ACCUMULATION FEATURES OF HALOGENATED PHENOLIC COMPOUNDS IN THE BLOOD OF PINNIPEDS FROM JAPANESE COASTAL WATERS

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Introduction

Several polychlorinated biphenyl (PCB) congeners are known to affect endocrine systems and neurodevelopment in humans and wildlife. 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 [1-2)]. 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 systems than for the parent PCBs, due to the structural similarity of OH-PCBs to thyroxine (T4) [1)]. TH plays critical roles in the development of central nervous system and brain function [3)].

Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants (BFRs) widely used in many consumer products. Detection of hydroxylated PBDEs (OH-PBDEs) in wildlife tissues [4-5)] and human blood [6)] indicates the formation of OH-PBDEs in animal tissues upon exposure to PBDEs [7-8)]. In particular, it has been demonstrated that the binding affinity to TTR of some OH-PBDEs isomers was much stronger than that of T4 [9)]. On the other hand, occurrence of higher concentrations of OH-PBDEs and methoxylated (MeO-) PBDEs than parent PBDEs in marine organisms has led to the finding that these compounds may be formed naturally by marine algae or cyanobacteria [10-11)]. There is considerable interest in the origin of OH-PBDEs in biota and abiotic environmental matrices.

MeO-PBDEs have also been found in various animals at concentrations sometimes greater than those of PBDE congeners [12)]. Among these, two abundant isomers (6MeO-BDE47 and 2′MeO-BDE68) have been found to be of natural origin [13)]. Since demethylation of MeO-PBDEs by cytochrome P450 (CYP) might be a significant pathway for the formation of OH-PBDEs rather than the metabolism of parent PBDEs in the organism [12).] These results brought a considerable interest about the origin of OH-PBDEs and MeO-PBDEs in biota. However, information on the accumulation features of brominated and chlorinated phenolic compounds in the blood of pinnipeds from Japanese coastal waters is limited. The present study attempted to elucidate residue levels and patterns of PCBs, OH-PCBs, PBDEs, OH-PBDEs, MeO-PBDEs and bromophenols (BPhs) in the blood of pinnipeds along the Japanese coastal waters.

Materials and methods

The whole blood samples were collected from four species of pinnipeds (*n* = 38) including northern fur seal (*Callorhinus ursinus*) (*n* = 10: male = 3, female = 7), spotted seal (*Phoca largha*) (*n* = 8: male = 6, female = 2), Steller sea lion (*Eumetopias jubatus*) (*n* = 18: male = 6, female = 12) and ribbon seal (*Phoca fasciata*) (*n* = 2: male = 1, female = 1), and comparison to related studies on cetaceans (harbour porpoise and Dall's porpoise) [14)] from northern Japanese coastal waters (Fig. 1). All the blood samples were collected in falcon polypropylene conical tubes, stored in the Environmental Specimen Bank (*es*-BANK) of Ehime University, Japan, at -25 °C and were used for analysis.

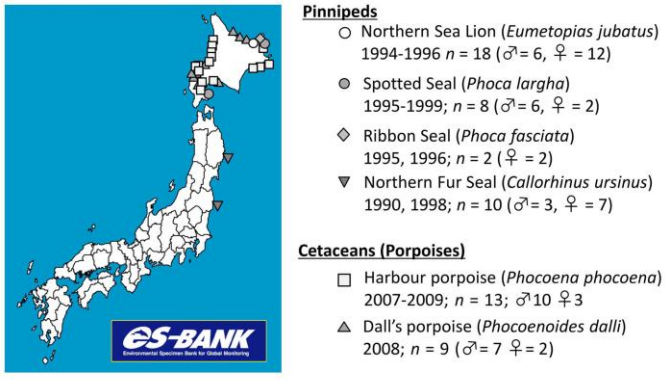

Fig. 1. Sampling locations of four species of pinnipeds, and comparison to related studies on cetaceans (harbour porpoise and Dall's porpoise) [14)] from northern Japanese coastal waters

Table 1. Median concentrations (min-max) of halogenated phenolic compounds and CB153 ratios in the whole blood from pinnipeds, and comparison to related studies on cetaceans from northern Japanese coastal waters

	Pinnipeds (pg/g whole blood wet wt)				Cetaceans (pg/g whole blood wet wt)[1]	
Species	Steller Sea Lion	Ribbon Seal	Spotted Seal	Northern Fur Seal	harbour porpoises	Dall's porpoises
Food Habit	Fish, Cephalopods	Fish, Cephalopods, krill	Fish, Cephalopods	Fish, Cephalopods	Fish, Cephalopods	Fish, Cephalopods
n (Sex)	10 (♂: 6, ♀: 4)	2 (♀: 2)	8 (♂: 6, ♀: 2)	10 (♂: 3, ♀: 7)	13 (♂: 10, ♀: 3)	17 (♂: 12, ♀: 5)
ΣPCBs	<100 (<100-7400)	<100	1800 (400-17000)	<100 (<100-7400)	6200 (800-69000)	37000 (15000-110000)
ΣOH-PCBs	11 (<0.6-46)	160 (69-250)	190 (71-2200)	43 (20-140)	8.3 (5.4-46)	58 (2.9-390)
ΣPBDEs	<100 (<100-500)	<100	<100 (<100-500)	<100 (<100-130)	200 (27-830)	230 (<100-3700)
ΣOH-PBDEs	7.6 (<0.6-38)	3.9 (2.4-5.4)	14 (8.2-30)	24 (6.2-39)	190 (100-580)	300 (22-1400)
ΣMeO-PBDEs	3.4 (<0.6-11)	13	13 (0.8-76)	30 (<0.6-150)	620 (55-1400)	1200 (1600-2700)
ΣBPhs	51 (10-690)	330 (300-360)	580 (380-800)	460 (140-1600)	110 (<0.6-270)	30 (<0.6-530)
ΣPCBs/CB153	2.34	NA[2]	2.38	2.49	3.84	5.02
ΣOH-PCBs/CB153	0.02	NA	0.26	0.09	0.07	0.02
ΣPBDEs/CB153	<0.05	NA	0.08	<0.05	0.67	1.35
ΣOH-PBDEs/CB153	0.02	NA	0.01	0.04	0.79	0.56
ΣMeO-PBDEs/CB153	0.008	NA	0.02	0.08	2.18	1.65

1. Cetacean data: Ochiai et al., 2011, Organohalogen compounds [14].
2. 2. CB153 of Ribbon seals was not quantified (<LOQ).

Organobrominated and organochlorinated compounds were extracted from each blood sample (5-10 g) with 50% methyl *t*-butyl ether (MTBE)/hexane. The organic phase was partitioned into neutral and hydroxylated compound fractions by 1 M potassium hydroxide (KOH) in 50% ethanol/water. The organic phase (containing PCBs, PBDEs, MeO-PBDEs) was passed through the gel permeation chromatography (GPC) and activated silica-gel column chromatography. PCBs and PBDEs were concentrated for GC/MS analysis. The alkaline phase (containing OH-PCBs, OH-PBDEs and BPhs) was acidified with sulfuric acid, and then the hydroxylated compounds were re-extracted with MTBE/hexane. The organic phases were passed through non-activated silica-gel column chromatography. OH-PCBs, OH-PBDEs, MeO-PBDEs and BPhs were derivatized using trimethylsilyldiazomethane. The derivatized solution was treated with activated silica-gel column chromatography. Identification and quantification of MeO-PCBs were performed using high-resolution GC/MS (JEOL JMS-800D, Japan).

Results and discussion

Residue levels of PCBs and OH-PCBs

High concentrations of PCBs were found in blood of spotted seals (1800 pg/g wet wt); these values were significantly higher than the concentrations found in Steller sea lions, and followed by northern fur seals and ribbon seals ($p < 0.05$) (Table 1). However, the PCB levels of pinnipeds in this study were lower than that of harbour seals (*Phoca vitulina*) living in the Norwegian waters (9300 pg/g wet wt) [15] and ringed seals (*Phoca hispida*) living in Svalbard coast (22000 pg/g wet wt) [16]. This result is probably reflecting the low contamination levels of PCBs around Hokkaido. These pinnipeds inhabit the coastal waters around Hokkaido where the human population density is the lowest among the Japanese prefectures (70.2 people/km^2, national census), and this may explain the lower human activities as well as lower levels of PCBs found in the pinnipeds. On the other hand, residue levels of PCBs in pinnipeds were 1–2 orders of magnitude lower than in the cetaceans, harbour porpoise (*Phocoena phocoena*) (6200 pg/g wet wt) and Dall's porpoise (*Phocoenoides dalli*) (37000 pg/g wet wt) inhabiting the same area with the pinnipeds in this study [14]. The dominant PCBs isomers identified in the blood of the sampled pinnipeds were CB153, followed by 138 118 and 99.

OH-PCBs were detected in the blood samples of all species analyzed in this study. High concentrations of OH-PCBs were found in the blood of spotted seals (180 pg/g wet wt), followed by ribbon seals (160 pg/g wet wt), northern fur seals (52 pg/g wet wt) and Steller sea lions (6.8 pg/g wet wt) (Table 1). The OH-PCBs levels of spotted seals and northern fur seals were significantly higher than Steller sea lions ($p < 0.05$). The OH-PCBs levels of pinnipeds in this study were lower than that of harbor seals living on the Norwegian coast (2400 pg/g ww) (Løken *et. al*., 2008) and ringed seals living in Svalbard waters (370 pg/g wet wt) (Routti *et. al*., 2008). On the other hand, residue levels of the OH-PCBs in pinnipeds were the same as those found in a harbour porpoise (8.3 pg/g wet wt) and Dall's porpoise (58 pg/g wet wt) [14].

Concentration ratios of OH-PCBs to PCBs (OH-PCBs/PCBs ratios) might show the alteration of metabolic capacity rates by a number of factors, which include exposure level of PCBs, induction of hepatic enzymes, and the TTR binding species-specificity of the blood. Total OH-PCB/PCBs ratios in pinnipeds were the same or higher than the cetaceans living in Japanese coastal waters [17]; however, the values were lower than those of terrestrial mammals [18] (Fig. 2). It is presumed that the metabolic capacity and/or binding affinity of OH-PCBs to TTR in pinnipeds may be higher than that of cetaceans.

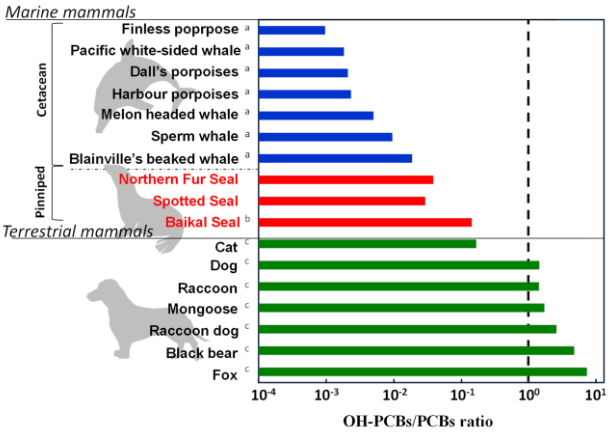

Fig. 2. Total OH-PCBs/PCBs ratios in the blood of marine and terrestrial mammals. a: Nomiyama et al., 2010 [17]; b: Imaeda et al., 2008 [21]; c: Mizukawa et al., 2011 [18].

OH-PCB isomer profiles

Among the OH-PCB isomers identified, 4OH-CB107 was predominant in the blood of ribbon seals and spotted seals (62% of the total OH-PCB concentrations). 4OH-CB107 was already detected as dominant isomer in seals [15-16], human [19] and other wildlife [16]. Furthermore, the predominant OH-PCBs isomers were 4′OH-CB101/120 (28%) in northern fur seals and 4OH-CB107 (24%) and 4OH-CB187 (20%) in Steller sea lions. These results reveal that the accumulation profiles of OH-PCB in pinnipeds blood are entirely different from the profiles found in other marine mammals; the cetacean species [17]. However, the predominant OH-PCB isomers detected in all pinnipeds species were similar in structure to T4 (T4-like OH-PCBs).

Residue levels of PBDEs, MeO-PBDEs, OH-PBDEs

Significantly lower concentrations of brominated compounds (PBDEs, MeO-PBDEs, OH-PBDEs) were found in the blood of pinnipeds than the levels found in two porpoise species living in the same area ($p < 0.05$) (Table 1). Residue levels of OH-PBDEs in the blood of Steller sea lions were significantly lower than northern fur seals and spotted seals ($p < 0.05$). Of the 28 OH-PBDE isomers analyzed, only two isomers (2′OH-BDE68 and 6OH-BDE47) could be consistently identified in all pinnipeds species. 6OH-BDE47 was detected in all samples. Similar profiles of OH-PBDEs have been reported previously in harbour porpoises (98%) and Dall's porpoises (96%) which are present in the same area with pinnipeds of this study [14]. 6OH-BDE47 has been reported as a marine natural products in red algae and fish [10-11]. Therefore, the origin of large percentage of 6OH-BDE47 detected in the blood of pinnipeds in this study might be from the natural products.

Although concentrations of PCBs and OH-PCBs found in pinnipeds of the present study were the same as in cetaceans living in the same coastal area, concentrations of brominated compounds (PBDEs, MeO-PBDEs, OH-PBDEs) found in pinnipeds of the present study were significantly lower than the levels in the cetaceans [16]. For comparison, we also calculated of ratios of brominated compounds to CB153 [20]. Concentration ratios of ΣPCBs/CB153 of Steller sea lions, spotted seals and northern fur seals (2.38-2.40) were almost the same as in the two porpoise species (3.84 and 5.02) (Table 1). Moreover, the ratio of ΣOH-PCBs/CB153 of Steller sea lions, spotted seals and northern fur seals (0.02-0.26) were also the same as in the two porpoise species (0.02 and 0.07). However, except for ribbon seals, ratios of ΣPBDEs/CB153 for pinnipeds were 2–3 orders lower than those of two porpoise species. Ratios of ΣOH-PBDEs/CB153 and ΣMeO-PBDEs/CB153 were also 2–3 orders lower than those of two porpoise species. This result suggests that metabolic and/or elimination capacity for organobromine compounds differ from organochlorine compounds.

In a recent study, the residue level of brominated 1′-Methyl-1,2′-bipyrroles (MBP) in pinnipeds was reported to differ significantly from toothed whales, but the residue levels of organochlorine compounds in pinnipeds were similar to those in toothed whales, even though both the groups eat similar diet, live in the same habitat, have similar blubber structure and thickness of blubber [20]. The authors indicated that this difference in accumulation pattern indicates that pinnipeds have an enhanced capability to degrade organobromine compounds relative to toothed whales. Since pinnipeds in this study are in the same trophic level as porpoise, it can be presumed that

pinnipeds accumulate less PBDEs, OH-PBDEs and MeOPBDEs or they might have a more enhanced capability to degrade those organobromine compounds than the two porpoise species.

Residue levels of BPhs

BPhs were found in all samples. High concentrations of BPhs were found in the blood of spotted seals (580 pg/g ww) followed by northern fur seals (460 pg/g ww), ribbon seal (330 pg/g ww) and Steller sea lion (51 pg/g ww) (Table 1). Of the 10 BPhs isomers measured, only two (2,4,6-BPh and penta-BPhs) could be identified in pinnipeds. 2,4,6-BPh was the dominant isomer in pinnipeds (spotted seal and ribbon seal: 99%, northern fur seal and Steller sea lion: 100%). Recent investigations reported that the concentrations of 2,4,6-BPh and 6OH-BDE47 showed significant positive correlations in cetaceans, which indicated that they share a common source or metabolic pathways [5)]. In this study, the concentrations of 2,4,6-BPhs and 6OH-BDE47 showed significant positive correlations in the blood of ringed seals. Since 6OH-BDE47 detected in this study was reported to be a natural product, the origin of large percentage of 2,4,6-BPh in pinnipeds suggest that this compund occurs in marine environment.

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