ORGANOHALOGEN CONTAMINANTS AND THEIR HYDOXYLATED METABOLITES IN THE BLOOD OF JAPANESE TERRESTRIAL MAMMALS

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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[1, 2].

OH-PBDEs are well known metabolites of PBDEs as well as natural products found in marine organisms like red algae and sponges[3, 4]. Recently it has also been found that some OH-PBDEs are formed through demethylation of methoxylated PBDEs (MeO-PBDEs) in some animals[5]. MeO-PBDEs also have been found as natural products in marine organisms[6]. Additionally, bioaccumulation has been reported in marine food webs[7]. It has also been suggested that some MeO-PBDEs are formed through methylation of OH-PBDEs[8]. Sometimes, concentrations of the natural MeO-/OH-PBDEs are greater than anthropogenic PBDEs.

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[9]. Moreover, beagle dogs quickly form metabolites of PCBs compared to cynomolgus monkeys[10]. On the other hand, drug-metabolizing enzymes are induced in raccoon dogs depending on the hepatic levels of contaminants, and metabolized PCBs and PBDEs[11]. Concentration of PBDEs in the red fox from Belgium were lower than those of voles and mice, the main prey species of the red fox[12]. This result shows the red fox has the strong metabolic capacity and eliminate lower brominated congeners[12]. Carnivorous species might have high-risk to metabolites of PCBs and PBDEs. However, information on status of PCBs and PBDEs metabolites in terrestrial mammals is limited.

The present study elucidates the accumulation features of PCBs, PBDEs, their hydroxylated metabolites and MeO-PBDEs, 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, and the origin of OH-PBDEs.

Materials and methods

Blood samples of terrestrial mammals [cats (*Felis silvestris catus*); dogs (*Canis lupus*); raccoons (*Procyon lotor*); foxes (*Vulpes vulpes japonica*); raccoon dogs (*Nyctereutes procyonoides*); masked palm civets (MP civet, *Paguma larvata*); badgers (*Meles meles*); small Asian mangoose (*Herpestes javanicus*)] were collected from various regions of Japan from 2006 to 2009. Animals collected in this study were 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) were 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, PBDEs and MeO-PBDEs was passed through activated silica-gel packed in a 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, OH-PBDEs and MeO-PBDEs, and DB-1MS for PCBs and PBDEs, J&W Scientific) and operated in electron impact and selected ion monitoring mode (EI-SIM).

Results and discussion

PCBs and OH-PCBs were detected in the blood of all the terrestrial mammals in this study. Metabolic capacity of PCBs in terrestrial mammals was found to vary widely among species. These variations could be due to species-specific metabolic capacity by phase I CYP and/or phase II conjugation enzymes, binding affinity to TTR, and exposure profiles of parent PCBs. Cats and raccoons showed specific accumulation pattern of OH-PCBs that is different from other mammals analyzed in this study. Especially, cats may preferentially metabolize lower chlorinated PCBs and retain the metabolites in the blood. They may not metabolize phenolic compounds due to lack of glucuronate conjugation ability[13]. Therefore, cats might have potentially high risk to some phenolic compounds including halogenated metabolites.

The concentration of PBDEs was found in higher concentrations in the blood of cats than in other species. The PBDE congener pattern in terrestrial mammals showed high proportion of BDE209. This might suggest the exposure through house dust and garbage in the terrestrial mammals. It was already reported that BDE209 does bioaccumulate in terrestrial top predators, such as the red fox[12]. Moreover, BDE209 was the dominant isomer in Japanese human blood[14]. PBDE profiles in human blood reflect recent exposure[15], considering that technical deca-BDE is in use in Japan at present. It is highly probable that Japanese wildlife is exposed to deca-BDE derived from technical products.

The concentration of OH-PBDEs observed in the cats and mongooses were 1 to 2 orders of magnitude higher than those in other species (Table 1). The dominant isomers of OH-PBDEs were 6OH-BDE47 and 2´OH-BDE68 accounting for up to 80% of total amount of OH-PBDEs in the blood of all the terrestrial species (Fig. 2). Especially, 6OH-BDE47 accumulation was prominent in all species. Although, these two abundant congeners were already reported as natural products in marine environment[3, 4], they were also accumulated in terrestrial mammals. 2´OH-BDE28 and 5OH-BDE47 were detected only in the blood of cats, and 4OH-BDE49 was detected in those of cats and foxes (Fig. 2). 2´OH-BDE28, 5OH-BDE47 and 4OH-BDE49 have been observed as metabolites in animals from *in vitro* experiments[16, 17]. It is suggested that cats and foxes metabolize PBDEs which then remains into the body. Moreover, 3OH-BDE154 was presented in the blood of foxes, raccoons and badgers (Fig. 2). Accumulation of 3OH-BDE154, probably the metabolite of BDE154, was found in the blood of wild terrestrial mammals for the first time. The structure of 3OH-BDE154 is similar to T4, in which binding of

Table 1 Arithmetic mean (±SD) of concentrations (pg/g wet wt) of total PBDEs, total OH-PBDEs, total MeO-PBDEs and total OH-PCBs in the blood of terrestrial and marine mammals.

Species	*n*	Total PCBs	Total PBDEs	Total OH-PCBs	Total OH-PBDEs	Total MeO-PBDEs
Cats	5	4600⊥6400	5500_12000	680⊥450	6900_6200	550⊥770
Dogs	2	180±140	9.8±14	260±320	10±0.53	81±120
Raccoons	6	700±1400	10±14	6900±2700	940±680	<10
Foxes	4	250±150	34±24	290±230	63±50	<10
Raccoon dogs	8	360±360	74±130	640±530	37±32	<10
MP civets	8	370=90	160±360	2300±4000	290±570	26±51
Badgers	6	160+290	5.5+16	1200+140	36+69	<10
Mongooses	2	27000±3500	1100±860	37000±4200	6700=3500	230±320
Finless porpoises[20,21]	7	33000±15000	480±550	40±15	590±590	700=1300
Beluga whale[20,21]	1	33000	270	150	53	410
Sperm whales[20,21]	2	22000±24000	520±280	140±40	2300±10	1000±940
Killer whales[20,21]	3	2100000±3100000	24000±26000	4200±4100	1400=1900	6200±6200
Common minke whales[20,21]	2	5300±100	760±900	360±17	410±140	580±27
Polar bears[22]	20	40000±9000	1200±100	1000000±130000	2900=1000	160±60

OH group at *para*-substituted position is adjacent to halogenated atoms at *meta*-positions. 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[18, 19]. Therefore, these species may compete with TH for binding to TTR, resulting in the disturbance of TH homeostasis.

Moreover, the 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[20] (Table 1). This result suggests that the origin of large percentage of OH-PBDEs from natural products, do exist in marine environment, and carnivore species may have OH-PBDEs accumulation feature differing from those of OH-PCBs. In contrast, the concentrations of OH-PBDEs in cats and mongooses were higher than marine mammals[20, 21] (Table 1). This might be due to higher accumulation of OH-PBDEs in cats and mongooses than in other species, easier metabolism of PBDEs, and demethylation of MeO-PBDEs which are also natural compounds in the marine environment[6]. Accumulation of MeO-PBDEs in the blood of terrestrial mammals were observed in mongooses, cats, dogs, MP civets and badgers (Fig. 3). High concentrations of MeO-PBDEs were found in the cats. Among the MeO-PBDE isomers, only 6MeO-BDE47 and 2´MeO-BDE68 were detected in the blood of animals. These two abundant MeO-PBDE isomers occur naturally in marine organisms[6]. Moreover, accumulation patterns of MeO-PBDEs are different among carnivorous species. Recent study indicates hydroxylation of anthropogenic PBDEs is negligible in some species[5]. Interconversion between MeO-PBDEs and OH-PBDEs is also reported in *in vivo* studies[8]. Possibly, some terrestrial species such as cats and mongooses may have high ability of interconversion, and demethylation of MeO-PBDEs might be considered a possible source of OH-PBDEs. Especially, cats eat fishes and they are the main ingredients of pet food in Japan. So, it can be presumed that pet cats are exposure to OH-/MeO-PBDEs originated from the marine environment. In future, further studies on the metabolism and toxicity of OH-PBDEs in various terrestrial mammals are needed.

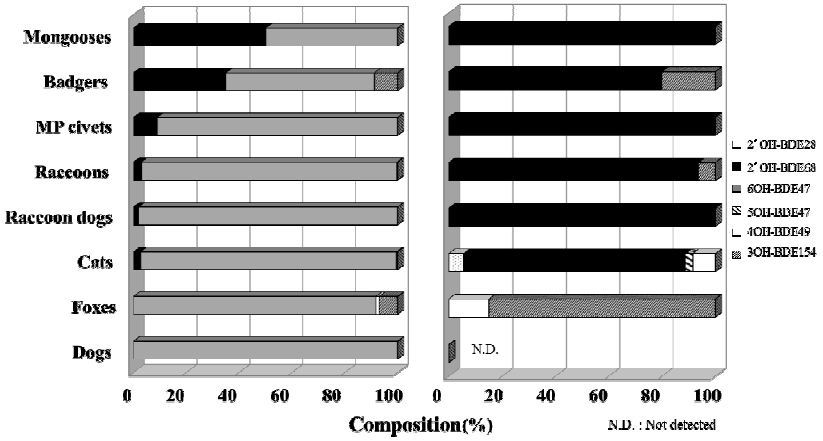

Fig. 2 (Left) Composition of OH-PBDEs homologues in the blood of Japanese terrestrial mammals. (Right) Composition of OH-PBDEs homologues except 6OH-BDE47 in the blood of Japanese terrestrial mammals.

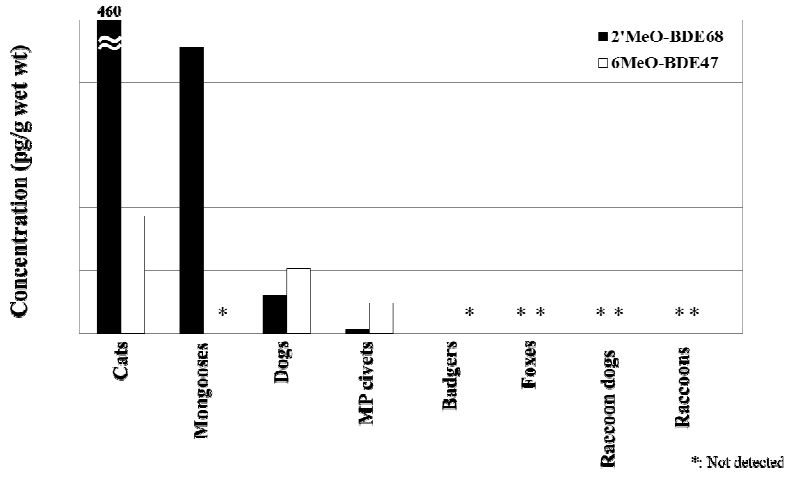

Fig. 3 Concentrations of MeO-PBDEs (pg/g wet wt) in the blood of terrestrial mammals.

The present study indicates that the metabolic capacity of PCBs and PBDEs in terrestrial mammals varies widely among species. Particularly, cats, raccoons and mongoose showed specific pattern of accumulation of hydroxylated compounds which is different from other mammals, suggesting that they are high-risk animals of hydroxylated

metabolites of PCBs and PBDEs.

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