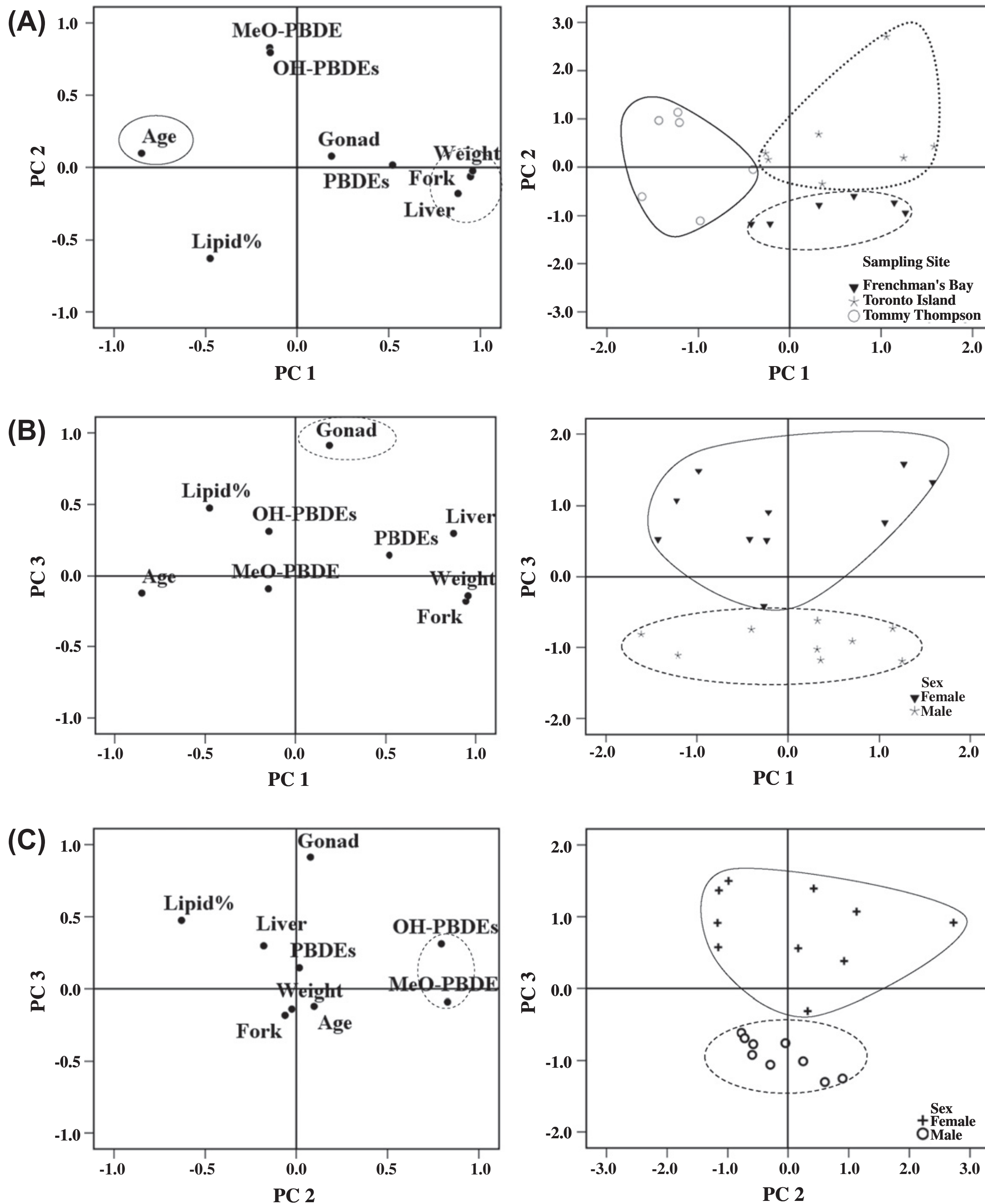

Fig. 1. Diagrams of dispersion related to the three components resulting from a principal component analysis (PCA): (A) PC 1 and PC 2, (B) PC 1 and PC 3, and (C) PC 2 and PC 3. Loading plots (left) contributions of each variable to each component. Scores plot (right) of all samples on each component.

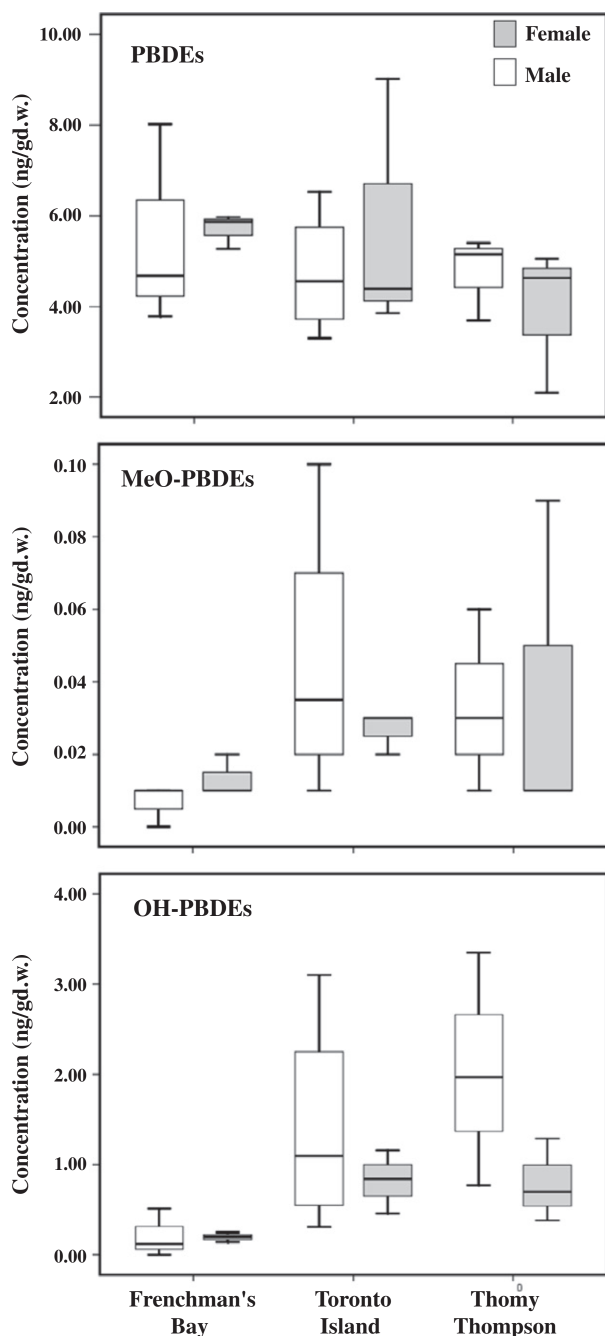

Fig. 2. Box and whisker plots obtained for (A) PBDEs (B) MeO-PBDEs, and (C) OH-PBDEs in the studied locations, distinguishing between male and female pooled samples. Upper edge of the box, line within the box and lower edge of the box, represents the 75th, 50th, and 25th percentiles. Vertical lines extend from the minimum to the maximum value.

sites. The higher MeO- and OH-PBDEs concentrations found near the city of Toronto (Toronto Island and Tommy Thompson Park) were plausibly due to the large population and greater usage of PBDEs in urban areas (Ueno et al., 2008).

The relationship between the first and third components was shown in Fig. 1B. Samples were labelled according to sex. Samples were clearly located in two poles for the third component, females on the positive side and males on the negative side. Females invest more energy into gonad weight compared to males. Similarly, females and males were located in both poles of the first factor indicating heterogeneity in age of the samples for both sexes. Fig. 1C represented second and third components. Samples were labelled according to sex. Both females and males were distributed in the negative and positive side of the second factor, thus no significant difference could be observed between females and males in terms of MeO- and OH-PBDE concentrations.

4.1. Potential sources of MeO- and OH-PBDEs in plasma of Brown Bullhead

Presence of MeO- and OH-PBDEs in the plasma could be derived from: (i) bioaccumulation from natural or anthropogenic sources, and/or (ii) metabolism.

4.1.1. Bioaccumulation from natural sources

MeO- and OH-PBDEs have been reported as natural products of marine organisms (Fu et al., 1995; Handayani et al., 1997; Cameron et al., 2000; Vetter et al., 2001; Marsh et al., 2004; Kierkegaard et al., 2004; Teuten et al., 2005; Malmvärn et al., 2005; Malmvärn et al., 2008). Naturally occurring MeO- and OH-PBDEs reported in the literature always have a methoxy or hydroxy group in the ortho position relative to the diphenyl ether bond (Marsh et al., 2004) whereas polybrominated diphenyl ether exposed mice and rats (Örn and Klasson-Wehler, 1998; Malmberg et al., 2005; Marsh et al., 2006; Qiu et al., 2007), have the methoxy or hydroxy group in the meta and para position also. Thus, findings of MeO- or OH-PBDEs with methyoxyl or hydroxyl groups in the meta or para positions may indicate PBDEs metabolism. To date, no natural freshwater sources of these compounds are known, although this does not preclude that a percentage of ortho hydroxylated or methoxylated PBDEs reported in this study (2′-OH-BDE-28, 2′-OH-BDE-68, 6-OH-BDE-47, 2′-MeO-BDE-28, 2′-MeO-BDE-68, and 6-MeO-BDE-47) could be due to unidentified freshwater sources.

4.1.2. Bioaccumulation from anthropogenic sources

MeO- and OH-PBDEs could also be originated from the reaction of PBDEs with $OH^{\cdot}$ formed from the UV degradation of ozone or from UV reactions with dissolved organic matter in the aquatic environment (Ueno et al., 2008), but this contribution is difficult to quantified with data obtained in this study. However, there were two factors in the results that indicated anthropogenic sources of OH-PBDEs near the city of Toronto: (i) the variation of total OH- and MeO-PBDEs among captured sites, and (ii) a shift in the ratio of 6-OH-BDE-47 to 4′-OH-BDE-49. As discussed previously, samples captured close to Toronto, presented statistically higher ($p < 0.05$) concentrations of total MeO and OH-PBDEs (0.039 and 0.034 ng g^{-1} w.w. for MeO-PBDEs, and 1.28 and 1.53 ng g^{-1} w.w. for OH-PBDEs; obtained at Toronto Island and Tommy Thompson Park, respectively) compared to those obtained at Frenchman Bay (0.01 ng g^{-1} w.w. for MeO-PBDEs and 0.27 ng g^{-1} w.w. for OH-PBDEs; mean). In addition there was a shift in the ratio of 6-OH-BDE-47 to 4′-OH-BDE-49 between pooled samples from the Frenchman's Bay (0.2 ± 0.1; mean ± SD) and samples captured near the city of Toronto (Toronto Island and Tommy Thompson Park) (4.2 ± 1.5; mean ± SD). Qiu et al. (2007) reported a ratio of 0.4 for these OH-PBDEs in mouse plasma exposed to DE-71. This ratio was similar to the one obtained in fish from the Frenchman's Bay, and suggests a metabolic source in these samples. On the other hand, enrichment of 6-OH-BDE-47 found in the samples near the city of Toronto could indicate bioaccumulation in samples collected near the city of Toronto.

4.1.3. Metabolism

Metabolic transformation could be indicated by significant correlation between precursors and metabolites (Wan et al., 2009). However total PBDE concentrations obtained in this study did not correlate neither with MeO-PBDEs nor OH-PBDEs, suggested

that metabolism was not the main source of OH- and MeO-PBDEs in the samples. However, significant correlation was found between total MeO-PBDEs and total OH-PBDEs ($p < 0.05$; $r = 0.454$). Interconversion of MeO-PBDEs and OH-PBDEs by formation of MeO-PBDEs from OH-PBDEs (Allard et al., 1987; Haglund et al., 1997) and vice versa (Wan et al., 2009) have been demonstrated, and could support this correlation. Nevertheless, since each congener could produce different OH- and/or MeO-PBDEs, metabolism should be evaluated by congeners.

Concentrations of 4′-OH-BDE-17, 2′-OH-BDE-68, and 6-OH-BDE-47 were well correlated ($p < 0.01$; $r > 0.646$), indicating a possible common origin. Qiu et al. (2007) proposed a metabolic pathway that produces these OH-PBDEs as metabolites of BDE-47 in mouse plasma after exposure to a PentaBDE commercial formulation (DE-71). However no correlation was obtained between 4′-OH-BDE-17, 2′-OH-BDE-68, and 6-OH-BDE-47 and BDE 47 to support this metabolic pathway in fish. Others metabolic pathways for 2′-OH-BDE-68 formation could be: (i) direct metabolic derivation of BDE-68, (ii) via a 1,2-shift of a bromine atom after epoxidation of BDE-49, or by (iii) debromination/hydroxylation of BDE-90 as shown in Fig. S1. Although samples were evaluated for 36 PBDEs, commercial mixture used for instrumental analysis did not include neither BDE-68 nor BDE-90. However, Valters et al. (2005) also indicated 2′-OH-BDE-68 in the plasma of fish from the Detroit River but was not found in exposed mice (Qiu et al., 2007) or rats (Marsh et al., 2006) which suggests bioaccumulation from natural or anthropogenic sources.

Good correlations were obtained between 4-OH-BDE-42 and 4′-OH-BDE-101 ($p < 0.01$; $r = 0.819$) and these with BDE-100, -153, -154, and -183 ($p < 0.05$, $r > 0.522$), see Table S6. Origin of 4′-OH-BDE-101 could be via a 1,2-shift of a bromine atom after epoxidation of the parent BDE-99 as shown in Fig. S2. In addition, Qiu et al. (2007) proposed the metabolic formation of 4-OH-BDE-42 from BDE-47 and reported that levels of BDE metabolites could be due to a debromination and hydroxylation of highly brominated PBDEs. Therefore, degradation of BDE-100, -153, -154, and -183 to BDE-47 and BDE-99 and their hydroxylation could explain the presence of 4-OH-BDE-42 and 4′-OH-BDE-101 in the plasma. In the same manner, debromination and hydroxylation of BDE-47 have been proposed as a potential origin for 2′-OH-BDE-28 and 4′-OH-BDE-17 in faeces (Marsh et al., 2006) and plasma (Qiu et al., 2007) of BDE-47 exposed rats (Marsh et al., 2006). However, we did not find any correlation between these OH-PBDEs and BDE-47 to support this hypothesis in Brown Bullhead.

In summary, this study detected PBDEs, MeO-, and OH-PBDEs in fish samples from Lake Ontario and the results demonstrate that these compounds are ubiquitous in biota. Results indicate that dietary intake represents the most important source, while contribution of PBDEs metabolism is low. Since up to date no freshwater sources for MeO- or OH-PBDEs have been reported, bioacumulated concentrations found in this study should be related to anthropogenic sources. Results also suggests that among others, sources related to human activities like human excretion, or oxidative processes occurring in the WWTP, could be important sources of OH-PBDEs and MeO-PBDEs. Further research is needed to evaluate the sources, fate, bioavailability, and toxicological significance of these compounds in freshwater fish.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.09.005.

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