ULTRATRACE ANALYSIS OF POLYCHLORINATED BIPHENYLS (PCBs) AND THEIR HYDROXYLATED METABOLITES (OH-PCBs) IN HUMAN SERUM AND CEREBROSPINAL FLUID (CSF) SAMPLES

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Introduction

As members of the group of halogenated aromatic hydrocarbons, polychlorinated biphenyls (PCBs) have received much attention over last 30 years because of their great potential for bioaccumulation and consecutive biological adverse effects. In biological system, PCBs taken in to the body and were metabolized into hydroxylated (OH-) or methylsulfonyl ($MeSO_2$-) forms. Particularly, metabolism of the individual components of PCBs proceeds via CYP450-mediated formation of arene oxide intermediates, which results in both OH- and $MeSO_2$- products, and mercapturic acid pathway (MAP) metabolites including products after C-S lyase cleavage of the alkyl sulfur carbon bond in the cysteinyl moiety, methylation of PCB methyl sulfides, and oxidation to PCB methyl sulfoxides and finally sulfones. Although ample evidence suggests toxicity of the parent compounds, information regarding toxicity of PCB metabolites is scarce. Especially, metabolites are likely to affect thyroid hormone, and it has already demonstrated that some OH-PCBs act as endocrine disrupters[1]. The study confirms that when thyroxin is added to the cultured neuron of the cerebellum it grows normally[2], however the growth is strongly obstructed when OH-PCB added[3]. Therefore, OH-PCBs attributed to impact the biochemical process of the brain is likely[4-5].

In the present study, we established pretreatment and high sensitivity analytical method of polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) in serum and cerebrospinal fluid (CSF) of humans for the first time. Analyzing serum and CSF samples from humans found unique because PCBs behavior and metabolism could be discerned. Furthermore, so far studies reported concentrations of OH-PCBs in wildlife samples obtained by HRGC-LRMS or GC-ECD data. In this study, we established cleanup and analytical methods by high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) using 1 mL of sample. Mainly, total PCBs and OH-PCBs in the CSF were extracted by specialized developed method[4]. Using this method, PCBs and OH-PCBs could be determined swiftly. Based on this method, major OH-PCB congeners were detected from human, serum, CSF, control serum and Rhesus monkey plasma. Present methodology developed based on the isotope dilution technique using OH-PCBs

standard and thus we suggest the present methodology could apply for ultra trace analysis of OH-PCBs as well as total PCBs in human samples.

Materials and Methods

Samples and Cleanup: Cerebrospinal fluid (CSF) and serum was collected from six individual volunteers from Ehime University Hospital by specialized surgeon. The human control serum (purchased from Wako Pure Chemicals, Japan) sample was analyzed as control whereas, Rhesus monkey plasma was analyzed as reference. The brief cleanup procedure of the PCBs and OH-PCBs analysis has been illustrated in Figure 1. The extraction followed by ethylation (a modified procedure[6] found to best suit method than the methylation or trimethylsilylation "TMS" that adopted in earlier procedures) process for the sample analysis that followed by mini multilayer silicagel column chromatography that preceded by the spike of injection (recovery) standards and HRGC-HRMS analysis.

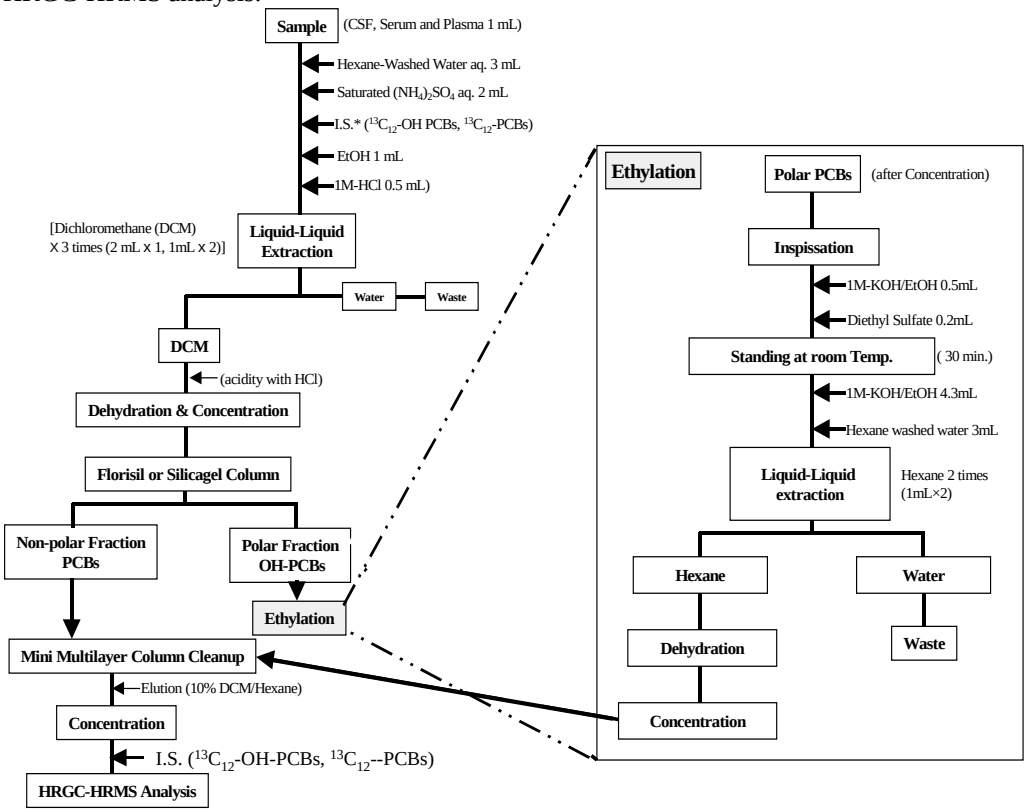

Figure 1. Schematic diagram of cleanup procedure for PCBs and its hydroxylated metabolites.

Identification and Quantification: Native and internal standards of OH-PCBs and PCBs were purchased from Wellington Laboratories, Canada. Micromass Autospec Ultima (HRGC-HRMS) was used for quantification of OH-PCBs and PCBs at resolution of >10,000 (10% valley) by isotope dilution technique with selected ion monitoring (SIM). For OH-PCBs, DB-5MS (60m x

0.32 mm i.d. [0.25 µm] J&W), on column injection was employed. For PCBs, HT8-PCB (60m x 0.25 mm i.d. SGE), was employed. The PCBs and OH-PCBs (ethyl derivative) was monitored in two predominant parents ions. The limit of quantification was <1 pg/g wet wt for OH-PCBs and PCBs. The laboratory blank also conducted in order to see any glass wares and solvents contamination. Blank contains <1 pg/g wet concentration for any congener (except HxCB-153 at 0.32 pg/g).

Results and Discussion

Methodology Development

Based on the newly developed method, OH-PCBs and PCBs (mainly penta through octa PCBs) can be determined using 1-mL of human sample with the detection limit of 1 pg/g for PCBs and OH-PCBs (HxCBs/HpCBs), while, 2 pg/g was noticed for OH-PCBs (PeCBs). The recovery for OH-PCBs was >90% while, 80-90% was noticed for PCBs due to the loss of MoCBs and DiCBs during cleanup. It should be indicated that for OH-PCBs, OH-DiCB through OH-HpCBs was spiked as internal standard but only OH-PCBs (PeCBs through HpCBs) was detected. Similarly, for PCBs MoCBs through DeCB was spiked as internal standards however, TrCBs through DeCB was detected.

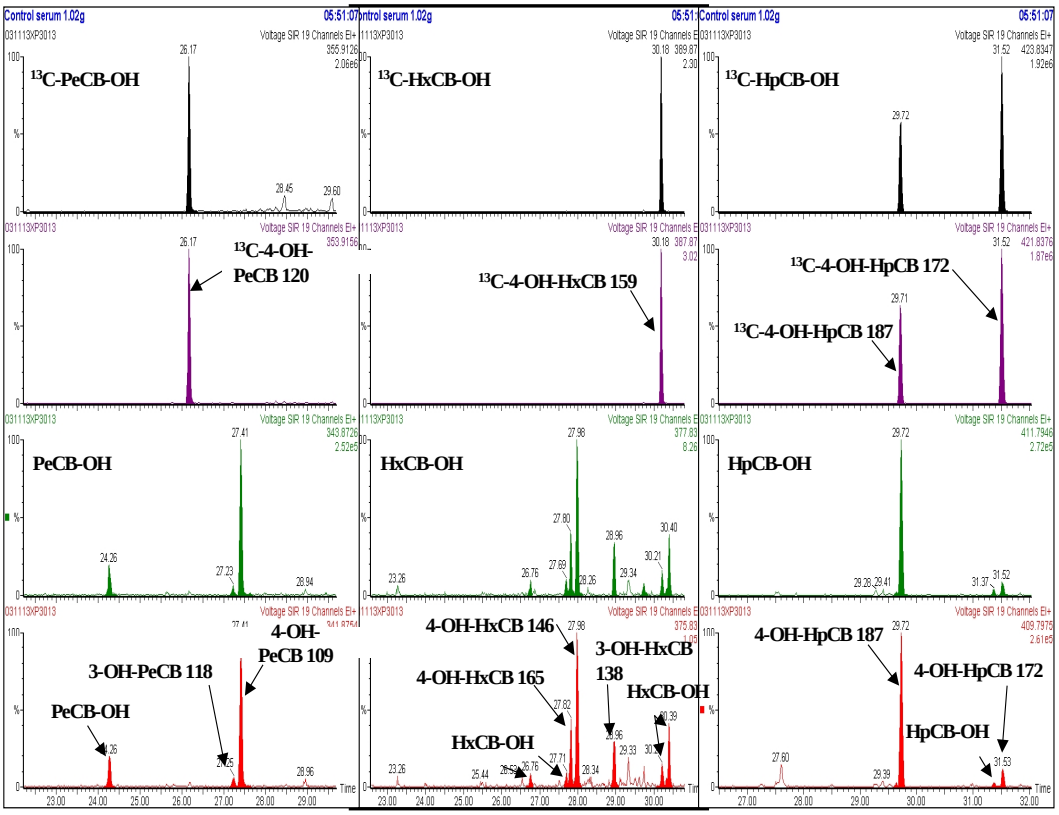

Figure 2. **HRGC-HRMS SIM chromatograms of OH-PCBs in control serum sample.**

The ethyl derivative seems to better than TMS for OH-PCBs. Particularly, internal standards of $^{13}C_{12}$-OTMS-PCBs fragment ions interfere to the native OTMS-PCBs. Furthermore, additional cleanup of TMS derivative found to impossible due to unstable derivative. Consequently, we established ethylation procedure (Figure 1) and these modified program seems to appropriate for OH-PCBs and PCBs analysis (Figure 2).

PCBs and OH-PCBs in Human Serum and CSF

In control serum, concentrations of PCBs(penta through octa PCBs) and OH-PCBs were 170 and 67 pg/g wet wt, respectively (Table 1). Approximately 28% of OH-PCBs were contributed to the PCBs burden in control human serum. This trend is similar to the human sample from Canada and Sweden as well as wildlife samples from remote North Pacific Ocean[7]. The predominant OH-PCBs was 3-OH-PeCB-109 and 4-OH-HpCB-187 congeners in serum. Similar accumulation trend are also reported in excreta of gray seal, common murre, intralumina uterine fluid of mice. The parent compound for 3-OH-PeCB-109 was PeCB-118/105, which is predominant PCB accumulant in biological samples. While, parent PCB congeners for 4-OH-HpCB-187 was HpCB-187/183 which is not predominant contaminants in biological samples and thus metabolism of HpCB-187/183 to 4-OH HpCB-187 seem to have specific protein binding activity after metabolism.

Table 1. Concentrations of total PCBs and OH-PCBs in serum and cerebrospinal fluid (CSF) samples of humans.

Total PCBs				OH-PCBs			
	Concentration (pg/g wet weight)				Concentration (pg/g wet weight)		
Congeners	CSF (n=6)	Serum (n=4)	Control Serum (n=1)	Congeners	CSF (n=6)	Serum (n=4)	Control Serum (n=1)
PeCB-118	ND-(0.55)*	63-280	14	3-OH-PeCB-118	ND-(1.2)*	2.1-14	(1.5)*
PeCB-99	ND-(0.40)*	51-120	8.4	4-OH-PeCB-109	ND-(1.3)*	25-67	22
PeCB-105	ND	12-54	3.0	OH-PeCB-(UK[a])	ND-2.6	13-52	5.9
HxCB-153	(0.41)*-3.2	430-1000	46	4-OH-HxCB-165	ND-1.9	4.9-12	1.7
HxCB-138	ND-1.0	200-410	28	4-OH-HxCB-146	(0.61)*-3.0	24-86	8.2
HxCB-163/164	ND-(0.82)*	71-200	7.7	4-OH-HxCB-138	ND-1.3	7.4-18	2.6
HpCB-180	ND-1.9	260-670	32	3-OH-HxCB-130	ND	(0.65)*-1.5	ND
HpCB-170	ND-(0.50)*	76-220	9.9	OH-HxCB-(UK[a])	ND-3.3	14-34	5.4
HpCB-182/187	ND-1.3	130-330	9.2	4-OH-HpCB-187	4.0-25	54-130	19
OCB-194	ND	33-110	5.9	4-OH-HpCB-172	ND-(0.81)*	4.1-9.7	1.7
OCB-199	ND-(0.34)*	45-130	6.0	OH-HpCB-(UK[a])	ND-1.3	9.7-38	(0.68)*

*Below the limit of quantification; [a]UK indicates unknown peak

ND: <0.3 pg/g wet weight for total PCBs, OH-HxCBs and OH-HpCBs; <0.6 pg/g wet weight for OH-PeCBs.

In CSF, concentration ranges of PCBs (penta through octa PCBs) and OH-PCBs in CSF were ND-7.4 and 4.0-38 pg/g, wet wt. respectively (Table 1). It is noteworthy that OH-PCBs was orders of magnitude greater than their parent congeners in CSF. Specific protein binding of hydroxylated tail of PCB with CSF could be possible explanation. 4-OH-HpCB-187 was abundant congener followed by 4-OH- HxCBs-146 and un known OH-HxCB in CSF (Table 1).

PCBs (penta through octa PCBs) and OH-PCBs were also determined in serum of same volunteer who provided CSF. Serum concentrations of penta through octa PCBs (1,400-3,500 pg/g wet wt) were orders of magnitude greater than OH-PCBs (160-460 pg/g wet wt). Approximately, 2.5 to

8.3% of serum OH-PCBs were found in CSF. These results suggests that least amount of blood serum concentration enter into CSF. The major serum OH-PCB congeners were 4-OH-HpCB-187 followed by 4-OH-PeCB-146, 4-OH-HxCB-109 which is similar to the results of CSF. Besides, 3 unknown OH-PCBs such as OH-PeCB, OH-HxCB and OH-HpCB peaks detected at considerable concentrations (Table 1). Hydroxylated PCBs found in serum and CSF have two structural elements in common; either a 4-hydroxy-3,5-dichlorophenyl ring (or more chlorine atoms in the ring) or a 3-hydroxy-2,4-dichlorophenyl ring (or more chlorine atoms in the ring) and chlorine atoms in at least 3- and 4-positions in the other phenyl ring. Hydroxylated PCBs are thus, in part; structurally similar to thyroxine and several OH-PCBs can compete with thyroxine for a binding site on the transport protein, transthyretin (TTR). Occurrence of OH-PCBs in CSF suggests they enter into the brain through blood-brain barrier.

OH-PCBs have been identified and quantified in blood from wildlife (5,900-33,000) and human blood plasma (238-1,750), breast milk (<1-5.0), maternal blood plasma (82-328), umbilical cord (103-788), cord blood plasma (35-271) on pg/g wet basis[7-8]. These studies reported that concentrations of OH-PCBs were greater or similar than the results obtained in our study. According to Sandau[9], OH-PCB was detected from whole blood with the total concentration were in the range of 0.117-11.6, and 0.161 on ng/g whole blood wet weight basis for the Inuit samples and southern population pool, respectively. Consequently, the magnitude of PCBs concentrations in humans reflects the local pollution sources.
In general, PCBs (DiCBs through DeCB) average concentrations (n=156) in blood of Japanese adults were 210,000 pg/g fat weight. The average dioxin-like PCBs were (25,000 fat weight) in same group of humans in which mono-ortho PCBs such as PeCB-118, 105 and HxCB-156 contributed to greater amount of total dioxin-like PCBs. Occurrence of OH-PCB-118 in human samples is of greater concern as it produce multitude of toxic effects.

Table 2. Concentrations of OH-PCBs in Rhesus monkey plasma samples.

Congeners	LOQ	Blank	Conc[a]
3-OH-PeCB-118	<2	<2	
4-OH-PeCB-109	<2	<2	
OH-PeCB-(UK[b])	<2	<2	3.4
4-OH-HxCB-165	<1	<1	2.3
4-OH-HxCB-146	<1	<1	58
4-OH-HxCB-138	<1	<1	
3-OH-HxCB-130	<1	<1	18
OH-HxCB-(UK[b])	<1	<1	
4-OH-HpCB-187	<1	<1	530
4-OH-HpCB-172	<1	<1	
OH-HpCB-(UK[b])	<1	<1	
Sum OH-PCBs			**612**

[a]denotes concentration on pg/g wet wt

[b]unknown peak; LOQ=limit of quantification

Concentrations of total OH-PCBs in plasma of Rhesus monkey were 612 pg/g (Table 2), which is several hundred time greater than human CSF samples. The contamination profiles of OH-PCBs in monkey plasma were similar with those of CSF with 4-OH-HpCB-187 was abundant congener which contribute about 86% of the total OH-PCBs. PCBs were not determined in monkey plasma and thus we cannot estimate percentage of PCBs metabolized but the levels were similar to some humans from Canada, Sweden. Slightly greater concentrations of OH-PCBs in plasma than the human serum samples suggest availability of specific binding protein as well as ecological niche of monkey and humans.

Among several congeners detected in human serum, CSF and monkey plasma, 4-OH-2,2',3,4',5,5',6-HpCB(187) and 4-OH-2,2',3,4',5,5'-HxCB(146) accounted greater mass, 70 to 90% of the total OH-PCBs. These two OH-PCBs were also major OH-PCBs in human blood[8]. The parent PCB congeners for these two OH-PCBs are most likely HpCB-180/CB-187 and HxCB-138/CB-153, respectively. The relatively great concentrations of OH-PCBs observed in Rhesus monkey plasma and the limited number of -OH PCB congeners indicate that these metabolites are selectively retained. These trends were similar to laysan and black-footed albatross from Northern Pacific Ocean. The retention of OH-PCB congeners may be influenced by the exposure profile of PCBs, metabolism rate, and protein binding specificity, Further work suggested to verify specificity and concentration levels of the OH-PCBs in humans

In General, metabolites of PCB are considered to be less toxic than their parent compounds. However, hydroxylated metabolites of 3,3',4,4'-TeCB(77) have a marked structural resemblance to thyroxine, the natural ligand for TTR and, therefore, competitively bind to TTR and can cause reductions in plasma tetraidothyroxine (T_4) levels and serum transport of vitamin A in rodents. Hydroxylated metabolites of PCBs have been shown *in vitro* to have binding affinities that are 10 times greater than TTR than for T_4. These results in persistent retention of these metabolites in blood of both humans exposed environmentally to PCBs. In addition, occurrence of OH-PCBs in CSF is of major concern as it can alter and modulate any signal that originated from brain. This is first of its kind of study with CSF which is plays a central role in human brain physiology. Further study with not only monitoring of OH-PCBs but also the binding affinity and any further intrinsic effects should be focused. Based on the limited sample size, it is difficult to conclude any effects of OH-PCBs to humans.

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