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Accumulation Features of Halogenated Phenolic Compounds in the Blood of Pinnipeds from Japanese Coastal Waters

Chika KANBARA[1], Kei NOMIYAMA[1], Hazuki MIZUKAWA[1], Akifumi EGUCHI[1], Tomohiko ISOBE[2], Tadasu K. YAMADA[3] and Shinsuke TANABE[1]

[1]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[2]*Senior Research Fellow Center, Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[3]*Department of Zoology, National Museum of Nature and Science, 3-23-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan*

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Abstract—The present study determined the residue levels and characteristic patterns of polychlorinated biphenyls (PCBs), hydroxylated PCBs (OH-PCBs), polybrominated diphenyl ethers (PBDEs), hydroxylated PBDEs (OH-PBDEs), methoxylated PBDEs (MeO-PBDEs) and bromophenols (BPhs) in the blood of pinnipeds collected in 1990–1999 from Japanese coastal waters. Except in ribbon seal, PCBs and OH-PCBs were detected in all species. Concentration ratios of OH-PCBs/PCBs in pinnipeds were the same or higher than cetaceans living in Japanese coastal waters, suggesting that the metabolic capacity of pinnipeds for organochlorines might be higher than cetaceans. In contrast, except for ribbon seal, PBDEs and MeO-PBDEs were detected in all species at extremely small amounts. Even though OH-PBDEs were detected in all species, concentrations of these compounds found in pinnipeds were significantly lower than in cetaceans. This result suggests that pinnipeds may have an enhanced ability to metabolize these natural products. BPhs were found in all species. However, BPhs concentrations in pinnipeds were higher than in cetacean species, and BPhs accumulation characteristics in pinnipeds were different from other brominated compounds and polychlorinated biphenyls.

Keywords: organobromines, organochlorines, metabolites, blood, pinnipeds

INTRODUCTION

Polychlorinated biphenyls (PCBs) congeners and their biological metabolites are known to affect endocrine systems in humans and wildlife (Safe, 1994). Despite their ban, PCBs persist widely in wildlife and human because of their lipophilic properties, low water solubilities, and strong tendencies to accumulate in higher organisms. Accumulated PCBs are known to have numerous adverse health effects including embryotoxicity, oncogenicity and endocrine disruption (Soto *et al*., 1995; Danse *et al*., 1997). In epidemiological and experimental studies, it has

already been shown that PCBs disrupt thyroid hormone (TH) homeostasis and the cerebral nervous system in human and rodents (Brouwer *et al.*, 1990).

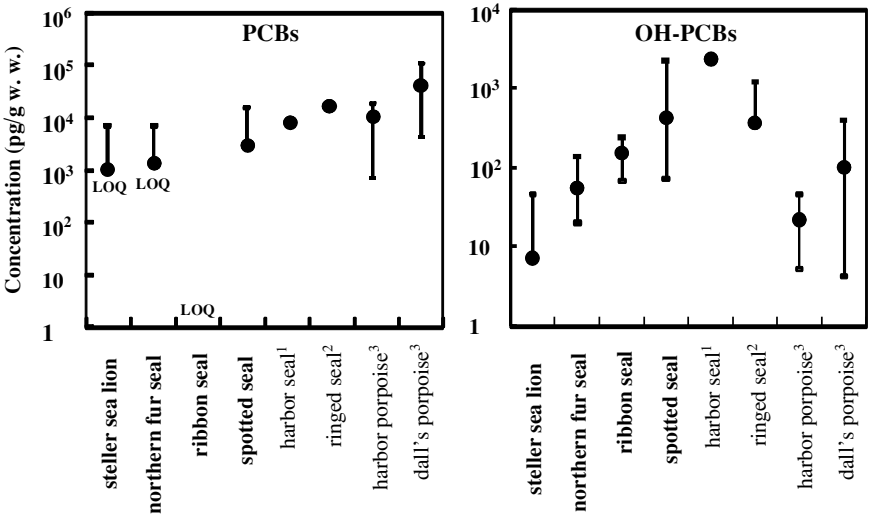
PCBs are metabolized to hydroxylated PCBs (OH-PCBs) by the cytochrome P450 monooxygenase enzyme systems that generally involve the oxide intermediates in the human body (Letcher *et al.*, 2000). Introduction of hydroxyl groups in the molecules increases polarities of PCB and facilitates excretion. If the hydroxyl group is in the *para*-position of a biphenyl structure and has adjacent chlorine atoms, the structure resembles thyroxin (T4) (Rickenbacher *et al.*, 1986). This structural similarity allows OH-PCBs to bind with a strong affinity to one of the TH transport proteins, transthyretin (TTR) (Brouwer *et al.*, 1990; Lans *et al.*, 1993), and disrupt TH and retinol (vitamin A) transportation (Brouwer *et al.*, 1986; Hallgren *et al.*, 2001). In particular, TH plays critical roles in the development of the central nervous system and brain function (Yen, 2001).

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFRs) used in textiles, furniture, and electronic and electrical items. PBDEs are persistent, bioaccumulative, and toxic, and have become widespread contaminants in the environment, human and wildlife worldwide (Hites, 2004; de Wit *et al.*, 2006). PBDEs have also been associated with various adverse effects on the endocrine and reproductive functions in marine mammals (Talsness, 2008). Detection of hydroxylated PBDEs (OH-PBDEs) in wildlife blood suggests the formation of these compounds in the liver of some terrestrial mammalian species upon exposure to PBDEs (Hakk and Letcher, 2003). In contrast, some OH-PBDE congeners are well known natural products found in marine organisms like red algae or cyanobacteria (Malmvärn *et al.*, 2005, 2008). Additionally, methoxylated PBDEs (MeO-PBDEs) and bromophenols (BPhs) are present in marine organisms (Covaci *et al.*, 2008). BPhs, which are in use as brominated flame retardants and bactericides were also reported to originate from both anthropogenic and natural sources (Gribble, 2000).

In recent study, PCBs and PBDEs were detected in fat of northern fur seals collected from northern Japan (Kajiwara *et al.*, 2004). They insisted that since PCBs compositions in fur seals showed no temporal variation, suggesting a continuous input of PCBs into the marine environment in significant quantities, and peak concentrations of PBDEs occurred later than organochlorines, it is essential to follow up the patterns of PBDEs pollution that may be of great concern in the future. 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 BPhs in the blood of pinnipeds caught 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

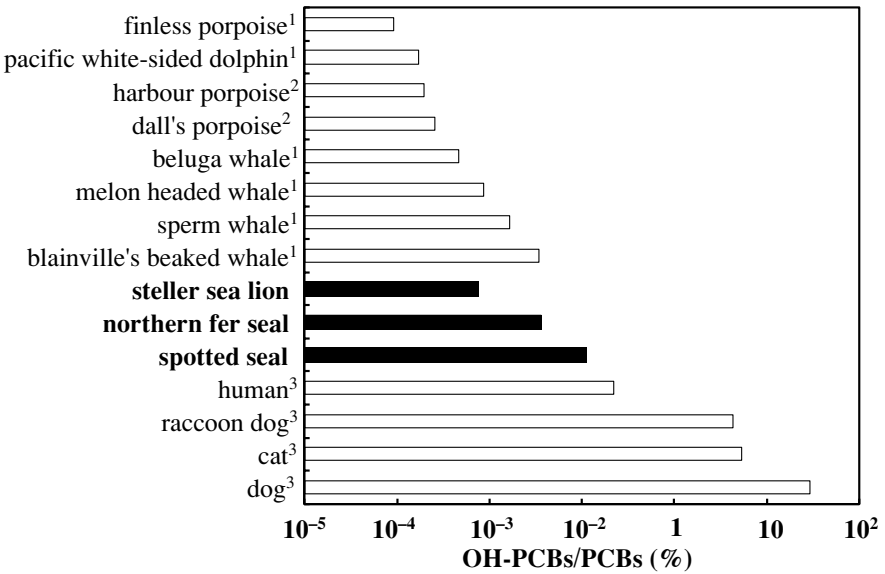


LOQ: the limit of quantification
1) Løken *et al*., 2008 2) Routti *et al*., 2008 3) Ochiai *et al*., 2011a

Fig. 1. Concentrations of PCBs and OH-PCBs in the blood of pinnipeds from Japanese coastal waters.

= 7), spotted seal (*Phoca largha*) (*n* = 8: male = 6, female = 2), stellar sea lion (*Eumetopias jubatus*) (*n* = 18: male = 6, female = 12) and ribbon seal (*Phoca fasciata*) (*n* = 2: male = 1, female = 1) during 1990–1999. All the blood samples were collected in falcon polypropylene conical tube and stored in the Environmental Specimen Bank (*es*-BANK) of Ehime University, Japan, at –25°C until they were analyzed.

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



1) Nomiyama *et al.*, 2010 2) Ochiai *et al.*, 2011a 3) Kunisue and Tanabe, 2009

Fig. 2. Comparison OH-PCBs/PCBs rations in the blood of pinnipeds from Japanese coastal waters with those of human and wildlife reported previously.

RESULTS AND DISCUSSION

Residue levels of PCBs and OH-PCBs

Except in ribbon seal (PCB levels were less than the limit of quantification (LOQ)), PCBs were detected in all species analyzed. High concentrations of PCBs were found in the blood of spotted seal; these values were significantly higher than the concentrations found in steller sea lion ($p < 0.05$), followed by northern fur seal and steller sea lion (Fig. 1). The PCBs levels of pinnipeds in this study were lower than that of harbor seal (*Phoca vitulina*) living in the Norwegian seas (Løken *et al.*, 2008) and ringed seal (*Phoca hispida*) living in Snalbard (Routti *et al.*, 2008). The smaller amount of PCBs usage into the environment in Hokkaido may be a possible reason for the low PCBs levels found in the present study.

On the other hand, residue levels of PCBs in pinnipeds were 1–2 orders of magnitude lower than in harbour porpoise (*Phocoena phocoena*) and Dall's porpoise (*Phocoenoides dalli*) which live in the same area with pinnipeds in this study (Ochiai *et al.*, 2011a). The dominant PCBs isomers identified in pinnipeds blood were CB153, followed by 138, 118 and 99, in that order.

OH-PCBs were detected in the blood samples of all species analyzed in this study. High concentrations of OH-PCBs were found in blood of spotted seal,

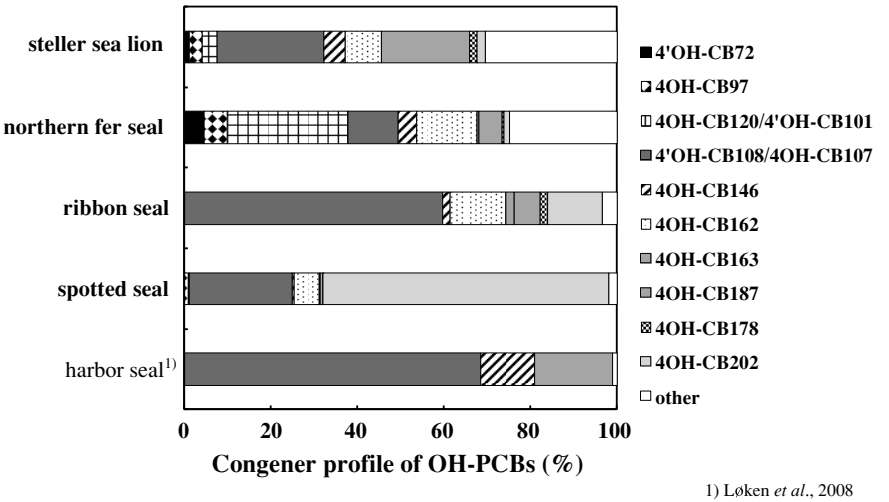


Fig. 3. OH-PCBs profiles in the blood of pinnipeds from Japanese coastal waters.

followed by ribbon seal, northern fur seal and steller sea lion (Fig. 1). The OH-PCBs levels of spotted seal and northern fur seal were significantly higher than steller sea lion ($p < 0.05$). The OH-PCBs levels of pinnipeds in this study were lower than that of harbor seal living in the Norwegian coastal area (Løken *et al*., 2008) and ringed seal living in Snalbard (Routti *et al*., 2008), whereas residue levels of the OH-PCBs in pinnipeds were the same as found in harbour porpoise (Ochiai *et al*., 2011a).

Concentration ratios of OH-PCBs to PCBs (OH-PCBs/PCBs) 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 OH-PCBs in the blood. Total OH-PCBs/PCBs ratios in pinnipeds were in the same levels or higher than in the cetaceans living in Japanese coastal waters (Nomiyama *et al*., 2010; Ochiai *et al*., 2011a); however, the values were lower than those of terrestrial mammals (Kunisue and Tanabe, 2009) (Fig. 2). It is presumed that metabolic capacity and/or binding affinity of OH-PCBs to TTR in pinnipeds may be higher than the cetaceans.

OH-PCB isomers profiles

Among the OH-PCB isomers identified, 4OH-CB107 and 4′OH-CB108 were predominant in the blood of ribbon seal and spotted seal (Fig. 3). 4OH-CB107 and 4′OH-CB108 were already detected as dominant isomers in seals (Løken *et al*., 2008; Routti *et al*., 2008) and other wildlife (Kunisue and Tanabe, 2009; Ochiai *et al*., 2011a). Furthermore, the predominant OH-PCB isomers were 4′OH-CB101/120 in northern fur seal and 4OH-CB107/4′OH-CB108 and 4OH-CB187 in steller sea lion (Fig. 3). These results reveal that the accumulation

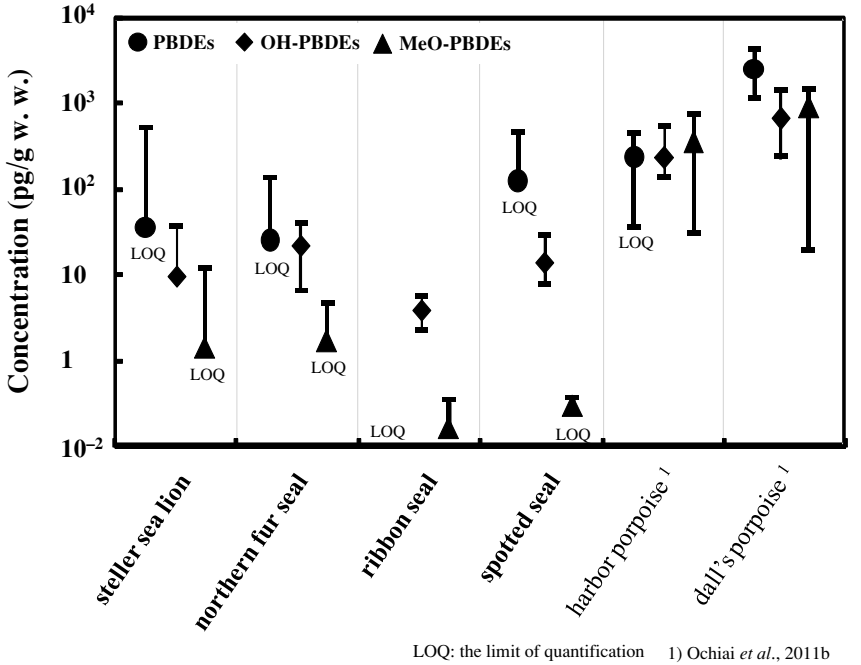


Fig. 4. Concentrations of PBDEs, MeO-PBDEs and OH-PBDEs in the blood of pinnipeds from Japanese coastal waters.

profiles of OH-PCB in pinnipeds blood are entirely different from profiles found in the other marine mammals, the cetaceans (Nomiyama *et al.*, 2010). However, the predominant OH-PCB isomers detected in all pinnipeds species were similar in structure to T4 (T4-like OH-PCBs). Because T4-like OH-PCBs are reported as having high binding affinity to TTR (Lans *et al.*, 1993), the influence that they may have on the homeostasis of the thyroid hormone should be a matter of concern.

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

Except in ribbon seal (PBDE levels were less than the LOQ), PBDEs were detected in all species at extremely small amounts. High concentrations of PBDEs were relatively found in blood of spotted seal, followed by steller sea lion and northern fur seal (Fig. 4).

MeO-PBDEs were also detected in all the species of the present study at extremely small amounts. Accumulation level of MeO-PBDEs found in the blood of steller sea lion were relatively higher than that in other pinnipeds in this study, and followed by northern fur seal, spotted seal and ribbon seal (Fig. 4).

OH-PBDEs were detected in all the species of the present study. OH-PBDEs

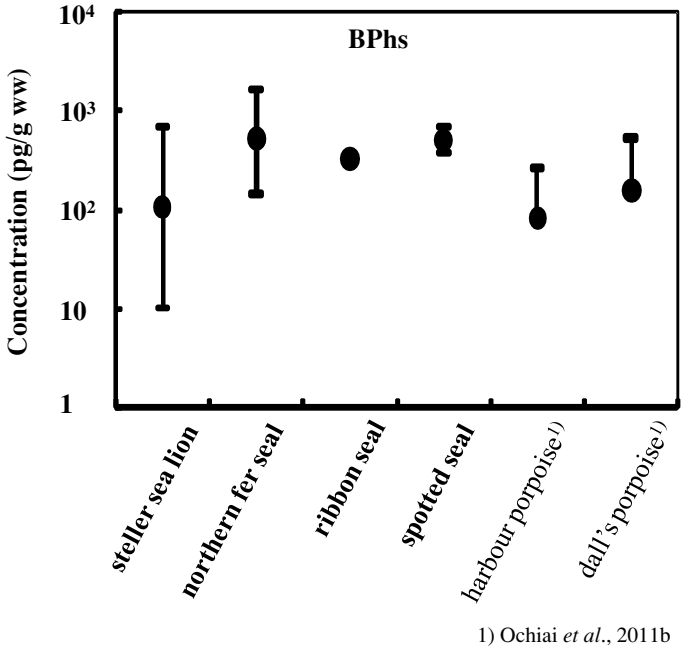


Fig. 5. Concentrations of BPhs in the blood of pinnipeds from Japanese coastal waters.

levels found in the blood of northern fur seal were relatively higher than that in other pinnipeds in this study, and followed by spotted seal, steller sea lion and ribbon seal (Fig. 4). Residue levels of OH-PBDEs in blood of steller sea lion was significantly lower than northern fur seal and spotted seal ($p < 0.05$). Of the 28 OH-PBDE isomers monitored, only two isomers (2′OH-BDE68 and 6OH-BDE47) could be consistently identified in all pinniped species. 6OH-BDE47 was detected in all samples. Similar profiles of OH-PBDEs have been reported previously in harbour porpoise and Dall's porpoise which live in the same area with pinnipeds of this study (Ochiai *et al*., 2011b). 6OH-BDE47 has been reported as a marine natural product in red algae and fish (Verreault *et al*., 2005; Malmvarn *et al*., 2008). Origin of large percentage of 6OH-BDE47 detected in 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 in same level found in cetaceans living in same coastal area, concentrations of PBDEs, MeO-PBDEs and OH-PBDEs found in pinnipeds of the present study were significantly lower than the levels in the cetaceans (Ochiai *et al*., 2011b). 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 as in toothed whales, even though both the groups eat similar diet, live in the same habitat, have similar blubber structure and thickness of blubber (Pangallo and Reddy, 2009). They indicated that this difference of accumulation pattern showed pinnipeds have an enhanced capability to degrade organobromine compounds relative to toothed whales. Since pinnipeds in this study are in the same trophic level with porpoise, pinnipeds accumulate less PBDEs, OH-PBDEs and MeO-PBDEs or they have more enhanced capability to degrade those organobromine compounds than porpoise.

Residue levels of BPhs

BPhs were found in all samples. High concentrations of BPhs were found in the blood of northern fur seal followed by spotted seal, ribbon seal and steller sea lion (Fig. 5). Of the 10 BPhs isomers monitored, only two isomers (2,4,6-BPh and penta-BPh) could be identified in pinnipeds. 2,4,6-BPh was the dominant isomer in pinnipeds. 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 (Nomiyama *et al.*, 2011). 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 indicated to be natural product, the origin of large percentage of 2,4,6-BPh in pinnipeds might be from natural products already present in the marine environment.

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C. Kanbara (e-mail: rokky05@agr.ehime-u.ac.jp)