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Concentration of Hydroxylated Polychlorinated Biphenyls (OH-PCBs) in the Blood of Yusho Patients in 2010

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Abstract Hydroxylated polychlorinated biphenyls (OH-PCBs) are formed as major metabolites of PCBs by cytochrome P450 enzyme-mediated oxidation. It has been reported that their total concentration in serum samples of Yusho patients ranged from 390 to 1300 pg/g.
We measured the concentration of OH-PCBs in blood collected from 183 Yusho patients living in Japan in 2010. The major OH-PCB metabolites were 4-OH-CB187 (ND-1300 pg/g-wet), 4-OH-CB146 + 3-OH-CB153 (8.4-1200 pg/g-wet), 4-OH-CB109 (ND-530 pg/g-wet) and 4'-OH-CB172 (ND-380 pg/g-wet). The total OH-PCBs ranged from 36 to 3800 pg/g-wet.
A positive relationship between the concentrations of OH-PCBs and PCBs was observed, but no significant relationship between the concentrations of OH-PCBs and PCDD/DFs was observed.

Key words : Yusho・Blood・OH-PCB

Introduction

Polychlorinated biphenyls (PCBs) are one of the persistent and bioaccumulative chemicals. Hydroxylated polychlorinated biphenyls (OH-PCBs) are well known as metabolites of PCBs formed by the cytochrome P450 enzyme-mediated oxidation of PCBs. Enomoto et al.[1)] investigated the concentrations of OH-PCBs in the Japanese human blood plasma reporting that the major congeners and levels were 4-OH-CB109 10-230 pg/g-wet, 4-OH-CB146 13-340 pg/g-wet and 4-OH-CB187 12-110 pg/g-wet. Linderholm et al.[2)]reported that the highest OH-PCB metabolite in serum samples from 9 Yusho patients was 4-OH-CB187 followed by 4-OH-CB146, 4-OH-CB109 and 4'-OH-CB120 ; further, that the total of 6 OH-PCB metabolites ranged between 390 and 1300 pg/g serum with a mean value of 780 pg/g serum.

Sato et al.[3)] measured the concentrations of OH-PCBs in human urine and blood sample taken from same person. They indicated that the pattern and order of detected OH-PCBs were different between blood and urine samples.

In this study, we measured the concentrations

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of OH-PCBs, PCBs, PCDDs and PCDFs in blood collected from 183 Yusho patients living in Japan in 2010, and we compared the blood concentrations of these compounds.

Materials and Methods

1. Sampling

The blood samples examined in this study were collected from 183 Yusho patients at their medical checkups in 2010 ; all gave their informed consent. 10 mL of blood samplings were collected using a vacuum blood-collecting device containing heparin and stored at 4℃ until analyze for concentrations of OH-PCBs, PCBs, PCDDs and PCDFs.

2. Chemicals and reagents

OH-PCBs standards were purchased from Wellington Laboratories, Inc., Ontario, Canada and Cambridge Isotope Laboratories, Inc., Massachusetts, US. These OH-PCBs standards are listed in Table 1. Each 1 mg/L standard solution was prepared by dilution with acetonitrile. Labeled standards of OH-[$^{13}C_{12}$]-PCBs, as internal standards, are listed in Table 2. 4-OH-2', 3, 3', 4', 5, 5'-HxCB (4'-OH-CB159) was used as a syringe spike. Acetonitrile, methanol, Ammonium acetate and ultra pure water of LC/MS grade were purchased from Wako Pure Chemical Industries, Tokyo, Japan. A cartridge of Envi-18 (500mg / 6mL glass tube) was purchased from Sigma-Aldrich, Inc., Missouri, US.

Table 1 OH-PCBs standards

Compounds	Abbreviations	
4-OH-2,3,3',4',5-PeCB	4-OH-CB109	4H109
3-OH-2,2',3',4,4',5-HxCB	3'-OH-CB138	3H138
4-OH-2,2',3,4',5,5'-HxCB	4-OH-CB146	4H146
4-OH-2,2',3,3',4',5,5'-HpCB	4'-OH-CB172	4H172
4-OH-2,2',3,4',5,5',6-HpCB	4-OH-CB187	4H187

Table 2 OH-[$^{13}C_{12}$]-PCBs for internal standards

Compounds	Abbreviations	
4-OH-2,3,3',4',5-PeCB	4-OH-CB109	M4H109
4-OH-2',3,4',5,5'-PeCB	4'-OH-CB120	M4H120
3-OH-2,2',3',4,4',5-HxCB	3'-OH-CB138	M3H138
4-OH-2,2',3,4',5,5'-HxCB	4-OH-CB146	M4H146
4-OH-2',3,3',4',5,5'-HxCB	4'-OH-CB159	M4H159
4-OH-2,2',3,3',4',5,5'-HpCB	4'-OH-CB172	M4H172
4-OH-2,2',3,4',5,5',6-HpCB	4-OH-CB187	M4H187

3. Sample preparation

Each 5g blood sample was loaded into an extraction cell filled with Isolute. After freeze-drying, OH-[$^{13}C_{12}$]-PCBs, [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs and [$^{13}C_{12}$]-Co-PCBs were added as internal standards. Acetone : n-hexane (1 : 4, v/v) was used as the extraction solvent for an accelerated solvent extractor (ASE-200, Thermo Scientific Dionex, California, US). After the extract was evaporated to near dryness, it was dissolved in n-hexane and treated with sulfuric acid overnight. The separated hexane layer was applied to a silver nitrate/silica gel column. The first fraction containing PCDDs, PCDFs and Co-PCBs was eluted with 15mL of n-hexane. OH-PCBs were eluted with 15mL of 50% dichloromethane/n-hexane as the second fraction. The eluate was concentrated to near dryness with a multiple sample concentrator, and dissolved in 2mL of methanol. After the methanol solution was loaded onto an Envi-18 cartridge with 4mL of methanol, the eluate was concentrated under nitrogen flow and transferred to an LC injection vial with 0.2mL of methanol. A flow chart of this method is shown in Fig. 1. The details of the sample preparations are described in another paper.[4)]

4. Determination of OH-PCBs

All LC/MS/MS analysis was performed using an Alliance 2695 series high-performance Liquid Chromatograph Separations Module equipped with Quattro micro API mass spectrometer (Waters Corporation, Massachusetts, US). An analytical column, L-column 2 ODS, 2.1 mm × 100 mm, 2 μm (CERI, Tokyo, Japan) was used under a linear gradient solvent condition with the flow

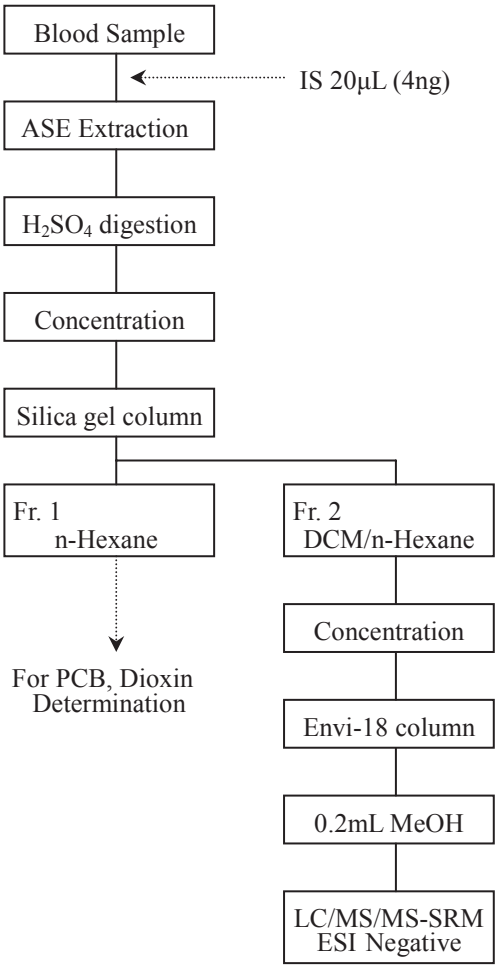


Fig. 1 Flow chart of the measurement method for OH-PCBs in blood samples

rate set at 0.2mL/min. The initial mobile phase was 40 : 60 methanol/2mM ammonium acetate in ultra pure water. The injection volume was 20 μL.

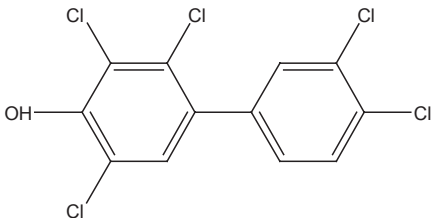
Detection was performed on a quadrupole analyzer operated in negative electrospray ionization (ESI-) and in selected reaction monitoring acquisition mode (SRM). Nitrogen was used as the cone and desolvation gas. The potential applied onto the capillary was 1.0 kV. Cone and collision potentials were optimized for each molecule. Argon was used as the collision gas. The details of the operating conditions for the LC/MS/MS measurements are described in another paper.[5)]

Results and Discussion

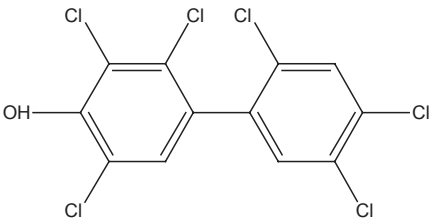
1. Analysis of OH-PCBs in blood samples

Peaks of 4-OH-CB109, 4-OH-CB146 + 3-OH-CB153, 4-OH-CB187 and 4'-OH-CB172 were detected. Figure 2 shows the structure of OH-PCBs detected in the blood of Yusho patients. These chemicals have the similar structure ; 3, 5-substituted chlorinated-4-hydroxybiphenyls.

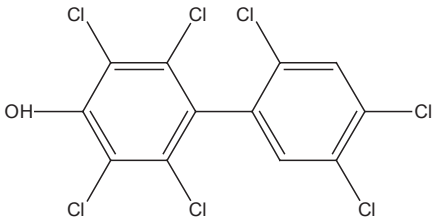
4-OH-CB146 and 3-OH-CB153 could not be separated in this study, while 3'-OH-CB138 could



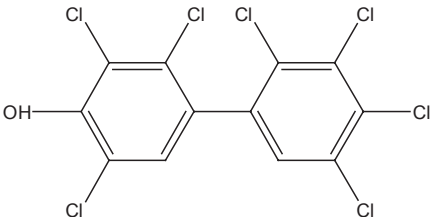
4-OH-2,3,3',4',5-PeCB (4-OH-CB109)



4-OH-2,2',3,4',5,5'-HxCB (4-OH-CB146)



4-OH-2,2',3,4',5,5',6-HpCB (4-OH-CB187)



4-OH-2,2',3,3',4',5,5'-HpCB (4'-OH-CB172)

Fig. 2 Structure of OH-PCBs detected in the blood of Yusho patients

Table 3 Concentrations of OH-PCBs, PCBs, PCDDs and PCDFs in the blood of Yusho patients collected in 2010 (pg/g-wet, n = 183)

Congeners	Mean	Median	Min.	Max.	SD	CV
4-OH-CB109	67	49	ND	530	68.8	1.02
4-OH-CB146 + 3-OH-CB153	96	71	8.4	1200	106	1.10
4-OH-CB187	120	77	ND	1300	131	1.12
4'-OH-CB172	29	20	ND	380	35.1	1.22
Total OH-PCBs	310	230	36	3800	340	1.09
Total PeCBs	150	130	11	830	111	0.734
Total HxCBs	1000	820	80	6300	785	0.774
Total HpCBs	840	620	86	6600	793	0.942
Total PCBs	2300	1800	210	15000	1800	0.799
Total PCDDs	2.4	2.0	0.49	7.5	1.32	0.558
Total PCDFs	0.70	0.31	0.034	5.5	0.922	1.32
Total PCDD/DFs	3.1	2.7	0.54	11	1.79	0.584
Total Co-PCBs	1.0	0.90	0.13	4.8	0.686	0.663
Total dioxins	4.1	3.8	0.74	13	2.23	0.545
Lipid (%)	0.25	0.25	0.15	0.62	0.0606	0.238

SD : Standard deviation ; CV : Coefficient of variation ; CB : chlorinated biphenyl ; Pe : penta ; Hx : hexa ; Hp : hepta; ND : not detected ; PCDD : polychlorinated dibenzo-p-dioxin; PCDF : polychlorinated dibenzofuran ; Co: coplanar.

not be observed because of low recovery. We suspected that 3'-OH-CB138 degrades under sulfuric acid treatment.

2. Concentrations of OH-PCBs in blood samples

Concentrations of OH-PCBs, PCBs, PCDDs and PCDFs in the blood of the 183 Yusho patients are summarized in Table 3. The major OH-PCB metabolite (range) was 4-OH-CB187 (ND-1300 pg/g-wet) followed by 4-OH-CB146 + 3-OH-CB153 (8.4-1200 pg/g-wet), 4-OH-CB109 (ND-530 pg/g-wet) and 4'-OH-CB172 (ND-380 pg/g-wet). The total of 4 OH-PCBs ranged between 36 and 3800 pg/g-wet with a mean value of 310 pg/g-wet. These results were in good agreement with those reported by Linderholm et al.

3. Relationship between the concentrations of OH-PCBs and related compounds

Figure 3 shows the relationship between the concentrations of OH-PCBs and PCBs in the blood of Yusho patients. A positive relationship between the concentrations of OH-PCBs and PCBs was observed. But, no significant relationship between the concentrations of OH-PCBs and PCDD/DFs was observed (Fig. 4). The concentrations of OH-PCBs and lipid were also not correlated (Fig. 5).

Figure 6 shows the relationships between the

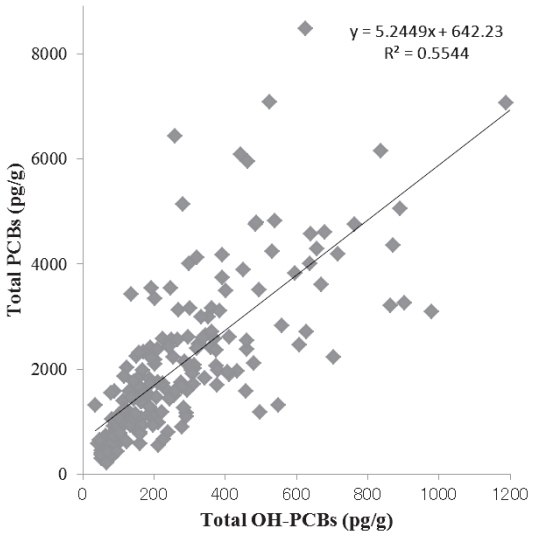


Fig. 3 Relationship between the concentrations of OH-PCBs and PCBs in the blood of Yusho patients

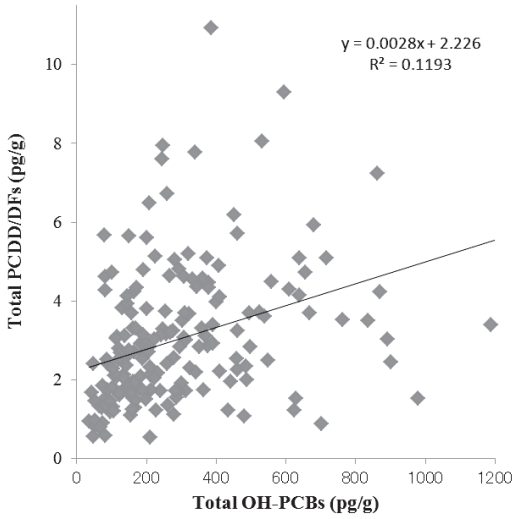


Fig. 4 Relationship between the concentrations of OH-PCBs and PCDD/DFs in the blood of Yusho patients

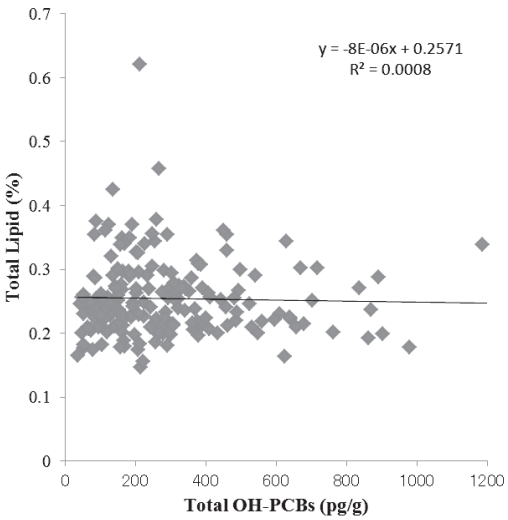


Fig. 5 Relationship between the concentrations of OH-PCBs and lipid in the blood of Yusho patients

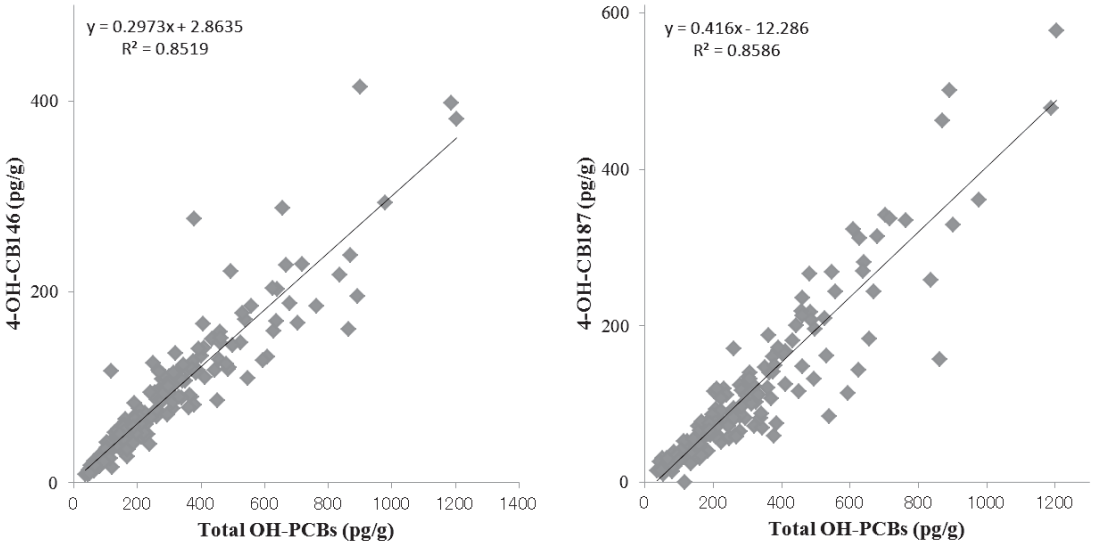


Fig. 6 Relationship between the concentrations of OH-PCBs and their congeners

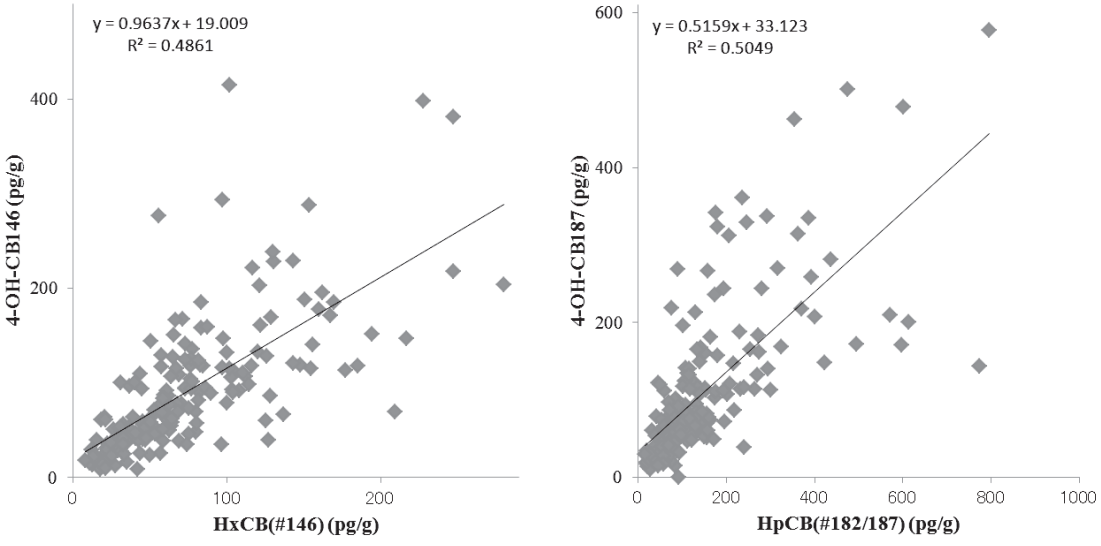


Fig. 7 Relationship between the concentrations of OH-PCB congeners and related PCBs

concentrations of OH-PCBs and their congeners (4-OH-CB146 and 4-OH-CB187). Significant relationships between the concentrations of OH-PCBs and their congeners were observed.

Figure 7 shows the relationships between the concentrations of OH-PCB congeners and related PCBs (4-OH-CB146/HxCB (#146) and 4-OH-CB187/HpCB (#182/187)). Positive relationships between the concentrations of OH-PCB congeners and related PCBs were observed.

In conclusion, we measured the blood samples from 183 Yusho patients for OH-PCBs using LC/MS/MS technique. The total OH-PCBs ranged from 36 to 3800 pg/g-wet. A positive relationship between the concentrations of OH-PCBs and PCBs was observed, but no significant relationship between the concentrations of OH-PCBs and PCDD/DFs was observed.

Acknowledgements

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References

1) Enomoto T, Nishino Y, Matsuda M, Kawano M and Wakimoto T : Hydroxylated polychlorinated biphenyl metabolites (HO-PCBs) in human blood plasma-Development of analytical methods and their residue in Japanese general population-J. Environ. Chem. 16 : 51-59, 2006.
2) Linderholm L, Masuda Y, Athanasiadou M and Bergman Å : PCB and PCB metabolites in serum from Yusho patients 37 years after accident. Organohalogen Comp. 69 : 2141-2144, 2007.
3) Sato N, Yonekubo J, Ezaki T, Suzuki M, Matsumura C, Haga Y and Nakano T : Measurement of Accumulation of Hydroxylated Polychlorinated Biphenyls (OH-PCBs) in Human Urine and Blood. Organohalogen Comp. 74 : 76-79, 2012.
4) Todaka T, Hirakawa H, Tobiishi K and Iida T : New protocol of dioxin analysis in human blood. Fukuoka Igaku Zasshi 94 : 148-157, 2003.
5) Tobiishi K, Suzuki S, Todaka T, Hirakawa H, Hori T, Kajiwara J, Hirata T, Iida T, Uchi H and Furue M : Improvement of Measurement Method for Hydroxylated Polychlorinated Biphenyls (OH-PCBs) in Blood Samples using LC/MS/MS. Fukuoka Igaku Zasshi 104 : 128-135, 2013.

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（和文抄録）

2010 年度油症認定患者血液中の水酸化ポリ塩化ビフェニル（OH-PCBs）濃度

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水酸化ポリ塩化ビフェニル（OH-PCBs）は，人体内における PCB の主要代謝物である．OH-PCBs は体内でチトクローム P450 酵素誘導により PCB から生成され，油症認定患者の血清から 390-1300pg/g の濃度で検出された報告がある．

2010 年度に 183 名の油症患者から採取した血液中の OH-PCBs 濃度を測定し，これら化合物の濃度を比較した．主要な PCB の代謝物は，4-OH-CB187（ND-1300 pg/g-wet），4-OH-CB146 + 3-OH-CB153（8.4-1200 pg/g-wet），4-OH-CB109（ND-530 pg/g-wet），4'-OH-CB172（ND-380 pg/g-wet）であり，その合計は 36-3800 pg/g-wet であった．

また，OH-PCBs と PCB 濃度には正の関係が認められたが，OH-PCBs と PCDD/DFs の濃度には有意な関係は認められなかった．