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Organohalogen Compounds and Their Metabolites in the Blood of Japanese Amberjack and Scalloped Hammerhead Shark from Japanese Coastal Waters

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Abstract—In the present study, we determined the residue levels and patterns of PCBs, OH-PCBs, PBDEs, OH-PBDEs and methoxylated PBDEs (MeO-PBDEs) in the blood collected from scalloped hammerhead shark (HS) (*Sphyrna lewini*) and Japanese amberjack (JA) (*Seriola quinqueradiata*) at Japanese coastal waters. The predominant OH-PCB congeners were lower-chlorinated OH-PCBs such as 6OH-CB2 and 2′OH-CB9 in JA. Exposure of JA to lower-chlorinated OH-PCBs might be from the ambient aquatic environment. In scalloped HS, 4,4′diOH-CB202, 4′OH-CB201 and 4OH-CB146 were the predominant congeners accounting for approximately 60% of the total OH-PCBs.

The predominant MeO-PBDE congeners were 6MeO-BDE47 followed by 2′MeO-BDE68 in both species. As for OH-PBDE congeners, 6OH-BDE47 was predominant followed by 2′OH-BDE68 in JA and HS. Residue levels of ∑MeO-PBDEs and ∑OH-PBDEs showed a significant positive correlation ($p = 0.029$, Spearman's rank correlation coefficients). This result suggests that MeO-PBDEs and OH-PBDEs share a common source or a metabolic pathway in fishes. Characteristic differences found in the profiles of OH-PCBs and OH-PBDEs in JA and HS show the need for further studies on the differences in exposure profiles, metabolic capacities and toxic effects in fish.

Keywords: OH-PCBs, OH-PBDEs, Japanese amberjack, scalloped hammerhead shark

INTRODUCTION

Several polychlorinated biphenyl (PCB) congeners are known to affect endocrine systems and neurodevelopment in humans and wildlife (Danse *et al*., 1997). It has been reported that PCBs disrupt thyroid hormone (TH) homeostasis in animals.

A possible mechanism involved in the disruption of TH homeostasis is the competitive binding of PCBs with TH transport protein, transthyretin (TTR), in blood (Ucán-Marin *et al.*, 2010). It has been demonstrated that the binding affinity to TTR was much stronger for hydroxylated PCBs (OH-PCBs), which are formed by oxidative metabolism of PCBs by cytochrome P450 (CYP) monooxygenases enzyme system, than for the parent PCBs, due to the structural similarity of OH-PCBs to thyroxine (T4).

Thyroid dysfunction (large goiters and thyroid hyperplasia) has been reported in salmonids for more than 30 years in the Great Lakes (Leatherland, 1998). It has been suggested that some thyroidogenic contaminants such as OH-PCBs might be one of the causes of thyroid dysfunction in fish (Campbell *et al.*, 2003). Buckman *et al.* (2007) showed that rainbow trout exposed to a mixture of PCBs bioformed OH-PCBs. Moreover, the influence of CYP induction on the rates of biotransformation of PCBs mixtures to OH-PCB isomers and homologue group was assessed (Buckman *et al.*, 2007). These results indicates that biotransformation of PCBs is the likely source for OH-PCBs in wild fish. However, there have been only few studies on the patterns and levels of OH-PCBs in marine fish species.

Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants (BFRs) widely used in many consumer products (Hites, 2004). Detection of hydroxylated PBDEs (OH-PBDEs) in wildlife (Valters *et al.*, 2005; Gebbink *et al.*, 2008; Houde *et al.*, 2009) suggests the formation of these compounds in some terrestrial mammalian species livers upon exposure to PBDEs (Qiu *et al.*, 2007). It has been demonstrated that the binding affinity to TTR of some OH-PBDEs congeners was much stronger than that of T4 (Ucán-Marin *et al.*, 2010). On the other hand, occurrence of higher concentrations of OH-PBDEs and methoxylated (MeO-) PBDEs than those of PBDEs in marine organisms has led to the finding that these compounds may be formed naturally by marine algae or cyanobacteria (Malmvärn *et al.*, 2008).

Demethylation of MeO-PBDEs by cytochrome P450 (CYP) can result in the formation of OH-PBDEs rather than the metabolism of parent PBDEs in some species, the possibility that some OH-PBDEs are formed with the demethylation of MeO-PBDEs was shown (Wan *et al.*, 2009). These results brought a considerable interest about the origin of OH-PBDEs and MeO-PBDEs in biota.

The present study elucidated the residue levels and patterns of mono- through nona-chlorinated homologues of OH-PCB congeners in the blood from scalloped hammerhead shark (HS) (*Sphyrna lewini*) and Japanese amberjack (JA) (*Seriola quinqueradiata*), two predatory species of fish. Moreover, residue levels and patterns of OH-PBDEs and MeO-PBDEs in the blood were also investigated.

MATERIALS AND METHODS

Collection of blood from Japanese amberjacks and scalloped hammerhead sharks

The whole blood samples were collected from HS (male: $n = 4$, female: $n = 3$; whole length: 1260–1930 mm) and JA (male: $n = 5$, female: $n = 10$; whole

length: 790–910 mm) at the Japanese coastal waters in 2008 and 2010. Blood were collected from fresh fish by cardiac puncture or from caudal vein. All the blood samples were collected in falcon polypropylene conical tube and stored in the Environmental Specimen Bank (*es*-BANK: http://esbank-ehime.com/) of Ehime University, Japan, at –25°C until they were used for analysis.

The authentic reference standards of 101 mono-OH-PCB congeners (mono- through nona-, methoxylated derivatives: MeO-PCBs) and 4,4′diOH-CB202 (4,4′dihydroxylated-CB202) are used for identification and quantification (Nomiyama *et al*., 2010a, b). The authentic reference standards of 20 OH-PBDEs (tri-through penta-methoxylated derivatives: MeO-PBDEs) used for identification and quantification were obtained from Cambridge Isotope Laboratories, Inc., (Andover, MA, USA), AccuStandard, Inc., and Wellington Laboratories, Inc. The extraction procedure of PCBs, OH-PCBs, PBDEs, MeO-PBDEs and OH-PBDEs in the blood was performed following the procedures described by Nomiyama *et al*. (2011a, b).

RESULTS AND DISCUSSION

Concentrations of PCBs and OH-PCBs

Figure 1A shows the concentrations of OH-PCBs and total PCBs in the blood of JA and HS. The mean concentrations (pg g^{-1} whole blood wet wt. base ± standard deviation: SD) of ∑PCBs in blood of JA were 2200 ± 1300 pg g^{-1} in female and 1800 ± 1300 pg g^{-1} in male. The levels in HS were 2700 ± 1700 pg g^{-1} in female and 4400 ± 4400 pg g^{-1} in male. Among the PCB congeners, CB153, CB101, CB99, and CB138 were predominant in the blood of JA. In the blood of HS, CB153 and CB138 were the predominant congeners.

In this study, 10 OH-PCB congeners were identified in the blood of JA, and 22 OH-PCB congeners were identified in HS. The mean concentrations of identified ∑OH-PCBs in the blood of JA were 85 ± 56 pg g^{-1} in female and 100 ± 77 pg g^{-1} in male. HS were 440 ± 210 pg g^{-1} in female and 300 ± 67 pg g^{-1} in male. OH-PCBs in HS were significantly higher than the concentrations found in JA ($p < 0.05$, Mann-Whitney *U*-test).

Among the OH-PCB congeners, lower-chlorinated OH-PCBs such as 6OH-CB2 and 2′OH-CB9 were predominant in the blood of JA. In contrast, higher-chlorinated OH-PCBs such as 4,4′diOH-CB202, 4′OH-CB201, 3′OH-CB182/ 3′OH-CB183 and 4OH-CB146 were the predominant congeners in HS. Ueno *et al*. (2007) reported that the lower-chlorinated OH-PCB congeners (1-3Cl) including 6OH-CB2 and 2′OH-CB9 were predominant in the surface water from sites in Lake Ontario, Canada. This result suggests that exposure of JA to lower-chlorinated OH-PCBs might be from the ambient aquatic environment, which indicates that JA do not possess any exceptional metabolic capacity for OH-PCB formation. The homolog patterns of OH-PCB congeners in JA and HS are shown in Fig. 2. The predominant homologues in JA were OH-di-PCBs and OH-mono-PCBs, and in HS were OH-octa-PCBs. The pattern of OH-PCB congeners found in the blood of HS was apparently different from that of JA. These observations

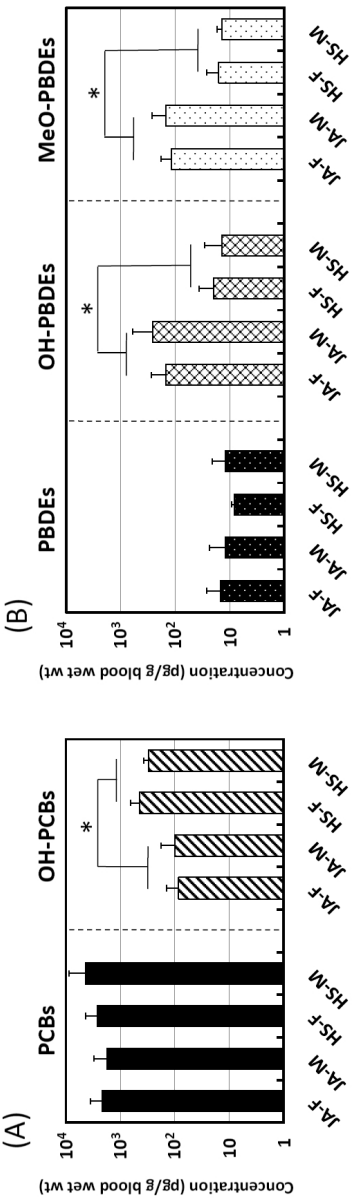

Fig. 1. Mean concentrations (pg/g blood wet wt) of (A) PCBs and OH-PCBs, (B) PBDEs, OH-PBDEs and MeO-PBDEs in the blood of Japanese amberjack and scalloped hammerhead shark. JA-F: Japanese amberjack female (n = 10), JA-M: Japanese amberjack male (n = 5), HS-F: hammerhead shark female (n = 3), HS-M: hammerhead shark male (n = 4). Error bars represent the standard deviation of the mean. The asterisk symbol (*) denotes significant differences ($p < 0.05$, Mann-Whitney U-test).

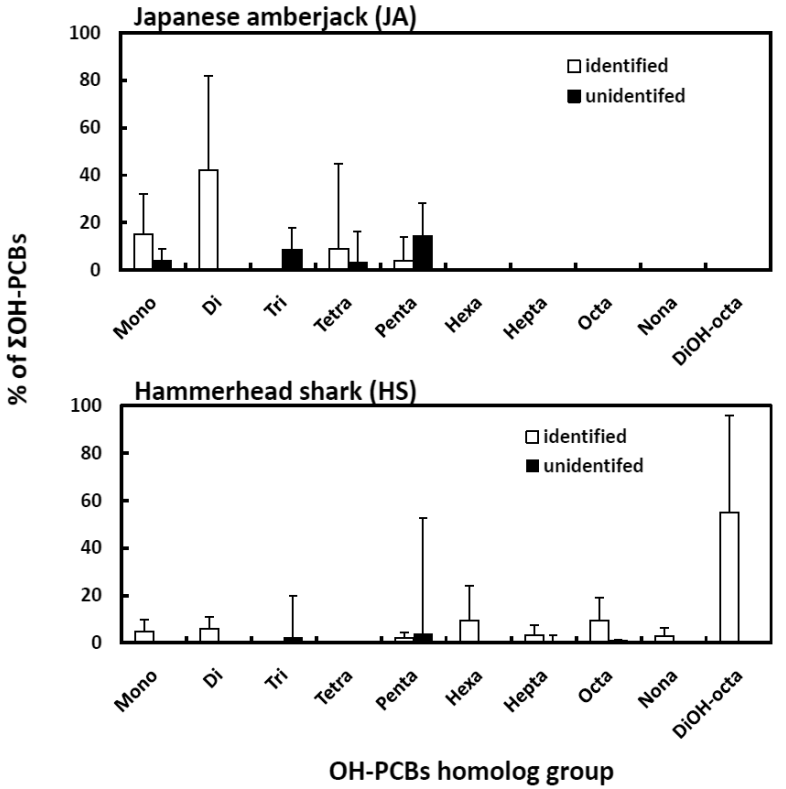

Fig. 2. The percentage of identified and unidentified OH-PCB congeners comprising the total OH-PCBs concentrations by homolog groups in the blood of Japanese amberjacks and scalloped hammerhead sharks at the Japanese coastal waters.

may indicate specific metabolic capacity (specific to higher-chlorinated PCBs) and/or exposure of HS to OH-octa-PCBs through the food-chain. Indeed, residues of higher-chlorinated OH-PCB congners (7-8Cl) were reported in the blood plasma of the Lake trout (*Salvelinus namaycush*) from the Great Lakes (Campbell *et al*., 2003). Likewise, octa- and nona-chlorinated OH-PCB congners have been exclusively detected in benthic-feeding white sucker (*Catostomus commersoni*) and common carp (*Cyprinus carpio*) from the Detroit River (Li *et al*., 2003). The specific fish prey of HS may have higher levels of OH-octa-PCBs. However, OH-octa-PCBs can also be formed from metabolism of PCBs, and 4OH-CB202 has been reported to be bioformed by rainbow trout exposed to a mixture of PCBs (including CB202) resulted in bioformed OH-PCB (including 4OH-CB202) congeners through the catalytic activity of CYP1B and/or CYP2B enzymes (Buckman *et al*., 2007). 4,4′diOH-CB202 has been found at fairly high

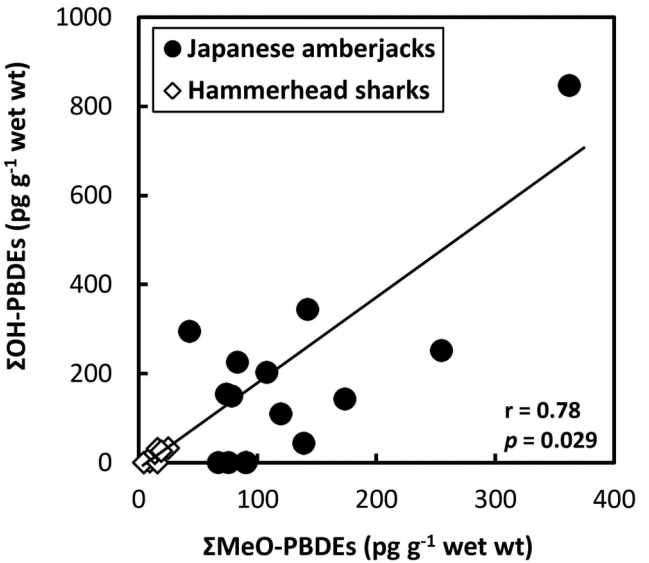

Fig. 3. Relationship the concentrations (pg/g blood wet wt) of ∑MeO-PBDEs and ∑OH-PBDEs (r = 0.78, p = 0.029) in the blood of Japanese amberjacks and scalloped hammerhead sharks. p value was calculated using Spearman's rank correlation.

concentrations in polar bears, which could have resulted by the efficient metabolism of PCBs (Gebbink *et al.*, 2008).

Accumulation features of PBDEs, MeO-PBDEs and OH-PBDEs

Figure 1B shows the concentrations of PBDEs, MeO-PBDEs and OH-PBDEs in the blood of JA and HS. The mean concentrations of ∑PBDEs in the blood of JA were 15 ± 12 pg g^{-1} in female and 12 ± 12 pg g^{-1} in male. The levels in HS were below the 8.3 ± 1.1 pg g^{-1} in female and 12 ± 8.8 pg g^{-1} in male.

MeO-PBDEs were detected in the blood of all JA and HS analyzed in this study. Of the 20 MeO-PBDEs (tri- to penta-) congeners monitored, tri- and penta-brominated MeO-BDEs were lower than the LOQ in both species. This result was in agreement with various recent studies on MeO-PBDEs in marine fish, which indicated that MeO-tetra-BDEs were present at much higher levels than the tri- or penta-BDEs (Covaci *et al.*, 2008). The mean concentrations (pg g^{-1} wet wt. ± SD) of ∑MeO-PBDEs in the blood of JA were 120 ± 62 pg g^{-1} in female and 150 ± 120 pg g^{-1} in male, and HS were 16 ± 11 pg g^{-1} in female and 14 ± 3.0 pg g^{-1} in male. The MeO-PBDEs levels of JA were significantly higher than that of HS ($p < 0.05$, Mann-Whitney U-test). These observations may indicate specific metabolic capacity (strong metabolic capacity of HS for MeO-PBDEs) and/or high exposure of JA to MeO-PBDEs through the food-chain.

6MeO-BDE47 was the predominant isomer, followed by 2′MeO-BDE68 and 6′MeO-BDE49. The 6MeO-BDE47 and 2′MeO-BDE68 have been reported as natural products (Malmvärn *et al*., 2008). These two abundant congeners accounted to mean 85% (6MeO-BDE47) and 15% (2′MeO-BDE68) of the ∑MeO-PBDEs concentrations in both species.

Among OH-PBDEs, only two congeners (2′OH-BDE68 and 6OH-BDE47) could be consistently measured above the LOQ in both species. The mean concentrations of ∑OH-PBDEs in the blood of JA were 150 ± 130 pg g^{-1} in female and 260 ± 340 pg g^{-1} in male, and the levels in HS were lower than the 20 ± 17 pg g^{-1} in female and 14 ± 15 pg g^{-1} in male. 6OH-BDE47 and 2′OH-BDE68 have already been reported as natural products present in red algae and cyanobacteria (Malmvärn *et al*., 2008). These two congeners accounted for about 66% (6OH-BDE47) and 33% (2′OH-BDE68) of ∑OH-PBDEs.

Correlations between MeO-PBDEs and OH-PBDEs

Positive correlations between MeO-PBDEs, OH-PBDEs are indicative of sources of OH-PBDEs, but not definitive. Figure 3 shows correlation between ∑MeO-PBDEs and ∑OH-PBDEs in the blood of JA and HS. ∑MeO-PBDEs and ∑OH-PBDEs showed significant positive correlation (Spearman's rank correlation coefficient: $r = 0.78$, $p = 0.029$, Spearman's rank correlation coefficients). However, ∑PBDEs and ∑OH-PBDEs showed no significant correlations ($p > 0.05$; data not shown). This result suggests that MeO-PBDEs and OH-PBDEs originate from a common source (i.e., biosynthesis by marine organisms), or metabolic pathway (i.e., interconversion of OH- and MeO-PBDEs) in JA and HS. This is consistent with results from the previous *in vitro* study; further supporting the hypothesis that 6MeO-BDE47 is a contributor to the formation of 6OH-BDE47 (Wan *et al*., 2009). The precise origin of OH-PBDEs in the blood of fish cannot be determined because there may be a contribution from MeO-PBDE metabolism as well as from natural accumulation of OH-PBDEs, in this study. However, considering the fact that 6OH-BDE47 and 6MeO-BDE47 are present in the marine environment and these marine fish, a large proportion of 6OH-BDE47 might have been accumulated directly from natural sources.

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