HYDROXYLATED POLYCHLORINATED BIPHENYLS IN THE BLOOD OF BAIKAL SEALS (*PUSA SIBIRICA*)

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Introduction

PCBs are persistent and bioaccumulative chemicals that have been found to reach elevated concentrations in high trophic animals such as aquatic mammals. We have investigated contamination status and temporal trends of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in the blubber of Baikal seals (*Pusa sibirica*) which are one among the top predators of Lake Baikal. This species accumulated high levels of PCDD/Fs and PCBs, and the concentrations of PCBs and PCDDs did not show any change between 1992 and 2005.[1] In addition, half lives of PCBs were estimated to be longer than those of PCDDs. These results imply that input of PCBs into Lake Baikal and exposure of Baikal seals to PCBs are still continuing. Concentrations of TEQs and PCBs in some of specimens collected in 2005 exceeded the LOAEL for immunosuppression reported for harbor seals and especially, the risk posed by PCBs seems to be still high.[1] From these results, it is evident that a detailed risk assessment of PCBs in Baikal seals is needed. It has been noted that PCBs disturb thyroid hormone (TH) homeostasis and cerebral nervous system in animals.[2, 3] As a possible mechanism involved in disturbing TH homeostasis, the competitive binding between PCBs and thyroxine (T4) to transthyretin (TTR) in blood is well known.[2] It has been demonstrated that the binding affinity to TTR was much stronger for hydroxylated polychlorinated biphenyls (OH-PCBs), which are formed by oxidative metabolism of PCBs by the cytochrome P450 monooxygenases, than for the parent PCBs.[2, 4] In addition, it was recently shown that extremely low doses of OH-PCBs suppressed TH-induced transcriptional activation of TH receptor (TR) in cerebellar cell line, implying the disturbance of cerebral nervous system by these metabolites.[5] These observation show that risk assessment of OH-PCBs is also important.

The objectives of this study are to understand the contamination status and characterization of PCBs and OH-PCBs in the blood of Baikal seals collected in 2005.

Materials and Methods

Baikal seals were collected by shooting from Lake Baikal in 2005 under license from the local government by shooting and were immediately dissected. The blood samples of 10 males (age: 2.5-41.5) were obtained and stored in Environmental Specimen Bank for Global Monitoring (*es*-BANK) of Ehime University [6] at -25 °C until

analysis.

OH-PCBs were analyzed as follows. Briefly, the blood sample (10g) was denatured with hydrochloric acid (HCL). 2-propanol was added, and then OH-PCBs were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane. $^{13}C_{12}$-labeled 4OH-T_3CB29, 4OH-T_4CB61, 4OH-P_5CB120, 4OH-H_6CB159, 4OH-H_7CB172 and 4OH-H_7CB187, and 20 $^{13}C_{12}$-labeled T_3-D_{10}CB congeners were spiked as internal standards. The organic phases were combined, evaporated and dissolved in hexane. 1M potassium hydroxide (KOH) in 50% ethanol/water was added and shaken. The partition process was repeated and the alkaline phases were combined. The remaining organic phase was concentrated and lipid was removed by gel permeation chromatography (GPC), and then passed through activated silica-gel packed in a glass column. PCBs were eluted with hexane and concentrated for gas chromatograph (GC; Agilent 6890)-mass spectrometry (MS; Agilent 5973) analysis. The combined alkaline phase was acidified with sulfuric acid, and then OH-PCBs were extracted twice with 50% MTBE/hexane. The organic phases were combined, evaporated and dissolved in hexane, and then passed through non-activated silica-gel packed in a glass column. OH-PCBs were eluted with 50% dichloromethane (DCM)/hexane, concentrated and dissolved in hexane. OH-PCBs in hexane were methylated by reaction with trimethylsilyldiazomethane. The derivatized solution was treated with sulfuric acid, and then passed through activated silica-gel packed in a glass column. CH_3O-PCBs were eluted with 10% DCM/hexane and concentrated. Identification and quantification of OH-PCBs were performed using GC (Agilent 6890)-high resolution MS (JEOL JMS-800D).

Unknown OH-PCBs were identified following the method described previously.[7]

Results and Discussion

OH-PCBs were detected in all the blood samples of Baikal seals in this study (Table 1). Concentrations of OH-PCBs including identified and unknown isomers were in the range of 3.5-16 ng/g wet wt. and the levels were about one order of magnitude lower than PCBs (9.9-130 ng/g wet wt.). Some studies on the residue levels of OH-PCBs have been conducted using blood of human and wildlife.[8-13] Concentrations of OH-PCBs in the blood of Baikal seals were considerably lower than those of polar bears, but relatively high compared with human and cetaceans.[8-10] When concentration ratio of OH-PCBs to PCBs (OH-PCBs/PCBs

Table 1. Concentration (ng/g wet wt.) of OH-PCBs and PCBs in blood of male Baikal seals (n = 10).

	Mean ± SD	Median	Range
age	17.4 ± 15.3	12	2.5 – 41.5
	OH-PCBs		
OH-tri-CB			
Unknown isomers [a]	0.035 ± 0.017	0.032	0.016 - 0.068
OH-tetra-CB			
Unknown isomers [b]	0.51 ± 0.26	0.42	0.24 - 0.98
OH-penta-CB			
Identified isomers	0.51 ± 0.36	0.35	0.21 - 1.3
Unknown isomers [c]	3.4 ± 1.6	3.1	1.6 - 6.4
Total	3.9 ± 2.0	3.4	1.8 - 7.7
OH-hexa-CB			
Identified isomers	0.79 ± 0.52	0.64	0.28 - 1.7
Unknown isomers [d]	0.95 ± 0.60	0.67	0.43 - 2.2
Total	1.7 ± 1.1	1.3	0.70 - 3.9
OH-hepta-CB			
Identified isomers	0.41 ± 0.28	0.30	0.17 - 1.1
Unknown isomers [e]	0.78 ± 0.46	0.64	0.30 - 1.8
Total	1.2 ± 0.74	0.93	0.46 - 2.8
OH-octa-CB			
Identified isomers	0.24 ± 0.21	0.14	0.066 - 0.66
Unknown isomers [f]	0.048 ± 0.039	0.031	0.014 - 0.12
Total	0.29 ± 0.25	0.17	0.079 - 0.76
Total identified isomers	**2.0 ± 1.3**	**1.4**	**0.74 - 4.8**
Total unknown isomers	**5.7 ± 2.9**	**4.8**	**2.7 - 11**
Total OH-PCBs	**7.7 ± 4.2**	**6.2**	**3.5 - 16**
	PCBs		
Tetra-CB	1.1 ± 0.49	1.1	0.42 - 1.8
Penta-CB	13 ± 8.7	12	3.5 - 30
Hexa-CB	27 ± 25	21	4.8 - 72
Hepta-CB	8.2 ± 8.6	5.1	1.1 - 25
Octa-CB	0.76 ± 0.95	0.4	< 0.12 - 2.8
Nona-CB	0.47 ± 0.66	0.19	< 0.12 - 2.0
Deca-CB	0.29 ± 0.16	0.26	< 0.12 - 0.51
Total PCBs	**51 ± 44**	**41**	**9.9 - 130**

[a]sum of 3 isomers; [b]sum of 20 isomers; [c]sumof 16 isomers
[d]sum of 24 isomers; [e]sum of 9 isomers; [f]sum of 2 isomers

ratio) were examined, relatively low values in Baikal seals were observed compared with other species including human (Fig. 1), suggesting poor metabolic capacity for PCBs in Baikal seals compared to other wildlife except for cetaceans and/or continuous exposure to PCBs in Lake Baikal.

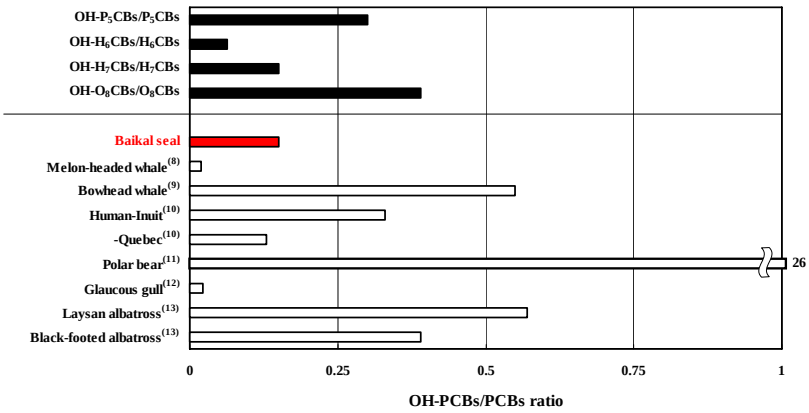

Fig. 1. Comparison of OH-PCBs/PCBs ratio in the blood of Baikal seals with those of human and wildlife reported previously. (8-13) Refferences cited.

In general, 4OH-CB120/101, 4OH-CB107/108, 4OH-CB146, 4OH-CB138, 4OH-CB187 and 4OH-CB172 have been detected as predominant congeners in blood of many species including human.[8-13] These patterns were also observed in this study (Fig. 2). As for unknown isomers, OH-T_4CBs, OH-P_5CBs, OH-H_6CBs and OH-H_7CBs in the blood of Baikal seals were more than 70% of total OH-PCBs; especially, OH-P_5CB levels were relatively high. When OH-PCB/PCB homologue ratios were calculated, OH-P_5CB/P_5CB and OH-O_8CB/O_8CB ratios were higher than the values for H_6- and H_7-chlorinated homologues (Fig. 1), suggesting a preferential accumulation of OH-P_5CBs and OH-O_8CBs in the blood of Baikal seals. However, no other information is available for pinniped species. In cetaceans, OH-P_5CB detected in the liver of beluga whale from Canadian Arctic and St Lawrence River account for 90% of total OH-PCB concentrations.[14] OH-P_5CB/P_5CB ratios were considerably higher than H_6- and H_7-chlorinated homologues in the blood of melon-headed whales.[8] But no information is available for OH-O_8CBs.

In the present study, unknown OH-PCB congeners were higher than identified OH-PCB congeners. Therefore the identification of these congeners is essential to assess adverse effects such as TH disturbance in Baikal seals.

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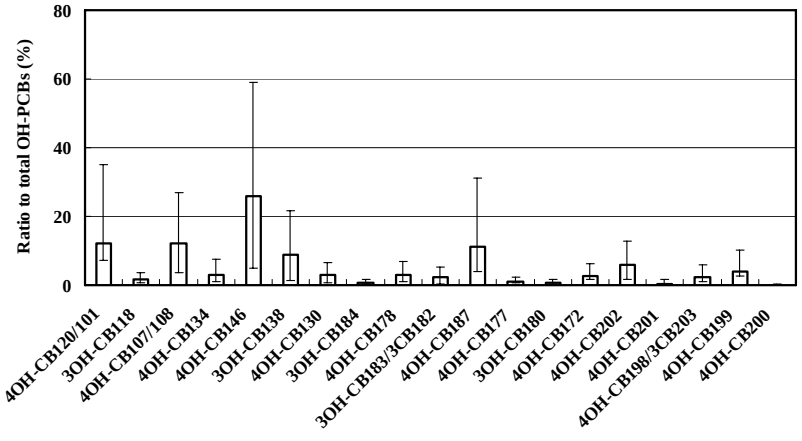

Fig. 2. Mean concentration ratio of the identified OH-PCBs to identified total OH-PCBs.

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