MATERNAL-INFANT TRANSFER OF POLYBROMINATED DIPHENYL ETHERS

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Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used brominated flame retardants (BFRs) in plastics of automobiles, textile industry, television, personal computer, electronic appliances etc. The amount of production world-wide has reached 40,000 tons in 1992. In 1992 world–wide production of PBDEs reached 40,000 tons raising serious concern over the dangers of environmental pollution by BFRs. The toxicity of PBDEs was reported to be an antagonist of thyroid-hormone (T4) [1, 2)] and inhibition to aryl hydrocarbon (Ah) receptor. Since PBDEs are structurally similar to PCBs and therefore they work as an antagonist [3)].

Polychlorinated biphenyl (PCBs) demonstrate biological stability and high lipophilicity. As a result, PCBs used in the past and released into the environment, have been transmitted through the food chain and accumlated in the human body over time. In Japan, approximately 58,000 tons of PCBs were produced with the grade name of Kanechlor in between 1954 and 1971.

In this study, all PCB congeners and 25 PBDE congeners (#17, #25, #28, #30, #32, #33, #35, #37, #47, #49, #66, #71, #75, #77, #85, #99, #100, #116, #119, #126, #138, #153, #154, #155, #166) were analyzed by the method that combines high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The purpose of this study was to investigate whether congener-specific PCBs and PBDEs were transferred from pregnant women to their infants.

Materials and Methods

Chemicals: Authentic PBDEs, PCBs, $^{13}C_{12}$-PBDEs and $^{13}C_{12}$-PCBs were obtained from Cambridge Isotope Laboratories, Inc. (MA, USA) and AccuStandard Co. (CT, USA). All solvents and reagents used were of DIOXIN or PCB-analysis grade.

Samples: The maternal blood, cord blood, placenta and breast milk were obtained from 4 pregnant women with proper consent, and were stored at –80ºC until analysis.

Preparation of samples

Lipid extraction

Maternal blood and Cord blood: About 10-15 g of whole blood was transferred to a 200 ml tube, and $^{13}C_{12}$-PCBs and $^{13}C_{12}$-PBDEs internal standards were added, and then a lipid fraction containing PCBs and PBDEs were obtained by extracting with saturated ammonium sulfate (12 ml), ethanol (6 ml) and n-hexane (18 ml), and repeated extraction by n-hexane for three more times. The extract was washed with ultra-pure water and the organic layer was dried over sodium sulfate and evaporated to dryness. The amount of lipid remaining was determined by gravimetric method.

Placenta: About 5 g of placenta was homogenized in the presence of the five fold of sodium sulfate and transferred to a 100 ml tube. Then, $^{13}C_{12}$-PCB and $^{13}C_{12}$-PBDEs internal standards were added, and then a lipid fraction containing PCBs and PBDEs were obtained by extracting with 50 ml of acetone/n-hexane (2:1 V/V) and repeated extraction by n-hexane for two more times. The extract was washed with ultra-pure water and the organic layer was dried over sodium sulfate and evaporated to dryness. The amount of lipid remaining was determined by gravimetric method.

Breast milk: About 5 g of breast milk was transferred to a 200 ml tube, and $^{13}C_{12}$-PCBs and $^{13}C_{12}$-PBDEs internal standards were added. And then a lipid fraction containing PCBs was obtained by extracting with saturated potassium oxalate (2 ml), diethyl ether (10 ml), ethanol (10 ml) and n-hexane (6 ml), and repeated extraction by n-hexane for three more times. The extracts were washed with 5% solution of sodium chloride and the organic layer was dried over sodium sulfate and evaporated to dryness. The amount of lipid remaining was determined by weight.

Clean-up and analysis of HRGC/HRMS

PBDEs: The clean-up method reported by Hirai T. *et al.* [4] was applied. An HRGC/HRMS instrument was used and consisted of an AutoSpec-Ultima NT (Micromass, Manchester, U.K.) and an HP-6890 Series gas chromatograph (Agilent Technologies Inc., CA, USA). The used column was an DB-17HT capillary column, 0.25 mm I.D. x 30 m (J&W Scientific, USA). The column temperature program was maintained at 180 ºC for 2 min, heated to 240 ºC at a rate of 3 ºC/min, heated to 320 ºC at a rate of 20 ºC/min, and maintained at 325 ºC for 5 min. The injection temperature was 240 ºC, the ion source temperature was maintained at 290 ºC, and the carrier gas (helium) flow rate was 1.0 ml/min. The ionizing energy and accelerating voltage were 35 eV and 8 kV, respectively. The resolution was about 10,000 throughout the study, and was carried out using selected ion mode (SIM).

PCBs: The clean-up method reported by Hirai T. *et al.* [5] was applied. An HRGC/HRMS instrument was described above. The used column was an HT8-PCB capillary column, 0.25 mm I.D. x 60 m (Kanto chemical Co., Inc., Tokyo, Japan).The column temperature program for the analysis of mono- to penta- CB congeners was maintained at 120 ºC, heated to 180 ºC at a rate of 20 ºC/min, heated to 252 ºC at a rate of 2 ºC/min, heated to 310 ºC at a rate of 50 ºC/min, and maintained at 310 ºC for 5 min. The column temperature program for the hexa- to deca- CB congeners was maintained at 120 ºC, heated to 180 ºC at a rate of 20 ºC/min, heated to 260 ºC at a rate of 2 ºC/min, heated to 310 ºC at a rate of 5 ºC/min, and maintained at 310 ºC for 5 min. The injection temperature was 290 ºC, the ion source temperature was maintained at 280 ºC, and the carrier gas (helium) flow rate was 1.0 ml/min. The ionizing energy and accelerating voltage were 35 eV and 8 kV, respectively. The resolution was about 11,000 throughout the study, and was carried out using SIM.

Results and Discussion

Few biological characteristics of the pregnant women were shown in Table 1. The observations elucidated that all subjects are in very good health conditions with a mean age of 29.3 and a pre-pregnant body mass index (BMI) of 20.7. The gestational age in all subjects is about 40 weeks (data not shown).

PBDEs: The individual concentrations of predominant congeners and sum of 25 PBDE congeners (Sum PBDEs) in maternal blood, cord blood, placenta and breast milk are summarized in Table 2. The Sum PBDEs concentrations were more or less equal among maternal blood and placenta with 1042±299 and 968±278, respectively on pg/g-lipid basis. On the other hand, slightly higher concentrations were revealed in breast milk (1509±898), and lower concentrations were revealed in cord blood (333±292) pg/g-lipid basis. Among 25 congeners, #47, #153, #100, #99, #28+#33 were predominated, accounting for more than 65% of the Sum PBDEs concentrations in these human samples.
PBDEs lacks cross-placental behavior and therefore shift rate to cord blood reveals some results. The low brominated congeners detected in placenta was higher than those levels in cord blood and maternal blood, indicating that the transfer of low brominated congeners to infants are minimized by the placenta.

PCBs: The concentration of the predominant PCB congeners and total PCB concentration in the maternal blood, cord blood, placenta and breast milk are summarized in Table 3. The concentration of total PCBs was almost the same among the maternal blood, placenta and breast milk at 93.6±10.5, 102.6±19.4 and 88.8±10.4, respectively on ng/g-lipid basis . On the other hand, it was about 40% lower in cord blood, being 58.0±15.3 pg/g lipid basis. The results indicate that PCBs haven't played cross-placental role as it blocked in placenta. Among all the PCB congeners, #153, #138, #180, #74, #99, #118 and #187 predominated, accounting for more than 59% of total PCBs concentrations in all these human samples. These predominant congeners had chlorine at the 2-, 4- and 5- positions of the phenyl-ring. #153 and #138 are contained in PCB products, in conformity with reports[6] that they are hard for humans to metabolize. It turns out that the congeners with chlorine at the 2-, 4-, and 5- positions also remain as the predominant congeners in these human samples.

References

1 Hallgern,S. and Darnerud, P.O. (1998). Organohalogen Compounds, 35, 391-394.
2 Manchester-Neesving, J.B., Valters, K., Sonzogni, W.C. (2001). Environ. Sci.Technol., 35, 1072-1077.
3 Meerts,I.A.T.M., Luijks, E.A.C., Marsh, G., Jakobsson, E., Bergman, A. (1998). Organohalogen Compounds, 37, 147-150.
4 Hirai T., Furutani H., Myoren M., Fujimine Y., Kodaira T., Hata J., Watanabe S. (2002). Organohalogen Compounds, 58, 277-280.
5 Hirai T., Fujimine Y., Watanabe S., Nakano T. (2003). Organohalogen Compounds, 63, 417-420.
6 Koga N, Kanamaru T, Oishi N, Kato Y, Kimura R, Haraguchi K, Masuda Y. (2001). Fukuoka Igaku Zasshi, 92, 167-176.

Table 1: General characteristics of the pregnant women

Subject No.	Age	Pre-pregnant BMI (kg/m^2)	Age at breast milk collection (days)	Parity
1	29	18	6	2
2	26	21	3	1
3	34	21	5	3
4	28	23	4	2
Mean	29	21	5	2

Table 2: Predominant and Sum PBDEs concentrations in the maternal blood, cord blood, placenta and breast milk

(pg/g-lipid basis)	Subject 1				(pg/g-lipid basis)	Subject 2			
	Placenta	Maternal Blood	Cord Blood	Breast Milk		Placenta	Maternal Blood	Cord Blood	Breast Milk
#28+#33	139	100	0.0	183	#28+#33	81	8.5	0.0	72
#47	499	461	335	1058	#47	166	125	38	218
#100	141	161	80	378	#100	38	63	11	76
#99	91	88	0.0	232	#99	40	39	0.0	69
#153	149	375	324	629	#153	350	534	165	222
Sum PBDEs	***1342***	***1344***	***763***	***2794***	***Sum PBDEs***	***864***	***770***	***214***	***703***

(pg/g-lipid basis)	Subject 3				(pg/g-lipid basis)	Subject 4			
	Placenta	Maternal Blood	Cord Blood	Breast Milk		Placenta	Maternal Blood	Cord Blood	Breast Milk
#28+#33	119	31	0.0	98	#28+#33	104	8.8	0.0	60
#47	163	178	44	344	#47	337	241	149	422
#100	56	87	53	122	#100	92	92	51	173
#99	38	94	0.0	78	#99	86	52	0.0	110
#153	73	625	18	486	#153	160	302	4.9	326
Sum PBDEs	***684***	***1253***	***114***	***1321***	***Sum PBDEs***	***982***	***799***	***239***	***1216***

Table 3: Predominant and total PCBs concentrations in the maternal blood, cord blood, placenta and breast milk

(ng/g-lipid basis)	Subject 1				(ng/g-lipid basis)	Subject 2			
	Placenta	Maternal Blood	Cord Blood	Breast Milk		Placenta	Maternal Blood	Cord Blood	Breast Milk
#28	0.80	0.74	0.90	0.83	#28	0.51	0.50	0.51	0.44
#74	2.4	2.7	2.2	2.7	#74	3.3	3.8	2.9	3.5
#118	4.8	5.2	4.7	4.7	#118	4.2	4.1	3.3	3.7
#99	3.8	4.2	3.1	4.0	#99	2.4	2.5	2.7	2.5
#138	8.3	10	8.1	10	#138	7.2	7.0	5.2	6.6
#146	2.1	3.0	2.2	3.4	#146	3.1	3.3	2.2	3.3
#153	16	22	16	22	#153	18	19	13	18
#156	1.6	2.0	1.3	1.9	#156	2.6	2.8	1.8	2.5
#163,164	3.4	4.0	4.3	4.2	#163,164	5.4	5.3	4.3	5.0
#170	2.7	3.4	2.4	2.8	#170	3.1	3.5	2.4	2.8
#180	9.4	12.7	8.6	10.5	#180	11.1	12.2	7.3	8.9
#182,187	5.5	7.0	4.1	6.2	#182,187	5.9	5.5	3.1	4.5
#194	1.3	1.8	1.0	1.5	#194	1.0	1.4	0.70	0.76
#201	1.5	1.8	1.2	1.4	#201	1.3	1.6	0.86	1.0
Total PCBs	**83**	**104**	**77**	**98**	**Total PCBs**	**88**	**90**	**61**	**77**

(ng/g-lipid basis)	Subject 3				(ng/g-lipid basis)	Subject 4			
	Placenta	Maternal Blood	Cord Blood	Breast Milk		Placenta	Maternal Blood	Cord Blood	Breast Milk
#28	1.9	1.0	1.0	1.4	#28	1.6	1.0	0.6	0.8
#74	6.2	4.0	3.2	5.3	#74	4.1	3.3	1.6	2.5
#118	10	6.2	5.4	8.1	#118	7.7	6.2	3.0	4.9
#99	5.7	3.3	2.7	4.8	#99	4.8	3.2	1.4	2.9
#138	12	8.3	6.0	8.7	#138	12	10	4.3	8.7
#146	2.9	2.2	1.5	3.2	#146	3.3	2.8	1.1	2.7
#153	22	16	11	20	#153	24	22	8.4	19
#156	2.6	1.9	1.1	2.3	#156	2.6	2.1	0.8	1.9
#163,164	5.0	3.6	2.7	4.5	#163,164	5.3	4.2	2.1	3.7
#170	3.6	2.7	1.5	2.7	#170	4.2	3.6	1.4	1.3
#180	12	8.1	4.6	8.7	#180	14	12	4.4	11
#182,187	5.7	3.8	2.1	4.4	#182,187	7.0	5.7	1.8	5.2
#194	1.4	1.2	0.46	1.1	#194	1.7	1.7	0.40	1.3
#201	1.6	1.2	0.54	1.3	#201	1.9	1.6	0.46	1.4
Total PCBs	**118**	**81**	**54**	**97**	**Total PCBs**	**120**	**100**	**40**	**84**