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POLYBROMINATED DIPHENYL ETHERS AND HYDROXYLATED AND METHOXYLATED ANALOGUES IN DETROIT RIVER FISH

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Introduction

Polybrominated diphenyl ethers (PBDEs) are the most important class of brominated flame retardants (BFRs) in terms of environmental occurrence and persistence in the tissues of wildlife and humans [1]. PBDEs have been shown to magnify in fish tissues [2]. Temporal studies over approximately the last 10 years have demonstrated exponentially increasing levels in lake trout (*Salvelinus namaycush*) muscle from areas in the Great Lakes system [3]. Like most species where PBDEs have been determined, congener profiles of residues in the tissues of exposed fish are predominantly composed of 2,2',4,4'-tetrabromoDE (BDE-47), 2,2',4,4',5-pentabromoDE (BDE-99), 2,2',4,4',6-pentabromoDE (BDE-100), 2,2',4,4',5,5'-hexabromoDE (BDE-153), 2,2',4,4',5',6-hexabromoDE (BDE-154) and 2,2',3,4,4',5',6-heptabromoDE (BDE-183).

The Detroit River is a channel connecting Lake Huron and Lake Erie via Lake St. Clair, and is an effluent-dominated waterway in a highly industrialized area of the Great Lakes. The sediments and aquatic biota, including fish, from the Detroit River contain high concentrations of persistent polyhalogenated contaminants including polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides (e.g., DDTs, chlorobenzenes and chlordanes) relative to other areas of the Great Lakes system [4]. Recently, PBDEs were reported in whole body homogenates of largemouth bass (*Micropterus salmoides*) and common carp (*Cyprinus carpio*) collected in summer 1999 from the western branch of the Trenton Channel , which is a section mid-way along the Detroit River and receives high levels of municipal and industrial effluents [5].

Knowledge of PBDE uptake, elimination and metabolism in fish is limited [6,7]. In northern pike (*Esox lucius*) dosed with ^{14}C-labeled BDE-47 six hydroxylated (HO) PBDE metabolites were detected in several tissues including blood [8,9]. Blood plasma of Swedish Atlantic salmon (*Salmo salar*), herring (*Clupea harengus*) and commercial fish oils were also found to contain HO-PBDEs and/or MeO-PBDEs [10,11]. HO-PBDEs likely result from cytochrome P450-mediated metabolism of PBDEs, although food chain accumulation may be possible. Since MeO-PBDEs are not produced commercially, it has been suggested, but not proven that MeO-PBDEs may be formed from HO-PBDEs via methylation in liver or intestinal microflora, or directly in sediments [6,11].

Despite studies illustrating that fish are generally slow to depurate higher chlorinated PCB congeners [4], we recently reported HO-PCBs and other halogenated phenolic compounds (HPCs) using GC-

μECD and low-resolution GC-MSD(EI) in the blood-plasma of 13 pelagic and benthic feeding fish from the Detroit River [12]. Total (Σ) HO-PCB concentrations were comparable, and in some cases exceeded those of, e.g., Σ-PCBs and Σ-chlordanes. Furthermore, a number of ECD peaks in the HPC fractions were not identified but appeared to be polybrominated. In the present study, we characterize and determine the congener patterns and relative amounts of PBDEs, HO-PBDEs and MeO-PBDEs in the blood-plasma of fish collected from the Trenton Channel-Gross Ile area of the Detroit River.

Materials and Methods

Three common carp and three largemouth bass were collected by gill net techniques in the vicinity of Grosse Ile in the Trenton Channel in August of 2001. The largemouth bass were 30-41 cm in length and 675-1230 grams in weight, and the carp were 37-71 cm in length and 1100-5240 grams in weight. All details regarding sample collections and chemical fraction isolation are extensively described in Li *et al.* [12]. Plasma samples from 3 individual fish for each species were pooled to obtain a suitable sample size of 4.0 grams. Plasma pools were spiked with neutral fraction (i.e., BDE-30, CB-85 and CB-122) and phenolic (HPC) fraction (i.e., 4'-HO-BDE17 and 4-OH-CB72) internal standards for assessment of the contaminant recovery efficiency. Plasma pools were then extracted and two fractions (100 μL each) were isolated, 1) a HPC fraction containing HO-PBDEs (subsequently derivatized to MeO-PBDE analogues), and 2) a neutral fraction containing PBDEs and MeO-PBDEs [12]. Using GC-μECD, the recovery efficiencies were >85% for CB-85, CB-122 and BDE-30 (neutral fraction), and >75% for 4'-HO-BDE17 and 4-OH-CB72 (HPC fraction).

Analysis of PBDEs, MeO-PBDEs and HO-PBDEs was accomplished by using the GC parameters described in Li *et al.* [12] coupled with high resolution-mass spectrometry in the electron impact mode (HRMS-EI) (resolution = 10,000). Appropriate SIR ions for the PBDEs and MeO-PBDEs (estimated instrumental detection limit of 1 pg) were first determined with the external standard mixtures of PBDEs (6 congeners, Table 1) and MeO-PBDEs (14 congeners, Table 2). These SIR ions were subsequently used for the analyte determinations in the samples. Quantitation ions were $[M]^+$ or $[M-2Br]^+$ and identify confirmation ions were $[M+2]^+$ or $[(M+2)-2Br]^+$, which reflect the ^{81}Br and ^{79}Br isotopic contributions (Tables 1 and 2). Mass chromatograms of the fish contaminant fraction were compared with PBDE (100 ng/mL each) and MeO-PBDE (1.0 ng/mL each, and obtained from Prof. Å. Bergman, Stockholm University, Sweden) external standard mixtures. The predominant congeners in the fractions were BDE-47 (neutral), 4'-MeO-BDE-49 (neutral) and 6-HO-BDE47 (HPC). The % concentrations of other analytes in the mass chromatograms of there fractions were relative to the predominant congeners (Tables 1 and 2). Plasma method blanks were also analyzed, although no significant background interference was observed in the neutral or HPC fractions. This project is supported by a research grant from the NSERC Canada and the Canada Research Chairs Program (to R.J. Letcher). We thank Dr. K. Drouillard and C. Busch (GLIER) for their assistance with sampling.

Results and Discussion

The concentration of BDE-47 is 1.8 ng/g (wet weight) and 3.4 ng/g (w.w.) in the carp and largemouth bass plasma, respectively. Rice *at al.* [5] reported a similar BDE-47 concentrations in whole body homogenates of carp (3.0 ± 0.5 ng/g w.w.) and largemouth bass (2.8 ± 2.0 ng/g w.w.) collected from the same area of the Detroit River, where BDE-47 accounted for about 55% of the total (Σ) PBDEs relative to < 10% for other congeners (i.e., BDE-99, -100, -153, -154, -181 and -183). MeO-PBDEs are not detectable in the neutral fraction from the present carp plasma. In the plasma of largemouth bass 4'-MeO-BDE49, 6-MeO-BDE47, 4-MeO-BDE42 and 6-MeO-BDE85 are identified, and all

with a concentration about the same as 4'-MeO-BDE49, which is 1.1 pg/g (w.w.), and just above the plasma detection limit (i.e.,<0.1 pg/g w.w. at a S/N ratio = 10) (Table 2). The presence of MeO-PBDEs in the blood plasma would suggest accumulation and/or enzyme-mediated methylation of the HO-PBDE analogues occurs in largemouth bass.

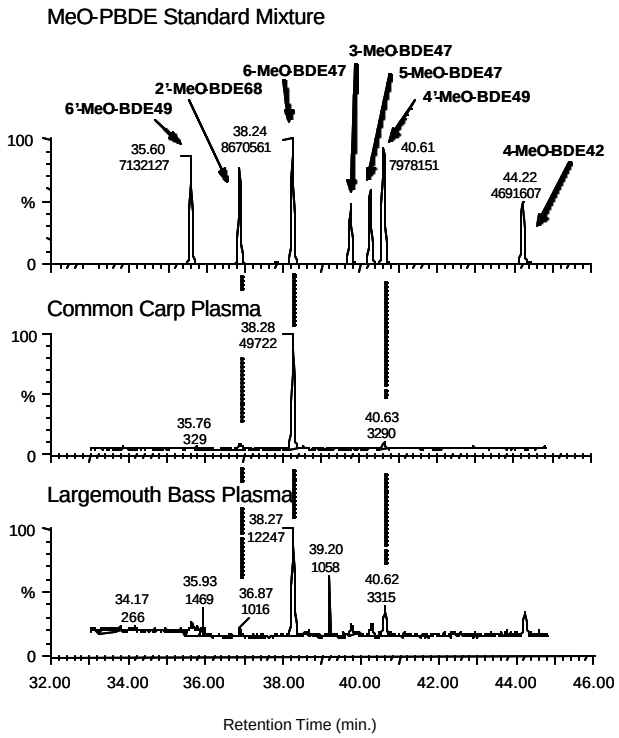

Figure 1. GC-HRMS(EI$^+$) mass chromatograms of tetrabrominated MeO-PBDE standards, and HO-PBDEs (derivatized to MeO-PBDEs) in phenolic fractions from the plasma from selected fish based on the $[M]^+$ at m/z 515.7217 (Table 2).

HO-PBDEs are identified in the plasma of the two fish species, but in each case the dominant congeners are tetrabrominated (Table 2 and Figure 1). The concentration of the predominant congener, 6-HO-BDE47, is 42.3 pg/g (w.w.) and 10.4 pg/g (w.w.) in carp and largemouth bass, respectively. The presence of 6-HO-BDE47, 4-HO-BDE42 and 4'-HO-BDE49 is consistent with HO-PBDE metabolites identified in studies on northern pike dosed with BDE-47 [8,9]. This would suggest that the present carp and largemouth bass are capable of metabolizing BDE-47 to HO-PBDEs. The 6HO-BDE47 concentration is 0.3% and 2.3% of the BDE-47 concentration in largemouth bass and carp plasma, respectively.

Analysis of the plasma of 11 other Detroit River fish species (i.e., black crappie, white bass, northern pike, longnose gar, bowfin, brown bullhead, channel catfish, freshwater drum, white sucker, bigmouth buffalo and lake sturgeon) all contain the predominant 6-HO-BDE-47 and 4'-HO-BDE49 congeners, each at concentrations in approximately the 10 to 100 pg/g (w.w.) range (not shown).

Table 1. The % concentration amount of major PBDE congeners identified in the neutral fraction from blood plasma of selected fish collected from the Detroit River in 2001.

PBDE Congener	HRMS Quant. Ion		% Amount	
			Common Carp	Largemouth Bass
BDE-47 [a]	$[M-2Br]^+$ (323.8785)		100	100
BDE-99	$[M-2Br]^+$ (403.7870)		6	102
BDE-100	$[M-2Br]^+$ (403.7870)		36	52
BDE-153	$[M-2Br]^+$ (481.6975)		n.d.	2
BDE-154	$[M-2Br]^+$ (481.6975)		3	3

[a] BDE-47 was the predominant congener in carp and largemouth bass, and thus assigned as 100% (See the text for specific BDE-47 concentrations.). BDE-181 and BDE-183 were not detected.

Table 2. The % concentration amount of MeO-PBDE (neutral) and HO-PBDE (HPC) congeners identified in the blood plasma of selected fish species collected from the Detroit River in 2001.

Congener	HRMS Quant. Ion	% Amount			
		Common Carp		Largemouth Bass	
		Neutral	HPC	neutral	HPC
2'-MeO-BDE68	$[M]^+$ (515.7217)	n.d.	4	n.d.	6
6-MeO-BDE47 [a]	$[M]^+$ (515.7217)	n.d.	100	70	100
3-MeO-BDE47	$[M]^+$ (515.7217)	n.d.	n.d.	n.d.	4
5-MeO-BDE47	$[M]^+$ (515.7217)	n.d.	n.d.	n.d.	8
4'-MeO-BDE49 [b]	$[M]^+$ (515.7217)	n.d.	6	100	25
4-MeO-BDE42	$[M]^+$ (515.7217)	n.d.	n.d.	31	15
6-MeO-BDE90	$[M]^+$ (593.6323)	n.d.	n.d.	n.d.	6
6-MeO-BDE99	$[M]^+$ (593.6323)	n.d.	n.d.	n.d.	7
2-MeO-BDE123	$[M]^+$ (593.6323)	n.d.	n.d.	n.d.	6
6-MeO-BDE85	$[M]^+$ (593.6323)	n.d.	n.d.	16	17

[a] 6-HO-BDE47 (derviatized to 6-MeO-BDE47 like all HO-PBDEs in the HPC fraction) is assigned as 100%, and [b] 4'-MeO-BDE49 (neutral fraction) is assigned as 100% (See text for concentrations.).

To our knowledge, this is the first report of HO-PBDE and MeO-PBDE contaminant residues in a marine or freshwater fish species from a North American ecosystem. HO-PBDEs appear to be formed metabolically but accumulation may be possible, and are in the pg/g w.w. range although lower than PBDE concentrations. However, the ratio of apparent HO-BDE metabolites of BDE-47 to BDE-47 concentrations, are similar to Σ-HO-PCB to Σ-PCB concentration ratios of 0.06 and 0.09 in the same carp and largemouth bass plasma, respectively [12]. Levels of PBDEs are increasing in Great Lakes biota [1,3], and endocrine activity and other potential toxicities have been shown for HO-PBDEs [6]. Therefore, exposure to circulating and bioavailable HO-PBDEs is a potential health concern in Detroit River fish and perhaps fish throughout the Great Lakes in areas of high PBDE loading.

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