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Hydroxylated Polychlorinated Biphenyls in the Blood of Cetacean Species 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*) and finless porpoises (*Neophocaena phocaenoides*) stranded along the Japanese coast during 2005–2006. Total concentrations of OH–PCBs including identified and unknown isomers were in the range of 26–330 pg/g wet wt. and the levels were 1–2 orders of magnitude lower than PCBs. The residue levels of OH–PCBs observed in the blood of two cetacean species were relatively lower than in humans and wildlife reported previously implying poor metabolic capacity for PCBs in these odontocete species. Unknown isomers were dominant among OH–P_5CBs and –H_6CBs in these cetacean blood samples; 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 same values for H_6- and H_7-chlorinated homologues, suggesting a preferential accumulation of OH–P_5CBs in cetacean bloods.

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). However, very little information on OH–PCBs is available on cetaceans. The present study attempted to elucidate the residue levels and patterns of OH–PCBs in the blood of cetaceans, melon-headed whales (*Peponocephala electra*) and finless porpoises (*Neophocaena phocaenoides*) stranded along the Japanese coast.

MATERIALS AND METHODS

The blood samples were collected from melon-headed whales (n = 9: male = 6, female = 3) and finless porpoises (n = 6: male = 3, female = 3) stranded along the coast of Chiba prefecture in Japan during 2005–2006. 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 *et al*., 2007), 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

Table 1. Concentrations of PCBs and OH–PCBs (pg/g wet wt.) in the blood of melon-headed whales and finless porpoises stranded along the coast of Chiba prefecture, Japan.

Species (Nomenclature)	Melon-headed whale (*Peponocephala electra*)								
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	Male
Body length (cm)	249	232	256	256	239	222	250	248	226
Stranded year					2006				
CB52	1200	52	520	630	670	1600	53	69	390
CB49	430	27	170	250	250	600	28	32	140
CB44	120	43	100	160	94	140	37	37	74
CB74	520	27	190	230	310	700	24	31	160
CB70	44	39	35	39	45	25	23	35	<10
T_4CBs	2400	190	1000	1300	1400	3100	160	210	760
CB95	1600	50	600	740	820	1900	60	74	500
CB101	2700	69	860	1100	1400	3000	100	130	730
CB99	2300	35	650	650	840	1800	62	72	490
CB119	49	<10	48	41	51	83	11	22	22
CB87	320	21	140	210	220	420	25	29	120
CB110	110	57	83	130	100	110	64	71	55
CB118	2600	55	830	990	1300	2600	84	100	650
CB105	740	21	260	310	390	820	28	34	210
P_5CBs	10000	320	3500	4100	5100	11000	440	530	2800
CB155	110	<10	22	35	52	96	<10	<10	31
CB151	690	11	240	240	280	520	27	25	170
CB149	3000	42	960	970	1100	2500	100	95	710
CB153	9000	100	2300	2100	2900	5800	340	270	1800
CB138	7500	110	2400	2100	2500	5000	290	240	1600
CB158	350	<10	120	98	130	270	<10	<10	84
CB128	580	<10	150	150	190	510	18	17	110
CB167	230	<10	74	87	110	200	10	<10	66
CB156	240	<10	74	85	120	220	16	14	66
CB157	140	<10	47	45	<10	100	<10	<10	32
H_6CBs	22000	260	6300	5900	7500	15000	790	670	4700
CB188	<10	<10	<10	<10	<10	<10	<10	<10	<10
CB178	480	<10	180	150	160	260	30	16	110
CB187	2700	35	730	600	790	1400	170	93	490
CB183	910	12	230	190	250	470	59	29	160
CB177	630	<10	200	170	190	360	<10	19	120
CB171	240	<10	66	58	71	140	<10	<10	46
CB180	3000	44	860	680	940	1700	240	120	560
CB191	53	<10	20	17	19	31	28	<10	70
CB170	1100	16	300	220	300	550	70	36	210
CB189	76	<10	19	19	32	<10	<10	<10	12
H_7CBs	9100	110	2600	2100	2800	4900	600	310	1800
CB202	170	<10	57	41	50	85	22	<10	32
CB201	110	<10	31	22	29	46	13	<10	17
CB199	420	13	170	120	130	210	70	24	89
CB194	320	<10	87	59	94	130	57	24	48
CB205	20	<10	<10	<10	10	<10	<10	<10	<10
O_8CBs	1000	13	340	240	320	480	160	48	190
Total PCBs	45000	890	14000	14000	17000	34000	2200	1800	10000

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 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.

Table 1. (continued)

Species (Nomenclature)	Finless porpoise (*Neophocaena phocaenoides*)					
Sample ID	M34068	M34056	M33764	M33765	M33774	M33771
Sex	Female	Female	Male	Female	Male	Male
Body length (cm)	180	150	114	102	114	117
Stranded year			2005			
CB52	1300	1100	710	690	650	1000
CB49	560	680	590	570	350	890
CB44	280	480	180	170	110	350
CB74	390	700	250	160	120	350
CB70	36	160	60	10	6.1	13
T_4CBs	2500	3200	1800	1600	1200	2600
CB95	1300	980	1100	1200	890	1400
CB101	2200	2100	1900	1600	950	2200
CB99	1800	1800	2200	2100	1500	2600
CB119	92	100	60	62	46	76
CB87	360	370	170	110	66	180
CB110	590	890	570	250	240	490
CB118	1900	2300	1300	950	670	1500
CB105	430	720	240	130	96	230
P_5CBs	8700	9300	7500	6400	4500	8700
CB155	110	92	<10	<10	<10	<10
CB151	570	440	740	720	480	700
CB149	2000	1800	2400	2200	1400	2600
CB153	6700	7100	9600	7600	4700	11000
CB138	6400	6100	4800	4000	2800	5900
CB158	360	360	290	250	190	270
CB128	600	650	240	330	160	330
CB167	170	230	130	75	58	120
CB156	81	230	80	<10	<10	<10
CB157	84	120	<10	<10	<10	<10
H_6CBs	17000	17000	18000	15000	9800	21000
CB188	<10	<10	60	52	28	37
CB178	400	400	510	370	280	730
CB187	2200	2500	4200	3200	1900	4600
CB183	680	890	1300	840	10	900
CB177	580	500	580	420	270	860
CB171	230	270	380	250	170	290
CB180	2400	3200	4500	2500	1500	2900
CB191	300	290	340	250	220	460
CB170	950	1200	1200	720	490	920
CB189	84	84	50	<10	<10	43
H_7CBs	7800	9400	13000	8600	4900	12000
CB202	150	190	270	130	97	230
CB201	89	130	180	72	56	120
CB199	390	530	730	310	240	550
CB194	350	670	960	350	260	500
CB205	30	49	40	<10	<10	21
O_8CBs	1000	1600	2200	862	653	1421
Total PCBs	37000	41000	43000	34000	22000	47000

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.

Table 1. (continued)

Species (Nomenclature)	Melon-headed whale (*Peponocephala electra*)								
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	Male
Body length (cm)	249	232	256	256	239	222	250	248	226
Stranded year					2006				
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_5CB[a]	72	23	98	200	170	200	33	40	110
Total OH–P_5CB	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_6CB[b]	19	4.0	40	78	51	54	12	14	39
TotalOH–H_6CB	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
4–OH–CB177	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Unknown OH–H_7CB[c]	0.67	<0.5	1.7	3.6	2.1	3.0	1.5	1.6	1.1
TotalOH–H_7CB	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

Species (Nomenclature)	Finless porpoise (*Neophocaena phocaenoides*)					
Sample ID	M34068	M34056	M33764	M33765	M33774	M33771
Sex	Female	Female	Male	Female	Male	Male
Body length (cm)	180	150	114	102	114	117
Stranded year			2005			
OH–PCBs						
4′OH–CB101/120	8.9	2.0	8	2.4	5.8	3.2
3′OH–CB118	5.0	1.0	2	<0.5	<0.5	<0.5
4OH–CB107/4′OH–CB108	18	1.2	3	2.5	5.6	4.0
Unknown OH–P_5CB[a]	77	18	95	30	65	35
Total OH–P_5CB	110	22	107	35	76	42
4OH–CB134	1.2	<0.5	<0.5	<0.5	<0.5	<0.5
4OH–CB146	1.5	<0.5	3	<0.5	<0.5	<0.5
3′OH–CB138	0.86	<0.5	1	<0.5	<0.5	<0.5
4′OH–CB130	1.9	<0.5	<0.5	<0.5	<0.5	<0.5
Unknown OH–H_6CB[b]	27	3.2	36	20	31	15
TotalOH–H_6CB	33	3.2	40	20	31	15
4OH–CB178	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
4OH–CB187	0.8	<0.5	0.6	<0.5	<0.5	<0.5
4′OH–CB172	1.7	<0.5	1.3	<0.5	<0.5	<0.5
4–OH–CB177	<0.5	<0.5	1.5	<0.5	<0.5	<0.5
Unknown OH–H_7CB[c]	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
TotalOH–H_7CB	2.5	<0.5	<0.5	<0.5	<0.5	<0.5
4′OH–CB199	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Total OH–O_8CB	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Total	140	26	150	54	110	57

[a]17 (Melon-headed whale) and 11 (Finless porpoise) isomers were quantified.
[b]14 (Melon-headed whale) and 9 (Finless porpoise) isomers were quantified.
[c]3 (Melon-headed whale) isomers were quantified.

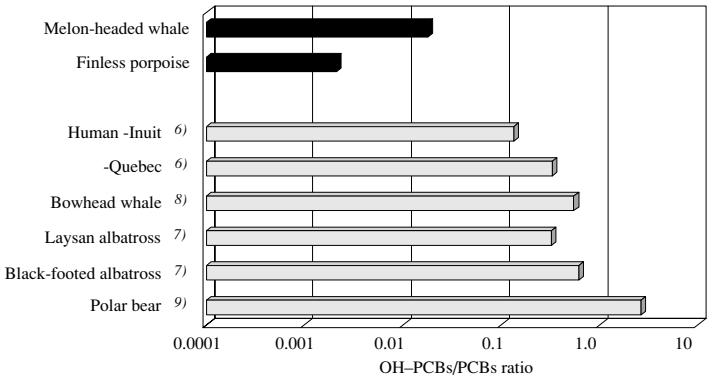

Fig. 1. Comparison of OH–PCBs/PCBs ratios in the blood of cetaceans; melon-headed shales and finless porpoises with those of human and wildlife reported previously.

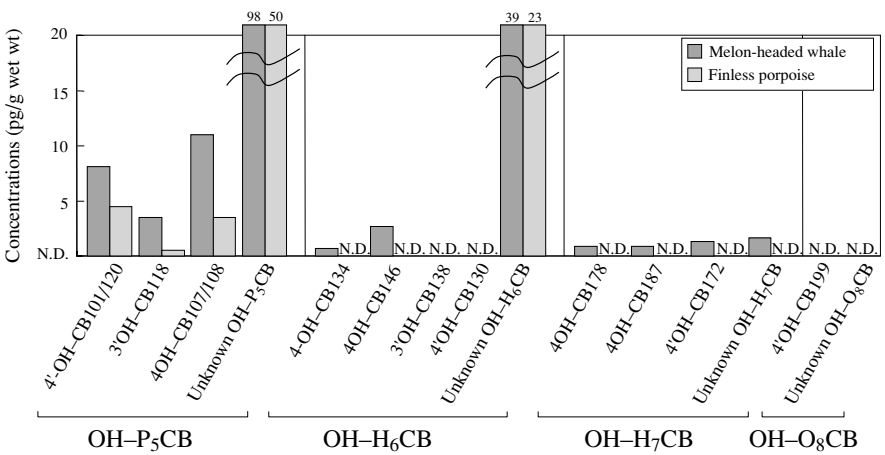

Fig. 2. Median concentrations of identified OH–PCBs isomers and unknown homologues detected in the blood of melon-headed whales and finless porpoises.

RESULTS AND DISCUSSION

Residue levels of PCBs and OH–PCBs

OH–PCBs were detected in all the blood samples of melon-headed whales and finless porpoises analyzed in this study (Table. 1). Concentrations of OH–PCBs including identified and unknown isomers were in the range of 26–330 pg/g wet wt. and were 1–2 orders of magnitude lower than PCBs (890–47000 pg/g wet wt.).

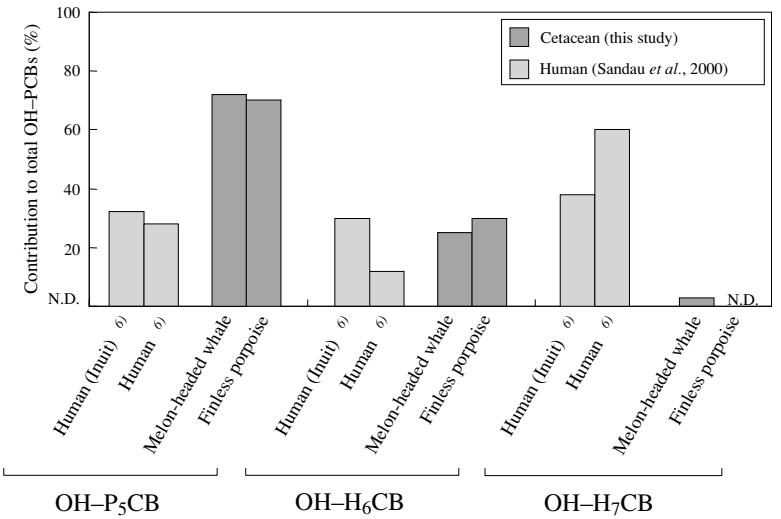

Fig. 3. Composition of OH–PCB homolog in the blood of cetaceans analyzed in this study and human reported previously.

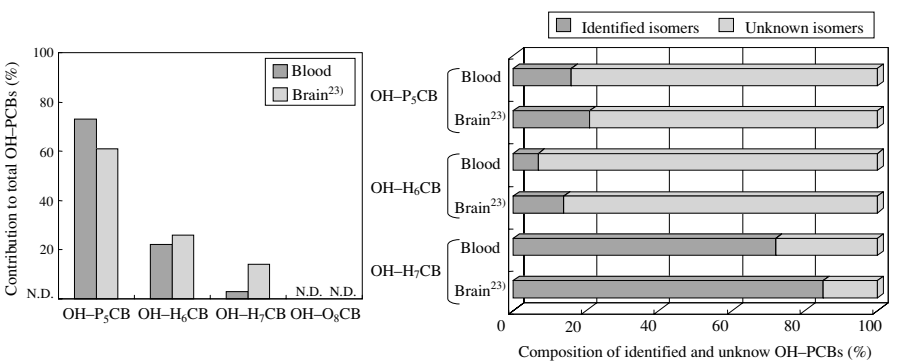

Fig. 4. OH–PCB homolog patterns and compositions of identified and unknown OH–PCBs in blood and brain of melon-headed whales.

The residue levels of OH–PCBs and concentration ratios of OH–PCBs to PCBs observed in the cetacean bloods in this study were relatively lower than in humans and other wildlife reported previously (Klasson-Wehler *et al.*, 1998; Sandau *et al.*, 2000; Hoekstra *et al.*, 2003; Gebbink *et al.*, 2005) (Fig. 1). This result indicates poor metabolic capacity for PCBs and possible specific function of transport proteins such as TTR in these odontocetes.

Accumulation features of OH–PCBs

Among the identified OH–P_5–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 cetacean blood (Fig. 2). These metabolites were also found in the 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, unknown isomers were dominant among OH–P_5CBs and –H_6CBs in cetacean blood; especially OH–P_5CB levels were relatively higher (Fig. 3), whereas predominant OH–H_6CB or –H_7CB isomers were found in humans reported previously (Sandau *et al.*, 2000). 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 in blood of these two species. 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). Considering these observations, it is highly plausible to believe that odontocete species including melon-headed whale and finless porpoise preferentially metabolize lower chlorinated PCBs and accumulate their hydroxylated metabolites in their liver and blood.

Comparison with brain tissue of melon-headed whales

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 (23). Among OH–PCB homologues detected in the blood of melon-headed whales, OH–P_5CBs were predominant followed by OH–H_6, H_7 and O_8CBs. This order was similar to that in the brain samples (Fig. 4), suggesting preferential metabolism of lower chlorinated PCBs and accumulation of their hydroxylated metabolites in the bodies of melon-headed whales and finless porpoises. Moreover, 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. These results might suggest a preferential transfer route for these metabolites into the brain via blood (Fig. 4). Hence, determination of lower chlorinated OH–PCBs and the identification of these unknown OH–PCBs are crucial to assess adverse effects on thyroid hormone homeostasis and cerebral nervous system in cetaceans.

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