ORGANOHALOGEN COMPOUNDS AND THEIR METABOLITES IN THE BLOOD OF PET DOGS AND CATS, AND IN PET FOOD

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Introduction

Organohalogen compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have been widely used for industrial applications since the 1960s and 1970s. As a consequence, PCB and PBDE levels have been increasing dramatically in the environment, wildlife and humans, because of their persistent properties and bioaccumulation characteristics[1,2]. Toxic effects of these compounds on endocrine systems and neurodevelopment are already well known[3,4]. It is suspected that the thyroid hormone homeostasis is disturbed by not only PCBs and PBDEs but also their hydroxylated metabolites[5]. Hydroxylated PCBs (OH-PCBs) are formed by the oxidative metabolism of PCBs by cytochrome P450 (CYP) monooxygenase enzyme systems. Hydroxylated PBDEs (OH-PBDEs) are well-known metabolites of PBDEs[6,7], and also natural substances found in marine organisms, such as red algae and sponges[8,9], and formed through demethylation of methoxylated PBDEs (MeO-PBDEs) [10].

Recently, a study found higher concentrations of PBDEs in pet cats than in human serum[11], and it has been hypothesized that increases of feline hyperthyroidism (HT) are related to increased PBDE exposure, with key routes of exposure being diet and ingestion of house dust. The PBDE levels in California house cats were approximately two fold higher than east coast cats[11,12]. On the other hand, dogs have much lower serum levels of PBDEs than cats due to their high metabolic capacity for these compounds[13]. Domestic pets, such as dogs and cats, who share their environment with their custodians can be biosentinels for human, especially infants, exposure to environmental contaminants. Moreover, increased thyroid hyperfunction disease may be linked to OH-PCBs and OH-PBDEs derived from metabolites of PCBs and PBDEs, diet, natural products, and/or demethylation of MeO-PBDEs. However, information on status of hydroxylated metabolites of PCBs and PBDEs in pet animals and pet food are limited.

The present study determined the concentrations and accumulation patterns of organohalogen contaminants such as PCBs and PBDEs and their metabolites (OH-PCBs, OH-PBDEs and MeO-PBDEs) in the whole blood of pet cats and pet dogs collected from veterinary hospital in Japan. Moreover, to estimate the dietary PCB and PBDE exposure levels and biotransformation to hydroxylated metabolites in pet animals, PCB, PBDE and their derivatives (OH-PCBs, OH-PBDEs, and MeO-PBDEs) in the representative dry and wet pet food samples were also analyzed.

Materials and methods

Sample collection

The blood of pet dog and cat samples were collected at Nakatsu veterinary surgery hospital in Osaka and Tao veterinary hospital in Hiroshima, Japan from 2009 to 2012. All of the pet animals custodians completed a questionnaire providing information about their animals (age, weight, sex etc), eating habits of pets (dry or wet food, frequency), and living area (indoor or outdoor). Commercially dry and wet pet food products were purchased in Ehime, Japan in 2010.

Analytical procedures

The analytical details are described in previous studies[14-16]. Briefly, whole blood (approximately 5 g) were denatured with 6 M HCl and homogenized with 2-propanol and 50% methyl t-butyl ether (MTBE)/hexane. The food samples (approximately 10 g) were crushed to pieces in a mortar and homogenized with 6 M HCl, 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 removal of fat by gel permeation chromatography (GPC), and eluted with 5% DCM/hexane and concentrated. Only in the neutral fraction of pet food samples,

lipids were removed using sulfuric acid before GPC. The KOH solution phase was acidified with sulfuric acid and re-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% H2O 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 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) was operated in electron impact and selected ion monitoring mode (EI-SIM). The higher brominated PBDEs (Octa-Deca BDEs) were quantified using GC (7890 series, Agilent)/MS (5975 series, Agilent).

Results and discussion

Residue levels and profile of PCBs and OH-PCBs in pet blood and food

Mean concentrations (on a wet weight basis: mean ± standard deviation) of total PCBs detected in pet dogs (18 ± 35 pg/g) and cats (93 ± 99 pg/g) are one order of magnitude lower than in stray cats and dogs reported previously[17] and household cats in US[12] (Table 1). In some of the dog and cat food, higher PCB levels were found than thoes detected in the pet blood. PCB concentrations of dry food both for dogs (140 ± 57 pg/g) and cats (640 ± 730 pg/g) were higher than in wet food (dog: 20 ± 26 pg/g, cat: 130 ± 160 pg/g) (Table 1.). It is suggested that PCBs in raw materials are accumulated and concentrated during the manufacturing process. Lower concentration of PCBs found in the dog suggests that dogs have higher metabolic capacity for PCBs. Penta- through hepta-chlorinated PCB congeners were predominant in pet blood and pet food.

Mean concentrations of OH-PCBs (on a wet weight basis: mean ± standard deviation) in the blood of dogs and cats were 220 ± 260 and 150 ± 80 pg/g, respectively (Table 1). However, OH-PCB levels in pet food (range: 0.52-1.3) were 2－3 orders lower than in blood (Table 1). Tri- and penta-chlorinated OH-PCB congeners, especially 4′OH-CB18, 4OH-CB25/31/4′OH-CB26, were predominant in cat blood. Accumulation of hexa- through octa-chlorinated OH-PCBs, especially 4OH-CB199, 4OH-CB202, has been found in dog blood. These results were similar to a previous terrestrial mammal study[17]. 4OH-CB79 was found in dry and wet pet food samples, though at very low level. Other congeners such as 4OH-CB97 and 4OH-CB107 were found in one dog and cat wet food at negligible level. These results indicate that the OH-PCBs detected in pet dogs and cats are metabolites of PCBs because pet food did not contain OH-PCBs.

Residue levels and profile of PBDEs, OH-PBDEs and MeO-PBDEs

PBDEs were found in ten of thirteen samples of dog blood and at six of eight cat blood samples. Mean concentration (on a wet weight basis: mean ± standard deviation) of total PBDEs in dog and cat blood were 140 ± 130 and 200 ± 170 pg/g, respectively (Table 1). The concentration of PBDEs in dog bloods was similar to the one previously detected in Japanese stray dogs[17] and lower than in the serum of pet dogs in US[13]. On the other hand, the PBDE levels in cat bloods was one to four orders lower than Japanese stray cats[17] and pet cat in US[11,12]. These results demonstrated that pet dogs and cats in US were exposed to high concentrations of PBDEs from furniture and household electrical appliance than in Japan, suggesting higher PBDE contamination in the indoor environment in US. The PBDE congener pattern in Japanese pet dogs and cats showed high proportion of BDE209. Moreover, BDE206 and BDE207 were also found in one pet dog blood although at very low concentrations (22 and 23 pg/g, respectively). The dog serum in US were dominated by BDE47, BDE153 and BDE209[13], and predominant PBDE congeners in US and Swedish cats were BDE47, BDE99 and BDE209[11,12,18]. Interestingly, in previous studies on pet cats and dogs, the presence of BDE209 was significantly associated with a dry food diet[11-13]. In this study, comparable levels of PBDEs in pet dry food (dog: 180 ± 42 pg/g, cat: 330 ± 250 pg/g) to pet blood were found (Table 1), and BDE209 was the dominant congener in pet food except cat wet food. Hence, high levels of BDE209 in the blood of pet dogs and cats may be a result of dry food consumption.

Concentrations of OH- and MeO-PBDEs in the bloods of pet dogs were 2.0 ± 4.1 pg/g and 7.3 ± 22 pg/g pg/g, respectively (Table 1), which were much lower than those of PBDEs. Moreover, mean concentration of OH-PBDEs in the dog food (dry: 3.4 ± 4.6 pg/g, wet: 1.2 ± 2.3 pg/g) were similar to the blood levels. On the other hand, mean concentration of MeO-PBDEs in the dog food (dry: 110 ± 180 pg/g, wet: 140 ± 270 pg/g) were two orders higher than in the blood.

Table 1. Mean concentrations ± standard deviation (pg g-1 whole blood wet wt.) of PCBs, PBDEs, OH-PCBs, OH-PBDEs, and MeO-PBDEs in the blood of pet dogs and cats and commercially pet food.

Species/sample no.	*n*	Total PCBs	Total PBDEs	Total OH-PCBs	Total OH-PBDEs	Total MeO-PBDEs
Dog Blood	13	18 ± 35	140 ± 130	220 ± 260	2.0 ± 4.1	5.0 ± 22
Cat Blood	8	93 ± 99	200 ± 170	150 ± 80	710 ± 560	270 ± 880
Dog Dry Food	4	140 ± 51	180 ± 42	0.94 ± 0.72	3.4 ± 4.6	110 ± 180
Dog Wet Food	4	20 ± 26	14 ± 23	0.99 ± 0.85	1.2 ± 2.3	140 ± 270
Cat Dry Food	4	640 ± 730	330 ± 250	0.52 ± 0.60	21 ± 21	870 ± 450
Cat Wet Food	4	130 ± 160	74 ± 140	1.3 ± 1.4	74 ± 62	4000 ± 1900

Cncentrations of OH-/MeO-PBDEs in the blood of pet cats were 710 ± 560 pg/g and 420 ± 880 pg/g pg/g, respectively (Table 1), which were much higher than dog blood, and also higher than those of PCBs, OH-PCBs, and PBDEs. Among the OH-/MeO-PBDE isomers, only 6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68 were detected in the blood of dogs and cats and pet food. These two abundant isomers are produced naturally by marine organisms such as sponges, algae, and cyanobacteria[9]. Especially, high concentrations of 6OH-BDE47 and 2′MeO-BDE68 were found in cat blood (Fig. 1), suggesting the possible presence of marine natural products in the raw materials of cat food.

Estimation of PBDEs, OH-PBDEs, and MeO-PBDEs exposure from pet food

In this study, low concentrations of OH-PBDEs in both dog blood and dog food were found. This result suggests that uptake of naturally produced OH-PBDEs within the dog food is very low. Low concentrations of MeO-PBDEs were detected in the blood of dogs, although higher concentrations of MeO-PBDEs than OH-PBDEs in dog food were found. It can be suggested that dogs may have high metabolic capacities for MeO-PBDEs through phase II reaction, or low TTR binding potencies of MeO-PBDEs in dogs.

MeO-PBDE levels in cat food (on a wet weight basis, mean ± standard deviation: 870 pg/g for dry type, 4000 pg/g for wet type) were one order higher than in cat blood (Table 1). However, OH-PBDE levels in cat food (dry: 21 ± 21 pg/g, wet: 74 ± 61 pg/g) were lower than those of the blood (Table 1). Moreover, the concentrations of 6MeO-BDE47 and 2′MeO-BDE68 in cat food containing seafood materials were higher than those of OH-PBDE levels (Fig. 1). These results suggest that pet cats are exposed to 6MeO-BDE47 and 2′MeO-BDE68 through cat food particularly those with added fish flavor, and a portion of 6OH-BDE47 in cat blood might be the result of the biotransformation from the naturally produced 6MeO-BDE47 via biogenic demethylation (Fig. 2). On the other hand, 2′OH-BDE68 level in the cat blood was lower than 2′MeO-BDE68 (Fig. 1). This result suspects that cats may have low binding affinity of 2′OH-BDE68 to TTR and/or low potency of demethylation for 2′MeO-BDE68. A recent study reported that OH-PBDEs have the ability to interact with thyroid hormone receptors[19], possibly disturbing thyroid function. Cats may not metabolize phenolic compounds due to lack of glucuronate conjugation ability[20]. Therefore, cats might have potentially high risk to some phenolic compounds including halogenated metabolites as a result of continuous exposure by feeding cat food containing seafood materials.

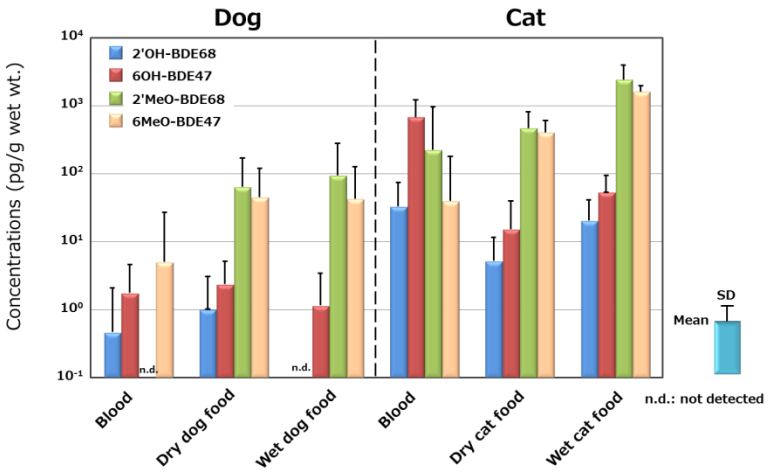

Fig. 1 Mean concentrations of OH-PBDEs and MeO-PBDEs in the blood of pet dogs and dog food (left), and the blood of pet cats and cat food (right).

This is the first study to analyze PCBs, PBDEs, OH-PCBs, OH-PBDEs and MeO-PBDEs in the blood of pet animals and pet food. Higher concentrations of 6OH-BDE47 have been found in the blood of pet cat than pet dogs. It is suggested that 6OH-BDE47 in the blood of cats are via intake from cat food, metabolites of PBDEs, and demethylation of 6MeO-PBDEs containing fish flavored cat food. These results indicate possibility of

induction of adverse health effects, especially feline HT, due to the consumption of cat feed made from fishery product. In future studies, we need to investigate the metabolic capacities to halogenated compounds including phase II conjugation reactions, and assess the toxicological risk posed by these hydroxylated metabolites.

Acknowledgements

We thank Prof. Annamalai Subramanian, Center for Marine Environmental Studies (CMES), Ehime University, Japan, for the critical reading of this manuscript. This study was supported by Research Fellowships of the Japan Society for the Promotion of Science (JSPS) for Young Scientists to Hazuki Mizukawa (23-4511) and Grants-in-Aid for Scientific Research (S) (No. 20221003).

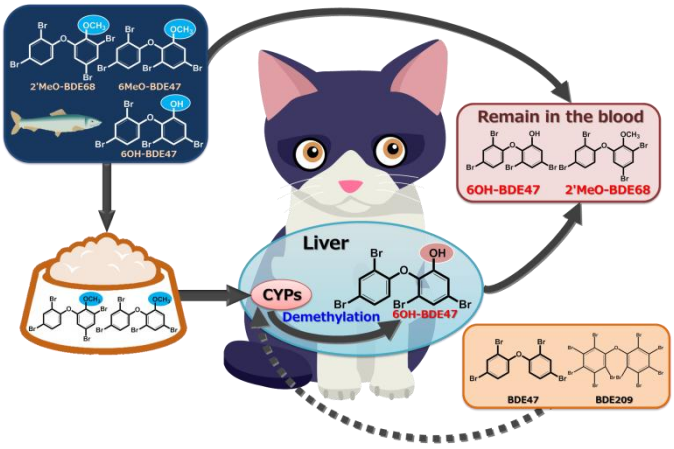

Fig. 2 Scheme of predicted biotransformation of 6MeO-BDE47 in pet cats.

References

1. Shaw SD, Kannan K. (2009) *Rev Environ Health*.24 (3): 157-229.
2. Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jorgensen EH, Sonne C, Verreault J, Vijayan MM, Gabrielsen GW. (2010) *Sci. Total. Environ.* 408 (15): 2995-3043.
3. Darnerud PO, Aune M, Larsson L, Hallgren S. (2007) *Chemosphere* 67 (9): 386-392.
4. Lehmler HJ, Harrad SJ, Huhnerfuss H, Kania-Korwel I, Lee CM, Lu Z, Wong CS. (2010) *Environ. Sci. Technol.* 44 (8): 2757-2766.
5. Qiu X, Bigsby RM, Hites RA. (2009) *Environ Health Perspect* 117 (1): 93-98.
6. Hakk H, Letcher RJ. (2003) *Environ. Int.* 29 (6): 801-828.
7. Malmberg T, Athanasiadou M, Marsh G, Brandt I, Bergman A. (2005) *Environ. Sci. Technol.* 39 (14): 5342-5348.
8. Gribble GW. (2003) *Chemosphere* 52 (2): 289-297.
9. Malmvarn A, Marsh G, Kautsky L, Athanasiadou M, Bergman A, Asplund L. (2005) *Environ. Sci. Technol.* 39 (9): 2990-2997.
10. Wan Y, Wiseman S, Chang H, Zhang X, Jones PD, Hecker M, Kannan K, Tanabe S, Hu J, Lam MH, Giesy JP. (2009) *Environ. Sci. Technol.* 43 (19): 7536-7542.
11. Guo W, Park JS, Wang Y, Gardner S, Baek C, Petreas M, Hooper K. (2012) *Environ. Toxicol. Chem.* 31 (2): 301-306.
12. Dye JA, Venier M, Zhu L, Ward CR, Hites RA, Birnbaum LS. (2007) *Environ. Sci. Technol.* 41 (18): 6350-6356.
13. Venier M, Hites RA. (2011) *Environ. Sci. Technol.* 45 (10): 4602-4608.
14. Nomiyama K, Murata S, Kunisue T, Yamada TK, Mizukawa H, Takahashi S, Tanabe S. (2010) *Environ. Sci. Technol.* 44 (10): 3732-3738.
15. Nomiyama K, Eguchi A, Mizukawa H, Ochiai M, Murata S, Someya M, Isobe T, Yamada TK, Tanabe S. (2011) *Environ. Pollut.* 159 (12): 3364-3373.
16. Eguchi A, Isobe T, Ramu K, Tanabe S. (2011) *Int. J. Environ. An. Ch.* 91 (4): 348-356
17. Mizukawa H, Nomiyama K, Nakatsu S, Yachimori S, Hayashi T, Tashiro Y, Tanabe S. (2011) *Organohalogen Compounds* 73: 1207-1210.
18. Norrgran J, Jones B, Lindquist NG, Bergman A. (2012) *Arch Environ Contam Toxicol.* on line.
19. Kojima H, Takeuchi S, Uramaru N, Sugihara K, Yoshida T, Kitamura S. (2009) *Environ Health Perspect* 117 (8): 1210-1218.
20. Watkins JB, 3rd Klaassen CD. (1986) *J. Anim. Sci.* 63 (3): 933-942.