Interdisciplinary Studies on Environmental Chemistry — Environmental Research in Asia,
Eds., Y. Obayashi, T. Isobe, A. Subramanian, S. Suzuki and S. Tanabe, pp. 55–66.

Hydroxylated Polychlorinated Biphenyls in the Blood of Cetaceans Stranded along the Japanese Coast

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(Received 29 January 2009; accepted 15 March 2009)

Abstract—The present study determined the residue levels and patterns of polychlorinated biphenyls (PCBs) and hydroxylated PCBs (OH–PCBs) including lower chlorinated isomers in the blood of melon-headed whales (*Peponocephala electra*), finless porpoises (*Neophocaena phocaenoides*), stejneger's beaked whales (*Mesoplodon stejnegeris*) stranded along the Japanese coast during 2005–2006. Total concentrations of OH–PCBs were in the range of 11–1400 pg/g wet wt. and the levels were 2–3 orders of magnitude lower than PCBs (the range: 6000–43000 pg/g wet wt). The residue levels of lower chlorinated OH–PCBs observed in the blood of three cetacean species were relatively higher than those of higher chlorinated isomers. Moreover, when OH–PCB/PCB homologue ratios were calculated, OH-P_3CB/P_3CB, OH-P_4CB/P_4CB and OH-P_5CB/P_5CB ratios were higher than the same values for H_6-, H_7-, O_8-chlorinated homologues, it might be suggests a preferential metabolism of lower chlorinated PCBs and accumulate but hardly eliminate them by conjugation reaction in cetacean bodies.

Keywords: PCBs, hydroxylated PCBs, blood, cetacean, Japanese coast

INTRODUCTION

PCBs are persistent and bioaccumulative chemicals that have been found to reach elevated concentrations in high-trophic animals such as marine mammals (Tanabe, 2002). It has been noted that PCBs disturb thyroid hormone (TH) homeostasis and cerebral nervous system in animals (Brouwer *et al*., 1995, 1998). A possible mechanism involved in disturbing TH homeostasis may be the competitive binding between PCBs and thyroxine (T4) to transthyretin (TTR) in blood (Brouwer *et al*., 1998). It has been demonstrated that the binding affinity to TTR was much stronger for hydroxylated PCBs (OH–PCBs), which are formed by oxidative metabolism of PCBs by the cytochrome P450 monooxygenases, than for the parent compounds due to the structural similarity of OH–PCBs to T4 (Brouwer *et al.*, 1998; Cheek *et al.*, 1999). Moreover, it has also been revealed through the competitive binding assay studies that the binding of *para*-substituted OH-high chlorinated PCB isomers with chlorine atoms on each of adjacent *meta*-positions to TTR was clearly higher and the binding affinity of several OH–PCB isomers were stronger than the affinity of T4, the natural ligand of TTR (Lans *et al.*, 1993; Cheek *et al.*, 1999; Meerts *et al.*, 2002). Therefore, such *para*-substituted OH–PCBs easily persist in blood at higher levels, in which a few OH–PCBs showed longer half-life than the respective parent PCB isomers exist (Sinjari and Darnerud, 1998; Sinjari *et al.*, 1998; Oberg *et al.*, 2002). OH–PCBs have also been detected in blood of several wildlife species, but the levels and patterns vary by species, possibly due to species-specific metabolic capacity by phase I CYP and/or phase II conjugation enzymes and binding affinity to TTR (Bergman *et al.*, 1994; Sinjari and Darnerud, 1998; Olsson *et al.*, 2000; Oberg *et al.*, 2002; Campbell *et al.*, 2003; Li *et al.*, 2003). In addition, in a recent study using reporter gene assays, it was shown that extremely low doses of OH–PCBs (10^{-10} M) suppressed T3-induced transcriptional activation of TR; the suppression of TR action by OH–PCBs was not likely due to the ligand competition with T3, implying that this mechanism may be involved in the disturbance of the cerebral nervous system by PCBs (Iwasaki *et al.*, 2002). In fact, little or no binding affinity of OH–PCBs to TR is observed in competitive binding assay examinations using human- and rat-TR (Cheek *et al.*, 1999; Gauger *et al.*, 2004; Kitamura *et al.*, 2005). More recently, it was indicated that OH–PCBs might suppress T3/TR mediated transcription directly through partial dissociation of TR/retinoid X receptor (RXR) from the thyroid hormone-response element (TRE) (Miyazaki *et al.*, 2004).

Because of such observations, investigations on residue levels of OH–PCBs in human and wildlife blood are increasing (Klasson-Wehler *et al.*, 1998; Sandau *et al.*, 2000; Hoekstra *et al.*, 2003; Gebbink *et al.*, 2005). A few information on OH–PCBs is available on cetaceans (McKinney *et al.*, 2006; Houde *et al.*, 2006; Murata *et al.*, 2007). Our previous study (Murata *et al.*, 2007) analyzed OH-P_5, H_6, H_7CBs in the blood of cetaceans; melon-headed whales (*Peponocephala electra*) and finless porpoises (*Neophocaena phocaenoides*) stranded along the Japanese coast showed that OH-P_5CB/P_5CB ratios were higher than the same values for H_6- and H_7-chlorinated homologues, when OH–PCB/PCB homologue ratios were calculated. Moreover, when compositions of OH–PCB homolog in melon-headed whales and finless porpoises were compared with those in humans (Sandau *et al.*, 2000), considerably higher proportions of OH-P_5CB were observed in this odontocete species, suggesting a preferential accumulation of OH-P_5CBs and implied the possibility of higher accumulations of lower chlorinated OH–PCBs; OH-T_3 and T_4CBs in cetacean blood. In fact, 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 (McKinney *et al.*, 2006). 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 (Houde *et al.*, 2006).

The present study attempted to elucidate the residue levels and patterns of OH–PCBs including low chlorinated isomers and correlations between OH–PCBs and PCBs in the blood of cetaceans stranded along the Japanese coasts.

MATERIALS AND METHODS

The blood samples were collected from melon-headed whales (n = 4: male = 3, female = 1) stranded along the coast of Chiba prefecture during 2006, finless porpoises (n = 3: male = 1, female = 2) stranded along the coast of Chiba prefecture during 2005 and stejneger's beaked whales (n = 3; male = 1, female = 2) stranded along the coast of Akita and Ishikawa prefecture in Japan during 2005. Samples were stored in the Environmental Specimen Bank (*es*-BANK) for Global Monitoring at Ehime University (Tanabe, 2006) at –20°C until analysis.

Analysis of OH–PCBs and PCBs were performed following the procedure reported previously (Kunisue and Tanabe, 2009), with slight modification. The blood sample (10 g) was denatured with HCl. $^{13}C_{12}$-labeled 4OH-T_3CB29, 4OH-T_4CB61, 4′OH-P_5CB120, 4′OH-H_6CB159, 4′OH-H_7CB172, 4OH-H_7CB187 (Wellington Laboratories, Canada), 4OH-T_4CB79, 4′OH-P_5CB107 and 4OH-H_6CB146 (Cambridge Isotope Laboratories, USA) were spiked as internal standards. 2-propanol was added, and then OH–PCBs were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane using polytron and supersonic wave. 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 the extract was then passed through activated silica-gel packed in a glass column. PCBs were eluted with hexane and added ^{13}C-labeled BDE139 as syringe spike, and concentrated for GC (Agilent 6890)–MS (Agilent 5973) analysis. The combined alkaline phase was concentrated and passed 5% water-impregnated silica-gel and gel permeation chromatography. Then OH–PCBs fraction was methylated by reaction with trimethylsilyldiazomethane over night. The derivatized solution was concentrated and passed through activated silica-gel packed in a glass column for clean-up. OH–PCBs were eluted with 10% dichloromethane/hexane and concentrated, and added ^{13}C-labeled CB77 and CB157 as the syringe spike. 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. 62 PCB isomers; CB-1, -3, -4, -8, -10, -15, -18, -19, -22, -28, -33, -37, -44, -49, -52, -54, -70, -74, -77, -81, -87, -95, -99, -101, -104, -105, -110, -114, -118, -119, -123, -126, -128, -138, -149, -151, -153, -155, -156, -157, -158, -167, -168, -169, -170,

Table 1. Concentrations of PCBs and OH–PCBs (pg/g wet wt) in whole blood of Melon-headed Whales, Finless Porpoises and Stejneger's beaked whales stranded along Japanese coasts.

Species (Nomenclature)	Melon-headed whale (*Peponocephala electra*)				Finless porpoise (*Neophocaena phocanoides*)			Stejneger s beaked whale (*Mesoplodon Stejnegeri*)		
Sample No.	060301-8	M34076	M34077	060302-25	M34056	M3468	M33771	M33770	M33773	M33777
Sex	M	M	M	F	F	F	M	F	F	F
Stranded year	2006				2005			2005		
3′OH-CB28	0.82	<0.5	<0.5	1.15	<0.5	<0.5	1.29	<0.5	<0.5	0.81
4OH-CB26	7.5	6.5	14	13	<0.5	<0.5	12	3.4	12	63
4′OH-CB25/4′CB26/4′CB31	6.3	2.1	5.5	7.8	<0.5	1.4	9.7	2.2	12	35
4OH-CB18	2.9	2.0	2.3	2.3	<0.5	2.0	4.6	3.30	18	<0.5
Total Unknown	24	8.9	10	18	<0.5	<0.5	37	12	54	130
OH-TriCB	41	19	32	41.9	<0.5	3.4	64.0	21	95	230
3OH-CB66	3.7	0.30	<0.5	1.97	<0.5	<0.5	3.7	6.7	8.0	11
4OH-CB70	23	7.5	24	2.57	<0.5	<0.5	23	59	53	99
3′OH-CB53	3.6	<0.5	<0.5	0.80	<0.5	<0.5	2.2	12	8.2	17
4′OH-CB65	0.74	<0.5	22.00	<0.5	<0.5	<0.5	3.7	<0.5	<0.5	0.55
3′OH-CB74	2.2	0.23	0.28	3.3	<0.5	0.90	1.2	9.5	<0.5	280
4′OH-CB72	2.5	2.0	2.4	1.6	<0.5	1.3	0.91	11	9	300
4′OH-CB61	<0.5	<0.5	2.8	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Total Unknown	31	12	43	12	2.5	19	45	51	110	130
OH-TeCB	80	22	94	22	2.5	21	80	150	180	840
4OH-CB120/101	8.3	5.1	6.6	0.62	4.9	3.2	2.6	18	21	28
3OH-CB118	16	7.4	16	0.8	2.9	3.9	23.3	20.5	13.7	33.7
4OH-CB107/108	22	19	23	3.9	0.45	1.1	0.48	84	40	60
4OH-CB97	5.2	6.3	6.3	3.5	0.46	1.0	0.48	35	24	46
4′OH-CB106	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1.5	<0.5	4.1
Total Unknown	52	8.1	49	1.9	<0.5	6.8	55	96	77	150
OH-PeCB	86	31	85	11	4.4	30	60	210	160	310

Species (Nomenclature)	Melon-headed whale (*Peponocephala electra*)				Finless porpoise (*Neophocaena phocanoides*)			Stejneger s beaked whale (*Mesoplodon Stejnegeri*)		
Sample No.	060301-8	M34076	M34077	060302-25	M34056	M3468	M33771	M33770	M33773	M33777
Sex	M	M	M	F	F	F	M	F	F	F
Stranded year	2006				2005			2005		
4OH-CB134	1.1	0.66	1.7	<0.5	<0.5	1.2	0.56	3.5	2.7	3.4
4OH-CB146	1.1	0.72	0.72	<0.5	<0.5	0.84	0.65	0.71	0.88	1.4
3OH-CB138	<0.5	<0.5	<0.5	<0.5	<0.5	0.70	<0.5	0.61	0.62	0.93
4OH-CB130	<0.5	0.58	0.58	<0.5	<0.5	2.3	0.679	0.41	<0.5	0.35
4OH-CB159	1.0	<0.5	<0.5	0.12	0.84	0.90	0.76	1.3	1.1	1.2
4OH-CB162	2.7	<0.5	<0.5	0.81	<0.5	0.76	0.54	5.7	5.3	10
3OH-CB153	3.1	2.1	4.8	<0.5	<0.5	0.99	3.1	3.0	2.1	4.0
Total Unknown	10	6.8	19	3.6	2.3	<0.5	6.4	<0.5	21	30
OH-HxCB	20	10	27	5.4	3.6	7.7	13	15	34	51
3OH-CB184	<0.5	0.70	0.70	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
4OH-CB178	1.3	1.4	1.6	<0.5	<0.5	<0.5	<0.5	1.038	0.701	0.791
3OH-CB183/3CB182	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
4OH-CB187	0.73	0.73	0.73	<0.5	<0.5	1.1	1.1	1.1	1.2	1.3
4OH-CB177	<0.5	<0.5	<0.5	3.3	<0.5	<0.5	0.663	<0.5	<0.5	<0.5
3OH-CB180	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
4OH-CB172	1.7	2.5	2.5	1.6	<0.5	1.4	1.2	1.1	1.2	1.5
4OH-CB193	0.90	<0.5	<0.5	0.694	<0.5	<0.5	0.55	0.49	0.74	0.71
Total Unknown	<0.5	<0.5	3.2	2.6	<0.5	4.3	<0.5	1.02	<0.5	<0.5
OH-HpCB	4.7	5.4	8.7	8.2	<0.5	6.8	3.5	4.7	3.8	4.2

Table 1. (continued).

Species (Nomenclature)	Melon-headed whale (*Peponocephala electra*)				Finless porpoise (*Neophocaena phocanoides*)			Stejneger s beaked whale (*Mesoplodon Stejnegeri*)		
Sample No.	060301-8	M34076	M34077	060302-25	M34056	M3468	M33771	M33770	M33773	M33777
Sex	M	M	M	F	F	F	M	F	F	F
Stranded year	2006				2005			2005		
4OH-CB199	<0.5	<0.5	<0.5	1.83	<0.5	<0.5	0.54	<0.5	<0.5	<0.5
OH-OcCB	<0.5	0.71	0.71	2.3	0.54	<0.5	1.0	0.65	0.65	1.0
Total OH–PCBs	230	89	250	90	11	69	220	400	480	1400
T_3CBs	65	140	190	300	200	300	120	250	310	330
T_4CBs	2400	1100	1500	670	1100	3000	1200	2500	2600	2700
P_5CBs	7100	2700	3500	1100	3200	8900	3900	10000	11000	13000
H_6CBs	9400	5400	5900	2100	5900	18000	9500	16000	17000	19000
H_7CBs	3200	2200	2000	1500	3300	8000	5400	6800	7200	7200
O_8CBs	300	230	180	340	530	1000	660	700	780	970
Total PCBs	23000	12000	13000	6000	14000	39000	21000	36000	39000	43000

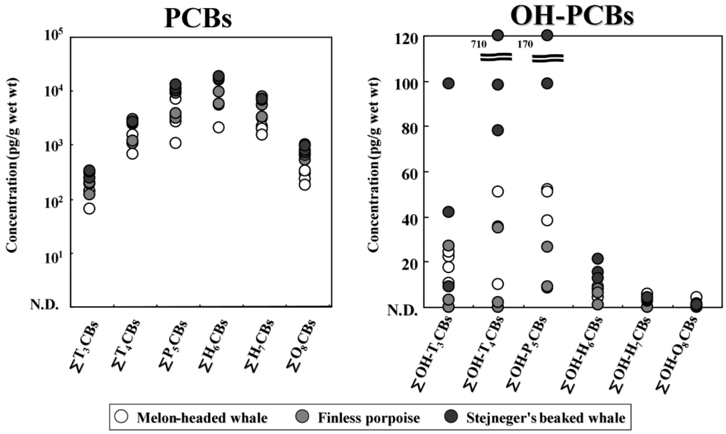

Fig. 1. Concentrations of PCBs and OH–PCBs in blood of various cetaceans stranded along the Japanese coast.

-171, -177, -178, -180, -183, -187, -188, -189, -191, -194, -199, -201, -202, -205, -206, -208, -209, and 53 OH–PCBs; 4′OH-CB25, 3′OH-CB28, 4OH-CB26, 4′OH-CB26, 3OH-CB25, 4′OH-CB20, 3OH-CB31, 4OH-CB31, 4′OH-CB35, 4′OH-CB18, 4OH-CB70, 3OH-CB66, 4OH-CB61, 4OH-CB79, 4′OH-CB61, 4′-OH-CB65, 3OH-CB53, 4′OH-CB72, 3′OH-CB74, 4OH-CB121, 4′-OH-CB106, 4OH-CB120, 2OH-CB114, 4OH-CB108, 4OH-CB97, 4OH-CB127, 4OH-CB101, 3OH-CB118, 4OH-CB107, 4OH-CB165, 4OH-CB153, 4OH-CB146, 4OH-CB130, 4OH-CB159, 4OH-CB162, 4OH-CB134, 3OH-CB138, 4OH-CB163, 3OH-CB184, 3OH-CB182, 4OH-CB187, 3OH-CB180, 4OH-CB172, 4OH-CB193, 4OH-CB178, 3OH-CB183, 4OH-CB177, 4OH-CB202, 3OH-CB203, 4OH-CB199, 4OH-CB201, 4OH-CB198, 4OH-CB200 were quantified and identified in this study.

RESULTS AND DISCUSSION

Residue levels of PCBs and OH–PCBs

OH–PCBs were detected in all the blood samples of various cetaceans, melon-headed whales, finless porpoises and stejneger's beaked whales analyzed in this study (Table 1, Fig. 1). Median concentrations of identified ∑OH–PCBs were 94 pg/g, 25 pg/g, 290 pg/g, wet wt. in melon headed-whales, finless porpoises, stejneger's beaked whales, respectively, and the levels were 2–3 orders of magnitude lower than PCBs (13000 pg/g, 21000 pg/g, 39000 pg/g wet wt., in melon headed-whales, finless porpoises, stejneger's beaked whales, respectively). The predominant PCBs were H_6-CBs, followed by P_5-CBs, H_7-CBs, T_4-CBs, O_8-CBs, T_3-CBs. Whereas, the predominant OH–PCBs were OH-P_5CBs, followed by OH-T_4CBs, OH-T_3CBs, OH-H_6CBs. OH-H_7CBs, OH-O_8CBs

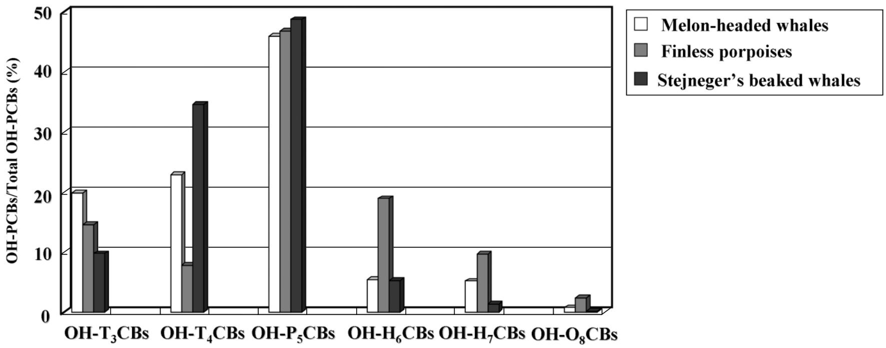

Fig. 2. Composition of OH–PCB homolog in blood of various cetaceans stranded along the Japanese coast.

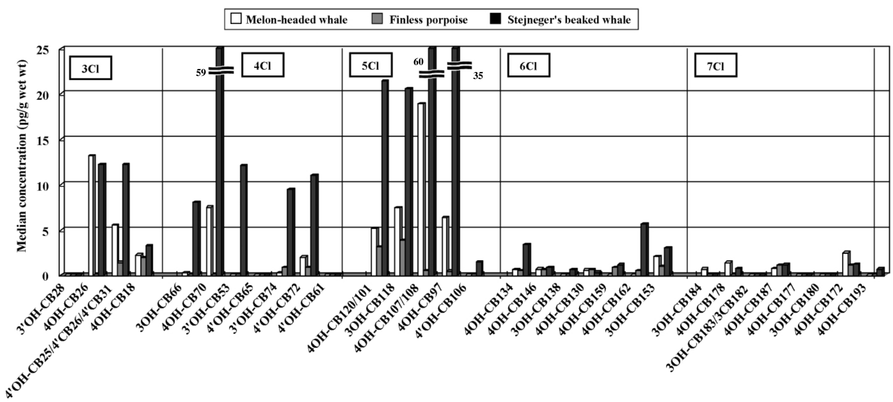

Fig. 3. Median concentrations of identified OH-T_3-H_7CBs isomers detected in blood of cetaceans stranded along the Japanese coast.

and OH-N_9CBs were detected in few specimens. This result showed the different accumulation pattern between PCBs and OH–PCBs. Concentrations of PCBs and OH–PCBs were relatively higher in stejneger's beaked whales than in melon-headed whales and finless porpoises.

Among the identified OH-P_5-H_7CB congeners, OH-P_5CBs were the predominant homologs, occupying about 50%, in blood samples of melon-headed whale, finless porpoise and stejneger's beaked whale (Fig. 2) followed by OH-T_4, T_3CBs, H_6CBs, H_7CBs and O_8CBs. Finless porpoises showed slightly different accumulation profile from those of the other two cetacean species. It might be due to the lower accumulation of OH-T_3, T_4CBs in finless porpoises. In addition, higher residue levels of OH-T_3-P_5CBs than OH-H_6-O_8CBs were also observed in bottlenose dolphin plasma from Western Atlantic and the Gulf of

Table 2. Median concentration ratios of OH–PCBs to PCBs in blood of melonheaded whales, Finless porpoises and Stejneger's beaked whales stranded along Japanese coasts.

Species	Melon-headed whale	Finless porpoise	Stejneger's beaked whale
4OH-CB18/CB18	0.0277	0.0111	0.0034
4OH-CB70/CB70	0.3344	—	0.9892
4′-CB101,120/CB101+CB118	0.0030	0.0016	0.0038
3′OH-CB118/CB118	0.0103	0.0035	0.0076
4OH-CB107,108/CB105+CB118	0.0156	0.0004	0.0148
4OH-CB146/CB138+CB153	0.0002	0.0001	0.0001
3OH-CB153/CB153	0.0010	0.0001	0.0005
4OH-CB187/CB183+CB187	0.0008	0.0004	0.0005
4′OH-CB172/CB170+CB180	0.0026	0.0005	0.0003

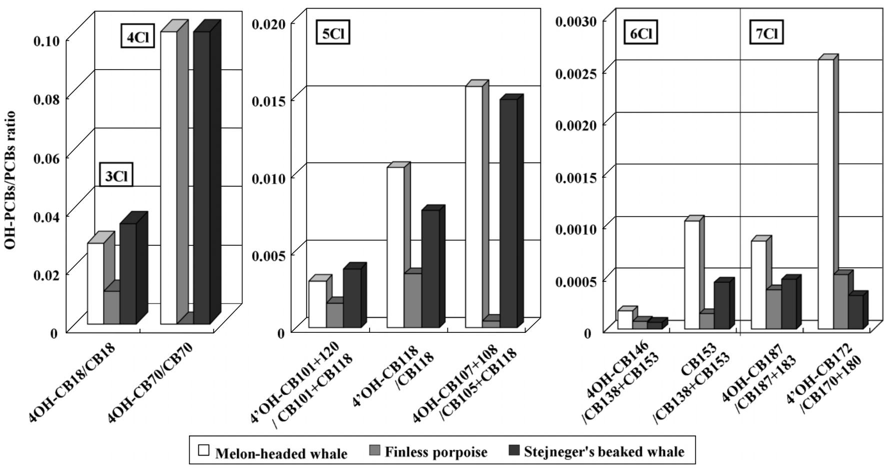

Fig. 4. Comparison of OH–PCBs/PCBs ratios in blood of various cetaceans; melon-headed whales, finless porpoises and stejneger's beaked whales stranded along the Japanese coast.

Mexico (Houde *et al.*, 2006). Considering these observations, it is highly plausible to believe that odontocete species including melon-headed whale, finless porpoise, stejneger's beaked whales and bottlenose dolphins preferentially metabolize lower chlorinated PCBs and accumulate their hydroxylated metabolites in their blood.

Accumulation features of OH–PCBs

Among the identified OH-T_3-H_7CB congeners, 4OH-CB18, 4OH-CB26, 4′OH-CB25/4′OH-CB26/4′OH-CB31, 3′CB74, 3′CB53, 4′CB72, 3′CB66, 4′CB70, 4OH-CB101, 4OH-CB120, 3OH-CB118, 4OH-CB107, 4OH-CB108, 4OH-CB97, 4′OH-CB106, 3′OH-CB138 and 3OH-CB153 were predominant in cetacean blood (Fig. 3). These OH-P_5 and H_6 CB metabolites have been detected in blood of humans and wildlife (Klasson-Wehler *et al.*, 1998; Sandau *et al.*, 2000; Hoekstra *et al.*, 2003; Gebbink *et al.*, 2005), possibly due to their structural similarity to T4. However, few reports show the isomer profiles of OH-T_3 and T_4CBs accumulated in cetacean blood (Houde *et al.*, 2006; Montie *et al.*, 2008). In this study, many OH-T_3- and T_4-PCBs were detected. These congeners show the structure having only one OH-group and one or more chlorines on one side of the phenyl ring. The chemical structure of these low chlorinated PCBs are different from those congeners reported having high binding affinity to TTR (Letcher *et al.*, 2000). However, the congeners (4′OH-CB61, 4′OH-CB65) substituted with only OH-group on one of the biphenyl rings were not detected in all samples. These congeners might be hardly accumulated in the blood due to their easy metabolism and weak binding affinity to TTR. Further studies evaluating the factors affecting accumulation of low chlorinated PCBs in cetaceans are needed.

Correlations between precursor PCBs and OH–PCBs

To elucidate the metabolic capacity, we estimated the ratios of precursor PCBs to OH–PCBs (Table 2, Fig. 4). Low chlorinated OH–PCBs/PCBs were 1–2 order higher than those of higher chlorinated congeners. This result implies that low chlorinated PCBs are relatively easily metabolized than higher chlorinated PCBs and low chlorinated OH–PCBs produced are easily accumulated in the body. Accumulation of OH–PCBs might be affected phase I activity (formation of PCBs to OH–PCBs) and phase II activity (formation of OH–PCBs to $(OH)_2$-PCBs and conjugation reaction) (Moriwaki and Nakano, 2006). Moreover, the intake of PCBs and OH–PCBs from the environment also might have some relation with the accumulation profiles in the blood of cetaceans. The study investigating relationship between accumulation of OH–PCBs and the activities of phase I and phase II are needed.

Acknowledgments—We thank the scientists and staff in Chiba, Akita and Ishikawa prefecture and National Museum of Nature and Science for help in sample collection. This study was supported by Global COE Program from the Ministry of Education, Culture, Sports, Science and Technology, Japan and Japan Society for the Promotion of Science.

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