NEW CASE EXAMPLES OF BIOTRANSFORMATION AND ELIMINATION OF POLYBROMINATED DIPHENYL ETHERS IN AQUATIC AND MARINE WILDLIFE

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Introduction

Polybrominated diphenyl ethers (PBDEs) have rapidly become an important class of persistent and bioaccumulating brominated flame retardants (BFRs) in the environment, and have received considerable research and regulatory attention [1]. Congener profiles of PBDE residues in the tissues aquatic and marine wildlife are composed to varying degrees of, e.g., 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), 2,2',3,4,4',5',6-heptabromoDE (BDE-183) and decabromoDE (BDE-209). Despite the considerable number of published studies on PBDEs in the environment in recent years, much less information is available on biotransformation and elimination, and general (pharmaco)toxicokinetics of these contaminants in aquatic and marine wildlife [2].

Recent studies on PBDE elimination, biotransformation and metabolite formation in wildlife have focused on fish species, and have illustrated that metabolism can occur via oxidative and debromination pathways [2]. The formation of hydroxylated (HO) PBDEs from exposure to PBDEs via cytochrome P450-mediated metabolism has been demonstrated, although full understanding of the sources, fate and bioaccumulative potential of these metabolites remain unclear for many species. In northern pike (*Esox lucius*) dosed with ^{14}C-labeled BDE-47, six HO-PBDE metabolites were detected in several tissues including blood [3]. Blood plasma of Swedish Atlantic salmon (*Salmo salar*), herring (*Clupea harengus*) and commercial fish oils were also found to contain HO-PBDEs [4]. More recently the dietary uptake and elimination of PBDE congeners, including BDE-209, has been reported in rainbow trout (*Oncorhynchus mykiss*) [5], lake trout (***Salvelinus namaycush***) [6] and common carp (*Cyprinus carpio*) [7,8,9].

The results of several case studies are presented illustrating new evidence for PBDE elimination and metabolism, and/or HO-PBDE metabolite formation in species, taxa and populations of aquatic and marine wildlife.

Materials and Methods

FISH CASE STUDY #1: The uptake, elimination and metabolism of PBDE congeners in captive treatment studies with common carp [7,8,9], and PBDE and HO-PBDE congener assessment in the plasma for common carp from the Detroit River [10].

AQUATIC FOOD WEB CASE STUDY #2: The bioaccumulation and dynamics of PBDEs and HO-PBDEs in a aquatic food web from Lake Huron (Great Lakes), burbot (*Lota lota*) - deepwater sculpin (*Myoxocephalus thompsoni*).

MARINE MAMMAL CASE STUDY #2: PBDEs, HO-PBDEs and xenobiotic-metabolizing enzymes in the liver of beluga whales (*Delphinapterus leucas*) from St. Lawrence River and Canadian arctic populations [11].

AVIAN CASE STUDY #3: PBDEs, HO-PBDEs and other BFRs in the eggs and plasma of glaucous gull (*Larus hyperboreus*) from Svalbard, Norway [12].

The methodology for PBDE and HO-PBDE determination in whole body fish homogenate, fish plasma, avian plasma and egg, and beluga liver has been described elsewhere [7,8,9,10,12]. Briefly, samples were spiked with the internal standards BDE-30 or BDE-71, and 4'-HO-BDE17 and 4-HO-CB72. After applicable extraction and/or lipoprotein rupturing in the case of plasma, phenolic and neutral fractions were obtained via liquid-liquid partitioning where the HO-PBDEs were deprotonated and isolated in an aqueous fraction, and subsequently protonated and re-extracted into an organic phase. The isolated phenolic fraction was subjected to diazomethane to derivatize the HO-PBDEs into methoxy (MeO) PBDE analogues. The neutral fractions and isolation of the PBDEs was accomplished using Florisil adsorption chromatography as described previously. Table 1 lists the PBDE and HO-PBDE congeners assessed in the samples, and for which authentic standards were available. Analysis of PBDEs and MeO-PBDE derivatives was accomplished using GC-electron capture negative ionization (ECNI)-MS in the selected ion mode (SIM) using the ^{79}Br and ^{81}Br isotope ions. Using an internal standard approach, the relative response factors of nine PBDE and ten MeO-PBDE congeners (Table 1) were used to quantify the analytes. Structural confirmations of congeners were also carried out in some cases by GC-ECNI)-MS fullscan (m/z 50 to 550 amu), or by GC-high resolution-mass spectrometry in the electron impact mode (HRMS-EI) (resolution = 10,000), and in SIM using the $[M+2]^+$ or $[(M+2)-2Br]^+$ ions, which reflect the ^{81}Br and ^{79}Br isotopic contributions. Recovery efficiencies were generally >85% for the PBDEs, and >75% for HO-PBDEs from the different matrices. We thank M. Alaee (Environment Canada) for the carrying out GC-(EI)HRMS analysis. We thank Drs G. March and Å. Bergman (Stockholm University, Sweden) for their generous supply of MeO-PBDE standards.

Results and Discussion

Representative results from case study #1 for common carp are presently discussed in some detail. The concentration of BDE-47 was 1.8 ng/g (wet weight) in the plasma of carp

from the Detroit River. BDE-47 accounted for about 55% of the total (Σ) PBDEs relative to < 10% for other congeners. HO-PBDEs were identified in the plasma of carp, and dominant congener was 6-HO-BDE-47 at 42.3 pg/g (w.w.) (Figure 1).

Table 1. PBDE and HO-PBDE congeners determined in the tissues for species of the four case studies.

PBDE Congeners	HO-PBDE Congeners
BDE-28	2'-HO-BDE-68
BDE-47	6-HO-BDE-47
BDE-99	3-HO-BDE-47
BDE-100	5-HO-BDE-47
BDE-138	4'-HO-BDE-49
BDE-153	4-HO-BDE-42
BDE-154	6-HO-BDE-90
BDE-183	6-HO-BDE-99
BDE-209	2-HO-BDE-123
	6-HO-BDE-85

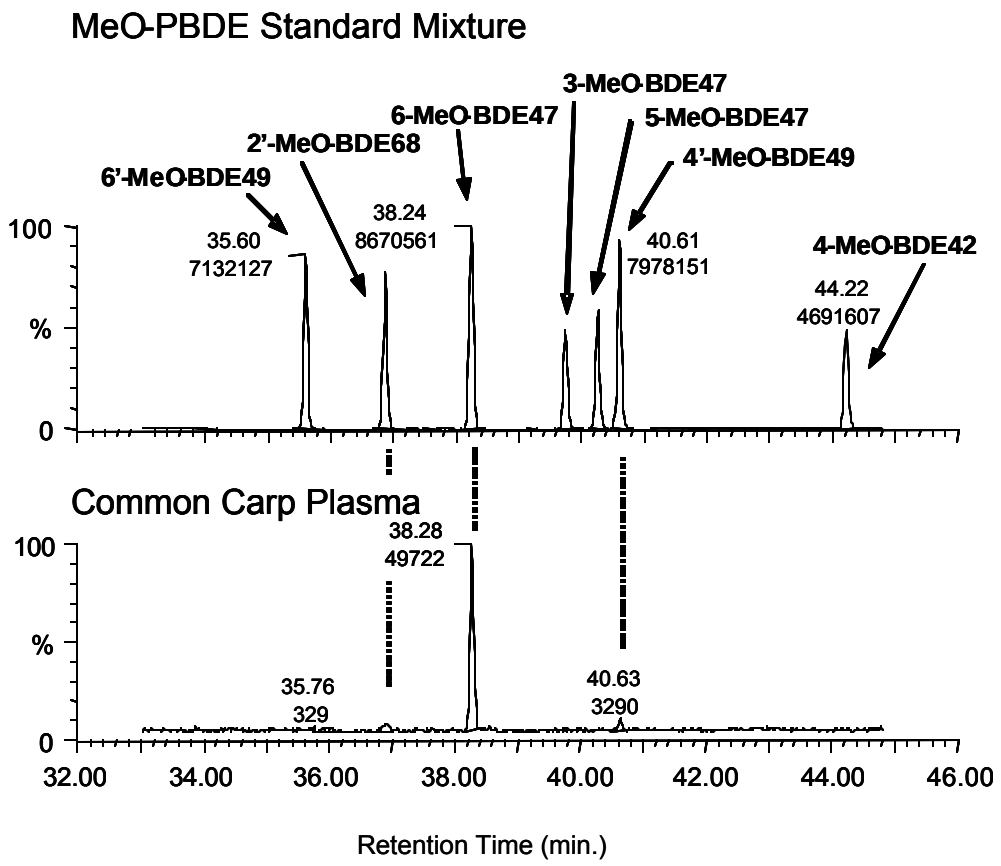

Figure 1. GC-HRMS(EI$^+$) mass chromatograms of tetrabrominated MeO-PBDE standards, and HO-PBDEs (derivatized to MeO-PBDEs) in phenolic fractions from the plasma from common carp based on the $[M]^+$ at m/z 515.7217.

The 6-HO-BDE-47 concentration was 2.3% of the BDE-47 concentration. Carp from the Detroit River are therefore apparently capable of metabolizing BDE-47 to HO-PBDEs. In studies on juvenile carp exposed to diets containing individual and mixtures of BDE congeners, elimination profiles of specific congeners showed the debromination of higher brominated BDEs to lower brominated BDEs, e.g., BDE-99 to BDE-47, BDE-183 to BDE-154, and BDE-209 to pentabrominated to octabrominated BDE metabolites [7,8,9]. However, unlike in the wild individuals, HO-PBDEs could not be detected in tissues (e.g., plasma) from carp in the captive treatment studies. HO-PBDEs were detected in both the plasma and eggs of glaucous gull from Svalbard [12], as well as in the liver of burbot and deepwater sculpin from the Great Lake, Lake Huron, and liver of beluga whale [11].

Conclusions

PBDE metabolism is a species-specific phenomenon and influenced by a complex number of biological and chemical factors. The present results demonstrate that PBDE metabolism/elimination occurs in various species from differing populations and taxonomy. Depending on the species and the congeners in question, mammalian, avian and fish species possess capacities or potential to metabolize PBDE congeners via a debromination or by oxidative pathways that can lead to the formation of HO-PBDE residues. HO-PBDEs of BDE-47 appear to be most common metabolite residues. The presence of HO-PBDE residues is also a likely a factor in PBDE-mediated toxicities in organisms in which they can be formed. More research is clearly necessary the pharmaco- and toxico-kinetics (metabolism) of PBDEs in wildlife.

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