Investigation Of The Main Source Of Polybrominated Diphenylethers In Human Breast Milk (The First Report)

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Introduction

We had been minutely analyzed samples of meal, breast milk and blood from 9 women, in order to investigate the main source of typical chlorinated organic pollutants including PCDDs, PCDFs and coplanar PCBs (Co-PCBs) in human breast milk[1,2)]. As the result, it was revealed that these pollutants were delivered from two sources of the meal and the accumulation-in-body. This new finding was able to break common senses in the environmental science field that " all of PCB and dioxin analogues in human breast milk originate in their storage-in-body" and "the breast milk is one of the most suitable index samples for human body contamination".

In this study, we focused on the polybrominated diphenyl ethers (PBDEs), one of the representative brominated flame retardant agents, whose close-up is taken greatly in the environmental science field in recent years, and investigated on the main sources of PBDEs in breast milk.

Materials and Methods

1) Brest milk samples for pollution change observation during breast-feeding on an empty stomach: Breast milk samples (ca. 30 mL) were obtained at the time of the start of breast-feeding on an empty from three nursing mothers (Mothers A, B and C) of Osaka and Hyogo prefectures, western Japan, in a period of September to October 2004. The numbers of samples are two samples from Mother A, six samples from Mother B and ten samples from Mother C, and a total of 18. In addition, the time zones of sampling from three mothers were 7 times before breakfast, 1 time before lunch and 1 time before dinner. **2) Breast milk and meal samples for change observation with the passage of time:** Thirty breast milk samples (ca. 30 mL) were obtained at a rate of once per every three hours on consecutive 4 days from Mother D of Hyogo prefecture in October, 2004. On the other hand, meal samples and between-meal snack samples were separately obtained at three time zones of morning time (from breakfast to lunch), daytime (from lunch to supper) and evening time (from supper to next breakfast) during a period of consecutive two days.

3) Analytical method:

2-1) Breast milk: About 20 g of sample was analyzed for PBDEs according to our previous methods[3)]. It was essentially composed of fat extraction, addition of $^{13}C_{12}$-laveled internal standards, alkaline decomposition, and clean up on a multi-layer silica gel column followed by an activate carbon-dispersed silica gel column. The cleaned up extract was analyzed in EI-SIM mode at a resolution of 10,000 using a Hewlett Packard 5890J GC-JEOL M700 MS.

2-2) Meal and between-meal snack samples: Meal or between-meal snack sample was dried in the decompression chamber for 3 days. After addition of $^{13}C_{12}$-laveled internal standards, the dried sample (ca. 100 g) was extracted with 400 ml of toluene for 4 hours under reflux. The extract was filtered, washed tree times with sulfuric acid, washed three times with water and dried over anhydrous sodium sulfate. After concentration, the extract was purified using a multi-layer silica gel column and an activate carbon-dispersed silica gel column, followed by HRGC/HRMS analysis.

Results and Discussion

1) Changes of fat content and PBDEs pollution in the breast milk during breast-feeding on an empty stomach time

We investigated about the breast milk samples obtained at the time of hungry before the breakfast, which the influence of a meal could almost disregard. In this case, it is expected that the PBDEs already accumulated in the inside of the mother body are generally discharged in the breast milk. In this study, however, the result of having differed from anticipation considerably was obtained.

Table 1. Changes of the fat content and PBEs pollution level in breast milk during breast-feeding on an empty stomach time

Mother	Sampling day	Time zone of sampling	Time portion of breast-feeding	Starting time of sampling	Fat content (%)	Ratio [a]	Conc. of PBDEs (pg/g fat)
A	4-Oct	Before B [b] (A-1)	First portion	6:30	1.60	2.24	266
			Last portion	7:20	3.59		146
B	24-Sep	Before B [b] (B-1)	First portion	8:30	1.70	1.89	104
			Last portion	9:15	3.22		239
	27-Sep	Before B [b] (B-2)	First portion	9:45	2.68	1.87	206
			Last portion	10:15	5.02		294
	30-Sep	Before B [b] (B-3)	First portion	9:00	1.91	1.79	435
			Last portion	9:40	3.42		176
	24, 27, 30-Sep	Average	First portion	-	2.10	1.85	248
			Last portion	-	3.89		236
C	27-Sep	Before B [b] (C-1)	First portion	7:20	2.55	1.73	513
			Last portion	7:50	4.41		482
	30-Sep	Before B [b] (C-2)	First portion	7:20	2.07	1.93	342
			Last portion	8:00	4.00		244
		Before L [c] (C-3)	First portion	12:45	3.32	2.25	555
			Last portion	13:15	5.23		876
		Before D [d] (C-4)	First portion	18:45	2.85	2.09	406
			Last portion	19:15	5.96		208
		Average	First portion	-	2.75	1.84	434
			Last portion	-	5.06		443
	1-Oct	Before B [b] (C-5)	First portion	6:50	1.70	1.94	398
			Last portion	7:25	3.20		441

a): Ratio of fat content in the first portion versus the last portion b) Before breakfast

c) Before lunch d) Before dinner

As shown in Table 1, in the case of Mother A, the fat content in breast milk increased 2.24 times (from 1.60% to 3.59%) during a short-time breast-feeding on an empty stomach time before breakfast. Such a phenomenon, which the fat content increases in the last portion compared with the first portion of breast-feeding, was also confirmed in the cases of Mothers B and C. In Mother B, the rate of increase was in the range of 1.79 to 1.89 with an average of 1.85 at a hungry-time before breakfast in three days in September 24 to 27. On the other hand, in Mother C, the similar increase of fat content at the time of hungry before lunch and before dinner (see Table 1). Although the cause of this new knowledge is unknown at present, when that investigation solves future pollution of breast milk, it is thought that it is an important subject.

As shown in Table 1, in the case of Mother A, the concentration of PBDEs reduced by half from 266 pg/g-fat to 146 pg/g-fat during a breast-feeding. The same thing was observed by B-3 on September 30 in Mother B, and by C-2 and C-4 on September 30 in Mother C. Conversely, however, the increase of PBDEs level was confirmed by B-1 in Mother B, and by C-3 and C-5 in Mother. On the other hand, the level of PBDEs was almost constant in C-1 of Mother C.

From these results, a big change of such concentration of PBDEs suggests the complicated action of PBDEs accumulated in the living body in spite of breast-feeding of a short time at the time of hungry.

2) Effect on the pollution level and composition ratio of PBDEs in the breast milk by meal

The PBDEs concentration in breast milk was also changed sharply temporally in the analysis result of breast milk sample of a total of 30 obtained for four days of continuation every about 3 hours from Mother D. With the fat base, the range of the concentration of PBDEs for four days became 68 - 947 pg/g (average: 364 pg/g), and was carrying out the thing difference 14 times at the maximum.

As a result of analyzing about six samples of meal and between-meal snack on the first experimental day which came to hand from Mother D, the PBDEs content per one sample was 81.7pg-374.3pg, and all the PBDEs intakes via a meal and between-meal snack in this examination date was 1,401 pg (see Table 2).

Table 2 Intake amounts of PBDEs via meal and between-meal snack on the first experiment day

Compound		Intake amount of PBDEs (pg/meal or Between-meal snack)						
		Time of meal or between-meal sanack						
		8:30	9:00	13:00	18:50	20:00	22:30	Total
TriBDE	(#17)	2.0	0.0	0.0	0.0	0.0	0.0	2.0
TeBDE	(#47)	8.4	17.6	18.9	56.7	11.2	0.7	113.5
PeBDE	(#99)	2.3	12.1	14.6	51.0	6.3	0.7	87.0
	(#100)	3.3	1.1	1.4	4.3	1.2	0.2	11.5
HxBDE	(#153)	30.4	41.6	19.6	103.3	19.7	15.8	230.4
	(#154)	6.5	5.3	2.5	19.6	4.5	5.3	43.7
HpBDE	(#181)	26.8	14.0	63.4	7.5	1.2	26.8	139.7
	(#183)	2.6	1.0	5.3	1.4	0.3	2.6	13.2
DeBDE	(#209)	110.0	92.0	248.5	99.6	37.4	172.4	759.9
Total PBDEs		192.3	184.7	374.2	343.4	81.8	224.5	1400.9

On the other hand, as shown in Table 3, the amount of PBDEs excretion per breast-feeding in the same day was 201.2 - 1,946 pg, and was sharply changed for every breast-feeding. Moreover, not only the difference of such an excretion amount but the composition ratio was changed remarkably. The total amount of excretion via breast milk was 6,063 pg. The intake via a meal was equivalent to 1/4 of the amount of excretion via breast milk. It is set also to one ninth even if it considers the absorption factor (it assumes as 50%) of PBDEs from the stomach and intestines.

Table 3 Excretion amount of PBDEs via breast milk on the first experiment day

Compound		Excretion amount of PBDEs (pg)							
		Time of breast-feeding							
		5:15	8:15	11:30	14:45	18:00	21:00	23:00	Total
TriBDE	(#17)	0.0	8.9	8.1	20.0	19.8	20.8	8.3	85.9
TeBDE	(#47)	33.1	55.1	93.7	415.8	308.4	461.4	196.1	1563.6
PeBDE	(#99)	14.0	10.4	11.6	25.5	22.7	43.7	21.0	148.9
	(#100)	17.9	21.2	14.2	230.0	128.8	194.2	105.9	712.2
HxBDE	(#153)	0.0	3.9	4.1	0.0	12.7	12.1	8.0	40.8
	(#154)	107.0	113.8	119.3	350.4	401.6	230.7	182.3	1505.1
HpBDE	(#181)	20.5	12.5	202.6	103.9	153.9	77.9	127.4	698.7
	(#183)	8.7	8.0	34.0	21.3	26.7	12.4	16.8	127.9
DeBDE	(#209)	0.0	0.0	48.2	778.8	0.0	0.0	352.6	1179.6
Total PBDEs		201.2	233.8	535.8	1945.7	1074.6	1053.2	1018.4	6062.7

On the other hand, in the result in another examination date, the total intake of PBDEs intake via a meal was 2,143pg. This intake was equivalent to one third of the above-mentioned amounts of excretion, and even if it considered the absorptivity from the stomach and intestines, it was set also to one sixth.

PBDEs are liposolubility substances and their absorption factors from the stomach and intestines are greatly influenced by the fat content of a meal. When a meal with a high fat content is eaten, it is suggested that the PBDEs intake via a meal may approach the amount of excretion via breast milk. Taking these things into consideration, it became clear that the PBDEs contamination in breast milk has received strongly the transient contamination depended on a meal course.

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