Species-specific differences in the accumulation features of organohalogen contaminants and their metabolites in the blood of Japanese terrestrial mammals

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ABSTRACT

Residue levels and patterns of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), their hydroxylated metabolites (OH-PCBs, OH-PBDEs), and methoxylated PBDEs (MeO-PBDEs) in the blood of various terrestrial mammals in Japan, including cats, raccoon dogs, dogs, masked palm civets, foxes, raccoons, badgers, and mongooses were determined. Tri- through penta-chlorinated OH-PCBs were predominant in cat blood, whereas hexa- through octa-chlorinated OH-PCBs were found in other species. High proportion of BDE209 was found in all species, suggesting exposure to municipal waste and soil containing higher levels of deca-BDE products. $6OH$-/MeO-BDE47 and $2'OH$-/MeO-BDE68 were dominant in all terrestrial mammals. This is first report on the detection of OH-/MeO-PBDEs in the blood of terrestrial mammals. High concentrations of OH-/MeO-PBDEs were found in cats, suggesting the intake of these compounds from seafood. Cats exhibited higher accumulation and specific patterns of OH-PCBs, OH-PBDEs, and MeO-PBDEs, they may be at a high risk from these metabolites.

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1. Introduction

Organohalogen compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental contaminants used in industrial applications and as synthetic flame retardants, respectively. Because of their persistence and bioaccumulation potential, these contaminants are widely distributed in the environment and accumulate in both aquatic and terrestrial food webs (Alaee et al., 2003; Law et al., 2006; Letcher et al., 2010). Adverse toxic effects of these contaminants have been widely reported, including neurotoxicity and endocrine disruption (Safe, 1994; Siddiqi et al., 2003).

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. These hydroxylated compounds are conjugated with glucuronate conjugate, glutathione conjugate, and sulfoconjugate and some of these conjugates,

especially glucuronate and glutathione conjugates, are eliminated from the body through phase II reaction. However, their structural similarity to thyroxine (T4) allows some OH-PCBs to bind competitively with thyroid hormone (TH) transport proteins, such as transthyretin (TTR), thyroxine-binding globulin (TBG), and albumin which is also transporting steroid hormones (Hall et al., 2003), and thus OH-PCBs are carried by blood to organs and tissues. It is well-known that two of the main toxic effects of these hydroxylated metabolites are disturbances to thyroid hormone (TH) homeostasis and the cerebral nervous system (Miyazaki et al., 2004; Purkey et al., 2004).

OH-PBDEs are well-known metabolites of PBDEs and also natural products found in marine organisms, such as red algae and sponges, which contain cyanobacteria (Gribble, 2000; Hakk and Letcher, 2003). Methoxylated PBDEs (MeO-PBDEs) have also been found in bivalves, salmon, several shark species, and beluga whales at concentrations higher than anthropogenic PBDEs (Haraguchi et al., 2009; Kelly et al., 2008). These MeO-PBDE congeners have been found as natural products in True's beaked whale (*Mesoplodon mirus*) (Teuten et al., 2005). Moreover, the bioaccumulation of MeO-PBDEs has been reported in marine food webs (Kelly et al., 2008). The possibility that some OH-PBDEs are formed through

the demethylation of MeO-PBDEs has been demonstrated in rats, chicken, and rainbow trout (Wan et al., 2009). Thus, the formation of OH-PBDEs may occur predominantly through the demethylation of MeO-PBDEs by CYPs, rather than by the metabolism of parent PBDEs. In contrast, it has also been suggested that some MeO-PBDEs are formed through the methylation of OH-PBDEs (Wan et al., 2010).

Hydroxylated metabolites of PCBs and PBDEs have been detected in the blood, liver, and brain of various marine mammals and birds (Gebbink et al., 2008; Jaspers et al., 2008; Kunisue and Tanabe, 2009; Nomiyama et al., 2011a, 2010a). Previous studies indicate that the metabolic capacity of organohalogen compounds differ widely among animal species (Kunisue and Tanabe, 2009; Verreault et al., 2008). However, information on the status of PCB and PBDE metabolites is limited in terrestrial mammals with the exception of humans. In particular, PBDE metabolites have not been examined in wild terrestrial mammals. However, Kunisue and Tanabe (2009) reported the OH-PCB residue levels in the blood of one specimen of a Japanese terrestrial mammal, although the individual congener patterns of lower-chlorinated OH-PCBs (3-4Cl) were not identified (Kunisue and Tanabe, 2009).

Carnivorous species are known to have a higher metabolic capacity for organohalogen compounds. The levels of PCB hydroxylated metabolites were found to be higher than parent PCBs in the blood of carnivorous species (Kunisue and Tanabe, 2009). Beagle dogs rapidly produce PCB metabolites compared to cynomolgus monkeys (Sipes et al., 1982). PBDE concentrations in the red fox from Belgium were lower than those of voles and mice, which are the main prey species of the red fox (Voorspoels et al., 2006). Furthermore, it was also reported that drug-metabolizing enzymes are induced depending on the hepatic levels of contaminants, which metabolize PCBs and PBDEs in raccoon dogs (Kunisue et al., 2008). These interesting observations suggest the need for studies on the OH-PCB residue levels in these species. It is also known that pet dogs have higher organochlorine metabolic and elimination capacities than pet cats (Kunisue et al., 2005). These studies on carnivorous species suggest that the toxicological risk of hydroxylated PCB and PBDE metabolites in the blood may vary among carnivorous species and some may be at a higher-risk from these metabolites.

The present study elucidated the accumulation features of PCBs, PBDEs, their hydroxylated metabolites, and MeO-PBDEs, as well as the species-specific metabolic capacities of different species by analyzing the blood samples of various carnivores collected in Japan including cats, raccoon dogs, dogs, masked palm civets (MP civet), foxes, raccoons, badgers, and mongooses. Furthermore, we attempted to estimate the high-risk species of hydroxylated metabolites in terrestrial mammals which can lead to further studies on risk assessment.

2. Materials and methods

2.1. Blood collection from carnivorous animals

Whole blood samples were collected from eight carnivorous species in various regions of Japan between 2006 and 2011, as shown in Table 1 and Table S1, i.e., cats (Felis silvestris catus), raccoon dogs (Nyctereutes procyonoides), dogs (Canis lupus familiaris), masked palm civets (MP civet, Paguma larvata), foxes (Vulpes vulpes japonica), raccoons (Procyon lotor), badgers (Meles meles), and small Asian mongoose (Herpestes javanicus). Animals collected during this study were wild, including stray cats and stray dogs, found dead in the street due to traffic accident related traumas or which were provided by hunters after mammalian pest control activities. Although nutritional status and lipid contents of these samples were not analyzed, there was no indication that any of the animals were severely malnourished. The blood samples were collected directly from the hearts by dissection of the terrestrial mammal cadavers. The samples from traffic accident cases included partially coagulated blood samples because cadavers were left for several hours

posthumously. The collected blood samples were stored at −25 °C in the Environmental Specimen Bank (es-BANK) of Ehime University, Japan until analysis (Tanabe, 2006).

2.2. Analytical procedures

The analytical details are described in Supplemental data and elsewhere (Nomiyama et al., 2011a, 2010a), and briefly summarized here. Denatured whole blood (approximately 10 g) was extracted with methyl t-butyl ether (MTBE)/hexane. $^{13}C_{12}$-labeled compounds (PCBs, PBDEs, OH-PCBs, and OH-PBDEs) were spiked as internal standards. After partition using potassium hydroxide (KOH), the neutral fraction containing PCBs, PBDEs, and MeO-PBDEs was passed through activated silica-gel after fat removal by gel permeation chromatography (GPC), and subsequent concentration. The KOH solution phase was re-extracted twice with MTBE/hexane. The organic fraction containing OH-PCBs and OH-PBDEs was passed through a column packed with 5% H_2O deactivated silica-gel, and derivatized overnight using trimethylsilyldiazomethane. The derivatized solution was passed through activated silica-gel after fat removal by GPC, and concentrated. The target compounds determined were tri- to deca-PCBs (56 congeners), tetra- to deca-PBDEs (11 congeners), tri- to octa-OH-PCBs (52 congeners), tri- to octa-OH-PBDEs (23 congeners), and tri- to penta-MeO-PBDEs (15 congeners). A complete list of the PCBs, PBDEs, their hydroxylated metabolites, and MeO-PBDE congeners is provided in the Supporting Information (Tables S2–S6).

Identification and quantification of PCB, tetra- to hepta-PBDEs, OH-PCBs, OH-PBDEs, and MeO-PBDEs were performed using a gas chromatograph (GC: 6890 series, Agilent)/high resolution (>10,000) mass spectrometer (HRMS: JMS-800D, JEOL). Octa- to deca-PBDEs were quantified using GC (7890 series, Agilent)/low resolution MS (5975 series, Agilent) and operated in the electron capture negative ionization (ECNI) mode. Detailed descriptions of the measurement condition are reported elsewhere (Eguchi et al., 2011; Nomiyama et al., 2011a, 2010a).

2.3. Quality assurance and quality control

Target compounds were quantified using the isotope dilution method for the corresponding $^{13}C_{12}$-internal standards according to a formula described previously (Nomiyama et al., 2011b). The following criteria were used: (a) the GC retention times matched that of the standard within ±0.1 min, (b) the signal to noise ratio (S/N) was greater than 10, and (c) the deviation of the ion intensity ratio was within 15% of the standards. One procedural blank was analyzed with each batch of 6–8 samples using the same protocol that was applied to the samples, to detect any possible contamination from solvents and glassware. The limit of quantification (LOQ) was defined as the concentration of target compounds that produced an S/N of 10. Recoveries for the $^{13}C_{12}$-labeled internal standard in this analytical procedure were within 85–105% for PCBs, 50–103% for PBDEs, 59–135% for OH-PCBs, 30–129% for OH-PBDEs and 50–115% for MeO-PBDEs.

2.4. Statistical analysis

The Mann–Whitney U-test was used to test significant differences of the concentrations of target compounds between species. Spearman's rank correlation coefficients were calculated to compare the concentrations of PCBs, OH-PCBs, PBDEs, OH-PBDEs, and MeO-PBDEs in each species. p-values < 0.05 were considered significant. All statistical analyses were carried out using Statcel 97.

3. Results and discussion

PCBs, PBDEs and MeO-PBDEs are neutral lipophilic compounds strongly distributed in the lipids. However, OH-PCBs and OH-PBDEs remain in the blood plasma by binding with a strong affinity to the thyroid hormone binding protein in the blood. In the present study, to compare OH-PCBs, OH-PBDEs and MeO-PBDEs levels, the concentrations in blood were expressed as pg g^{-1} whole blood wet wt. base. Total OH-PCBs and total OH-PBDEs in present study shows only the congeners for which standards are available in our laboratory and which are shown in Tables S3 and S5.

3.1. Accumulation features of PCBs and OH-PCBs

In this study, 1 of 10 dogs, 1 of 13 raccoons, and 2 of 6 badgers had PCB levels below the detection limit. High concentrations of total PCBs were found in mongooses and MP civets than other species ($p \leq 0.05$) (Table 1 and Table S2). Hexa- and hepta-chlorinated PCBs congeners were predominant in the blood of all terrestrial mammals (Fig. 1A). The concentrations of PCBs in