Supporting Information

MS Title:

Regional Trend and Tissue Distribution of Brominated Flame Retardants and Persistent Organochlorines in Raccoon Dogs (*Nyctereutes procyonoides*) from Japan

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Chemical analysis. Analysis of PBDEs (BDE 15, 28, 47, 99, 100, 153, 154, 183, 196, 197, 206, 207, and 209) and HBCDs (α-, β-, and γ-HBCD) was performed following the procedures described by Ueno et al. (*25*) and Isobe et al. (*26*) with slight modifications. Briefly, 20 g of liver or 6 g of adipose tissue was ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of hexane and diethyl ether. $^{13}C_{12}$-labelled PBDEs (BDE 15, 28, 47, 99, 153, 154, 183, 197, 207, and 209) and $^{13}C_{12}$-labelled HBCDs (α-, β-, and γ-HBCD) were spiked into an aliquot of the extract as internal standards (IS). Lipids in this extract were removed by gel permeation chromatography (GPC) using Bio-Bead S-X 3 (Bio-Rad Laboratories, USA). The GPC fraction including PBDEs and HBCDs was concentrated and passed through activated silica gel (Wakogel DX: Wako Pure Chemical Industries Ltd., Japan) packed in a glass column. PBDEs were eluted with 5 % dichloromethane (DCM) in hexane, followed by the elution of HBCDs with 25 % DCM/hexane. $^{13}C_{12}$-labelled BDE 139 was added to the final PBDEs solution prior to gas chromatograph (GC)-mass selective detector (MS) analysis to calculate the recovery rates of IS. Quantification was performed using a GC (Agilent 6890N)-MS (Agilent 5973N) for di- to hepta-BDEs, and a GC (Agilent 6890N)-MS (JEOL GCmate II) for octa- to deca-BDEs, using electron ionization with selective ion monitoring (EI-SIM) mode. Analytical conditions of GC-MS were previously described by Ueno et al. (*25*). PBDE congeners were quantified using the isotope dilution method to the corresponding $^{13}C_{12}$-labelled congeners (BDE 100, 196, and 206 were quantified using $^{13}C_{12}$-BDE 99, 197, and 207, respectively). Recoveries of $^{13}C_{12}$-labelled BDEs were within 60-110%.

The HBCDs fraction was concentrated and deuterated HBCDs (α-, β-, γ-HBCD-d_{18}) were spiked prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. LC (Waters 2795) separation of α-, β-, and γ-HBCD isomers was achieved with an Extend-C18 column (2.1mm i.d. x 150mm, 5μm). The mobile phase consisted of water/acetonitrile/methanol (20:30:50) and gradually changed to acetonitrile/methanol (30:70). The MS/MS (Micromass Quattro micro^{TM} API) analysis was performed using negative electrospray ionization (ESI) with multiple reaction monitoring mode (MRM). Native α-, β-, and γ-HBCD isomers were quantified from the mean value of the response at two MRM transitions (*i.e. m/z* 640.6 > 81 and 642.6 > 81) corrected against the response of $^{13}C_{12}$-labelled HBCDs (*m/z* 652.6 > 81). Performance of the instrument and effect of matrices in sample extract was evaluated by response of α-, β-, γ-HBCD-d_{18} (*m/z* 658.6 > 81 MRM transition). Recoveries of $^{13}C_{12}$-labelled HBCDs were within 70-110%.

Prior to chemical analysis, we checked the recovery rates of IS added before (liver : n = 3, adipose tissue : n = 3) or after (liver : n = 3, adipose tissue : n = 3) the extraction by using the same liver or adipose tissue samples. As a result, we obtained similar results of IS recovery rates (before extraction : PBDEs =

60-105%, HBCDs = 68-105%; after extraction : PBDEs = 62-115%, HBCDs = 70-115%). In addition, native concentrations in the samples quantified by isotope dilution method were also similar between both these methods. Thus, it appears certain that sufficiently adequate recovery of PBDEs and HBCDs from liver or adipose tissue is achieved by using Soxhlet-extraction for 8 hours.

Another aliquot of the extract was subjected to analysis of OCs including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs), and hexachlorobenzene (HCB). The analytical method of OCs in RDs has been already described (*20*). In this study, data on OCs in RD livers from Kanagawa were cited from Kunisue *et al*. (*20*).

Lipid contents were determined by measuring the total nonvolatile solvent extractable material in subsamples taken from the original extracts. The concentrations of organohalogen compounds were expressed on lipid weight basis.

QA/QC. The peaks that meet the following criteria were identified and quantified as target compounds: 1) the retention time match that of the standard compound within ±0.1 min, 2) the signal-to-noise ratio (S/N) is higher than 10:1, 3) the deviation of ion intensity ratio is within 15 % of that of the standard compound. Procedural blanks were analyzed simultaneously with every batch of five samples to check for interferences or contamination from solvent and glassware. Extremely low peaks of BDE 47 and 209 below S/N of 2:1 were found in a few blanks (the peak areas of these congeners were almost same within the batches) and hence concentrations in samples of these batches were corrected by subtracting the blank values. The limit of quantification (LOQ) was defined as the amount of target compound that resulted in S/N of 10:1. Values below LOQ were treated as zero for calculating total, mean, and median concentrations, and standard deviations, and for statistical analyses.

Our laboratory participated in the Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissues organized by the National Institute of Standards and Technology (NIST: Gaithersburg, MD, USA) and Marine Mammal Health and Stranding Response Program of the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (Silver Spring, MD, USA). Standard reference material SRM 1945 (whale blubber) was analyzed for selected PBDE and PCB congeners, and persistent OC pesticides. The deviation of our data in comparison to reference values were within 0-12 % for PBDE congeners, 1.3-57 % for PCB congeners, and 0.5-20 % for OC pesticides.

FIGURE S1. Map showing the sampling prefectures.

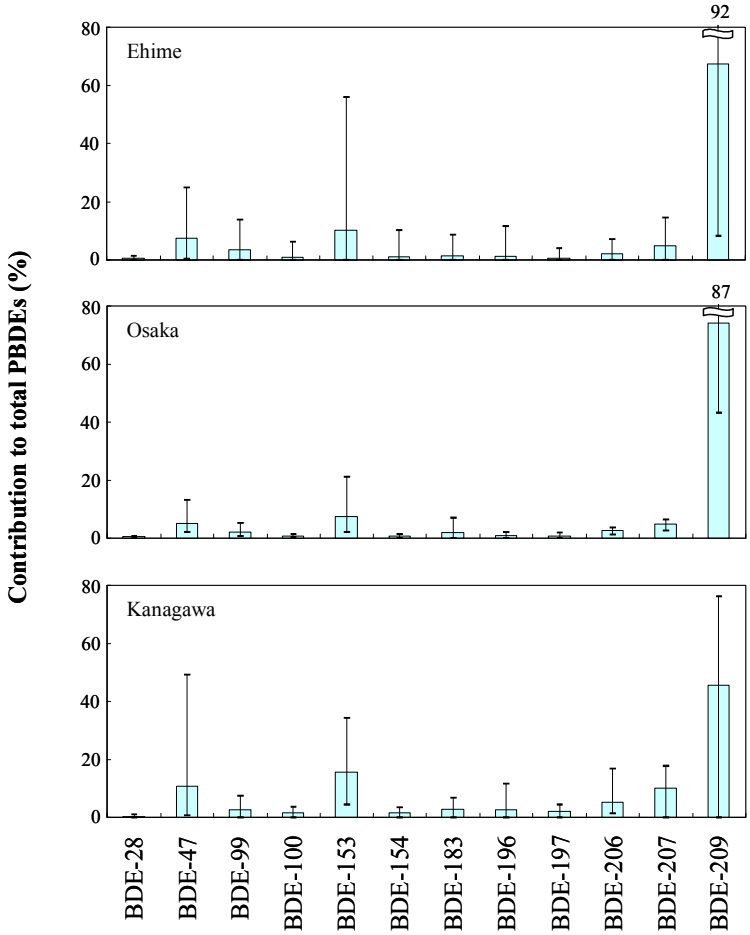

FIGURE S2. Composition of PBDEs in raccoon dog livers analyzed in this study. Error bars show ranges.

TABLE S1. Comparison of Concentrations (ng/g lipid wt.) of PBDEs and HBCDs in Raccoon Dogs Analyzed in This Study with Those in Japanese Human, Polar Bears and Other Wild Terrestrial Mammals Reported Elsewhere

Species	Tissue	Location	Year	*n*	Sex	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE196	BDE197	BDE206	BDE207	BDE209	α-HBCD	β-HBCD	γ-HBCD	Ref.
Raccoon dog	Liver	Japan	2001-2006	39	male/female	0.074	1.6	0.66	0.41	5.1	0.51	0.84	0.53	0.51	1.1	3.3	24	0.97	0.24	1.0	This study
(*Nyctereutes procyonoides*)	Adipose			8	male/female	0.13	3.0	0.97	0.34	4.3	0.35	1.3	0.29	0.24	0.59	0.95	5.9	1.8	0.040	0.059	
Human	Adipose	Japan	2003-2004	28	male/female	0.27	1.3	0.20	0.56	2.5	0.16	0.14	0.16	0.79	0.089	0.62	1.4	NA	NA	NA	(*17*)
Red fox	Liver	Belgium	2003-2004	30	male/female	ND	0.33	0.11	0.05	1.2	0.02	0.61	NA	NA	NA	NA	27 [a]	NA	NA	NA	(*29*)
(*Vulpes vulpes*)	Adipose			27	male/female	ND	0.23	0.33	0.05	0.76	0.04	0.37	NA	NA	NA	NA	ND	NA	NA	NA	
Wild rodents	Liver	Belgium	2001	63	-	ND	3.0	2.3	0.39	1.9	ND	1.5	NA	NA	NA	NA	ND	NA	NA	NA	(*33*)
(wood mice; *Apodemus sylvaticus*)	Muscle			63		ND	3.3	2.2	0.58	1.4	ND	1.3	NA	NA	NA	NA	ND	NA	NA	NA	
(bank voles; *Clethrionomys glareolus*)																					
Polar bear	Adipose	Svalbard	1999-2002	15	female	ND	41	2.3	0.87	3.2	ND	ND	NA	NA	NA	NA	ND	44 [b]			(*34*)
(*Ursus maritimus*)		East Greenland	1999-2002	44	female	ND	51	3.9	1.5	12	ND	ND	NA	NA	NA	NA	ND	45 [b]			
		Bering-Chukchi Sea	1994-2002	8	female	ND	4.9	0.78	0.20	0.11	ND	ND	NA	NA	NA	NA	ND	0.40 [b]			

[a] *n*=12

[b] Total

NA = Not analyzed.

ND = Not detected (trace level)