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Residue Levels of OH–PCBs and PCBs in the Blood of Baikal Seals (*Pusa sibirica*)

Daisuke IMAEDA[1], Kei NOMIYAMA[1], Tatsuya KUNISUE[1,2], Hisato IWATA[1], Oyuna TSYDENOVA[1], Shin TAKAHASHI[1], Masao AMANO[3], Evgeny A. PETROV[4], Valeriy B. BATOEV[5] and Shinsuke TANABE[1]

[1]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[2]*Wadworth Center, New York State Department of Health, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, U.S.A.*
[3]*Department of Animal Sciences, Teikyo University of Science and Technology, 2525 Yatsusawa Uenohara, Yamanashi 409-0193, Japan*
[4]*The Eastern-Siberian Scientific and Production Fisheries Center, "VOSTSIBRYBCENTER", Ulan-Ude, Buryatia 670034, Russia*
[5]*Baikal Institute of Nature Management, Siberian Branch of Russian Academy of Sciences, Ulan-Ude, Buryatia 670047, Russia*

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Abstract—The present study determined the residue levels and patterns of polychlorinated biphenyls (PCBs) and hydroxylated PCB (OH–PCBs) congeners in the blood of Baikal seals (*Pusa sibirica*) collected in 2005 from Lake Baikal, Russia. Concentrations of OH–PCBs were in the range of 0.71–4.6 ng/g wet wt. and the levels were one to two orders of magnitude lower than PCBs (6.4–130 ng/g wet wt.). Concentrations of high-chlorinated OH–PCBs (OH–H_6 to O_8CBs) were 80% of total OH–PCBs, suggesting risk by high-chlorinated OH–PCBs in Baikal seals. When OH–PCB/PCB homologue ratios were calculated, OH–O_8CB/O_8CB ratios were higher than the values of T_4- and H_7-chlorinated homologues, suggesting preferential accumulation of OH–O_8CBs in the blood of Baikal seals. When concentration ratios of OH–PCB to PCB (OH–PCB/PCB ratios) were examined, relatively low values in Baikal seals (OH–PCB/PCB ratios = 0.047) were observed when compared with other species, suggesting poor metabolic capacity for PCBs in Baikal seals. OH–PCB/PCB ratios of terrestrial mammals were the highest, followed by avian species, pinniped species and cetacean species. From the present results on hydroxylated PCB metabolites in Baikal seals, it is evident that concentration ratios of metabolites to parent compounds can be used as indicators of xenobiotic metabolism in organisms.

Keywords: PCBs, hydroxylated PCBs, blood, Baikal seal

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*). This species accumulated high levels of PCDD/Fs and PCBs, and the levels of PCBs and PCDDs did not vary much between 1992 and 2005 (Imaeda *et al.*, 2009). 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 the specimens collected in 2005 exceeded the lowest observed adverse effect level (LOAEL) for immunosuppression reported for harbor seals and especially, the risk posed by PCBs seems to be still high (Imaeda *et al.*, 2009). 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 (Brouwer *et al.*, 1995, 1998). As a possible mechanism involved in disturbing TH homeostasis, the competitive binding between PCBs and thyroxine (T_4) to transthyretin (TTR) in blood is well known (Brouwer *et al.*, 1998). 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 (Brouwer *et al.*, 1998; Cheek *et al.*, 1999). 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 (Iwasaki *et al.*, 2002). These observation show that risk assessment of OH–PCBs is also important.

MATERIALS AND METHODS

Baikal seals were collected from Lake Baikal by shooting, in 2005 under license from the local government and were immediately dissected. The blood samples of 10 males (age: 2.5–41.5) were collected and stored in the Environmental Specimen Bank for Global Monitoring (*es*-BANK) of Ehime University, Japan (Tanabe, 2006) at –25°C until analysis.

Analytical procedure of OH–PCBs and PCBs in blood samples were performed following previous reports (Kunisue *et al.*, 2007; Kunisue and Tanabe, 2009). Briefly, the blood sample (10 g) 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

Table 1. Concentrations of OH–PCBs in the blood of Baikal seals.

	Mean ± SD	Median	Range
OH–PCBs*			
ΣOH–T_3CBs	7.2 ± 3.7	6.0	3.4–17
ΣOH–T_4CBs	44 ± 19	39	23–91
ΣOH–P_5CBs	310 ± 220	230	150–980
ΣOH–H_6CBs	790 ± 580	580	260–2100
ΣOH–H_7CBs	440 ± 270	320	160–1100
ΣOH–O_8CBs	220 ± 160	160	60–560
ΣOH–PCBs	1800 ± 1200	1500	710–4600
PCBs**			
ΣT_3CBs	<0.13		
ΣT_4CBs	0.57 ± 0.30	0.40	0.22–1.2
ΣP_5CB	11 ± 8.1	8.2	2.8–31
ΣH_6CB	20 ± 21	12	2.7–70
ΣH_7CB	6.1 ± 7.0	3.8	0.65–24
ΣO_8CB	0.78 ± 0.97	0.50	<0.13–3.6
ΣN_9CB	0.41 ± 0.59	0.19	<0.13–2.2
ΣD_{10}CB	<0.13		<0.13–0.55
ΣPCBs	39 ± 38	26	6.4–130

*pg/g wet wt.
**ng/g wet wt.

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 5% water-containing 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. Lipid of the derivatized solution was removed by GPC, 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).

RESULTS AND DISCUSSION

OH–PCBs were detected in all the blood samples of Baikal seals in this study (Table 1). Concentrations of OH–PCBs were in the range of 0.71–4.6 ng/g wet wt.

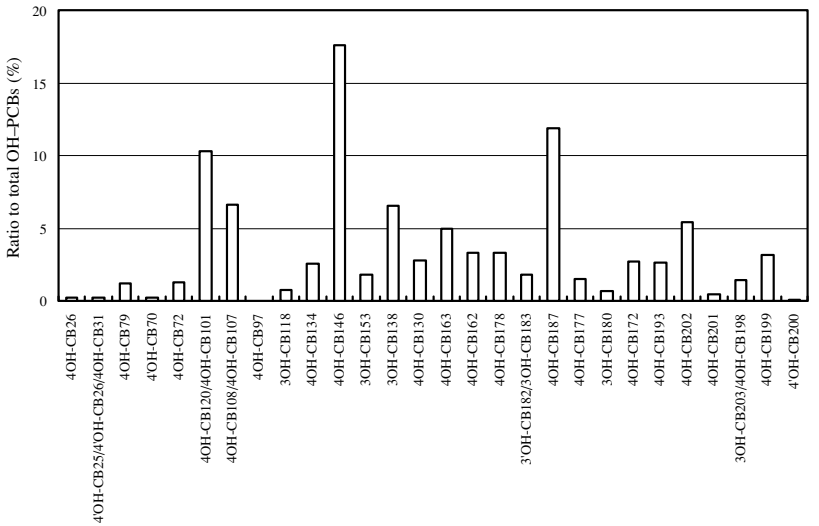

Fig. 1. Median Concentration ratio of OH–PCBs to total OH–PCBs.

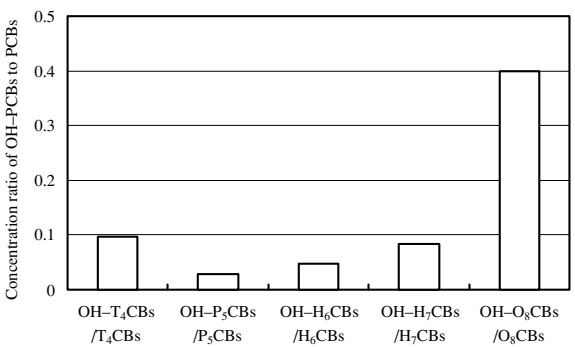

Fig. 2. Median concentration ratio of OH–PCB homologues to PCB homologues in the blood of Baikal seals.

and the levels were one to two orders of magnitude lower than PCBs (6.4–130 ng/g wet wt.). Concentrations of OH–H_6CBs (790 pg/g wet wt.) were dominant, followed by OH–H_7CBs (440 pg/g wet wt.), OH–P_5CBs (310 pg/g wet wt.), OH–O_8CBs (220 pg/g wet wt.), OH–T_4CBs (44 pg/g wet wt.) and OH–T_3CBs (7.2 pg/g wet wt.). Concentrations of high-chlorinated OH–PCBs (OH–H_6 to O_8CBs) were 80% to total OH–PCBs, suggesting risk by high-chlorinated OH–PCBs in Baikal seals. In composition of OH–PCBs, 4OH-CB146 were dominant, followed by 4OH-CB187, 4′OH-CB120/101, 3OH-CB138, 4OH-CB163, 4OH-CB107/

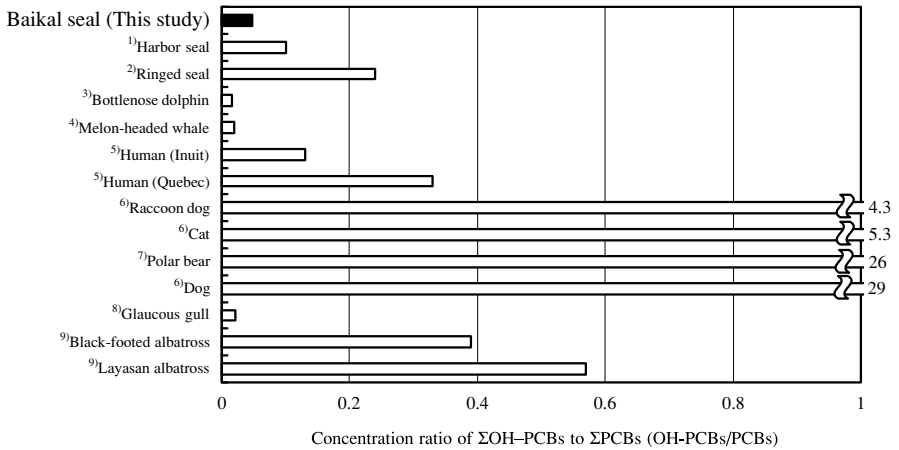

Fig. 3. Comparison of OH–PCBs/PCBs ratio in the blood of Baikal seals with those of human and wildlife reported previously. 1) Weijs *et al*., 2009; 2) Routti *et al*., 2008; 3) Houde *et al*., 2006; 4) Murata *et al*., 2007; 5) Sandau *et al*., 2000; 6) Kunisue and Tanabe, 2009; 7) Gebbink *et al*., 2008; 8) Verreault *et al*., 2007; 9) Klasson-Wehler *et al*., 1998.

4′OH-CB108 and 4OH-CB202 (Fig. 1). 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 (Klasson-Wehler *et al*., 1998; Sandau *et al*., 2000; Hoekstra *et al*., 2003; Houde *et al*., 2006; Murata *et al*., 2007; Verreault *et al*., 2007; Gebbink *et al*., 2008). These results were consistent with this study. Some studies on the residue levels of OH–PCBs were conducted using blood of human and wildlife (Klasson-Wehler *et al*., 1998; Sandau *et al*., 2000; Hoekstra *et al*., 2003; Houde *et al*., 2006; Murata *et al*., 2007; Verreault *et al*., 2007; Gebbink *et al*., 2008). 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 (Sandau *et al*., 2000; Houde *et al*., 2006; Murata *et al*., 2007; Gebbink *et al*., 2008).

When concentration ratios of OH–PCB homologue to PCB homologue were calculated, OH–O_8CB/O_8CB ratios were higher than the values for T_4- and H_7-chlorinated homologues (Fig. 2), suggesting a preferential accumulation of OH–O_8CBs and a preferential metabolism of O_8CBs in Baikal seals. Especially, contribution of 4OH-CB202 and 4OH-CB199 were dominant in OH–O_8CBs. However, no other information is available on pinniped species.

When concentration ratios of OH–PCB to PCB (OH–PCB/PCB ratios) were examined, relatively low values in Baikal seals (OH–PCB/PCB ratios = 0.047) were observed compared with other species including human (Fig. 3), suggesting poor metabolic capacity for PCBs in Baikal seals. OH–PCB/PCB ratios of terrestrial mammals were extremely higher than those of other animals (Gebbink *et al*., 2008; Kunisue and Tanabe, 2009). Ratios of OH–PCB/PCB observed in

Baikal seals were comparable with those of cetaceans (Fig. 3). From these results, it can be generally stand that the metabolic capacities of PCBs in terrestrial mammals were strongest, followed by avian species, pinniped species and cetacean species. The results of the analysis of hydroxylated PCB metabolites were consistent with our previous report (Tanabe, 2006). Therefore, it can be stated that the concentration ratios of metabolites to parent compounds can be indicator of xenobiotic metabolism in each organisms.

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D. Imaeda, K. Nomiyama, T. Kunisue, H. Iwata, O. Tsydenova, S. Takahashi, M. Amano, E. A. Petrov, V. B. Batoev and S. Tanabe (e-mail: shinsuke@agr.ehime-u.ac.jp)