BROMINATED FLAME RETARDANTS IN RACCOON DOGS FROM JAPAN – A MONITORING SURVEY OF WILD TERRESTRIAL MAMMALS

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Abstract

Brominated flame retardants, such as PBDEs and HBCDs, as well as organochlorines, including CHLs, PCBs, DDTs, HCHs, HCB, were determined in a wild terrestrial mammal, raccoon dog (*Nyctereutes procyonoides*), collected from Ehime, Osaka and Kanagawa prefectures in Japan during 2001-2003. Organohalogens were detected in all samples analyzed. CHLs were the highest followed by PCBs > DDTs ≥ HCHs > PBDEs > HCB ≥ HBCDs. Significant differences in the concentrations of CHLs and DDTs in raccoon dogs were observed among the three prefectures, but not for other organochlorine compounds. This could be due to regional-specific use of CHLs and DDTs in the past. Residue levels of hepta-, octa- and nona-BDEs in raccoon dogs from Kanagawa Prefecture were higher than those of Ehime and Osaka, indicating that raccoon dogs from Kanagawa were exposed to these BDE congeners originating from a technical octa-BDE mixture. On the other hand, HBCDs were not detected in raccoon dogs from Kanagawa, whereas these contaminants were found in Ehime and Osaka, suggesting sources of pollution in these two prefectures.

Introduction

Contamination by organochlorines (OCs) in marine mammals and predatory birds is still prominent and hence their toxic impacts to high trophic wildlife are of great concern. In recent years, environmental pollution by brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) have been drawing public attention due to their persistence, bioaccumulative nature, and possible adverse effects on humans and wildlife. Both these chemicals are used as additive flame retardants in a wide variety of commercial products such as plastics, textiles, and electronic appliances, though use of OCs has already been terminated in most of the countries. Although several reports on the environmental behavior and fate of PBDEs have been published[1,2], only limited information on other BFRs such as HBCDs are available so far[3]. Since statistical data demonstrated that Asian countries shared about 40% and 23% of the global PBDEs and HBCDs consumption in 2001, respectively[4], it is necessary to investigate its contamination status in Asia, especially in Japan. Our group previously investigated the contamination status of OCs in raccoon dogs from Kanagawa prefecture, Japan[5,6]. As a result of those studies, we have found TEQs-dependent hepatic sequestration of PCDD/DF congeners, oxychlordane and *p,p'*-DDD in raccoon dogs. These results indicated that wild terrestrial mammals in Japan are exposed to relatively high levels of OCs such as dioxins and CHLs. But the regional trend of OC contamination in raccoon
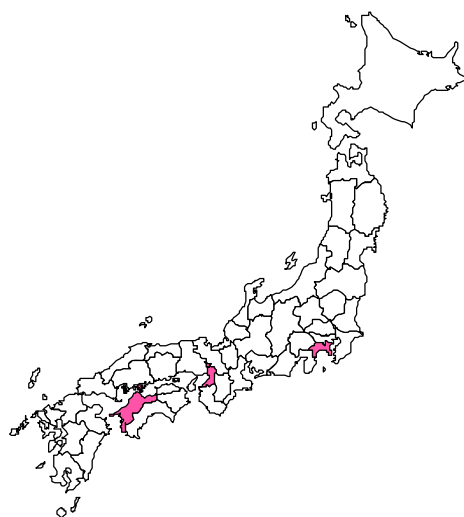

Figure 1. Sampling locations of raccoon dogs in Japan. The samples were collected from colored prefectures.

dogs is unknown. Furthermore, no information is available on PBDEs and on HBCDs in Japanese terrestrial mammals. The purpose of this study was to elucidate the contamination status and accumulation features of organohalogen compounds in a wild terrestrial mammal, raccoon dog, collected from Ehime, Osaka and Kanagawa prefectures in Japan.

Materials and Methods
Samples
Livers of raccoon dog were collected from Ehime (*n*=21; male=12, female=9), Osaka (*n*=8; male=5, female=3) and Kanagawa (*n*=10; male=5, female=5) prefectures during 2001-2003 (Figure 1). All samples were stored in Environmental Specimen Bank (*es*-BANK) of Ehime University[7] at -20℃ until analysis.

Chemical Analysis
OCs (PCBs, DDTs, HCHs, CHLs and HCB) and BFRs (PBDEs and HBCDs) were analyzed following the methods described previously[8,9]. Liver samples were Soxhlet extracted with diethyl ether/hexane solution. The extract was purified and fractionated using a gel permeation chromatography and an activated silica gel column. Identification and quantification of OCs, PBDEs and HBCDs were achieved using GC-ECD, GC-MS, and LC-MS-MS, respectively. Concentrations are expressed as ng/g lipid weight unless otherwise stated.

Table 1. Concentrations (ng/g lipid wt.) of Organohalogen Compounds in Raccoon Dogs from Japan

	Ehime (Western local prefecture)			Kanagawa (Eastern metropolis) [a]			Osaka (Western metropolis)		
	Liver (*n* = 21)		% of samples	Liver (*n* = 10)		% of samples	Liver (*n* = 8)		% of samples
	Mean ±SD	(Range)	detected	Mean ±SD	(Range)	detected	Mean ±SD	(Range)	detected
Lipid (%)	4.0 ±0.62	(2.6-5.1)		3.3 ±0.5	(2.6-4.2)		3.8 ±1.2	(2.8-5.8)	
BDE28	**0.097** ±0.27	(<0.01-1.3)	62	**0.019** ±0.044	(<0.01-0.13)	20	**0.083** ±0.052	(<0.01-0.18)	88
BDE47	**1.4** ±2.2	(0.03-8.2)	100	**2.2** ±1.5	(0.17-5.2) [c]	100	**1.5** ±1.2	(0.60-3.6)	100
BDE99	**0.53** ±0.86	(<0.01-3.7)	81	**1.1** ±1.0	(<0.01-3.3) [c]	90	**0.48** ±0.20	(0.25-0.76)	100
BDE100	**0.28** ±1.0	(<0.01-4.8)	48	**0.90** ±1.1	(<0.01-3.7) [d]	90	**0.15** ±0.10	(0.051-0.33)	100
BDE153	**3.5** ±9.5	(<0.01-43)	76	**11** ±13	(0.12-37) [c]	100	**2.0** ±1.3	(0.31-4.3)	100
BDE154	**0.44** ±1.7	(<0.01-8.0)	62	**0.98** ±1.1	(<0.01-3.4) [c, d]	90	**0.11** ±0.082	(<0.01-0.26)	88
BDE183	**0.25** ±0.55	(<0.01-2.4)	52	**2.4** ±3.3	(<0.01-10) [c]	80	**0.38** ±0.59	(<0.01-1.7)	50
BDE196	**0.22** ±0.51	(<0.02-1.8)	48	**1.4** ±1.6	(<0.02-4.8)	80	**0.20** ±0.15	(<0.02-0.93)	75
BDE197	**0.076** ±0.1	(<0.02-0.36)	52	**1.7** ±2.5	(<0.02-8.1) [c]	90	**0.12** ±0.13	(<0.02-0.28)	50
BDE206	**0.47** ±0.48	(<0.02-1.8)	76	**2.4** ±3.0	(0.059-9.4) [c]	100	**0.87** ±0.55	(0.16-1.9) [e]	100
BDE207	**0.90** ±0.95	(<0.02-2.8)	95	**9.6** ±12	(<0.02-32) [c]	90	**1.5** ±0.84	(0.48-3.0) [e]	100
BDE209	**16** ±18	(1.1-67)	100	**39** ±49	(<0.1-160)	90	**28** ±23	(5.8-76)	100
Σ PBDEs	**24** ±25	(1.4-87)	100	**73** ±83	(0.36-250)	100	**35** ±25	(13-87)	100
α-HBCD	**0.94** ±2.1	(<0.005-7.6)	91	**1.0** ±3.3	(<0.005-10)	10	**0.95** ±0.20	(0.51-1.2)	100
β-HBCD	**0.42** ±0.88	(<0.005-3.7)	62	<0.005 [b]		0	**0.13** ±0.26	(<0.005-0.71)	25
γ-HBCD	**1.9** ±4.8	(<0.005-20)	52	**0.004** ±0.014	(<0.005-0.044)	10	**0.29** ±0.36	(0.029-1.0)	100
Σ HBCDs	**3.3** ±7.1	(<0.005-29)	91	**1.0** ±3.3	(<0.005-10)	10	**1.4** ±0.7	(0.73-2.9)	100
Σ CHLs	**4400** ±9100	(180-43000)	100	**8200** ±7600	(170-22000) [c]	100	**8700** ±6300	(2000-20000) [e]	100
Σ PCBs	**200** ±370	(15-1700)	100	**390** ±410	(24-1200)	100	**130** ±100	(38-350)	100
Σ DDTs	**46** ±73	(0.45-290)	100	**210** ±270	(6.4-800) [c, d]	100	**29** ±25	(5.1-84)	100
Σ HCHs	**41** ±59	(6.5-250)	100	**67** ±74	(6.3-230)	100	**94** ±68	(25-220) [e]	100
HCB	**4.5** ±13	(0.78-61)	100	**2.7** ±2.0	(0.54-6.2)	100	**1.4** ±0.78	(0.57-2.5)	100

SD = standard deviation

The concentrations below detection limit were treated as zero for arithmetic mean values.

[a] Organochlorine data were cited from Kunisue et al. (2007).

[b] Concentrations in all the samples were below detection limit.

[c] Concentrations in liver samples from Kanagawa were significantly higher than those from Ehime.

[d] Concentrations in liver samples from Kanagawa were significantly higher than those from Osaka.

[e] Concentrations in liver samples from Osaka were significantly higher than those from Ehime.

Results and Discussion

Organohalogen compounds were detected in all raccoon dog samples analyzed (Table 1). CHLs (170-43000 ng/g lipid) were dominant, followed by PCBs (15-1700 ng/g lipid) > DDTs (2.1-290 ng/g) ≥ HCHs (6.3-250 ng/g) > PBDEs (0.36-250 ng/g) > HCB (0.54-61 ng/g) ≥ HBCDs (<0.005-29 ng/g). No significant difference between sexes was observed. This may be a result of the short lactation period in this species, which is consistent with previous studies[5,6]. It is well known that the concentration ranges in PCBs and DDTs are usually one to two orders of magnitude higher than CHLs in marine mammals and avian species from Japan[10,11]. Concentrations of CHLs, however, were higher than PCBs and DDTs in raccoon dogs, which indicate specific exposure of CHLs to terrestrial wildlife in Japan where CHLs had been used mainly as a termiticide for houses until 1986. In addition, levels of CHLs in raccoon dogs from Kanagawa and Osaka were significantly higher than those of Ehime, while concentrations of DDTs were significantly higher in raccoon dogs from Kanagawa compared to Osaka and Ehime. This could be due to regional-specific use of CHLs and DDTs in the past. Since information on contamination levels of BFRs in terrestrial wildlife is limited, it is difficult to compare with other studies. Concentrations of PBDEs in the present study were comparable to those in wild rodents (*Apodemus sylvaticus* and *Clethrionomys glareolus*) and slightly higher than those in red foxes (*Vulpes vulpes*) from Belgium[12,13]. BDE-47 and BDE-99 in raccoon dogs were lower than those in polar bears (*Ursus maritimus*) from Svalbard, whereas levels of BDE-153 were comparable[14]. Among PBDE congeners analyzed, BDE-209 was predominant in all the specimens (Table 1), which is consistent with the fact that deca-BDE mixture is the only product commercially available in Japan[15]. Although the exposure route is still unclear, uptake from soil could be one of the major routes because BDE-209 is usually a predominant congener in soils. This phenomenon is also supported by our previous study on dioxins in wild terrestrial mammals[5]. No regional difference in concentrations of total PBDEs was observed, whereas levels of tetra- to nona-BDE congeners, especially BDE-100, -154 and -197, were higher in raccoon dogs from Kanagawa than those from the other two prefectures (Figure 2). Higher levels of these congeners in Kanagawa suggest pollution sources of tetra- and octa-BDE technical mixtures in this region. HBCDs were also found in most of the specimens from Ehime and Osaka prefectures, but only in one sample from
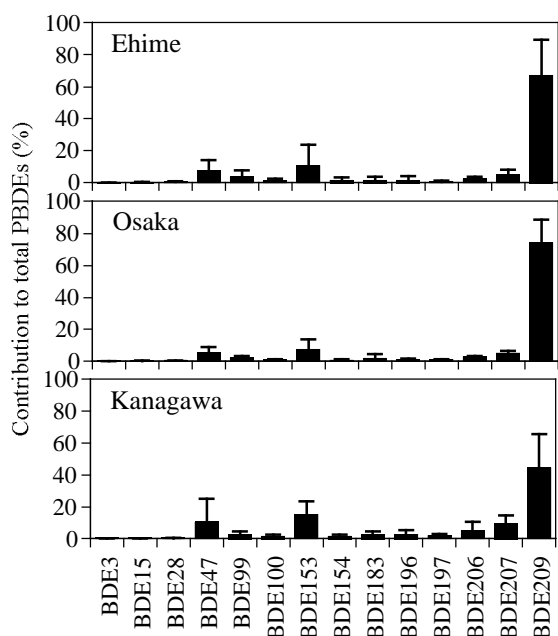

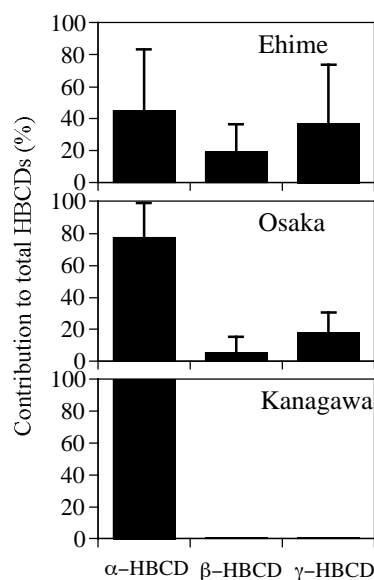**Figure 2. Relative congener profile of PBDEs in raccoon dogs.**

Figure 3. Relative isomeric composition of HBCDs in raccoon dogs.

Kanagawa. This suggests regional differences in contamination of the terrestrial environment by HBCDs. Isomeric composition of α- and γ-HBCD varied among samples (Figure 3). γ-HBCD is the dominant isomer in technical HBCD mixture, whereas α-HBCD accumulates in higher trophic organisms[16]. In addition, the half-life of γ-HBCD is shorter than α-HBCD suggesting α-HBCD is more persistent in the environment[17]. Therefore, higher levels of γ-HBCD in raccoon dogs from Ehime and Osaka implies continuous exposure of wild terrestrial mammals to commercial HBCD mixture.

Acknowledgements

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