Environmental Science & Technology

Article

pubs.acs.org/est

Organohalogen Compounds in Pet Dog and Cat: Do Pets Biotransform Natural Brominated Products in Food to Harmful Hydroxlated Substances?

Hazuki Mizukawa,[†,⊥] Kei Nomiyama,[*,†] Susumu Nakatsu,[‡] Hisato Iwata,[†] Jean Yoo,[†] Akira Kubota,[§] Miyuki Yamamoto,[†] Mayumi Ishizuka,[∥] Yoshinori Ikenaka,[∥] Shouta M. M. Nakayama,[∥] Tatsuya Kunisue,[†] and Shinsuke Tanabe[†]

[†]Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama, Ehime 790-8577, Japan

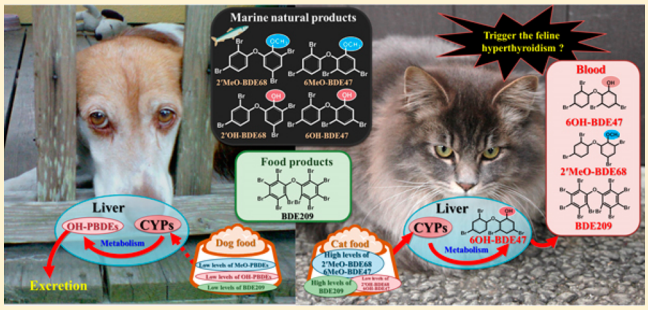
[‡]Nakatsu Veterinary Surgery, 2-2-5, Shorinjichonishi, Sakai-ku, Sakai-shi, Osaka 590-0960, Japan

[§]Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

[∥]Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan

Supporting Information

ABSTRACT: There are growing concerns about the increase in hyperthyroidism in pet cats due to exposure to organohalogen contaminants and their hydroxylated metabolites. This study investigated the blood contaminants polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) and their hydroxylated and methoxylated derivatives (OH-PCBs, OH-PBDEs, and MeO-PBDEs), in pet dogs and cats. We also measured the residue levels of these compounds in commercially available pet foods. Chemical analyses of PCBs and OH-PCBs showed that the OH-PCB levels were 1 to 2 orders of magnitude lower in cat and dog food products than in their blood, suggesting that the origin of OH-PCBs in pet dogs and cats is PCBs ingested with their food. The major congeners of OH-/MeO-PBDEs identified in both pet food products and blood were natural products (6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68) from marine organisms. In particular, higher concentrations of 6OH-BDE47 than 2′OH-BDE68 and two MeO-PBDE congeners were observed in the cat blood, although MeO-BDEs were dominant in cat foods, suggesting the efficient biotransformation of 6OH-BDE47 from 6MeO-BDE47 in cats. We performed in vitro demethylation experiments to confirm the biotransformation of MeO-PBDEs to OH-PBDEs using liver microsomes. The results showed that 6MeO-BDE47 and 2′MeO-BDE68 were demethylated to 6OH-BDE47 and 2′OH-BDE68 in both animals, whereas no hydroxylated metabolite from BDE47 was detected. The present study suggests that pet cats are exposed to MeO-PBDEs through cat food products containing fish flavors and that the OH-PBDEs in cat blood are derived from the CYP-dependent demethylation of naturally occurring MeO-PBDE congeners, not from the hydroxylation of PBDEs.



INTRODUCTION

Organohalogen compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are widely used in industry. Because of their persistence and high bioaccumulative potency, PCBs and PBDEs have been detected in both animal species and humans at significant levels.[1,2] These halogenated contaminants adversely affect the endocrine system and neurodevelopment.[3] Moreover, hydroxylated metabolites of PCBs (OH-PCBs) and PBDEs (OH-PBDEs) disrupt thyroid hormone (TH) homeostasis.[4] OH-PCBs and OH-PBDEs are formed in the phase I metabolic pathway, which is mediated by the cytochrome P450 (CYP) monooxygenase system.[5,6] OH-PBDE congeners such as 6OH-BDE47 and 2′OH-BDE68 are produced by marine sponges, cyanobacteria, and algae.[7−9] Studies of the Japanese medaka (*Oryzias latipes*) have reported that OH-PBDEs are formed by the demethylation of the methoxylated PBDEs (MeO-PBDEs) which occur naturally in the marine organisms mentioned above.[10] These hydroxylated metabolites are structurally similar to thyroxin (T_4), thus bind to the TH transport protein transthyretin (TTR) with a much higher affinity than their parent compounds. The effects of OH-PCBs

Received: August 31, 2015
Revised: November 23, 2015
Accepted: December 2, 2015
Published: December 2, 2015

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

and OH-PBDEs are thus of concern, because they have been detected in the tissues of a variety of animals.[11−14]

Pet dogs and cats might be exposed to environmental contaminants including PCBs and PBDEs.[15−18] One previous study investigated organochlorine compound residues in cats and dogs from a wide area of Southern Italy and reported that PCB concentrations were higher in cats than in dogs.[15] In addition, concentrations of PBDEs in the serum of the dogs were significantly lesser than those measured in the serum of cats in the U.S.[16] These results suggested that differences of size class, dietary exposure, and/or xenobiotic metabolizing systems exist between the species. Other studies have detected higher levels of PBDEs in the sera of pet cats than in the sera of humans.[18−20] Moreover, evidence suggests that the main routes of exposure to PBDEs for pet cats are diet and ingested contaminated house dust.[18,20,21] Cats are expected to have higher exposure to PBDE because of increased intake of house dust from their grooming behavior.[20,22] Several reports have hypothesized that increases in feline hyperthyroidism (FH) might be associated with increased exposure to PBDEs.[18,19] A more recent study has suggested that hyperthyroid cats have higher serum concentrations of PBDEs (BDE99, BDE153, and BDE183) and CB153 than euthyroid cats.[23] The number of cats diagnosed with FH has increased significantly over the last three decades, and the multiple risk factors for FH suggest that its pathogenesis involves exposure to goitrogens, including PBDEs.[24,25] The increased incidence of FH might be linked to the incorporation of phenolic metabolites such as OH-PCBs and OH-PBDEs.[19,20] Conversely, hyperthyroidism in dogs is very rare and is a iatrogenic disease caused by the medical treatment of hypothyroidism, for example, by the excess administration of an ergogenic thyroid hormone.

Terrestrial carnivorous species have a higher metabolic capacity for organohalogen compounds than marine mammals. Moreover, the levels of PBDEs and OH-PBDEs measured in the blood of cats have been shown to be higher than those of other carnivorous species.[26,27] In particular, high levels of 6OH-BDE47 and 2′OH-BDE68 were found in the blood of cats, suggesting the ingestion of the natural compounds from seafood. Conversely, low concentrations of these natural compounds were found in the blood of dogs. These results suggest either that dogs metabolize the natural compounds more rapidly than cats or that dogs are exposed to much lower levels of the compounds.[26] Our previous study suggested that the different residue levels of these compounds found among carnivorous species indicate a high risk of 6OH-BDE47 and 2′OH-BDE68 in cats[26] and that the metabolic capacities and binding affinities with specific proteins such as TTR are different in dogs and cats. However, there have been no reports of the biotransformation of organohalogen compounds to hydroxylated metabolites in dogs and cats. In addition, our previous study showed that lower-chlorinated OH-PCB congeners (3−5 Cl) were predominant (more than 80% to the total OH-PCBs) in cat blood, whereas higher-chlorinated OH-PCBs (6−8 Cl) were major congeners in the blood of other carnivorous species.[26] These findings suggest that halogenated phenolic compounds are preferentially retained in the blood of cats, because they do not undergo robust phase II conjugation. This hypothesis is consistent with the fact that the UDP-glucuronosyltransferase UGT1A6 is lacking in cats.[28] Nevertheless, the differences between the activities of enzymes that metabolize PCBs and PBDEs in dogs and cats remain unknown, and the data on exposure levels to these contaminants through pet food products remain insufficient. Considering that the increased incidence of FH may be responsible for the incorporation of phenolic compounds such as OH-PCBs and OH-PBDEs, more intensive study is necessary to assess the exposure and residue levels of these hydroxylated metabolites and to investigate their formation processes in these animals. However, only limited information is available on the levels of the metabolites of PCBs and PBDEs in pet animals and their food.[16,23]

The present study determined the levels and accumulation patterns of PCBs, PBDEs, and their metabolites (OH-PCBs, OH-PBDEs, and MeO-PBDEs) in the blood of pet cats and dogs collected from a veterinary hospital in Japan. To estimate the exposure routes to these chemicals, we determined the levels of dietary exposure of these pets to PCBs, PBDEs, and their derivatives from representative samples of dry and wet pet food products. In addition, we conducted in vitro demethylation experiments to confirm the biotransformation of MeO-PBDEs to OH-PBDEs in the livers of dogs and cats. Finally, we compared the biotransformation capacity of PCBs and PBDEs in dogs and cats.

EXPERIMENTAL SECTION

Sample Collection. Blood samples from pet dogs (n = 17) and cats (n = 11) were collected at the Nakatsu Veterinary Surgery in Osaka and the Tao Veterinary Hospital in Hiroshima, Japan, during 2009−2012. The pets were brought to the veterinary hospitals for clinical treatments such as surgical procedures (for lymphoma and pyometra, neutering etc.), but excluding FH. The owners of the pets completed a questionnaire, providing information about age, sex, weight, housing conditions, eating habits (dry or wet food), and housing environment (indoors or outdoors) (Table S1). Commercial dry and wet pet food products of the most popular brands in Japan were purchased from Japanese pet shops in 2010. The details of the pet food samples (n = 16) are presented in Table S2.

The pooled liver microsome from ten healthy beagle dogs used for the in vitro demethylation experiments was purchased from Life Technologies (Carlsbad, CA). We collected fresh liver samples (within 30 min of the donor's death) from three domestic cats, with the cooperation of Nakatsu Veterinary Surgery in Osaka, Japan (Table S3). These cats were euthanized using pentobarbital sodium, because of incurable (and painful) cases or diseases. Owners provided consent for the harvesting of the livers. For the chemical analyses, liver samples were flash-frozen in liquid nitrogen and stored at −80 °C and blood and food samples were stored at −20 °C. The samples were transferred to and stored at the Environmental Specimen Bank for Global Monitoring (*es*-BANK) at Ehime University, Japan.[29]

Chemicals. The standards for the 62 PCB and 52 OH-PCB (methoxylated derivatives; MeO-PCBs) congeners are described in the Supporting Information (SI) and Table S4. The standards for the 9 PBDE congeners (BDE47, 99, 100, 153, 154, 183, 196, 197, 206, 207, and 209) were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada). MeO-PBDE congeners (methoxylated derivatives; MeO-PBDEs) were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada), Accu Standard, Inc. (New Haven, CT), and Cambridge Isotope Laboratories Inc. Details of the nine PBDE and 28 MeO-PBDE (OH-PBDEs derivatives) congeners are presented in the SI and in Table S5. ^{13}C-labeled tri- to hepta-

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

Table 1. Median Concentrations (pg g^{-1} Wet Weight) And Range (Minimum to Maximum) of total PCBs, OH-PCBs, PBDEs, OH-PBDEs, MeO-PBDEs and Their Major Congeners in the Whole Blood of Pet Dogs and Cats, And Pet Food Products

	dog blood	dog dry food	dog wet food	cat blood	cat dry food	cat wet food
total PCBs[a]	<7.4 (<7.4−120)	120 (100−210)[e]	13 (<7.4−55)	48 (<7.4−260)	350 (130−1700)[e]	72 (20−350)
total OH-PCBs[b]	120 (9.4−820)	0.99 (<0.60−1.8)	0.95 (<0.60−2.1)	93 (38−290)	0.5 (<0.60−1.1)	0.86 (<0.60−3.3)
BDE47	< 4.2	< 4.2 (<4.2−42)	< 4.2	< 4.2	8.6 (<4.2−96)	4.3 (<4.2−14)
BDE209	100 (<4.2−280)	150 (130−170)	< 4.2 (<4.2−47)	160 (<4.2−490)	210 (160−510)	< 4.2 (<4.2−76)
total PBDEs[c]	100 < 4.2−300)	170 (140−240)[e]	4.2 (<4.2−48)	180 (<4.2−490)	210 (190−710)[e]	7.1 (<4.2−280)
6OH-BDE47	<1.0 (<1.0−8.0)	2.0 (<1.0−5.5)	< 1.0 (<1.0−4.6)	290 (100−1500)	4.7 (<1.0−52)	40 (19−110)
2′OH-BDE68	<1.0 (<1.0−5.6)	< 1.0 (<1.0−4.1)	< 1.0	14 (<0.60−98)	3.2 (<1.0−15)	14 (3.9−50)
total OH-PBDEs[d]	<1.0 (<1.0−14)	2.0 (<1.0−9.6)	< 1.0 (<1.0−4.6)	300 (100−1600)[f,g]	11 (7.7−52)	54 (23−160)
6MeO-BDE47	<1.0 (<1.0−65)	12 (<1.0−160)	< 1.0 (<1.0−170)	< 1.0 (<1.0−310)	380 (200−670)	1600 (1300−1900)
2′MeO-BDE68	<1.0	15 (<1.0−230)	< 1.0 (<1.0−380)	< 1.0 (<1.0−1700)	350 (200−970)	2000 (1200−4500)
total MeO-PBDEs[d]	<1.0 (<1.0−65)	26 (<1.0−380)	< 1.0 (<1.0−550)	< 1.0 (<1.0−2000)	840 (410−1400)[g]	3600 (2500−6400)[g]

[a]Total PCBs shows the sum of 62 PCB congeners (mono to deca-Cl). [b]Total OH-PCBs shows the sum of 52 PCB congeners (tri to octa-Cl). [c]Total PBDEs shows the sum of 11 PBDE congeners. [d]Total OH- and MeO-PBDEs shows the sum of 28 MeO-PBDEs. Details of the PCBs, OH-PCBs, PBDEs, OH-PBDEs, and MeO-PBDEs congeners are presented in the SI and Table S6−S10. [e]Significantly ($p < 0.05$) different between dry food and wet food. [f]Significantly ($p < 0.05$) different between dog blood and cat blood. [g]Significantly ($p < 0.05$) different between Total OH-PBDEs and Total MeO-PBDEs.

chlorinated OH-PCBs (4OH-CB29, 4′OH-CB61, 4OH-CB79, 4′OH-CB120, 4OH-CB107, 4′OH-CB159, 4OH-CB146, 4′OH-CB172, and 4OH-CB187), ^{13}C-labeled tetra- and penta-brominated OH-PBDEs (6OH-BDE47, 6′OH-BDE99, and 6′OH-BDE100), ^{13}C-labeled PCBs (CB28, CB52, CB95, CB101, CB105, CB118, CB138, CB153, CB156, CB157, CB167, CB170, CB178, CB180, CB189, CB194, CB202, CB206, and CB208), and ^{13}C-labeled PBDEs (BDE3, BDE15, BDE28, BDE47, BDE99, BDE153, BDE154, BDE183, BDE196, BDE197, BDE206, BDE207, and BDE209) were spiked as internal standards obtained from Wellington Laboratories Inc. (Guelph, ON, Canada).

Analysis of PCBs, PBDEs, OH-PCBs, OH-PBDEs, and MeO-PBDEs in the Blood. The analytical methods for PCBs, OH-PCBs, PBDEs, OH-PBDEs, and MeO-PBDEs have been reported elsewhere.[30,31] Briefly, a whole blood sample (approximately 3−5 g) in which ^{13}C-labeled internal standards were spiked was denatured with 6 M HCl and homogenized with 2-propanol and 50% methyl t-butyl ether (MTBE)/hexane. After centrifugation, the organic phase was partitioned into neutral and phenolic fractions using 1 M KOH in 50% ethanol/water. After lipids in the neutral fraction had been removed using gel permeation chromatography (GPC), the GPC fraction containing PCBs, PBDEs, and MeO-PBDEs was passed through an activated silica gel column. The phenolic fraction was acidified with sulfuric acid and re-extracted twice with 50% MTBE/hexane. The extracted solution containing OH-PCBs and OH-PBDEs was passed through a column packed with inactivated silica gel (5% H_2O deactivated). Moreover, OH-PCBs and OH-PBDEs were eluted with 50% DCM/hexane (100 mL), concentrated, dissolved in hexane (1 mL), and then derivatized to methylated compounds (MeO-PCBs and MeO-PBDEs) overnight using trimethylsilyldiazomethane. The derivatized solution was passed through an activated silica gel column after the lipids had been removed by GPC, and MeO-PCBs and MeO-PBDEs were eluted with 10% DCM/hexane. A gas chromatograph (GC, 6890 series, Agilent) coupled to a high-resolution (>10 000) mass spectrometer (HRMS, JMS-800D, JEOL) was used to identify and quantify the target organohalogen compounds. Highly brominated PBDEs (Octa-deca BDEs) were quantified using a GC (6890 series, Agilent)/MS (5973N, Agilent).[32] Electron impact and selected ion monitoring mode (EI-SIM) was used for the GC/MS analyses.

Analysis of PCBs, PBDEs, OH-PCBs, OH-PBDEs, and MeO-PBDEs in Pet Food Products. The contents of the organohalogen compounds in the pet food samples were analyzed using a previously reported method.[30,31] A pet food sample (approximately 10 g) was crushed using a mortar and homogenizer and then extracted with 6 M HCl, 2-propanol, and 50% MTBE/hexane in the same way as the blood samples. The details are described in the SI.

Quality Assurance and Quality Control. All organohalogen compounds were quantified using the isotope dilution method with the corresponding $^{13}C_{12}$-internal standards.[26,31] The details of QA/QC are given in the SI.

Preparation of Cat Liver Microsomes and Analysis of Proteins. The preparation of the liver microsomes followed previously published methods.[33,34] Briefly, 100−200 mg of excised livers was homogenized in 5 vol. of cold homogenization buffer (50 mM Tris-HCl, 0.15 M KCl, pH 7.4−7.5) with a Teflon-glass homogenizer (10 passes), and the homogenized liver samples were centrifuged for 10 min at 750*g*. After centrifugation, the nuclear pellet was removed and the supernatant was centrifuged at 12 000*g* for 10 min at 4 °C. The recovered supernatant was further centrifuged at 105 000*g* for 90 min at 4 °C. The microsomal pellet recovered from the centrifugation was resuspended in 1 vol. of resuspension buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, 20% (v/v) glycerol, pH 7.4−7.5). An aliquot of each microsome fraction was used for the measurement of protein content using a bicinchoninic acid (BCA) assay kit. More details are provided in the SI.

Analysis of CYP Levels and Enzyme Activities. The level of CYPs in the cat liver microsomes was determined from the sodium dithionite-reduced CO difference spectrum at approximately 450 and 490 nm (91 mM^{-1} cm^{-1} extinction coefficient). The CYP spectra were analyzed as described in the SI. Measurements of alkoxyresorufin *O*-dealkylase (AROD) activities in microsomal fractions and Western blotting were

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

Table 2. Protein Concentrations (mg g^{-1}) and AROD Activities (pmol/min/pmol CYP) in the Dog- And Cat-Liver Microsomes

samples	protein (mg g^{-1})	total CYP (pmol mg^{-1} protein)	MROD	EROD	PROD	BROD
			(pmol/min/pmol CYP)			
Dog[a]						
mean ± SD	20[b]	426[b]	0.033 ± 0.0046	0.14 ± 0.010	0.0045 ± 0.00023	0.088 ± 0.0058
Cat						
1	13	236	0.045	0.63	0.029	0.026
2	14	174	0.038	0.46	0.025	0.027
3	14	134	0.023	0.21	0.024	0.019
mean ± SD	14	181 ± 51	0.035 ± 0.011	0.43 ± 0.21	0.026 ± 0.0026	0.024 ± 0.0045

[a]Data are from triplicate analysis using a beagle dog liver microsome. [b]Data was given by Life Technologies (Carlsbad, CA) where the liver microsome was purchased.

performed with minor modifications of published methods.[33] Details are provided in the SI.

In Vitro Assay of Biotransformation of PBDEs and MeO-PBDEs. The reaction mixture (1 mL final volume) contained the buffer (80 mM NaH_2PO_4, 6 mM $MgCl_2$, 1 mM Na_2EDTA, pH 8.0), 10 ng of BDE47 (purity >98%) or a mixture of 6MeO-BDE47 (>98%) and 2′MeO-BDE68 (>98%) in 2% DMSO, and the microsomal suspension (200 pmol of CYPs). For the control sample, the reaction mixture contained only the buffer and the microsome. The mixture solution was preincubated at 37 °C for 10 min, and the CYP-dependent reaction was initiated by adding NADPH-regenerating solutions (50 μL of solution A and 10 μL of solution B) (BD Biosciences, NU). The solution was incubated for 180 min in a shaking (90 rpm) water bath at 37 °C. The negative control reaction mixture contained the buffer, BDE47 or MeO-PBDEs, and microsomes without the NADPH-regenerating solution. After incubation, the reaction was stopped by adding 1 mL of ice-cold methanol. All assays were performed in triplicate. The methods used for the analysis of PBDEs, OH-PBDEs, and MeO-PBDEs in the reaction mixture have been reported elsewhere.[30,31]

Statistical Analysis. The Mann−Whitney U-test was used to test the statistical significance of differences in the levels of target compounds between species. Spearman's rank correlation coefficients were calculated to evaluate the relationship between the concentrations of PCBs, OH-PCBs, PBDEs, OH-PBDEs, and MeO-PBDEs in each species. A p-value of <0.05 was considered significant. All statistical analyses were performed using Statcel 97 (OMS Ltd. Japan).

RESULTS AND DISCUSSION

Residue Levels and Profiles of Organohalogen Compounds. OH-PCBs and OH-PBDEs circulate in the blood by binding strongly to TH transport proteins, whereas PCBs, PBDEs, and MeO-PBDEs are neutral lipophilic compounds that are mostly stored in lipids. Concentrations of all target compounds detected in the blood are expressed here as wet weights to permit comparisons of blood and food product concentrations. Table 1 presents the median and range concentrations (wet weight) of PCBs, OH-PCBs, PBDEs, OH-PBDEs, and MeO-PBDEs in the blood of pet dogs and cats and in commercial pet food products. The congener levels of these compounds (mean ± SD, median, and detection frequency) are summarized in Tables S6−S10.

AROD Activities and Expression of CYP Proteins in Dog and Cat Liver Microsomes. The total CYP levels (pmol mg^{-1} protein) in the hepatic microsomes of three cats (Cat 1−3) and one dog were 134−236 pmol mg^{-1} and 426 pmol mg^{-1}, respectively (Table 2). The hepatic microsomal AROD activities (pmol/min/pmol CYP) of the dog microsome were characterized by higher activities of ethoxyresorufin-*O*-deethylase (EROD), followed by benzyloxyresorufin-*O*-debenzylase (BROD), methoxyresorufin-*O*-demethylase (MROD), and entoxyresorufin-*O*-deethylase (PROD) (Table 2). In the cat microsomes, the EROD activities were higher than other AROD activities and the mean value (0.43 ± 0.21) of EROD was three times higher than that observed in the dog microsome (0.14 ± 0.01: triplicate analysis). An earlier study has reported higher activities of EROD as a catalytic marker of CYP1A in liver microsomes from dogs exposed to a PCB mixture (Aroclor 1248), although PROD activity as a catalytic marker of CYP2B was low.[35]

Our group previously suggested that cats may preferentially metabolize lower-chlorinated PCBs and retain their hydroxylated metabolites in the blood.[26] This may be because of the low metabolic capacity for PCBs and the low activity of CYP2B, which may be responsible for the metabolism of higher-chlorinated PCBs in cats.[26] Therefore, to determine whether dogs and cats express CYP1A and CYP2B proteins in their livers, we performed Western blot analyses (Figure S3) using an antihuman CYP1A1 and an antidog CYP2B11 polyclonal antibody. The results showed a clear band of CYP1A1 protein at the site of its expected molecular weight in the liver microsomes of cats. In the dog liver microsomes, there was a band of CYP1A1 protein with its expected molecular weight. In the case of CYP2B, the dog liver microsomes showed a strong single band of CYP2B protein, whereas the cat liver microsomes presented a weak single band of CYP2B with its expected molecular weight. These results indicate that the livers of both animals express CYP1A and CYP2B proteins, which may be involved in the metabolism of PCBs. However, the CYP subfamily involved in the metabolism of PCBs in the cat is not clear from past studies. Although information on feline CYP activity is limited, a previous report showed that the metabolic activities of the CYP2C subfamily in the cat were less inhibited by Tolbutamide than those of other species (human, horse, and dog), compared with the CYP1A, 2A, 2D, 2E, and 3A subfamily.[36] Further studies of the activities of the CYP subfamily involved in the metabolism of PCBs in the cat microsome are required.

PCBs and OH-PCBs. PCBs were detected in 10 cat blood samples at concentrations above the limit of quantification (LOQ) with a detection rate of 91%, whereas the detection rate of PCBs in the dog blood samples was 41%. Median PCB levels in the cat blood samples (48 pg g^{-1}) were 1 order of magnitude

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

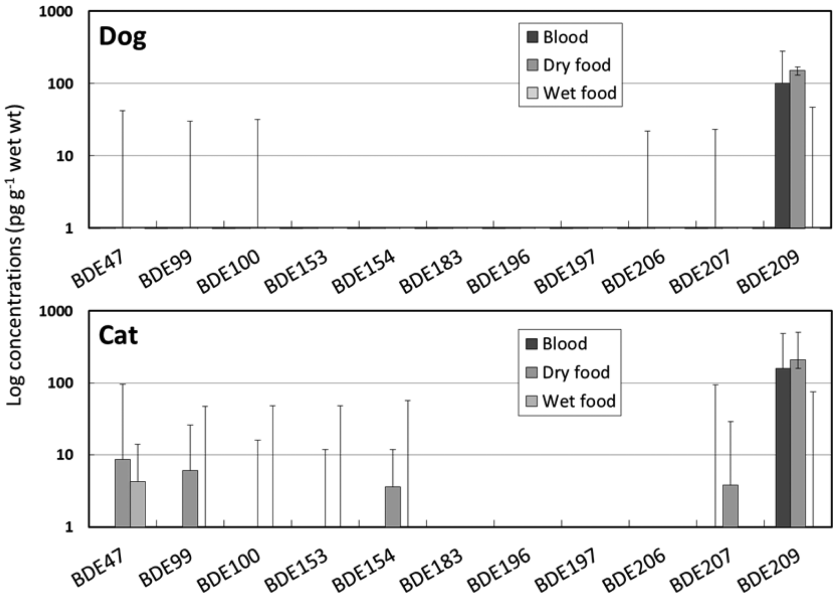


Figure 1. Median concentrations of PBDEs congeners in the pet dog and cat blood, and pet food products. Error bars indicate ranges (minimum to maximum levels).

lower than those of stray cats in Japan and pet cats in the U.S.[19,20] Concentrations of PCBs in the dog blood samples from Japan (median: < 7.4 pg g^{-1}) were lower than those from Southern Italy.[15] The lower detection frequency of PCBs observed in pet dogs than in cats might be attributed to the lower exposure levels. The PCB levels found in dry and wet dog food products were significantly lower than those in dry and wet cat food products (Table 1 and Table S6). Interestingly, PCB levels in dry pet food products (median: 120 pg g^{-1} for dogs and 350 pg g^{-1} for cats) were significantly higher than those of wet pet food products (median: 13 pg g^{-1} for dogs and 72 pg g^{-1} for cats) ($p < 0.05$), implying that PCBs in raw materials are concentrated during the manufacture of dry pet food products (Table S6). When the congener profiles of PCBs were examined, CB180, CB209, CB153, and CB206 were predominant in the pet dog blood but relatively high concentrations of lower-chlorinated congeners including CB28, in addition to CB153, CB138, and CB180, were found in dry dog food products (Figure S1). Conversely, penta- and hexa-chlorinated congeners were present in both the cat blood and cat food. These results suggest that dogs quickly metabolize lower-chlorinated PCBs.[26]

OH-PCBs were detected in the blood samples of both dogs and cats (median: 120 pg g^{-1} for dogs and 93 pg g^{-1} for cats), whereas only a few OH-PCB congeners at extremely low levels were found in the pet food products. This clearly suggests the biotransformation of PCBs to OH-PCBs in dogs and cats. However, the congener profiles of the OH-PCBs were different for dogs and cats (Figure S2 and Table S7). Tri- to penta-chlorinated OH-PCB congeners (particularly 4′OH-CB18, 4OH-CB25/31/4′OH-CB26) in the cat blood accounted for approximately 90% of the OH-PCBs. In contrast, hexa- to octa-chlorinated OH-PCBs (in particular, 4OH-CB199 and 4OH-CB202) in the dog blood accounted for >90% of the OH-PCBs (Table S7). These results are consistent with published results.[26] The difference in the congener patterns may be attributed to the species-specific metabolic capacity of phase I CYP and/or phase II conjugation enzymes, and the binding affinity to TH transport proteins such as TTR. It has been reported that the binding affinity of lower-chlorinated OH-PCBs to TTR is weak, and that they can be easily eliminated through phase II conjugation enzymes in the dog.[26] Taking these observations together, it is highly likely that cats preferentially metabolize lower-chlorinated PCBs, and retain their hydroxylated metabolites in the blood.

PBDEs. PBDEs were detected in 12 dog and eight cat blood samples at median concentrations (wet weight) of 100 pg g^{-1} and 180 pg g^{-1}, respectively (Table 1 and Table S8). No significant differences were found in the PBDE levels in the blood of the pet dogs and cats. Conversely, the residual levels of PBDEs in the cat blood collected from Japan were 1−3 orders of magnitude lower than those reported for the serum of pet cats in the U.S.[19,20] In addition, the concentration of PBDEs in the dog blood from Japan was $^1/_8$ of that of American pet dogs.[16] These results suggest that pet dogs and cats in Japan are exposed to low levels of PBDEs from furniture and household electrical appliances, and also suggest lower PBDE contamination of indoor environments in Japan than in the U.S.

The profiles of PBDE congeners in Japanese pet dogs and cats showed a high proportion of BDE209 (Figure 1). BDE206 and BDE207 were also detected in a few blood samples at extremely low levels, but the concentrations of other congeners were below LOQ (Table S8). It has been reported from the U.S. and Sweden that BDE47, 99, 153 in addition to BDE209, were predominant congeners in pet dog and cat serum.[16,19,20,37] However, the median level of BDE47 and 153 in the blood of pet dogs and cats in Japan was below the LOQ, reflecting past restrictions on the use of tetra-BDE and octa-BDE products in Japan.[38,39]

PBDEs were detected in the dry pet food products analyzed (median: 170 pg g^{-1} for dogs and 210 pg g^{-1} for cats) with BDE209 as the dominant congener (>80% of total PBDEs) (Figure 1 and Table S8). No statistically significant differences were found between PBDE concentrations in the dog dry food and cat dry food. PBDE detection rates in the wet dog and cat foods were 25% and 50%, respectively, which was lower than those in the dry food. These results suggest that the elevated BDE209 levels observed in the blood of pet dogs and cats were

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

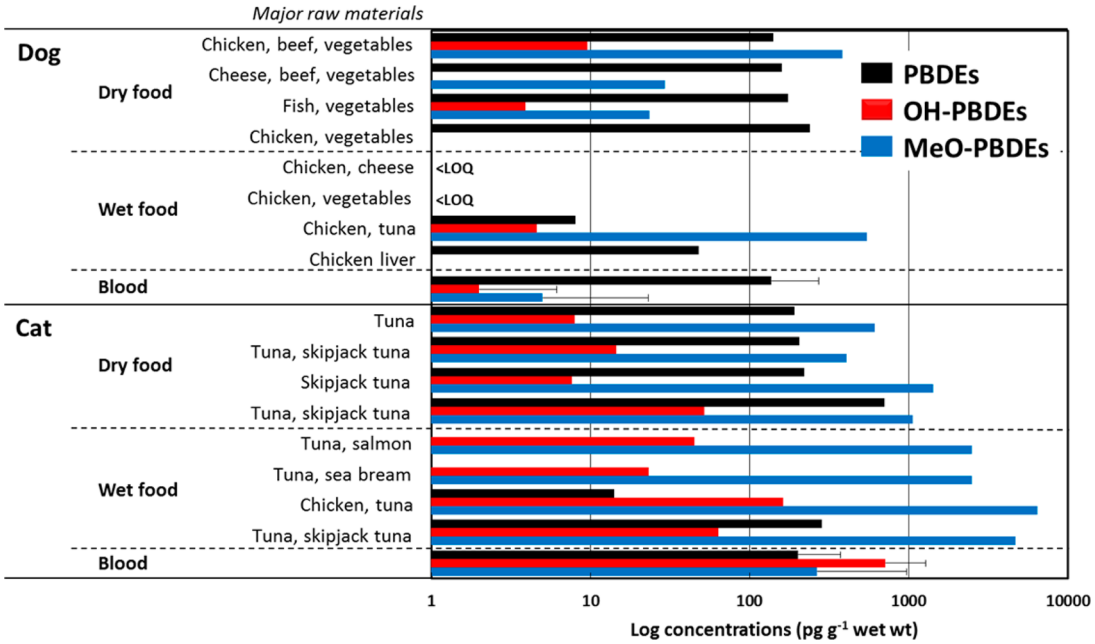


Figure 2. Concentrations (pg g^{-1} wet wt) of PBDEs, OH-PBDEs, and MeO-PBDEs in the dry and wet pet food products and dog and cat blood. Error bars of dog and cat blood indicate standard deviation (SD).

caused by the consumption of dry food products. However, other studies have reported that BDE209 is a dominant congener in house dust in both Japan and the U.S.[26,40,41] Thus, house dust may also be a source of the high BDE209 levels found in these pet animals. In the wet cat food products, BDE99, BDE100, BDE153, BDE154, and BDE209 were detected at the similar levels. This may reflect the congener composition of the fish which are used as raw materials. A comparable composition of these PBDEs has been found in skipjack tuna collected from Asian offshore waters, which is one of the most popular ingredients of cat food.[42]

OH-PBDEs and MeO-PBDEs. Concentrations of OH-PBDEs (detection rate: 29%) and MeO-PBDEs (detection rate: 5.9%) were below the LOQ in more than half of the dog blood samples. Conversely, OH-PBDEs were detected in all the cat blood samples, although the detection rate of MeO-PBDEs was 45% for the cat blood. A possible explanation for the lower detection frequencies of these brominated compounds in the dog blood could be their lower concentrations in the dog foods than in the cat foods (Table S9 and S10). Interestingly, elevated levels of MeO-PBDEs were found in wet cat food products, in which fish meats are the primary material (Figure 2). Of the OH-PBDE and MeO-PBDE congeners analyzed in this study, only 6OH-/MeO-BDE47 and 2′OH-/MeO-BDE68 were detected in the blood and pet food products. It is well-known that these congeners are produced naturally by marine organisms.[43,44] Assuming that fish accumulate these brominated compounds, especially, 6MeO-BDE47 and 2′MeO-BDE68, the wet cat food made from fish meat would also be expected to show a high concentration. These results suggest that pet cats are exposed to 6MeO-BDE47 and 2′MeO-BDE68 through food products, in particular, those which contain fish (Figure 2). However, higher concentrations and detection rates of 6MeO-BDE47 and 2′MeO-BDE68 in the cat blood were found, compared with 6OH-BDE47 and 2′OH-BDE68 (Table S9 and S10). In particular, 6OH-BDE47 levels in the blood of pet cats were significantly higher than 2′OH-BDE68 levels in the cat blood and 6OH-BDE47 levels in wet cat foods ($p < 0.05$). These results suggest the efficient biotransformation of 6OH-BDE47 from the natural product 6MeO-BDE47, probably by demethylation enzymes, in cats. Previous studies have confirmed the biotransformation of 6OH-BDE47 from 6MeO-BDE47 but have not detected the hydroxylation of PBDEs by microsomes.[10,44] MeO-PBDEs contribute to the formation of OH-PBDEs in vitro using rainbow trout, chicken, and rat hepatic microsomes.[44] Recently, it was reported that 6MeO-BDE47 was formed as a biotransformation product of 6OH-BDE47 in an in vivo study using Japanese medaka. These observations may indicate a more complex interrelation between OH-PBDEs and MeO-PBDEs in aquatic organisms.[10]

Demethylation Pathway of MeO-PBDEs by In Vitro Assay in Dog and Cat Liver Microsomes. As aforementioned, a high proportion of the OH-PBDEs detected in cat blood may be accounted for by the direct ingestion of cat food, as well as by biotransformation of MeO-PBDEs.[10,44] To estimate the demethylation potency of MeO-PBDEs by CYPs, we conducted an in vitro assay of the dog and cat liver microsomes. After 180 min incubation, demethylation of 6MeO-BDE47 and 2′MeO-BDE68 was observed in all the microsomes tested. The demethylation rates were calculated from the ratio of the amount of OH-PBDEs formed to the dosage amount of MeO-PBDEs. The demethylation rates of 6MeO-BDE47 and 2′MeO-BDE68 are shown in Figure 3.

In the cat microsomes, the estimated demethylation rates of 6MeO-BDE47 were in the range 6.7−18%, and higher than those of 2′MeO-BDE68 (0−5.0%). The order of demethylation rates of 6MeO-BDE47 estimated for each cat microsome (Cat 1 > Cat 2 > Cat 3) was consistent with that of AROD activities (Table 2). These results indicate the preferential formation of 6OH-BDE47 from 6MeO-BDE47 by CYP catalytic activities, and support the observation of higher 6OH-BDE47 levels in the blood of pet cats (Table 2), compared with 2′OH-BDE68. This may also be explained by the binding affinity of the TH transport protein for 6OH-BDE47 in the blood,[45] and by the

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

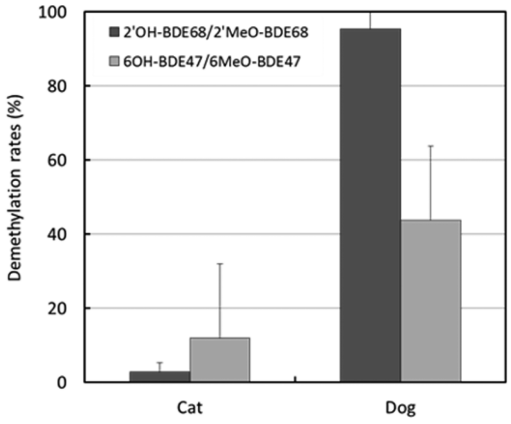


Figure 3. Demethylation rates (2′OH-BDE68/2′MeO-BDE68 and 6OH-BDE47/6MeO-BDE47) estimated from the in vitro assay using the dog and cat liver microsomes (+SD). Data on cat liver microsomes and beagle dog liver microsomes are from triplicate analysis.

weak ability to further metabolize this compound in the liver via a phase II conjugation reaction.[46] The toxicological implications of cat exposure to 6OH-BDE47 remain unknown. However, 6OH-BDE47 is of particular interest because it triggers a variety of toxic effects such as interruption of oxidative phosphorylation[47] and inhibition of estradiol-sulfotransferase[45] and is neurotoxic[48] in exposed wildlife and humans. A recent study showed that the hydroxylated metabolite 6OH-BDE47 is more potent in disturbing Ca^{2+} homeostasis and neurotransmitter release than the parent compound BDE47. This result suggests that bioactivation by metabolism adds considerably to the neurotoxic potential of PBDEs.[48] Further investigation is necessary to determine whether the accumulation of 6OH-BDE47 is associated with neurotoxicity and TH homeostasis in cats.

In the dog microsome, 2′MeO-BDE68 was mostly demethylated to 2′OH-BDE68 (95%) and the production rate of 6OH-BDE47 (44%) was also higher than the rate observed in the cat microsomes (Figure 3). These results indicate that dogs have a higher MeO-PBDE demethylation capacity than cats. However, 2′OH-BDE68 and 6OH-BDE47 were undetectable in the blood of pet dogs (Table 1 and Table S9). The low levels of MeO-BDEs in dog foods may be a contributing factor (Table S10); and may also be attributed to the efficient conjugation metabolism of these OH-BDEs in dogs because of their high phase II enzymatic activity.[46] The differences in CYP-mediated demethylation of MeO-BDEs to OH-BDEs may influence the levels of OH-BDEs in different mammalian species.[44] To our knowledge, no data are available on the biotransformation of 2′MeO-BDE68 to 2′OH-BDE68 by in vitro and in vivo assays, but a recent in vivo study using rainbow trout suggested that the demethylation of 6MeO-BDE47 to 6OH-BDE47 might be mainly catalyzed by a member of the CYP2 family.[49]

Using the same in vitro assays, we also investigated whether 6OH-BDE47 is formed by the hydroxylation of BDE47. After BDE47 was added to the cat- and dog-liver microsomes and incubated for 180 min, no hydroxylated metabolites of BDE47, including 6OH-BDE47, were detected in this study (data not shown). However, several previous studies on BDE47 metabolism have reported the detection of OH-PBDEs.[45,50,51] The level of PBDE used for exposure in those experiments was expressed as pg/g wet weight range, but OH-PBDEs were detected at <1% of the loaded amount of parent BDE47.[45,50] Qiu et al. (2007) reported, based on a exposure test with mice, that the hydroxylation of BDE47 occurred preferentially at the *para*-position of the phenyl ring with an NIH-shift of the original *para*-bromine atom.[51] In that case, 6OH-BDE47 was detected at a low level (6%). These results indicate that BDE47 is hydroxylated at the para-position rather than the ortho- or meta-positions. From these observations, we suggest that the hydroxylated metabolites of anthropogenic PBDEs in cats and dogs are present in negligible amounts. Thus, the primary exposure route of these two major OH-PBDEs in dogs and cats may be demethylation from MeO-PBDEs, not the hydroxylation of PBDEs.

To the best of our knowledge, this is the first in vitro assay to demonstrate the formation of OH-PBDEs from MeO-PBDEs using cat- and dog-liver microsomes. Our results suggest that pet cats routinely ingest natural MeO-PBDEs in cat food products containing fish and retain their demethylated metabolites, OH-PBDEs, in the blood for a prolonged time. Further studies are needed to clarify the toxic effects of OH-PBDEs such as 6OH-BDE47 on thyroid homeostasis and to establish the relationship between the exposure level and occurrence frequency of FH in pet cats.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04216.

Detailed information as follows: experimental procedures; biological information for individual dogs and cats (Table S1); information on dry and wet pet food products (Table S2); information on the fresh liver samples of cats (Table S3); the authentic reference standards of OH-PCBs, OH-PBDEs, and MeO-PBDEs (Tables S4 and S5); levels of PCB congeners (Table S6), OH-PCB congeners (Table S7), PBDE congeners (Table S8), OH-PBDE congeners (Table S9), and MeO-PBDE congeners (Table S10) in the blood of pet dogs and cats and pet food products; congener profiles of PCBs in the blood of pet dogs and cats, and pet food products (Figure S1); concentrations of OH-PCB congeners in the blood of pet dogs and cats, and pet food products (Figure S2); and immunoblot analyses of dog and cat hepatic microsomes using antihuman CYP1A1 and antidog CYP2B11 polyclonal antibodies (Figure S3) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +81-89-927-8171; e-mail: keinomi@agr.ehime-u.ac.jp.

Present Address

⊥(H.M.) Department of Environmental Veterinary Science, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid (KAKENHI) for Scientific Research (S) (No. 26220103), Young Scientists (A) (No. 25701014), Young Scientists (B) (No. 15K16132),

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

Scientific Research (A) (No. 25241013) and (B) (No. 25281050) from the Japan Society for the Promotion of Science (JSPS). The authors would like to thank Enago (www.enago.jp) for the English language review.

REFERENCES

(1) Shaw, S. D.; Kannan, K. Polybrominated diphenyl ethers in marine ecosystems of the American continents: foresight from current knowledge. *Rev. Environ. Health* **2009**, *24* (3), 157−229.
(2) Letcher, R. J.; Bustnes, J. O.; Dietz, R.; Jenssen, B. M.; Jorgensen, E. H.; Sonne, C.; Verreault, J.; Vijayan, M. M.; Gabrielsen, G. W. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Sci. Total Environ.* **2010**, *408* (15), 2995−3043.
(3) Darnerud, P. O.; Aune, M.; Larsson, L.; Hallgren, S. Plasma PBDE and thyroxine levels in rats exposed to Bromkal or BDE-47. *Chemosphere* **2007**, *67* (9), S386−392.
(4) Qiu, X.; Bigsby, R. M.; Hites, R. A. Hydroxylated metabolites of polybrominated diphenyl ethers in human blood samples from the United States. *Environ. Health Perspect.* **2009**, *117* (1), 93−98.
(5) Hakk, H.; Letcher, R. J. Metabolism in the toxicokinetics and fate of brominated flame retardants–a review. *Environ. Int.* **2003**, *29* (6), 801−828.
(6) Malmberg, T.; Athanasiadou, M.; Marsh, G.; Brandt, I.; Bergman, A. Identification of hydroxylated polybrominated diphenyl ether metabolites in blood plasma from polybrominated diphenyl ether exposed rats. *Environ. Sci. Technol.* **2005**, *39* (14), 5342−5348.
(7) Malmvarn, A.; Marsh, G.; Kautsky, L.; Athanasiadou, M.; Bergman, A.; Asplund, L. Hydroxylated and Methoxylated Brominated Diphenyl Ethers in the Red Algae Ceramium tenuicorne and Blue Mussels from the Baltic Sea. *Environ. Sci. Technol.* **2005**, *39* (9), 2990−2997.
(8) Malmvarn, A.; Zebuhr, Y.; Kautsky, L.; Bergman, K.; Asplund, L. Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-p-dioxins in red alga and cyanobacteria living in the Baltic Sea. *Chemosphere* **2008**, *72* (6), 910−916.
(9) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Van Soest, R. W.; Kunzmann, A. Four new bioactive polybrominated diphenyl ethers of the sponge Dysidea herbacea from West Sumatra, Indonesia. *J. Nat. Prod.* **1997**, *60* (12), 1313−1316.
(10) Wan, Y.; Liu, F.; Wiseman, S.; Zhang, X.; Chang, H.; Hecker, M.; Jones, P. D.; Lam, M. H.; Giesy, J. P. Interconversion of hydroxylated and methoxylated polybrominated diphenyl ethers in Japanese medaka. *Environ. Sci. Technol.* **2010**, *44* (22), 8729−8735.
(11) Gebbink, W. A.; Sonne, C.; Dietz, R.; Kirkegaard, M.; Riget, F. F.; Born, E. W.; Muir, D. C. G.; Letcher, R. J. Tissue-specific congener composition of organohalogen and metabolite contaminants in east Greenland polar bears (*Ursus maritimus*). *Environ. Pollut.* **2008**, *152* (3), 621−629.
(12) Jaspers, V. L.; Dirtu, A. C.; Eens, M.; Neels, H.; Covaci, A. Predatory bird species show different patterns of hydroxylated polychlorinated biphenyls (HO-PCBs) and polychlorinated biphenyls (PCBs). *Environ. Sci. Technol.* **2008**, *42* (9), 3465−3471.
(13) Nomiyama, K.; Uchiyama, Y.; Horiuchi, S.; Eguchi, A.; Mizukawa, H.; Horai-Hirata, S.; Shinohara, R.; Tanabe, S. Organohalogen compounds and their metabolites in the blood of Japanese amberjack (*Seriola quinqueradiata*) and scalloped hammerhead shark (*Sphyrna lewini*) from Japanese coastal waters. *Chemosphere* **2011**, *85* (3), 315−321. DOI: 10.1016/j.chemosphere.2011.06.092.
(14) Houde, M.; Pacepavicius, G.; Darling, C.; Fair, P. A.; Alaee, M.; Bossart, G. D.; Solomon, K. R.; Letcher, R. J.; Bergman, Å.; Marsh, G.; Muir, D. C. Polybrominated diphenyl ethers and their hydroxylated analogs in plasma of bottlenose dolphins (*Tursiops truncatus*) from the United States east coast. *Environ. Toxicol. Chem.* **2009**, *28*, 2061−2068.
(15) Storelli, M. M.; Storelli, A.; Barone, G.; Franchini, D. Accumulation of polychlorinated biphenyls and organochlorine pesticide in pet cats and dogs: assessment of toxicological status. *Sci. Total Environ.* **2009**, *408* (1), 64−68.
(16) Venier, M.; Hites, R. A. Flame retardants in the serum of pet dogs and in their food. *Environ. Sci. Technol.* **2011**, *45* (10), 4602−4608.
(17) Ali, N.; Malik, R. N.; Mehdi, T.; Eqani, S. A.; Javeed, A.; Neels, H.; Covaci, A. Organohalogenated contaminants (OHCs) in the serum and hair of pet cats and dogs: biosentinels of indoor pollution. *Sci. Total Environ.* **2013**, *449*, 29−36.
(18) Chow, K.; Hearn, L. K.; Zuber, M.; Beatty, J. A.; Mueller, J. F.; Barrs, V. R. Evaluation of polybrominated diphenyl ethers (PBDEs) in matched cat sera and house dust samples: investigation of a potential link between PBDEs and spontaneous feline hyperthyroidism. *Environ. Res.* **2015**, *136*, 173−179.
(19) Dye, J. A.; Venier, M.; Zhu, L.; Ward, C. R.; Hites, R. A.; Birnbaum, L. S. Elevated PBDE Levels in Pet Cats: Sentinels for Humans? *Environ. Sci. Technol.* **2007**, *41* (18), 6350−6356.
(20) Guo, W.; Park, J. S.; Wang, Y.; Gardner, S.; Baek, C.; Petreas, M.; Hooper, K. High polybrominated diphenyl ether levels in California house cats: house dust a primary source? *Environ. Toxicol. Chem.* **2012**, *31* (2), 301−306.
(21) Mensching, D. A.; Slater, M.; Scott, J. W.; Ferguson, D. C.; Beasley, V. R. The Feline Thyroid Gland: A Model for Endocrine Disruption by Polybrominated Diphenyl Ethers (PBDEs)? *J. Toxicol. Environ. Health, Part A* **2012**, *75* (4), 201−212.
(22) Dirtu, A. C.; Niessen, S. J. M.; Jorens, P. G.; Covaci, A. Organohalogenated contaminants in domestic cats' plasma in relation to spontaneous acromegaly and type 2 diabetes mellitus: a clue for endocrine disruption in humans? *Environ. Int.* **2013**, *57−58*, 60−67.
(23) Norrgran, J.; Jones, B.; Bignert, A.; Athanassiadis, I.; Bergman, Å. Higher PBDE Serum Concentrations May Be Associated with Feline Hyperthyroidism in Swedish Cats. *Environ. Sci. Technol.* **2015**, *49* (8), 5107−5114.
(24) Peterson, M. Hyperthyroidism in cats: what's causing this epidemic of thyroid disease and can we prevent it? *J. Feline Med. Surg.* **2012**, *14* (11), 804−818.
(25) Peterson, M. E.; Ward, C. R. Etiopathologic Findings of Hyperthyroidism in Cats. *Vet. Clin. Small Anim.* **2007**, *37* (4), 633−645.
(26) Mizukawa, H.; Nomiyama, K.; Nakatsu, S.; Yachimori, S.; Hayashi, T.; Tashiro, Y.; Nagano, Y.; Tanabe, S. Species-specific differences in the accumulation features of organohalogen contaminants and their metabolites in the blood of Japanese terrestrial mammals. *Environ. Pollut.* **2013**, *174*, 28−37.
(27) Nomiyama, K.; Kanbara, C.; Ochiai, M.; Eguchi, A.; Mizukawa, H.; Isobe, T.; Matsuishi, T.; Yamada, T. K.; Tanabe, S. Halogenated phenolic contaminants in the blood of marine mammals from Japanese coastal waters. *Mar. Environ. Res.* **2014**, *93*, 15−22.
(28) Court, M. H.; Greenblatt, D. J. Molecular basis for deficient acetaminophen glucuronidation in cats. An interspecies comparison of enzyme kinetics in liver microsomes. *Biochem. Pharmacol.* **1997**, *53* (7), 1041−1047.
(29) Tanabe, S.; Ramu, K. Monitoring temporal and spatial trends of legacy and emerging contaminants in marine environment: results from the environmental specimen bank (es-BANK) of Ehime University, Japan. *Mar. Pollut. Bull.* **2012**, *64* (7), 1459−1474.
(30) Eguchi, A.; Nomiyama, K.; Ochiai, M.; Mizukawa, H.; Nagano, Y.; Nakagawa, K.; Tanaka, K.; Miyagawa, H.; Tanabe, S. Simultaneous detection of multiple hydroxylated polychlorinated biphenyls from a complex tissue matrix using gas chromatography/isotope dilution mass spectrometry. *Talanta* **2014**, *118*, 253−261.
(31) Nomiyama, K.; Eguchi, A.; Mizukawa, H.; Ochiai, M.; Murata, S.; Someya, M.; Isobe, T.; Yamada, T. K.; Tanabe, S. Anthropogenic and naturally occurring polybrominated phenolic compounds in the blood of cetaceans stranded along Japanese coastal waters. *Environ. Pollut.* **2011**, *159* (12), 3364−3373.
(32) Eguchi, A.; Isobe, T.; Ramu, K.; Tanabe, S. Optimisation of the analytical method for octa-, nona- and deca-brominated diphenyl ethers using gas chromatography-quadrupole mass spectrometry and isotope dilution. *Int. J. Environ. Anal. Chem.* **2011**, *91* (4), 348−356.

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452

(33) Kubota, A.; Iwata, H.; Tanabe, S.; Yoneda, K.; Tobata, S. Hepatic CYP1A induction by dioxin-like compounds, and congener-specific metabolism and sequestration in wild common cormorants from Lake Biwa, Japan. *Environ. Sci. Technol.* **2005**, *39* (10), 3611−3619.
(34) Watanabe, M. X.; Kunisue, T.; Ueda, N.; Nose, M.; Tanabe, S.; Iwata, H. Toxicokinetics of dioxins and other organochlorine compounds in Japanese people: association with hepatic CYP1A2 expression levels. *Environ. Int.* **2013**, *53*, 53−61.
(35) Korytko, P. J.; Casey, A. C.; Bush, B.; Quimby, F. W. Induction of hepatic cytochromes P450 in dogs exposed to a chronic low dose of polychlorinated biphenyls. *Toxicol. Sci.* **1999**, *47* (1), 52−61.
(36) Chauret, N.; Gauthier, A.; Martin, J.; Nicoll-Griffith, D. A. *In vitro* comparison of cytochrome P450-mediated metabolic activities in human, dog, cat, and horse. *Chest* **1997**, *25* (10), 1130−1136.
(37) Norrgran, J.; Jones, B.; Lindquist, N. G.; Bergman, A. Decabromobiphenyl, polybrominated diphenyl ethers, and brominated phenolic compounds in serum of cats diagnosed with the endocrine disease feline hyperthyroidism. *Arch. Environ. Contam. Toxicol.* **2012**, *63* (1), 161−168.
(38) Watanabe, I.; Sakai, S. Environmental release and behavior of brominated flame retardants. *Environ. Int.* **2003**, *29* (6), 665−682.
(39) Kunisue, T.; Takayanagi, N.; Isobe, T.; Takahashi, S.; Nakatsu, S.; Tsubota, T.; Okumoto, K.; Bushisue, S.; Shindo, K.; Tanabe, S. Regional trend and tissue distribution of brominated flame retardants and persistent organochlorines in raccoon dogs (Nyctereutes procyonoides) from Japan. *Environ. Sci. Technol.* **2008**, *42* (3), 685−691.
(40) Stapleton, H. M.; Dodder, N. G.; Offenberg, J. H.; Schantz, M. M.; Wise, S. A. Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environ. Sci. Technol.* **2005**, *39* (4), 925−931.
(41) Suzuki, G.; Kida, A.; Sakai, S.-i.; Takigami, H. Existence State of Bromine as an Indicator of the Source of Brominated Flame Retardants in Indoor Dust. *Environ. Sci. Technol.* **2009**, *43* (5), 1437−1442.
(42) Ueno, D.; Kajiwara, N.; Tanaka, H.; Subramanian, A.; Fillmann, G.; Lam, P. K.; Zheng, G. J.; Muchitar, M.; Razak, H.; Prudente, M.; Chung, K. H.; Tanabe, S. Global pollution monitoring of polybrominated diphenyl ethers using skipjack tuna as a bioindicator. *Environ. Sci. Technol.* **2004**, *38* (8), 2312−2316.
(43) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* **2005**, *307* (5711), 917−920.
(44) Wan, Y.; Wiseman, S.; Chang, H.; Zhang, X.; Jones, P. D.; Hecker, M.; Kannan, K.; Tanabe, S.; Hu, J.; Lam, M. H.; Giesy, J. P. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants? *Environ. Sci. Technol.* **2009**, *43* (19), 7536−7542.
(45) Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Murk, A. J.; Visser, T. J.; Van Velzen, M. J.; Brouwer, A.; Bergman, A. Biotransformation of brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). *Mol. Nutr. Food Res.* **2008**, *52* (2), 284−298.
(46) Kakehi, M.; Ikenaka, Y.; Nakayama, S. M. M.; Kawai. Y. K.; Watanabe. K. P.; Mizukawa, H.; Nomiyama, K.; Tanabe, S.; Ishizuka, M.; UGT Xenobiotic metabolizing activity and genetic evolution in Pinniped species. *Toxicol. Sci.* **2015**, *147*, 360; DOI: 10.1093/toxsci/kfv144.
(47) van Boxtel, A. L.; Kamstra, J. H.; Cenijn, P. H.; Pieterse, B.; Wagner, J. M.; Antink, M.; Krab, K.; van der Burg, B.; Marsh, G.; Brouwer, A.; Legler, J. Microarray analysis reveals a mechanism of phenolic polybrominated diphenylether toxicity in zebrafish. *Environ. Sci. Technol.* **2008**, *42*, 1773−1779.
(48) Dingemans, M. M.; van den Berg, M.; Westerink, R. H. Neurotoxicity of brominated flame retardants: (in) direct effects of parent and hydroxylated polybrominated diphenyl ethers on the (developing) nervous system. *Environ. Health Perspect* **2011**, *119* (7), 900−907.
(49) Liu, F.; Wiseman, S.; Wan, Y.; Doering, J. A.; Hecker, M.; Lam, M. H.; Giesy, J. P. Multi-species comparison of the mechanism of biotransformation of MeO-BDEs to OH-BDEs in fish. *Aquat. Toxicol.* **2012**, *114−115*, 182−188.
(50) Stapleton, H. M.; Kelly, S. M.; Pei, R.; Letcher, R. J.; Gunsch, C. Metabolism of polybrominated diphenyl ethers (PBDEs) by human hepatocytes in vitro. *Environ. Health Perspect.* **2009**, *117* (2), 197−202.
(51) Qiu, X. H.; Mercado-Feliciano, M.; Bigsby, R. M.; Hites, R. A. *Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture*. *Environ. Health Perspect.* **2007**, *115*, 1052−1058.

DOI: 10.1021/acs.est.5b04216
Environ. Sci. Technol. 2016, 50, 444−452