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Accumulation of Halogenated Phenolic Compounds in Small Toothed Whales

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Abstract—The present study examined the accumulation features of hydroxylated polychlorinated biphenyls (OH-PCBs) and hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in the blood of harbor porpoises (*Phocoena phocoena*), Dall's porpoises (*Phocoenoides dalli*) and finless porpoises (*Neophocaena phocaenoides*) stranded or bycaught along the Japanese coastal waters. Concentrations of OH-PCBs ranged between 24–61 pg/g wet wt., approximately three orders of magnitude lower than that of PCBs. There was a positive correlation between the levels of PCBs and OH-PCBs ($p < 0.001$), and this result suggests metabolic formation of OH-PCBs from PCBs in porpoises. In contrast, OH-PBDEs concentrations were found to be comparable with those of PBDEs, and the levels of PBDEs and OH-PBDEs were not related to each other. The results indicate that OH-PBDEs have alternative source(s) other than the metabolic formation from PBDEs. Among the 24 OH-PBDE congeners analyzed, 6OH-BDE47 was predominant, followed by 2′OH-BDE68. These two congeners were reported to be biosynthesized in the marine environment; thus higher abundance of OH-PBDEs found in porpoises is likely to be the result of the intake of naturally produced OH-PBDEs, apart from the metabolism of PBDEs in the bodies of porpoises.

Keywords: OH-PCBs, OH-PBDEs, halogenated phenolic compounds, metabolite, marine mammal, porpoise

INTRODUCTION

Toothed whales are top predators in marine ecosystems and are known to bioaccumulate anthropogenic organohalogen pollutants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Tanabe, 2002;

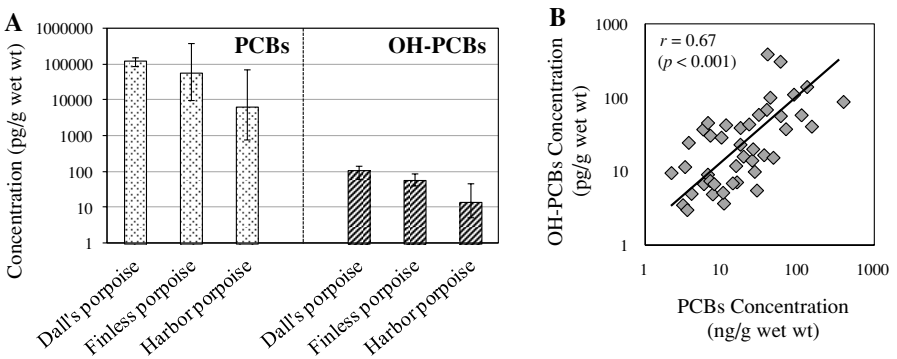

Fig. 1. Median concentrations of PCBs and OH-PCBs in Dall's porpoises, finless porpoises and harbor porpoises (A) and correlation between the levels of PCBs and OH-PCBs (B).

Hites, 2004; Law *et al.*, 2006). In cetacean livers, PCBs and PBDEs can be metabolized into their respective hydroxylated metabolites, OH-PCBs and OH-PBDEs, via cytochrome P450 monooxygenases (CYPs) (Bergman *et al.*, 1994). Hydroxylated metabolites are further transformed by Phase II conjugation enzymes and readily excreted, except for the congeners which resemble thyroid hormones (thyroxine; T_4). These congeners can compete with T_4 to bind thyroid hormone transport protein (transthyretin; TTR), circulate in blood and potentially disturb thyroid hormone homeostasis and cerebral nervous system (Lans *et al.*, 1993; Cheek *et al.*, 1999; Meerts *et al.*, 2002, 2004; Purkey *et al.*, 2004; Kimura-Kuroda *et al.*, 2007).

Recent studies have shown that OH-PBDEs have alternative sources other than metabolic formation from PBDEs. They have been identified as natural products synthesized from marine organisms such as marine sponges (Fu *et al.*, 1995; Bowden *et al.*, 2000) and algae (Asplund *et al.*, 2001; Malmvärn *et al.*, 2008). It is also reported that OH-PBDEs can be formed by their methoxylated analogs, MeO-PBDEs (Wan *et al.*, 2009), which are also natural compounds formed in marine environment (Teuten *et al.*, 2005; Malmvärn *et al.*, 2005).

The present study investigated the accumulation features of OH-PCBs and OH-PBDEs in the blood of harbor porpoises (*Phocoena phocoena*), Dall's porpoises (*Phocoenoides dalli*) and finless porpoises (*Neophocaena phocaenoides*) stranded or bycaught along the Japanese coastal waters.

MATERIALS AND METHODS

The blood samples were collected from the hearts or the blood vessels of harbor porpoises, finless porpoises and Dall's porpoises stranded or bycaught along the Japanese coastal waters during 2005–2010. Samples were stored in the Environmental Specimen Bank (*es*-BANK) at Ehime University, Japan, at –25°C until analyses.

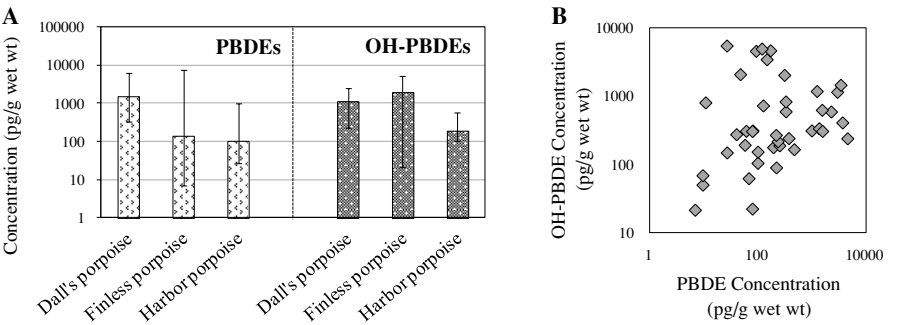

Fig. 2. Median concentrations of PBDEs and OH-PBDEs in Dall's porpoises, finless porpoises and harbor porpoises (A) and correlation between the levels of PBDEs and OH-PBDEs (B).

62 PCB (mono- to deca-), 52 OH-PCB (tri- to octa-), 42 PBDE (mono- to deca-), 24 OH-PBDE (tri- to octa-) congeners were analyzed. The extraction and cleanup methods for PCBs and OH-PCBs (Nomiyama *et al*., 2010) as well as for PBDEs and OH-PBDEs (Nomiyama *et al*., in press) were described elsewhere. Briefly, the blood sample (10 g) was denatured with HCl and extracted with methyl *t*-butyl ether (MTBE)/hexane (1:1). The organic phase was partitioned into neutral and phenolic fractions with KOH solution (50% ethanol/H_2O). The neutral fraction containing PCBs and PBDEs was cleaned up with gel permeation chromatography (GPC) and activated silica-gel column for gas chromatography/mass spectrometry (GC/MS) analysis. The phenolic phase was acidified and re-extracted with MTBE/hexane to obtain OH-PCBs and OH-PBDEs, and then passed through a deactivated silica-gel column (5% H_2O). OH-PCBs/-PBDEs were then derivatized with trimethylsilyldiazomethane (TMSDS) for overnight at 20°C. The derivatized solution was further cleaned up by using gel permeation chromatography (GPC) and an activated silica-gel column. OH-PCBs/-PBDEs were determined as MeO-PCBs/-PBDEs using high-resolution GC/MS.

RESULTS AND DISCUSSION

Levels of OH-PCBs

The median OH-PCBs level was the highest in Dall's pospoise (109 pg/g wet wt), followed by finless porpoises (56 pg/g wet wt) and harbor porpoises (14 pg/g wet wt) (Fig. 1A). Interspecies comparison of the OH-PCBs concentrations showed a similar trend compared with PCBs, where Dall's porpoises had the highest median concentration of 120,000 pg/g wet wt, followed by finless and harbor porpoises (59,000 and 6,500 pg/g wet wt, respectively) (Fig. 1A). PCBs and OH-PCBs concentrations had a positive correlation (Spearman's rank correlation coefficient: $r = 0.67$, $p < 0.001$) (Fig. 1B), suggesting that a large percentages of OH-PCBs in porpoise blood were metabolically formed from PCBs, rather than being taken through their diet.

Levels of OH-PBDEs

Finless porpoises had the highest median OH-PBDEs level of 2,000 pg/g wet wt, followed by Dall's porpoises (1,100 pg/g wet wt) and harbor porpoises (190 pg/g wet wt) (Fig. 2A). On the other hand, the median PBDEs level was the highest in Dall's porpoises (1,600 pg/g wet wt), and the level was significantly higher than that of finless porpoises (140 pg/g wet wt) and harbor porpoises (100 pg/g wet wt) (ManneWhitney U-test: $p < 0.05$) (Fig. 2A). While OH-PCBs levels were related with their parent compounds (i.e., PCBs), no correlation was found between the levels of OH-PBDEs and PBDEs (Fig. 2B). These results suggest that the levels of OH-PBDEs have alternative sources other than metabolic formation from PBDEs.

Accumulation Features of OH-PBDEs

Among the 24 OH-PBDE congeners analyzed, 6OH-BDE47 was the most abundant, accounting for more than 95% of the total OH-PBDEs concentrations and detected in all the samples. 6OH-BDE47 was reported to be a natural product (Malmvärn *et al.*, 2005) although it is a minor metabolite in rats exposed to PBDEs (Malmberg *et al.*, 2005; Marsh *et al.*, 2006). 2′OH-BDE68 was the second most abundant congener, comprising 1–3% of the total concentration, which is also biosynthesized by marine organisms (Malmvärn *et al.*, 2005).

In Dall's porpoises, several other congeners including 4′OH-BDE49 (8 pg/g wet wt), 4′OH-BDE101 (8 pg/g wet wt), 2′OH-BDE28 (6 pg/g wet wt) were also identified. Among them, 4′OH-BDE49 and 2′OH-BDE28 were reported to be one of the metabolic products of BDE47 (Marsh *et al.*, 2006; Qiu *et al.*, 2007), but so far not detectable in matural synthesis. Since the levels of PBDEs and PCBs in Dall's porpoises were exceeded the other two species, the exposure to higher levels of contaminants possibly led to induction of CYP enzymes (Erratico *et al.*, 2011), thus leading to the formation of 4′OH-BDE49 and 2′OH-BDE28. From the results, OH-PBDEs found in porpoise blood can be considered to have both natural and anthropogenic origins, although natural compounds were dominant.

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