ANALYSIS OF ALL 209 POLYCHLORINATED BIPHENYLS CONGENERS IN HUMAN POOLED BLOOD SAMPLE IN JAPAN

Tetsuya Hirai*, Yoshinori Fujimine*, Syunkichi Watanabe* and Takeshi Nakano**

*Otsuka Life Science Initiative, Otsuka Pharmaceutical Co,Ltd. 224-18, Ebisuno Hiraishi, Kawauchi-cho, Tokushima 771-0195, Japan.
** Environmental Safety Division Hyogo Pref. Inst. of Public Health & Env. Sci.3-1-27, Yukihira-cho, Suma-ku, Kobe 654-0037, Japan

Introduction

All 209 Polychlorinated biphenyl (PCB) congeners were analyzed by high resolution gas chromatography / high resolution mass spectrometer (HRGC/HRMS) in pooled blood sample from 10 healthy volunteers in Japan. The 89 PCB congeners were detected in pooled blood sample. Total PCB levels of human pooled blood sample were 936 ± 2.3 pg / g on a whole blood basis.

Measuring all PCB congeners has an advantage to provide detailed information regarding the congener distribution within the blood sample, which can be compared to congener patterns in other matrices. The specific analysis of all PCB congeners is especially useful in evaluating PCB exposure.

The objectives of this study were: To validate analytical method for detecting all PCB congeners in human blood. To measure levels of all PCB congeners in pooled blood sample from adult healthy volunteers in Japan.

Materials and Methods

Standards

Authentic PCBs and $^{13}C_{12}$-PCBs were obtained from Cambridge Isotope Laboratories, Inc. (U.S.A.) and AccuStandard Co. (U.S.A.). All solvents and reagents used were of dioxin-analysis grade.

Samples

Pooled sample of whole blood was collected from 10 healthy volunteers in Japan. As for this sample, informed concent was obtained. The pooled sample was stored at –20 until analysis.

Preparation of samples for PCBs analysis

About 15 g of whole blood was transferred to a 100 ml glass tube, and 100 pg $^{13}C_{12}$-PCBs were added as described above. This sample was saponified by 10 ml of 2 mol/L KOH/EtOH containing 10 % H_2O for 2 hr with shaking. The PCBs were partitioned and extracted with 15 ml of n-hexane (2 times). The extract was cleaned up on a multi-layer column containing Na_2SO_4 (0.9 g), silica (0.5 g), $AgNO_3$-silica (1.8 g), silica (0.5 g), 44 %(W/W) H_2SO_4-silica (2.7 g), silica (0.5 g) and Na_2SO_4 (0.9 g). Before loading of the sample, the column was washed with 60 ml of n-hexane. The sample was loaded on the column and was eluted with 100 ml of 10 % methylene chloride / n-hexane at a flow-rate of 2.5 ml/min. About 10 µl of n-decane was added to the first fraction containig the PCBs, and evaporated at 40 on a rotary evaporator to about 0.5 ml.

PCBs fraction was evaporated on a rotary evaporator to *ca.* 0.5 ml and transferred to a GC autosampler vial tube. The remaining solvent was evaporated under the stream of nitrogen. The walls of the flask were flushed throughly with small volumes of methylene chloride, typically decreasing from 20 to 40 µl. This solution was added to 50 pg of the each syringe spike ($^{13}C_{12}$-PCBs). The sample was injected in 1µl volume and analyzed for PCBs.

Analysis of PCBs

We used the instrument of HRGC/HRMS, which consisted of an AutoSpec-Ultima (Micromass, U.K.) and a HP-6890 Series gas chromatograph (Agilent Technologies, Inc., U.S.A.). The used column was a HT8-PCB capillary column[1)], 0.25 mm I.D. x 60 m (Kanto Chemical Co., INC., Japan) The column temperature program for mono to penta PCB congeners was maintained at 120 , heated to 180 at a rate of 20 /min., heated to 252 at a rate of 2 /min., heated to 310 at a rate of 50 /min., and maintained at 310 for 5 min. and the column temperature program for hexa to deca PCB congeners was maintained at 120 , heated to 180 at a rate of 20 /min., heated to 260 at a rate of 2 /min., heated to 310 at a rate of 5 /min., and maintained at 310 for 5 min.. The injection temperature was 290 , ion source temperature was maintained at 280 , and the carrier gas (helium) rate was 1.0 ml/min. The ionizing energy and accelerating voltage were 35 eV and 7.5 kV, respectively. The resolution was about 12,000 throughout the work, and carried out according to an SIM.

Results and Discussion

The reproducibility test for PCBs using pooled blood sample

We divided the pooled blood sample into four and performed the quantitative reproducibility test of individual PCB congeners. Table 1 shows the average, S.D. and C.V. (%) values obtained by HRGC/HRMS analysis. The method has good reproducibility as mention in Table 1, showing C.V. (%) in the range of 0.2 – 10 % for PCBs.

Level of PCBs in whole pooled blood sample.

PCBs level in blood are summarized in Table 1. Total PCB levels of human pooled blood sample were 936 ± 2.3 pg / g on a whole blood basis. The 89 PCB congeners were detected in pooled blood. Among the 89 PCB congeners, the contribution of #118, #138, #153 and #180 were the most, accounting for more than 48 % of the total level. These predominant congeners had been substituting by chlorine at 2-, 4- and 5- positions of phenyl-ring, as reported in human breast milk sample[1)]. The PCB congeners pattern in pooled blood sample quantified in this study appears to be similar to those of the breast milk reported[2,3)]. These results may be an evidence of the behavior of intaked PCB congeners in human body as metabolite pathway and its transmission ability for the biomembrane furthermore.

References

1. Matsumura C., Tsurukawa M., Nakano T., Ezaki T. and Ohashi M. (2002) Journal of Environmental Chemistry, 12(4), 855-866.
2. Nakano T., Tanno K., Kitamoto H., Matsumura C., Goto M., Majima Y., Masho R., Tohyama C., Okuno T. (2002) Organohalogen Compounds, 55, 339.
3. Konishi Y, Kuwabara K, Hori S. (2001) Arch Environ Contam Toxicol, 40(4), 571.

Table 1-1 Quantitative Variances of PCBs Extracted from 15ml of Human Whole Pooled Blood

Congener	Bz#	Concentrations (pg/g whole blood)				AVG.	S.D.	C.V.(%)
		1st	2nd	3rd	4th			
2,2'-DiCB	*#4*	*0.14*	*0.14*	*0.15*	*0.14*	***0.14***	***0.0031***	***2.2***
2,3-, 2,4'-DiCB	#5,#8	0.49	0.52	0.51	0.53	**0.51**	**0.017**	**3.4**
3,3'-DiCB	#11	4.0	4.2	4.2	4.3	**4.2**	**0.11**	**2.7**
Total-DiCB		**4.6**	**4.9**	**4.8**	**4.9**	**4.8**	**0.13**	**2.7**
3,4,4'-TriCB	*#37*	*0.08*	*0.06*	*0.07*	*0.08*	***0.072***	***0.0072***	***10***
2,2',5-TriCB	#18	0.35	0.41	0.37	0.42	**0.39**	**0.035**	**9.1**
2,4,4'-TriCB	#28	5.0	5.6	5.7	5.7	**5.5**	**0.31**	**5.7**
2,4',5-TriCB	#31	0.46	0.53	0.53	0.51	**0.51**	**0.033**	**6.5**
Total-TriCB		**5.9**	**6.6**	**6.6**	**6.7**	**6.5**	**0.36**	**5.6**
3,3',4,4'-TetraCB	*#77*	*0.13*	*0.13*	*0.11*	*0.11*	***0.12***	***0.012***	***10***
3,4,4',5-TetraCB	*#81*	*0.059*	*0.057*	*0.050*	*0.050*	***0.054***	***0.0044***	***8.2***
2,2',3,5'-TetraCB	#44	0.35	0.40	0.37	0.40	**0.38**	**0.026**	**6.8**
2,2',4,5'-TetraCB	#49	0.51	0.46	0.46	0.45	**0.47**	**0.028**	**5.9**
2,2',5,5'-, 2,3',4,6-TetraCB	#52,69	1.9	2.0	1.9	1.9	**1.9**	**0.053**	**2.8**
2,3,3',4'-TetraCB	#56	0.91	0.98	0.91	0.87	**0.92**	**0.046**	**5.1**
2,3,4,4'-TetraCB	#60	2.1	2.2	2.4	2.2	**2.21**	**0.11**	**5.0**
2,3,4,6-TetraCB	#62	0.97	0.95	0.87	0.95	**0.93**	**0.042**	**4.5**
2,3,4',5-TetraCB	#63	0.31	0.31	0.36	0.33	**0.33**	**0.023**	**7.1**
2,3',4,5'-TetraCB	#68	0.32	0.29	0.27	0.29	**0.29**	**0.019**	**6.3**
2,3',4,4'-TetraCB	#66	6.5	6.8	6.4	6.7	**6.6**	**0.16**	**2.5**
2,4,4',5-TetraCB	#74	42	45	44	44	**44**	**1.1**	**2.4**
Total-TetraCB		**56**	**59**	**58**	**58**	**58**	**1.2**	**2.1**
2,3,3',4,4'-PentaCB	*#105*	*14*	*14*	*14*	*14*	***14***	***0.21***	***1.5***
2,3,4,4',5-PentaCB	*#114*	*6.3*	*6.1*	*6.4*	*5.9*	***6.2***	***0.20***	***3.2***
2,3',4,4',5-PentaCB	*#118*	*71*	*75*	*73*	*71*	***73***	***1.5***	***2.1***
2',3,4,4',5-PentaCB	*#123*	*1.3*	*1.2*	*1.3*	*1.2*	***1.2***	***0.036***	***2.9***
3,3',4,4',5-PentaCB	*#126*	*0.49*	*0.46*	*0.47*	*0.46*	***0.47***	***0.014***	***2.9***
2,2',3,4,4'-PentaCB	#85	0.41	0.42	0.37	0.45	**0.42**	**0.033**	**7.9**
2,2',3,4,5'-, 2,3,4,4',6-PentaCB	#87,115	0.95	0.98	0.81	0.99	**0.93**	**0.080**	**8.6**
2,2',3,5,5'-PentaCB	#92	2.0	2.1	1.9	2.0	**2.0**	**0.049**	**2.4**
2,2',3,5',6-, 2,2',3',4,6-PentaCB	#95, 98	1.4	1.7	1.5	1.4	**1.5**	**0.15**	**10**
2,2',3',4,5-, 2,3,4',5,6-PentaCB	#97, 117	2.8	3.0	2.7	2.7	**2.8**	**0.13**	**4.8**
2,2',4,4',5-PentaCB	#99	34	36	34	34	**34**	**1.2**	**3.5**
2,2',4,5,5'-PentaCB	#101	5.0	5.6	4.9	5.0	**5.1**	**0.29**	**5.7**
2,3,3',4,5'- 2,3,3',4',5-PentaCB	#107,108	2.8	2.7	2.8	2.7	**2.8**	**0.067**	**2.4**
2,3,3',4',6-, 2,3',4,5,5'-PentaCB	#110,120	1.4	1.5	1.6	1.5	**1.5**	**0.081**	**5.5**
2,3,3',5,5'-PentaCB	#111	0.37	0.42	0.40	0.40	**0.40**	**0.021**	**5.4**
Total-PentaCB		**144**	**151**	**146**	**144**	**146**	**3.0**	**2.0**

Human Blood (g)	15.1	15.1	15.0	15.0

Table 1-2

Congener	Bz#	Concentrations (pg/g whole blood)				AVG.	S.D.	C.V.(%)
		1st	2nd	3rd	4th			
2,2',4,4',6,6'-HexaCB	*#155*	*0.040*	*0.040*	*0.040*	*0.040*	***0.040***	***0.00017***	***0.43***
2,3,3',4,4',5-HexaCB	*#156*	*21*	*22*	*22*	*22*	***22***	***0.66***	***3.1***
2,3,3',4,4',5'-HexaCB	*#157*	*5.3*	*5.2*	*5.3*	*5.3*	***5.3***	***0.048***	***0.91***
2,3',4,4',5,5'-HexaCB	*#167*	*8.1*	*8.1*	*8.3*	*8.2*	***8.2***	***0.078***	***1.0***
3,3',4,4',5,5'-HexaCB	*#169*	*0.23*	*0.24*	*0.25*	*0.23*	***0.24***	***0.0099***	***4.2***
2,2',3,3',4,4'-HexaCB	#128	0.83	0.80	0.86	0.91	**0.85**	**0.05**	**5.3**
2,2',3,3',4,5'-HexaCB	#130	3.9	4.0	3.9	4.0	**3.9**	**0.048**	**1.2**
2,2',3,3',5,5'-HexaCB	#133	3.1	3.2	3.2	3.2	**3.2**	**0.060**	**1.9**
2,2',3,3',5,6'-HexaCB	#135	0.54	0.55	0.55	0.54	**0.55**	**0.0065**	**1.2**
2,2',3,4,4',5-HexaCB	#137	5.6	5.4	5.7	5.4	**5.5**	**0.14**	**2.5**
2,2',3,4,4',5'-HexaCB	#138	73	73	74	75	**74**	**0.75**	**1.0**
2,2',3,4,5,5'-HexaCB	#141	0.44	0.45	0.43	0.45	**0.44**	**0.0071**	**1.6**
2,2',3,4',5,5'-HexaCB	#146	28	27	27	28	**27**	**0.28**	**1.0**
2,2',3,4',5,6-HexaCB	#147	0.79	0.82	0.79	0.79	**0.80**	**0.015**	**1.8**
2,2',3,4',5',6-HexaCB	#149	1.1	1.1	1.1	1.1	**1.1**	**0.022**	**2.0**
2,2',3,5,5',6-HexaCB	#151	1.8	1.9	1.9	1.9	**1.9**	**0.029**	**1.6**
2,2',4,4',5,5'-HexaCB	#153	188	186	185	180	**185**	**3.2**	**1.8**
2,3,3',4,4',6-HexaCB	#158	1.2	1.2	1.2	1.2	**1.2**	**0.024**	**2.0**
2,3,3',4',5,6-, 2,3,3',4',5',6-HexaCB	#163,164	43	42	44	43	**43**	**0.53**	**1.2**
2,3,4,4',5,6-HexaCB	#166	1.0	1.1	1.1	1.1	**1.0**	**0.017**	**1.6**
Total-HexaCB		**386**	**384**	**386**	**382**	**385**	**2.0**	**0.51**
2,,3,3',4,4',5,5'-HeptaCB	*#189*	*2.6*	*2.5*	*2.6*	*2.6*	***2.6***	***0.029***	***1.1***
2,2',3,3',4,4',5-HeptaCB	#170	32	955	31	31	**262**	**461.75**	**175.99**
2,2',3,3',4,4',6-HeptaCB	#171	3.7	3.7	3.6	3.7	**3.7**	**0.035**	**1.0**
2,2',3,3',4,5,5'-HeptaCB	#172	6.3	6.2	6.2	6.2	**6.3**	**0.048**	**0.77**
2,2',3,3',4,5,6'-HeptaCB	#174	0.44	0.42	0.41	0.46	**0.43**	**0.023**	**5.4**
2,2',3,3',4,5',6-HeptaCB	#175	0.76	0.73	0.73	0.65	**0.72**	**0.048**	**6.8**
2,2',3,3',4',5,6-HeptaCB	#177	10	11	10	10	**10**	**0.18**	**1.8**
2,2',3,3',5,5',6-HeptaCB	#178	11	10	10	11	**11**	**0.21**	**1.9**
2,2',3,3',5,6,6'-HeptaCB	#179	0.39	0.40	0.39	0.40	**0.40**	**0.0078**	**2.0**
2,2',3,4,4',5,5'-HeptaCB	#180	124	120	120	122	**121**	**1.8**	**1.5**
2,2',3,4,4',5,6'- , 2,2',3,4',5,5',6-HeptaCB	#182,187	58	57	56	58	**57**	**0.87**	**1.5**
2,2',3,4,4',5',6-HeptaCB	#183	13	12	12	13	**13**	**0.20**	**1.6**
2,3,3',4,4',5,6-HeptaCB	#190	7.3	7.2	7.1	7.4	**7.3**	**0.14**	**1.9**
2,3,3',4,4',5',6-HeptaCB	#191	1.6	1.6	1.6	1.6	**1.6**	**0.0082**	**0.51**
Total-HeptaCB		**270**	**265**	**263**	**268**	**267**	**3.3**	**1.25**
2,2',3,3',5,5',6,6'-OctaCB	*#202*	*6.1*	*6.0*	*6.1*	*6.0*	***6.1***	***0.055***	***0.91***
2,3,3',4,4',5,5',6-OctaCB	*#205*	*0.64*	*0.66*	*0.71*	*0.71*	***0.68***	***0.032***	***4.7***
2,2',3,3',4,4',5,5'-OctaCB	#194	16	15	15	16	**16**	**0.42**	**2.7**
2,2',3,3',4,4',5,6'-OctaCB	#195	3.1	3.1	3.1	3.1	**3.1**	**0.0062**	**0.20**
2,2',3,3',4,4',5',6- 2,2',3,4,4',5,5',6-OctaCB	#196,#203	15	15	15	15	**15**	**0.21**	**1.4**
2,2',3,3',4,5',6,6'-OctaCB	#200	0.88	0.81	0.87	0.89	**0.86**	**0.039**	**4.6**
2,2',3,3',4,5,5',6'-OctaCB	#201	18	18	19	19	**18**	**0.30**	**1.6**
Total-OctaCB		**60**	**58**	**60**	**61**	**60**	**1.0**	**1.6**
2,2',3,3',4,4',5,5',6-NonaCB	*#206*	*4.4*	*4.1*	*4.2*	*4.3*	***4.2***	***0.13***	***3.0***
2,2',3,3',4,5,5',6,6'-NonaCB	*#208*	*1.9*	*1.9*	*1.8*	*1.9*	***1.9***	***0.072***	***3.8***
2,2',3,3',4,4',5,6,6'-NonaCB	#207	0.90	0.78	0.83	0.81	**0.83**	**0.049**	**5.9**
Total-NonaCB		**7.1**	**6.8**	**6.8**	**7.1**	**6.9**	**0.17**	**2.5**
DecaCB	#209	3.2	3.3	3.1	3.2	**3.2**	**0.083**	**2.6**
Total-DecaCB		**3.2**	**3.3**	**3.1**	**3.2**	**3.2**	**0.083**	**2.6**
Total PCBs		**937**	**939**	**935**	**934**	**936**	**2.3**	**0.24**

The 120 PCB congeners were not detected.