HYDROXYLATED POLYCHLORINATED BIPHENYLS IN THE BLOOD OF MELON-HEADED WHALES (*PEPONOCEPHALA ELECTRA*) STRANDED ALONG THE JAPANESE COAST

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Abstract

The present study determined the residue levels and patterns of polychlorinated biphenyls (PCBs) and hydroxylated PCBs (OH-PCBs) in the blood of melon-headed whales (*Peponocephala electra*) stranded along the Japanese coast during 2006. Concentrations of OH-PCBs including identified and unknown isomers were in the range of 30-330 pg/g wet wt. and the levels were 1-2 orders of magnitude lower than PCBs. The residue levels of OH-PCBs observed in melon-headed whale blood were relatively lower than in humans and wildlife reported previously, implying poor metabolic capacity for PCBs in this odontocete. Unknown isomers were dominant among OH-P_5CBs and -H_6CBs in melon-headed whale blood; especially OH-P_5CB levels were considerably higher. When OH-PCB/PCB homologue ratios were calculated, OH-P_5CB/P_5CB ratios were higher than the values of H_6- and H_7-chlorinated homologues, suggesting a preferential accumulation of OH-P_5CBs in melon-headed whale blood.

Introduction

PCBs are persistent and bioaccumulative chemicals that have been found to reach elevated concentrations in high-trophic animals such as marine mammals.[1] 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 OH-PCBs, which are formed by oxidative metabolism of PCBs by the cytochrome P450 monooxygenases, than for the parent compounds.[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] Because of these observations, investigations on residue levels of OH-PCBs in human and wildlife blood are increasing.[6-9] However, less information on OH-PCBs in cetaceans is available despite accumulation of elevated PCB levels in their bodies. It has been also found that the residue levels and patterns of OH-PCBs vary in different animal species,[6-9] possibly because of species-specific metabolic capacity by phase I CYP and/or phase II conjugation enzymes, binding affinity to TTR, and exposure profiles to PCBs.

Our group recently detected OH-PCBs from the brain of three cetaceans stranded along the Japanese coast and demonstrated that more OH-PCB congeners and higher OH-PCBs/PCBs concentration ratios were found in melon-headed whales than in striped dolphins (*Stenella coeruleoalba*) and finless porpoises (*Neophocaena phocaenoides*),[10] indicating that this species may be at higher risk by OH-PCBs. The present study attempted to elucidate the residue levels and patterns of OH-PCBs in the blood of melon-headed whales stranded along the Japanese coast.

Materials and Methods

The blood samples were collected from nine melon-headed whales (*Peponocephala electra*) stranded along the coast of Chiba prefecture in Japan during 2006. Samples were stored in Environmental Specimen Bank (*es*-BANK) for Global Monitoring of Ehime University [11] at -20 °C until analysis.

Analysis of OH-PCBs and PCBs was performed following the procedure reported previously,[10] with slight modification. The blood sample (10 g) was denatured with HCl. $^{13}C_{12}$-labeled 4'OH-P_5CB120, 4'OH-H_6CB159, 4'OH-H_7CB172, and 4OH-H_7CB187, and 17 $^{13}C_{12}$-labeled T_3-O_8CB congeners were spiked as internal standards. 2-propanol was added, and then OH-PCBs were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane. The organic phases were combined, evaporated and dissolved in hexane. 1 M KOH in 50 % ethanol/H_2O 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, and then passed through activated silica-gel packed in a glass column. PCBs were eluted with hexane and concentrated for GC (Agilent 6890) - 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. OH-PCBs in the organic phase were methylated by reaction with trimethylsilyldiazomethane. The derivatized solution was concentrated and passed through activated silica-gel packed in a glass column. CH_3O-PCBs were eluted with 10% dichloromethane/hexane and concentrated. Identification and quantification of OH-PCBs were performed using GC (Agilent 6890) - high-resolution MS (JEOL JMS-800D). The peaks, which were within 10% of the theoretical ratio of two monitor ions and were more than 10 times of noise (S/N > 10), were also quantified as unknown OH-PCB isomers. All the OH-PCB and PCB congeners in samples were quantified using isotope dilution method to $^{13}C_{12}$-internal standards. Recoveries for $^{13}C_{12}$-labeled OH-PCBs and PCBs were within 50-80 % and 80-100 %, respectively.

Results and Discussion

OH-PCBs were detected in all the blood samples of melon-headed whales analyzed in this study (Table 1). Concentrations of OH-PCBs including identified and unknown isomers were in the range of 30-330 pg/g wet wt. and the levels were 1-2 orders of magnitude lower than PCBs (890-45000 pg/g wet wt.). The residue levels of OH-PCBs observed in melon-headed whale blood were relatively lower than in humans and wildlife reported previously.[6-9] When concentration ratios of OH-PCBs to PCBs (OH-PCBs/PCBs ratio) were examined, lower values in melon-headed whales were shown compared with those in blood of humans and other wildlife [6-9] (Fig. 1). This result indicates poor metabolic capacity for PCBs and possibly specific function of transport proteins such as TTR in this odontocete.

Among the identified OH-P_5CB, OH-H_6CB, and OH-H_7CB congeners, 4'OH-CB101/120, 4OH-CB107/4'OH-CB108, 4OH-CB146, 4OH-CB178, 4OH-CB187, and 4'OH-CB172 were predominant in melon-headed whale blood (Table 1). These metabolites have been detected in blood of humans and wildlife.[6-9] However, unknown isomers were dominant among OH-P_5CBs and -H_6CBs in melon-headed whale blood; especially OH-P_5CB levels were relatively higher, whereas predominant OH-H_6CB or -H_7CB isomers were found in humans and wildlife reported previously.[6-9] When OH-PCB/PCB homologue ratios were calculated, OH-P_5CB/P_5CB ratios were considerably higher than the values for H_6- and H_7-chlorinated homologues (Table 2), suggesting a preferential accumulation of OH-P_5CBs in melon-headed whale blood. Such a trend has been reported also in other odontocete species. OH-P_5CB detected in beluga whale (*Delphinapterus Leucus*) livers from Canadian Arctic and St. Lawrence River accounted for 90 % of total OH-PCB concentrations.[12] In addition, higher residue levels of OH-T_3-P_5CBs than OH-H_6-O_8CBs were observed in bottlenose dolphin (*Tursiops truncatus*) plasma from Western Atlantic and the Gulf

of Mexico.[13] Considering these observations, it is highly possible that odontocete species including melon-headed whale preferentially metabolize lower chlorinated PCBs and accumulate their hydroxylated metabolites in their liver and blood.

Table 1. Concentrations (pg/g wet wt.) of OH-PCBs and PCBs in the blood of melon-headed whales (*Peponocephala electra*) stranded along the Japanese coast during 2006

Sample ID	M34072	M34074	M34076	M34077	060301-6	060301-8	060302-25	060301-2	060302-2
Sex	Male	Female	Male	Male	Male	Male	Female	Female	Unknown
Body length (cm)	249	232	256	256	239	222	250	248	Not measured
OH-PCBs									
4'OH-CB101/120	8.0	<0.5	8.1	13	20	19	1.5	3.1	9.2
3'OH-CB118	5.0	<0.5	2.7	4.2	5.3	6.0	<0.5	<0.5	<0.5
4OH-CB107/4'OH-CB108	11	2.7	10	16	19	21	0.77	1.1	13
Unknown OH-P_5CBs [a]	72	23	98	200	170	200	33	40	110
Total OH-P_5CBs	96	26	120	240	210	250	35	44	130
4OH-CB134	1.2	<0.5	0.68	1.0	1.2	2.0	<0.5	<0.5	<0.5
4OH-CB146	2.5	<0.5	3.2	6.4	3.5	6.4	<0.5	<0.5	2.7
3'OH-CB138	0.86	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
4'OH-CB130	<0.5	<0.5	<0.5	0.68	0.80	0.61	<0.5	<0.5	<0.5
Unknown OH-H_6CBs [b]	19	4.0	40	78	51	54	12	14	39
Total OH-H_6CBs	23	4.0	44	86	57	63	12	14	42
4OH-CB178	0.92	<0.5	1.2	1.9	1.6	2.2	0.54	<0.5	<0.5
4OH-CB187	0.50	0.60	0.90	0.70	0.90	0.70	2.7	4.0	1.0
4'OH-CB172	1.0	<0.5	1.6	3.0	1.9	2.1	0.70	0.5	1.3
Unknown OH-H_7CBs [c]	0.67	<0.5	1.7	3.6	2.1	3.0	1.5	1.6	1.1
Total OH-H_7CBs	3.1	0.60	5.4	9.2	6.5	8.0	5.5	6.1	3.4
4'OH-CB199	<0.5	<0.5	0.64	<0.5	<0.5	<0.5	1.7	0.96	<0.5
Total OH-O_8CB	<0.5	<0.5	0.64	<0.5	<0.5	<0.5	1.7	0.96	<0.5
Total	120	30	170	330	280	320	54	64	180
PCBs									
T_4CBs	2400	190	1000	1300	1400	3100	160	210	760
P_5CBs	10000	320	3500	4100	5100	11000	440	530	2800
H_6CBs	22000	260	6300	5900	7500	15000	790	670	4700
H_7CBs	9100	110	2600	2100	2800	4900	600	310	1800
O_8CBs	1000	13	340	240	320	480	160	48	190
Total	45000	890	14000	14000	17000	34000	2200	1800	10000

[a] 17 isomers were quantified.

[b] 14 isomers were quantified.

[c] 3 isomers were quantified.

Our group recently detected OH-PCBs from the brain of melon-headed whales and demonstrated that unknown OH-P_5CB and -H_6CBs were considerably higher than identified congeners, also in the brain.[10] Predominant unknown OH-P_5CB and -H_6CB isomers in melon-headed whale blood analyzed in this study were identical with those detected in the brain of this species, suggesting an alternative transfer route for these metabolites into the brain via blood. Hence, the identification of these unknown OH-PCBs is essential to assess adverse effects on thyroid hormone homeostasis and cerebral nervous system.

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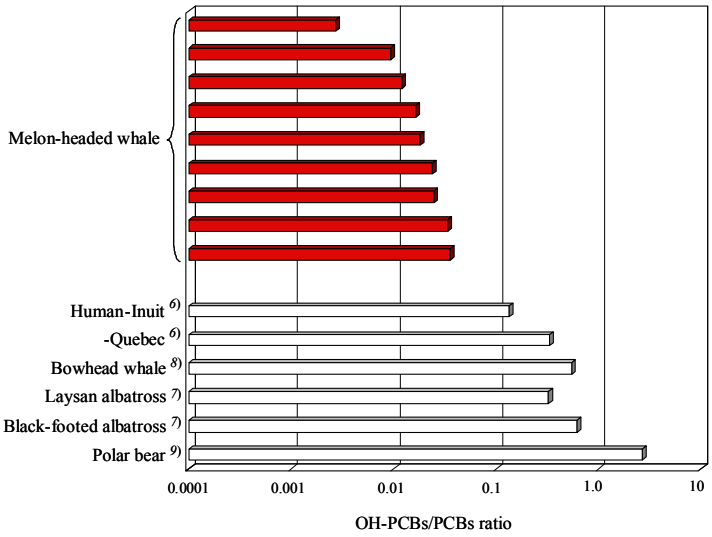

Fig. 1. Comparison of OH-PCBs/PCBs ratios in the blood of melon-headed whales with those of human and wildlife reported previously. *6-9*) References cited.

Table 2. Concentration ratios of OH-PCBs to PCBs in the blood of melon-headed whales

	Concentration ratio		
	Mean	Median	Range
OH-P_5CBs/P_5CBs	0.051	0.046	0.010-0.083
OH-H_6CBs/H_6CBs	0.010	0.009	0.001-0.020
OH-H_7CBs/H_7CBs	0.005	0.002	0.0003-0.019
OH-PCBs/PCBs	0.019	0.018	0.003-0.035

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