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

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(Received 29 January 2009; accepted 24 March 2009)

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–H6 to O8CBs) 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–O8CB/O8CB ratios were higher than the values of T4- and H7-chlorinated homologues, suggesting preferential accumulation of OH–O8CBs 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 (T4) 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 monoxygenases, 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. 13C12-labeled 4OH-T3CB29, 4OH-T4CB61, 4OH-P5CB120, 4OH–H6CB159, 4OH–H7CB172 and 4OH–H7CB187, and 20 13C12-labeled T3-D10CB 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
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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. CH3O–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.

<table>
<thead>
<tr>
<th>OH–PCBs*</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣOH–T3CBs</td>
<td>7.2 ± 3.7</td>
<td>6.0</td>
<td>3.4–17</td>
</tr>
<tr>
<td>ΣOH–T4CBs</td>
<td>44 ± 19</td>
<td>39</td>
<td>23–91</td>
</tr>
<tr>
<td>ΣOH–P5CBs</td>
<td>310 ± 220</td>
<td>230</td>
<td>150–980</td>
</tr>
<tr>
<td>ΣOH–H6CBs</td>
<td>790 ± 580</td>
<td>580</td>
<td>260–2100</td>
</tr>
<tr>
<td>ΣOH–H7CBs</td>
<td>440 ± 270</td>
<td>320</td>
<td>160–1100</td>
</tr>
<tr>
<td>ΣOH–O8CBs</td>
<td>220 ± 160</td>
<td>160</td>
<td>60–560</td>
</tr>
<tr>
<td>ΣOH–PCBs</td>
<td>1800 ± 1200</td>
<td>1500</td>
<td>710–4600</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCBs**</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣT3CBs</td>
<td>&lt;0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣT4CBs</td>
<td>0.57 ± 0.30</td>
<td>0.40</td>
<td>0.22–1.2</td>
</tr>
<tr>
<td>ΣP5CB</td>
<td>11 ± 8.1</td>
<td>8.2</td>
<td>2.8–31</td>
</tr>
<tr>
<td>ΣH6CB</td>
<td>20 ± 21</td>
<td>12</td>
<td>2.7–70</td>
</tr>
<tr>
<td>ΣH7CB</td>
<td>6.1 ± 7.0</td>
<td>3.8</td>
<td>0.65–24</td>
</tr>
<tr>
<td>ΣO8CB</td>
<td>0.78 ± 0.97</td>
<td>0.50</td>
<td>&lt;0.13–3.6</td>
</tr>
<tr>
<td>ΣN9CB</td>
<td>0.41 ± 0.59</td>
<td>0.19</td>
<td>&lt;0.13–2.2</td>
</tr>
<tr>
<td>ΣD10CB</td>
<td>&lt;0.13</td>
<td></td>
<td>&lt;0.13–0.55</td>
</tr>
<tr>
<td>ΣPCBs</td>
<td>39 ± 38</td>
<td>26</td>
<td>6.4–130</td>
</tr>
</tbody>
</table>

*pg/g wet wt.
**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 OH–H_6_CBs (790 pg/g wet wt.) were dominant, followed by OH–H_7_CBs (440 pg/g wet wt.), OH–P_5_CBs (310 pg/g wet wt.), OH–O_8_CBs (220 pg/g wet wt.), OH–T_4_CBs (44 pg/g wet wt.) and OH–T_3_CBs (7.2 pg/g wet wt.). Concentrations of high-chlorinated OH–PCBs (OH–H_{6} \text{ to } O_{8} CBs) were 80% to total OH–PCBs, suggesting risk by high-chlorinated OH–PCBs in Baikal seals. In composition of OH–PCBs, 4OH–CB_{146} were dominant, followed by 4OH–CB_{187}, 4′OH–CB_{120}/101, 3OH–CB_{138}, 4OH–CB_{163}, 4OH–CB_{107}/
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In general, 4\text{OH}-CB108 and 4\text{OH}-CB202 (Fig. 1) 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 \text{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 \text{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 \text{OH}-PCB homologue to PCB homologue were calculated, \text{OH}-O\text{O}_3CB/O\text{O}_3CB ratios were higher than the values for T\text{r}- and H\text{r}-chlorinated homologues (Fig. 2), suggesting a preferential accumulation of \text{OH}-O\text{O}_3CBs and a preferential metabolism of O\text{O}_3CBs in Baikal seals. Especially, contribution of 4\text{OH}-CB202 and 4\text{OH}-CB199 were dominant in \text{OH}-O\text{O}_3CBs. However, no other information is available on pinniped species.

When concentration ratios of \text{OH}-\text{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.

Acknowledgments—This study was supported by the “Global COE Program” from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) and Japan Society for the Promotion of Science (JSPS) and Grant-in-Aid for Scientific Research (S) (No. 20221003) from JSPS.

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