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Accumulation Features of Organohalogen Metabolites in the Blood of Japanese Terrestrial Mammals

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Abstract—Accumulation features of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and their hydroxylated metabolites (OH-PCBs, OH-PBDEs) were analyzed in the blood of various carnivorous mammals collected from Japan. PCBs and OH-PCBs were detected in all the blood samples analyzed in this study. Lower chlorinated OH-PCB congeners were predominant in raccoon and cat blood, and higher chlorinated OH-PCBs have been observed in other animals. PBDEs and OH-PBDEs were found in higher concentrations in the blood of cats than in other species. The dominant isomers of OH-PBDEs were 6OH-BDE47 and 2′OH-BDE68 in all species. The concentrations of OH-PBDEs in terrestrial mammals except cats and mongooses were lower than in marine mammals. However, higher OH-PBDE levels in cats and mongooses than in marine mammals were found. It is possible that higher levels of OH-PBDEs in cats and mongooses can be attributed to higher metabolic capacity of PBDEs and/or the intake of OH-PBDEs from food such as fishes. The present study indicates that the metabolic capacity for PCBs and PBDEs in terrestrial mammals have wide variations among species. Particularly, cats, raccoons and mongoose showed specific pattern of accumulation for hydroxylated compounds which is different from other animals, suggesting that they might be higher risk from hydroxylated metabolites.

Keywords: terrestrial mammals, blood, organohalogen compounds, metabolites, Japan

INTRODUCTION

Hydroxylated metabolites of persistent chemicals such as hydroxylated

polychlorinated biphenyls (OH-PCBs) and hydroxylated polybrominated diphenyl ethers (OH-PBDEs) are formed by oxidative metabolism of PCBs and PBDEs by cytochrome P450 monooxygenases (CYPs) in the liver. Then hydroxylated compounds undergo conjugation reactions (e.g., glucuronidation, glutathione conjugation, sulfoconjugation), and eliminated from the body. However, the structural similarity to thyroxine (T4) allows some OH-PCBs and OH-PBDEs to bind competitively with the transthyretin (TTR) of thyroid hormone transport protein. It is well known that two of the toxic effects of these hydroxylated metabolites are the disturbance on thyroid hormone homeostasis, and on cerebral nervous system (Miyazaki *et al*., 2004; Purkey *et al*., 2004).

OH-PBDEs are well known metabolites of PBDEs as well as natural products found in marine organisms like red algae and sponges (Gribble, 2000; Hakk and Letcher, 2003). Recently it has also been found that some OH-PBDEs are formed by demethylation of methoxylated PBDEs (MeO-PBDEs) in marine organisms and it has not been shown in terrestrial animals (Wan *et al*., 2009). MeO-PBDEs also have been found as natural products in marine organisms (Teuten *et al*., 2005).

Carnivorous species are known to have higher metabolic capacity for organohalogen compounds. Pet dogs have higher metabolic and elimination capacities for organochlorines than pet cats (Kunisue *et al*., 2005). Beagle dogs form metabolites of PCBs quickly when compared to cynomolgus monkeys (Sipes *et al*., 1982). Moreover, drug-metabolizing enzymes are induced in raccoon dogs depending on the hepatic levels of contaminants, and metabolized PCBs and PBDEs (Kunisue *et al*., 2008). Concentration of PBDEs in the red fox from Belgium were lower than those of the main prey species, voles and mice, indicating that the red fox has a strong metabolic capacity and eliminate lower brominated congeners (Voorspoels *et al*., 2006). These results suggest that carnivorous species might be at high-risk by the metabolites of PCBs and PBDEs. However, information on status of PCBs and PBDEs metabolites in terrestrial mammals are limited.

The present study elucidates the accumulation features of PCBs, PBDEs, their hydroxylated metabolites, and specific differences in metabolic capacities of different species by analyzing the blood samples of various carnivores such as cats, dogs, raccoon dogs, masked palm civets, foxes, raccoons, mongooses and badgers collected from Japan. Furthermore, we attempted to assess the risk by PCBs and PBDEs metabolism in a comparative biological perspective.

MATERIALS AND METHODS

Blood samples of terrestrial mammals [cats (*Felis silvestris catus*) (n = 5); dogs (*Canis lupus*) (n = 9); raccoons (*Procyon lotor*) (n = 9); foxes (*Vulpes vulpes japonica*) (n = 4); raccoon dogs (*Nyctereutes procyonoides*) (n = 10); masked palm civets (MP civet, *Paguma larvata*) (n = 8); badgers (*Meles meles*) (n = 6); and small Asian mongoose (*Herpestes javanicus*) (pooled: n = 2)] were collected from various regions of Japan from 2006 to 2009. Animals collected in this study

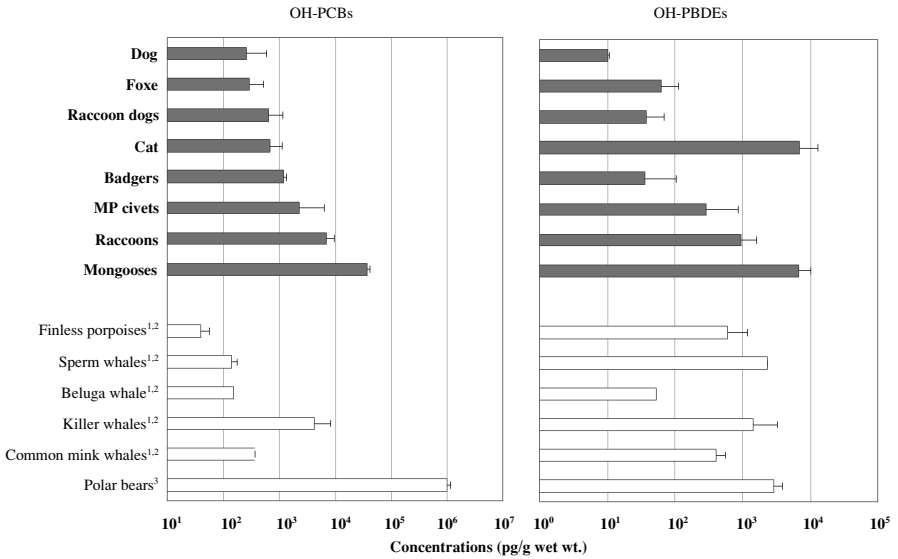

Fig. 1. Concentrations of OH-PCBs and OH-PBDEs (pg/g wet wt.) in the blood of terrestrial (this study) and marine mammals (1: Nomiyama *et al*., 2010a; 2: Nomiyama *et al*., 2011; 3: Gebbink *et al*., 2008). The bars represent standard deviation.

were wild animals, including stray cats and stray dogs, which found dead due to causes including traffic accidents and mammalian pest control activities. The blood samples were collected directly from the heart.

Whole blood (10 g) was denatured with 6 M HCl and homogenized with 2-propanol and 50% methyl *t*-butyl ether (MTBE)/hexane. Neutral and phenolic fractions were partitioned using 1 M KOH in 50% ethanol/water. The neutral fraction containing PCBs and PBDEs was passed through activated silica-gel packed in a glass column after the fat was removed by gel permeation chromatography (GPC) and eluted with 5% DCM/hexane and concentrated. The KOH solution phase was acidified with sulfuric acid and extracted twice with 50% MTBE/hexane. The organic fraction containing OH-PCBs and OH-PBDEs was passed through a column packed with inactivated silica-gel (5% H_2O deactivated) and eluted with 50% dichloromethane (DCM)/hexane and derivatized overnight by using trimethylsilyldiazomethane. The derivatized solution was passed through activated silica-gel packed in a column after the fat was removed by GPC then eluted with 10% DCM/hexane and concentrated. Identification and quantification were made using a gas chromatograph (GC: 6890 series, Agilent) coupled with high resolution (10,000) mass spectrometer (HRMS: JMS-800D, JEOL). GC-HRMS was equipped with a capillary column (DB-5MS for OH-PCBs and OH-PBDEs, and DB-1MS for PCBs and PBDEs, J&W Scientific) and operated in electron impact and selected ion monitoring mode (EI-SIM).

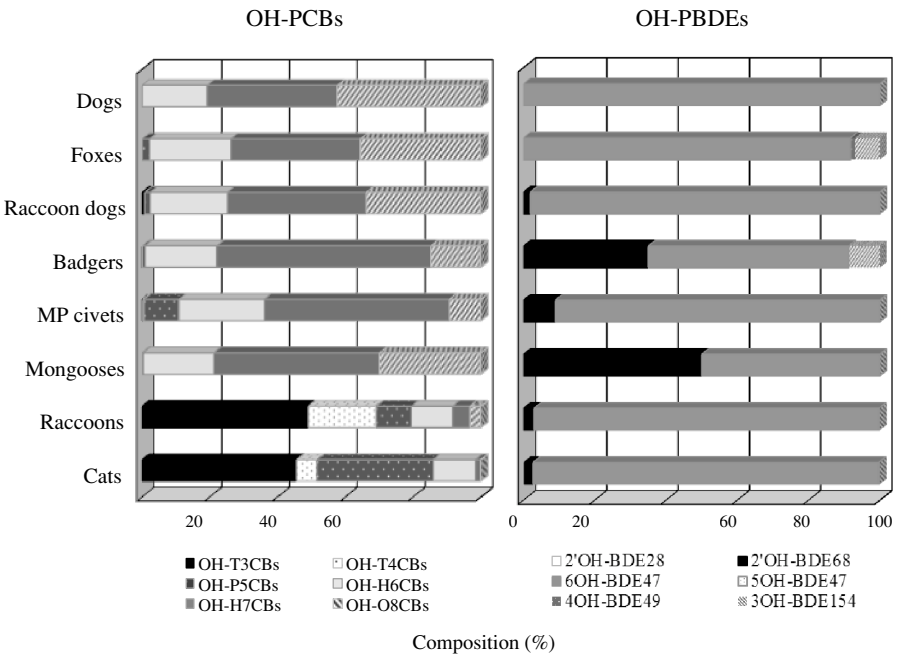

Fig. 2. Compositions of OH-PCB and OH-PBDE homologues in the blood of terrestrial mammals collected from Japan.

RESULTS AND DISCUSSION

PCBs and OH-PCBs were detected in the blood of all the terrestrial mammals in this study. The highest concentration of OH-PCBs was detected in the blood of mongooses (37000 pg/g) followed by MP civets (2300 pg/g), foxes (2300 pg/g), raccoons (1100 pg/g), badgers (590 pg/g), cats (550 pg/g), raccoon dogs (350 pg/g), and dogs (160 pg/g) (Fig. 1). Tri- to penta-chlorinated OH-PCB congeners were predominant in raccoons and cats; on the other hand, hexa- to octa-chlorinated OH-PCBs have been observed in other animals (Fig. 2). These variations might be due to species-specific metabolic capacity by phase I CYP and/or phase II conjugation enzymes, binding affinity to TTR, and exposure profiles to parent PCBs. The concentration ratios of OH-PCBs to PCBs (OH-PCBs/PCBs) were calculated and the highest value was found in foxes, followed by badgers, raccoons, mongooses, dogs, raccoon dogs, MP civets, and cats (Fig. 3). The OH-PCBs/PCBs ratios in the blood of cats (0.12) and MP civets (0.12) were similar to that reported for human blood (0.22–0.37) (Nomiyama *et al.*, 2010b). Meanwhile, the ratios observed in the blood of other terrestrial mammals (1.1–9.1) were one order of magnitude higher than those in human and 2 to 4 orders of magnitude higher than in marine mammals (0.0009–0.069) (Kunisue and Tanabe, 2009; Nomiyama *et al.*, 2010a), but the values were lower than those

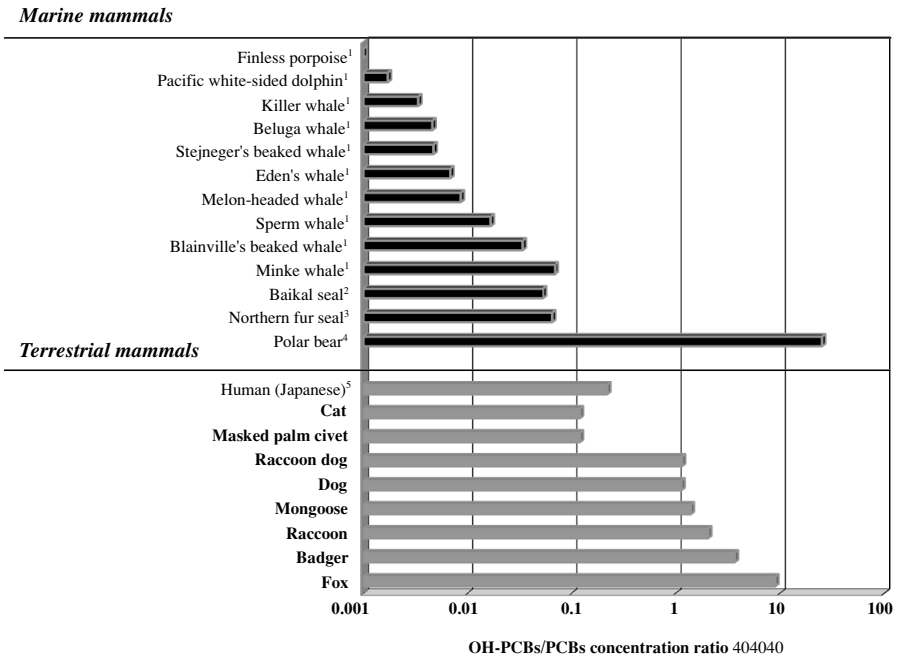

Fig. 3. Concentration ratios of OH-PCBs to PCBs in the blood of marine and terrestrial mammals. (1: Nomiyama *et al*., 2010a, 2: Imaeda *et al*., 2008, 3: Kunisue and Tanabe, 2009, 4: Gebbink *et al*., 2008, 5: Nomiyama *et al*., 2010b).

in polar bear blood (Gebbink *et al*., 2008). It is presumed that this could be due to higher metabolic capacity and/or binding affinity of OH-PCBs to TTR in these terrestrial mammals. Especially, cats may preferentially metabolize lower chlorinated PCBs and retain the hydroxylated metabolites in blood. They may not metabolize phenolic compounds due to lack of glucuronate conjugation ability (Watkins and Klaassen, 1986). Therefore, it is estimated that cats have potentially high risk to some phenolic compounds including halogenated metabolites.

Higher concentrations of OH-PBDEs were observed in the cats (6900 pg/g) and mongooses (6700 pg/g) followed by raccoons (960 pg/g), MP civets (290 pg/g), foxes (63 pg/g), raccoon dogs (63 pg/g), badgers (36 pg/g) and dogs (3.6 pg/g) (Fig. 1). The dominant isomers of OH-PBDEs were 6OH-BDE47 and 2′OH-BDE68 accounting for up to 80% of the total amount of OH-PBDEs in the blood of all the terrestrial mammals (Fig. 2). Especially, accumulation of 6OH-BDE47 was prominent in all species. Although, these two abundant congeners were already reported as natural products in the marine environment (Gribble, 2000; Hakk and Letcher, 2003; Nomiyama *et al*., 2011), they were also accumulated in terrestrial mammals. 2′OH-BDE28 and 5OH-BDE47 were detected only in the blood of cats, and 4′OH-BDE49 was detected in those of cats and foxes (Fig. 4).

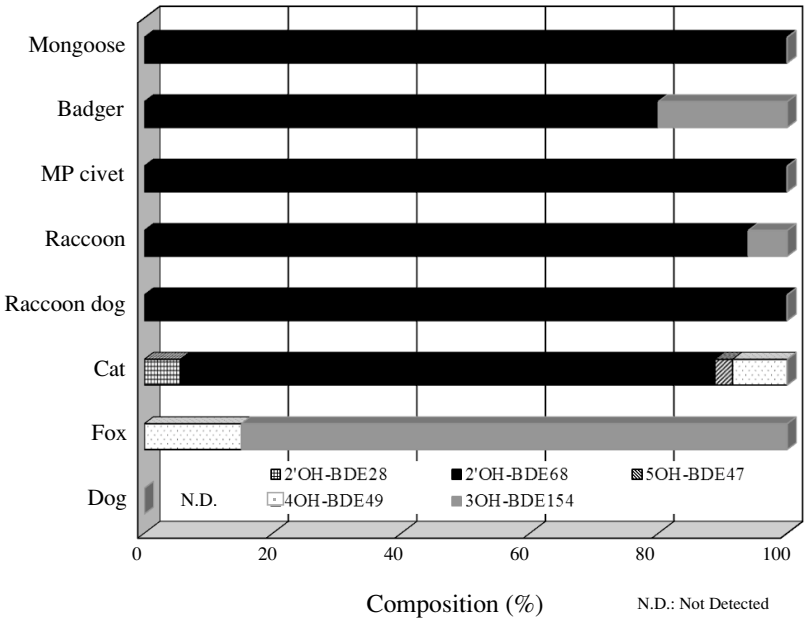

Fig. 4. OH-PBDE congener patterns except 6OH-BDE47 in the blood of terrestrial mammals.

2′OH-BDE28, 5OH-BDE47 and 4′OH-BDE49 have been observed as metabolites of PBDEs in animals from *in vitro* experiments (Marsh *et al*., 2006; Qiu *et al*., 2007). It is suggested that 4′OH-BDE49 in the cats and foxes could be the metabolites of BDE47. Moreover, 3OH-BDE154 was found in the blood of foxes, raccoons and badgers (Fig. 4). Accumulation of 3OH-BDE154, probably the metabolite of BDE154, is found in the blood of wild terrestrial mammals for the first time, in the present study. The structure of 3OH-BDE154 is similar to T4, in which the binding of OH group at *meta*-substituted position is adjacent to brominated atoms. It was reported that 3OH-BDE47 and 4OH-BDE90, which also have similar structures as T4, have higher TTR-binding potencies, and markedly inhibited the binding of T3 to TRα and acted as TH-like agents (Hamers *et al*., 2008; Kitamura *et al*., 2008).

Moreover, concentrations of OH-PBDEs in terrestrial mammals except cats and mongooses were lower than in marine mammals, although the concentrations of OH-PCBs in terrestrial mammals were one to three orders of magnitude higher than those of cetacean species (Fig. 1). This result suggests that the origin of large percentage of OH-PBDEs in cats and mongooses may be from marine natural products synthesized by algae, sponges and cyanobacteria, and also accumulation of OH-PBDEs may be low in carnivorous species. In contrast, OH-PBDEs levels in cats and mongooses were higher than in marine mammals (Fig. 1). This might be due to higher accumulation of OH-PBDEs in cats and mongooses than in other

carnivorous species, easier metabolism of PBDEs, and demethylation of MeO-PBDEs which are also natural compounds in the marine environment (Teuten *et al*., 2005). Especially, cats prefer to eat fishes (Houpt and Smith, 1981) and they are likely used for main ingredients of pet food. So, it can be presumed that cats are highly exposed to OH- and MeO-PBDEs originating from the marine environment through the pet food, although no experimental evidence is available. In future, further studies on the metabolism and toxicity of OH-PBDEs in various terrestrial mammals and analysis of pet food are needed.

The present study indicates that the metabolic capacity and characteristic of PCBs and PBDEs in terrestrial mammals varies widely among species. Particularly, cats, raccoons and mongoose showed specific accumulation patterns for OH-PCBs and OH-PBDEs, suggesting that they are high-risk animals of hydroxylated metabolites of PCBs and PBDEs.

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