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Polychlorinated Biphenyls and Hydroxylated Polychlorinated Biphenyls in the Blood of Toothed and Baleen Whales Stranded along Japanese Coastal Waters

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Abstract—In this study, we determined the residue levels and patterns of polychlorinated biphenyls (PCBs) and hydroxylated PCBs (OH-PCBs) in the blood from eight species of toothed whales and three species of baleen whales stranded along the Japanese coast during 1999–2007. Penta- through hepta-chlorinated congeners were the dominant homolog groups in all cetaceans. In contrast, specific differences in the distribution of dominant OH-PCB isomers and homologs were found among the cetacean species. In the seven species of toothed whales (except sperm whales), the predominant homolog group was OH-penta-PCBs followed by OH-tetra-PCBs and OH-tri-PCBs. The predominant OH-PCB isomers were *para*-OH-PCBs such as 4OH-CB26, 4′OH-CB25/4′OH-CB26/4OH-CB31, 4OH-CB70, 4′OH-CB72, 4′OH-CB97, 4′OH-CB101/4′OH-CB120, and 4OH-CB107/4′OH-CB108 in toothed whales. In three baleen whales (minke whale, Bryde's whale, and humpback whale) and in sperm whale (which is a toothed whale), OH-octa-PCB (4OH-CB202) was the predominant homolog group accounting for 40–80% of the total OH-PCB concentrations. These results reveal that the accumulation profiles of OH-PCBs in cetacean blood are clearly different from the profiles found in other mammals. This study is the first report to comprehensively document the accumulation profiles of OH-PCBs in cetaceans.

Keywords: OH-PCBs, PCBs, cetacean, toothed whale, baleen whale

INTRODUCTION

Polychlorinated biphenyls (PCBs) are known to elicit an array of toxic effects including endocrine disruption in humans and wildlife (Safe, 1994). In particular, it has been reported that PCBs disrupt thyroid hormone (TH) homeostasis in animals (Brouwer *et al*., 1998). A possible mechanism involved in the disruption

of TH homeostasis is competitive binding of PCBs with TH transport protein, transthyretin (TTR), in blood (Lans *et al.*, 1993). 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 T4 (Brouwer *et al.*, 1998). This structural similarity allows OH-PCBs to bind with a strong affinity to TTR, and disrupt TH homeostasis and retinol (vitamin A) transportation (Lans *et al.*, 1993; Hallgren *et al.*, 2001).

Our preliminary study on penta- through hepta-chlorinated OH-PCB congeners in the blood of melon-headed whales (*Peponocephala electra*) and finless porpoises (*Neophocaena phocaenoides*) stranded along Japanese coastal waters showed that OH-penta-PCB/penta-PCB ratios were higher than the ratios for hexa- and hepta-chlorinated homolog groups (Murata *et al.*, 2007). Moreover, considerably higher proportions of OH-penta-PCBs were found in melon-headed whales and finless porpoises than in humans (Nomiyama *et al.*, 2010). These results clearly indicate preferential accumulation of OH-penta-PCBs and suggest accumulation of less-chlorinated OH-PCBs (3–4 chlorines) in cetacean blood.

The present study elucidated residue levels and patterns of tri- through octa-chlorinated homologues of OH-PCB congeners and examined correlations between OH-PCBs and PCBs in the blood from eight species of toothed whales and three species of baleen whales stranded along the Japanese coast during 1999–2007.

MATERIALS AND METHODS

Collection of blood from toothed and baleen whales

The whole blood samples were collected from eleven species of cetaceans (n = 55) including melon-headed whale (*Peponocephala electra*) (n = 14: male = 7, female = 7), finless porpoise (*Neophocaena phocaenoides*) (n = 7: male = 4, female = 3), Stejneger's beaked whale (*Mesoplodon stejnegeris*) (n = 12: male = 5, female = 7), pacific white-sided dolphin (*Lagenorhynchus obliquidens*) (n = 7: male = 5, female = 2), Blainville's beaked whale (*Mesoplodon densirostris*) (n = 4: male = 2, female = 2), killer whale (*Orcinus orca*) (n = 3: female = 2, unknown = 1), beluga whale (*Delphinapterus leucas*) (n = 1: male = 1), sperm whale (*Physeter macrocephalus*) (n = 2: male = 2), Bryde's whale (*Balaenoptera brydei*) (n = 2: male = 1, female = 1), common minke whale (*Balaenoptera acutorostrata*) (n = 2: male = 1, female = 1) and humpback whale (*Megaptera novaengliae*) (n = 1: female = 1) stranded along the Japanese coast during 1999–2007 (Fig. 1). All the blood samples, stored in the Environmental Specimen Bank (*es*-BANK) of Ehime University, Japan, at –25°C were used for analysis (Tanabe, 2006).

Measurements of PCBs and OH-PCBs in whole blood

The extraction of procedure used in this study is similar to that described by

Kunisue and Tanabe (2009). The whole blood sample (10 g) was denatured with 6 M hydrochloric acid (HCl). $^{13}C_{12}$-labeled OH-PCBs and $^{13}C_{12}$-labeled PCBs (Wellington Laboratories, Canada) were spiked as surrogate internal standards. 2-propanol was added, and then OH-PCBs were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane by a homogenizer. After centrifugation, the organic phase was combined and washed with 5% NaCl prepared in hexane-washed water. The organic phase was evaporated by a rotary evaporator and dissolved in hexane. 1 M potassium hydroxide (KOH) in 50% ethanol/water (20 mL) was added and shaken. The partition process of PCBs and OH-PCBs was repeated and the alkaline phases were combined. The lipid in the organic phase containing PCBs was removed by gel permeation chromatography (GPC) with Bio-Bead S-X3 (Bio-Rad Laboratories, Hercules CA). First fraction containing lipid was discarded, and the following fraction containing PCBs was collected, concentrated and passed through activated 3 g of silica-gel (Wako-gel S-1: Wako Pure Chemical Industries Ltd., Japan) packed in a glass column. PCBs were eluted with 5% DCM/hexane and concentrated and $^{13}C_{12}$-labeled BDE139 was added as a syringe spike for gas chromatography/mass spectrometry (GC: Agilent 6890)/MS: Agilent 5973N) analysis. The KOH solution phase containing OH-PCBs was acidified (pH 2) with sulfuric acid. Then OH-PCBs were extracted twice with 50% MTBE/hexane (60 mL). The phases were separated and the organic phase was combined and evaporated by a rotary evaporator. The solvent-evaporated residue was dissolved in hexane and passed through a glass column packed with 3 g inactivated silica-gel (Wako-gel S-1, 5% H_2O deactivated). The OH-PCBs fraction was eluted with 50% DCM/hexane (100 mL), concentrated and dissolved in hexane (1 mL). The OH-PCBs in hexane were derivatized (methylation) by using trimethylsilyldiazomethane (overnight at 20°C). The derivatized solution was treated by GPC and then passed through 3 g activated silica-gel packed in a glass column. MeO-PCBs fraction was eluted with 10% DCM/hexane and this fraction was concentrated to near dryness. $^{13}C_{12}$-labeled CB77 and CB157 were then added as syringe spikes. Identification and quantification of MeO-PCBs isomers were performed using a HP-6890 gas chromatograph (HRGC: Agilent Technologies Inc., CA, USA) coupled with a MS-800D high-resolution mass spectrometer (JEOL, Japan).

RESULTS AND DISCUSSION

Residue levels of PCBs and OH-PCBs

Except for humpback whale (PCB levels were less than the limit of quantification), PCBs were detected in all cetacean blood samples (Fig. 1). High concentrations of PCBs were found in blood of killer whales; these values were significantly higher than the concentrations found in other cetaceans ($p < 0.01$). OH-PCBs were detected in the blood samples of all cetaceans analyzed in this study (Fig. 1). In killer whales significantly higher concentrations of OH-PCBs were detected than in other cetaceans ($p < 0.05$). OH-PCBs in cetaceans are

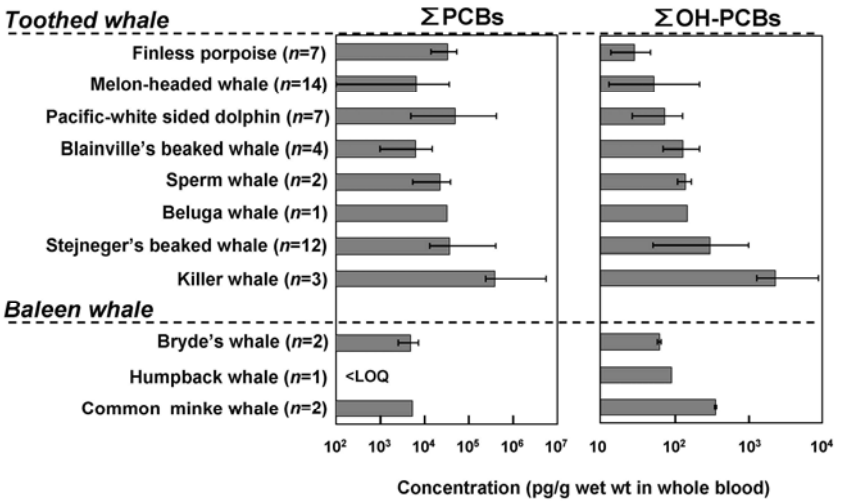

Fig. 1. Median concentrations of total PCBs and total OH-PCBs (pg/g wet wt in whole blood) in the blood of toothed and baleen whales stranded along Japanese coastal waters. Error bars indicate range (maximum to minimum concentrations). Concentration of total PCBs in humpback whale was lower than the limit of quantification (LOQ).

expected to be formed from the metabolism of PCBs in liver. CYP induction by the high accumulation of PCBs in killer whales has been suggested to yield OH-PCBs (Montie *et al*., 2008). Furthermore, it can be presumed that the prey species of cetaceans, such as other marine mammals and fishes, can accumulate not only PCBs but also OH-PCBs (Campbell *et al*., 2003; Montie *et al*., 2008; Weijs *et al*., 2009); thus exposure to OH-PCBs in cetaceans can arise from food chain transfer in addition to metabolic processes. High concentrations of OH-PCBs were also detected in baleen whales, especially in common minke whales. This was surprising and no explanation is available at the moment, although species-specific metabolism, bioaccumulation, food-chain transfer are plausible reasons.

Accumulation profiles of OH-PCB congeners

The homolog patterns of PCB and OH-PCB congeners in toothed and baleen whales are shown in Fig. 2. The dominant PCB congeners identified in cetacean blood were hexa-PCBs, followed by penta-, hepta-, tetra-, octa- and tri-CBs in that decreasing order. The homolog profiles of OH-PCB congeners were different among the cetacean species. In five species of toothed whales (melon-headed whale, Stejneger's beaked whale, pacific white-sided dolphin, Blainville's beaked whale, and killer whale), the predominant homologues were OH-penta-PCBs followed by OH-tetra-PCBs and OH-tri-PCBs. The predominant homologues of finless porpoise and beluga whale were OH-penta-PCBs followed by OH-hexa-

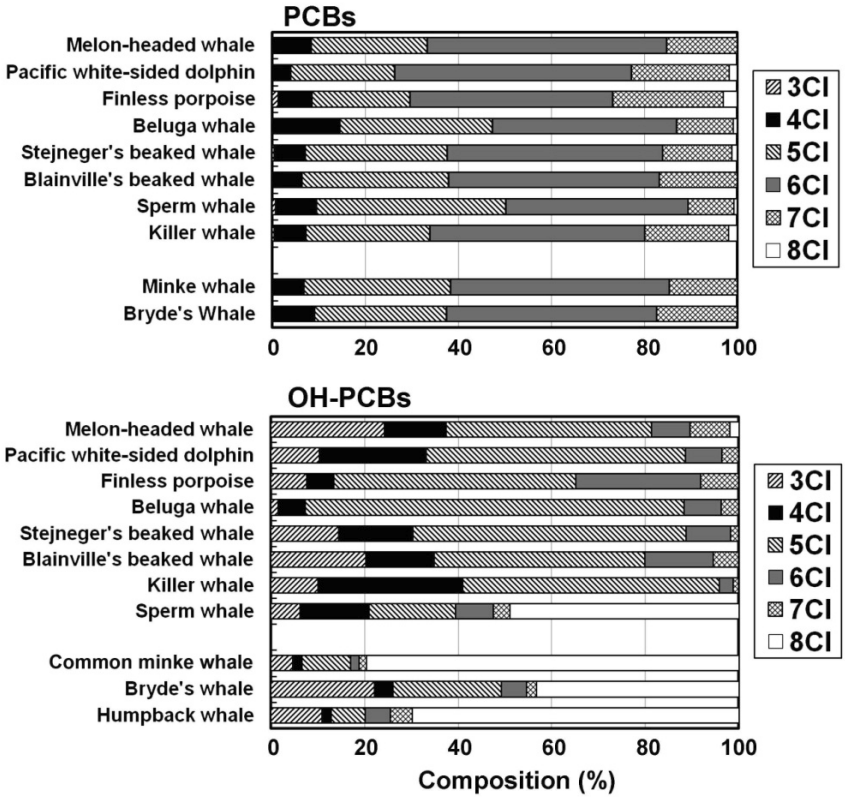

Fig. 2. Congener compositions (%) of PCBs and OH-PCBs in the blood of toothed and baleen whales stranded along Japanese coastal waters.

PCBs and OH-tri-PCBs. These lower-chlorinated OH-PCBs (3–5 chlorines) accounted for about 60–80% of the total OH-PCB concentrations in the blood of toothed whales. The profiles of OH-PCBs in toothed whales were similar to those reported earlier for bottle-nosed dolphins from US coastal waters (Houde *et al*., 2006; Montie *et al*., 2008). Considering these observations, it is highly plausible that toothed whales preferentially metabolize lower-chlorinated PCBs and accumulate their OH-metabolites in blood. This is due to the low metabolic capacity to PCBs and low activity of CYP2B-like enzymes in cetaceans (White *et al*., 2000; Boon *et al*., 2001). When concentration ratios of ∑OH-PCBs to ∑PCBs (∑OH-PCBs/∑PCBs) were calculated, relatively lower values were observed in cetaceans (finless porpoise: 0.001 - minke whale: 0.056) than in terrestrial mammals (human: 0.37 - dog: 29), further suggesting poor metabolic capacity for PCBs in cetaceans (Fig. 3) (White *et al*., 2000; Boon *et al*., 2001). Interestingly, in three baleen whales (common minke whales, Bryde's whales,

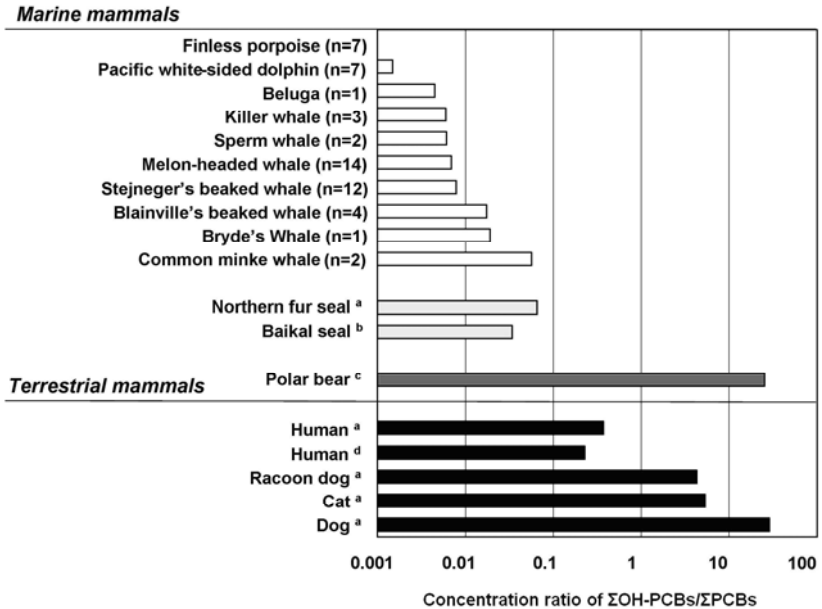

Fig. 3. Comparison of concentration ratios of total OH-PCBs/PCBs in the blood of marine mammals and terrestrial mammals. References for OH-PCBs/PCBs ratios are (a) Kunisue and Tanabe, 2009, (b) Imaeda *et al.*, 2008, (c) Gebbink *et al.*, 2008, (d) Nomiyama *et al.*, 2010.

and humpback whale) and in sperm whales, OH-octa-PCBs were the predominant homologues, accounting for 40–80% of total OH-PCB concentrations, a trend similar to that reported for dogs and raccoon dogs (Kunisue and Tanabe, 2009). These observations may suggest specific metabolic capacity (specific to higher-chlorinated PCBs) and exposure of these species of whales to OH-octa-PCBs through the foodweb (Campbell *et al.*, 2003). Previous study (Niimi *et al.*, 2007) on the activities of phenobarbital (PB)-type isozymes and 3-methylcholanthrene (MC)-type isozymes indicated that common minke whales have relatively high PB type (CYP 2B and 2C type) activity. A few studies have shown that CYP2B enzyme activity is minimal or inactive in toothed whales (Boon *et al.*, 2001). Elevated 4OH-CB187/CB183 + 187 and 4′OH-CB199/CB199 ratios were recently reported in blood of polar bears and it was suggested that these PCB metabolites were formed through the catalytic activity of CYP2B and/or CYP2D enzymes (Verreault *et al.*, 2008). Moreover, it is demonstrated that PB-type isozymes, CYP2B and/or CYP2C subfamilies, play a primary role in the metabolism of CB153 in dogs (Ariyoshi *et al.*, 1992). Based on these findings, it is likely that CYP2C-type enzymes are responsible for the metabolism of higher-chlorinated OH-PCBs in baleen whales and sperm whales.

Accumulation features of OH-PCB isomers

Among the OH-PCB isomers identified, 4OH-CB26, 4′OH-CB25/4′OH-CB26/4OH-CB31, 4OH-CB70, 4′OH-CB72, 4′OH-CB97, 4′OH-CB101/4′OH-CB120, and 4OH-CB107/4′OH-CB108 were the predominant ones in the blood of toothed whales. These tri- through penta-chlorinated OH-PCB isomers were detected at relatively higher levels than the hexa- through octa-chlorinated OH-PCB isomers. In recent studies, 4OH-CB107, 4OH-CB146, 3OH-CB153, and 4OH-CB187 were detected as dominant isomers in blood of humans and wildlife (Kunisue and Tanabe, 2009). These studies suggested that OH-PCBs having *para*-OH-group with two adjacent chlorine atoms have high binding affinity to TTR, due to their structural similarity to T4. However, the predominant OH-PCB isomers detected in cetacean blood, in this study, were not similar in structure to T4. The predominant 3–4 chlorinated OH-PCB isomers in cetaceans had one chlorine atom adjacent to OH-group on the phenyl rings (3,5,5′-triiodothyronine (T3)-like OH-PCBs), and the chemical structures of these OH-PCBs are different from those congeners reported as having high binding affinity to TTR. Concentrations of these OH-PCB isomers were 17–70% of the identified OH-PCB concentrations. This result indicates that the accumulation profiles of OH-PCB isomers in cetacean blood are clearly different from those in other mammals and birds (Kunisue and Tanabe, 2009). In three baleen species (minke whales, Bryde's whales, and humpback whale) and in sperm whales (which is a toothed whale), 4OH-CB202 was detected as a major congener at concentrations 1–2 orders of magnitude higher than that found in other toothed whales.

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