INVESTIGATION OF THE MAIN SOURCE OF HALOGENATED ENVIRONMENTAL POLLUTANTS IN HUMAN BREAST MILK (THE FOURTH REPORT) -INFLUENCE BY FAST-

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Abstract
The concentration of dioxin analogues in the breast milk in three nursing mothers in Hyogo prefecture, Japan, was considerably changed in a short time of a few hours. Moreover, the composition ratio of dioxin analogues was also changed sharply. This result emphasizes that the contamination of dioxin analogues in breast milk is greatly influenced by dietary intake. On the other hand, an influence on the pollution levels of dioxin analogues in human breast milk by fast for 5 nursing mothers was investigate. As a result, it became clear that PCDDs/DFs contamination decreased considerably by fast. However, the pollution level of Co-PCB was reveled to be hardly receiving the influence by fast.

Introduction
We had already shown clearly that dioxin analogues including PCDDs, PCDFs and Co-PCBs in human breast milk were all delivered from two sources of the meal and the accumulation-in-body[1,2]. Furthermore, as for the result of having been well similar, the polybrominated diphenyl ethers (PBDEs) investigation was also observed, showing the pollution level of PBDEs in the breast milk to change remarkably during a short-time breast-feeding of ca. 30 mins, and the intake of PBDEs via a meal to be equivalent to 1/4 - 1/3 of the amount of excretion via breast milk[3].
These our findings are able to break common senses in the environmental science field that “all of persistent halogenated environmental pollutants such as PCBs, dioxin analogues, DDT, HCHs, Dieldrins, PBDEs 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 influence on the pollution levels of dioxin analogues in human breast milk by fast following last year[4] for nursing mothers in Hyogo prefecture, Japan, in order to clarify further influence on their pollution of breast milk by dietary intake.

Materials and Methods
1) 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 or 5 days from three nursing mothers (Mothers A, B and C) of Hyogo prefecture in 2005. 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.
2) Brest milk samples for pollution change observation by fast: Breast milk samples were obtained at the time of once per every three hours on consecutive 4 days or 5 days from five nursing mothers (Mothers D, E, F, G and H) of Hyogo prefecture in 2005. These five mothers abstained from food on and after the 3rd day after breast milk sampling start.
3) Analytical method:
3-1) Breast milk: About 20 g of sample was analyzed for dioxin analogues according to our previous methods[2]. 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.

3-2) 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) Time alteration on pollution levels of dioxin analogues in breast milk

Breast milk samples were obtained at a rate of once per every ca. 3 hrs on consecutive 4 days or 5 days from Mother A, B and C. The concentration of dioxin analogues (abbreviated as Dioxins) in breast milk changed quite a lot in a short time of a few hours. For example, in the case of Mother A, the range of the pollution level in the 1st experiment was a minimum level of 1.48 pg TEQ/g lipid at 23:30 to a maximum level of 7.47 pg TEQ/g lipid at 17:00. The ratios of max./min. were 5.0 in the 1st day, 3.4 in the 2nd day, 5.2 in the 3rd day and 1.9 in the 4th day, respectively. The similar result was observed in Mothers B and A, showing the ratios of max./min. to be 1.5 in the 3rd day to 4.7 in the 1st day in the former, and 1.3 in the 1st day to 8.0 in the 3rd day in the latter, respectively.

In addition, the time alteration of the composition ratios of PCDDs, PCDFs and Co-PCBs in the total TEQ was also quite large. A shown in Fig. 1, in the case of Mother A, the composition ratios in the 1st day were in a wide range of 8.7 to 48.3% in PCDDs, 25.9 to 37.1% in PCDFs and 20.9 to 60.3% in Co-PCBs, respectively. Such a big composition change was observed in through the 2nd day to 4th day. In addition, the similar result was seen also in the cases of Mothers B and C.

As shown in Table 1, a big variation per day of the concentration of Dioxins in breast milk was also seen in all three mothers. In Mother A, the coefficient of variation (CV) in the period of the four experimental days, 47.8% in PCDDs, 13.2% in PCDFs, 7.31% in Co-PCBs, and 24.3% in the total, respectively. Such a similar result were recognized in the case of Mothers B and C, showing the C.V. to be 11.3% in PCDDs, 41.1% in PCDFs, 126% in Co-PCBs, and 32.4% in the total in the former, and 87.0% in PCDDs, 33.7% in PCDFs, 11.9% in Co-PCBs, and 13.3% in the total in the latter. As well as the concentration, a big variation per day of the composition ratio of Dioxins was also in all three mothers. For example, the CV of PCDDs, PCDFs and Co-PCBs were in 20 - 67.9%, 16.1 - 54.5% and 10.3 –

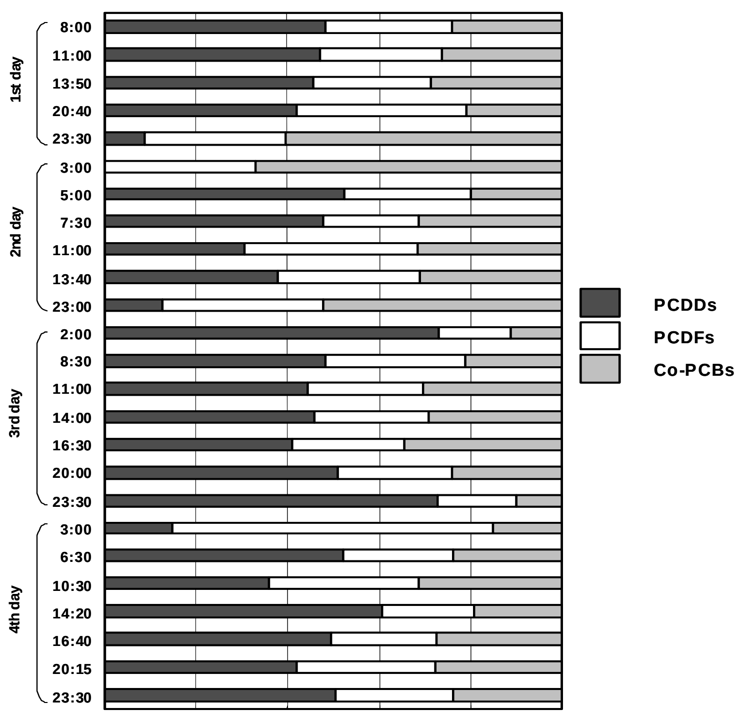

Fig. 1. Time alteration of the composition ratio of Dioxins in breast milk in Mother A

165%, respectively.

Taking the analytical result of meal samples into consideration, thus changing sharply the concentration and the composition ratio of Dioxins contained in breast milk or a meal emphasizes anew that the Dioxins pollution in breast milk has received greatly not only reflecting for their storage-in-body accumulation but the influence by a meal.

Table 1. Time alteration on pollution levels of dioxin analogues in breast milk in four mothers

Subject	Experimental day	Concentration (pgTEQ/g lipid)				Composition ratio (%)			
		PCDDs	PCDFs	Co-PCBs	Total	PCDDs	PCDFs	Co-PCBs	Total
Mother A	1st	2.14	1.63	1.51	5.28	40.5	30.9	28.6	100.0
	2nd	1.54	1.23	1.46	4.23	36.4	29.1	34.5	100.0
	3rd	4.44	1.60	1.42	7.46	59.5	21.4	19.0	100.0
	4th	2.41	1.66	1.27	5.34	45.1	31.1	23.8	100.0
	Mean	2.63	1.53	1.42	5.58	45.4	28.1	26.5	100.0
	CV (%)	47.8	13.2	7.31	24.3	22.2	16.1	25.0	0.0
Mother B	1st	17.4	3.59	1.47	22.5	77.5	16.0	6.54	100.0
	2nd	14.4	1.91	0.573	16.9	85.3	11.3	3.39	100.0
	3rd	12.9	1.57	1.19	15.7	82.4	10.0	7.60	100.0
	4th	16.0	1.45	6.71	24.2	66.2	6.00	27.8	100.0
	5th	16.0	1.97	16.1	34.1	47.0	5.78	47.3	100.0
	Mean	15.3	2.10	5.21	22.6	77.8	10.8	11.3	100.0
	CV (%)	11.3	41.1	126	32.4	20.0	38.9	165	0.0
Mother C	1st	0.984	0.484	5.47	6.94	14.2	6.98	78.8	100.0
	2nd	0.506	0.557	5.06	6.12	8.26	9.10	82.6	100.0
	3rd	1.71	0.604	5.44	7.75	22.1	7.79	70.2	100.0
	4th	0.114	0.317	5.95	6.38	1.79	4.97	93.2	100.0
	5th	0.334	0.835	4.28	5.45	6.13	15.3	78.5	100.0
	Mean	0.730	0.559	5.24	6.53	11.6	7.21	81.2	100.0
	CV (%)	87.0	33.7	11.9	13.3	67.9	54.5	10.3	0.0

2) Influence on the pollution level of Dioxins in breast milk by fasting

The breast milk samples were obtained from five mothers (Mothers D – H) for consecutive 4 days or 5 days. Although these mothers ate a meal for 2 days on the 1st and 2nd experimental day, and abstained from food for two days of the 3rd to 4th experimental day or three days of the 3rd to 5th day.

In the case of Mothers A - C who was taking in the meal through the experimental period, the fat content in breast milk changed to some extent with the CV of 10.6 to 21.7% (Table 2). On the hand, in the case of Mother D, the fat content suited the upward tendency by fast. However, such an upward tendency was not accepted by other 4 mothers (Mothers E – H). When these results were judged comprehensively, it turned out that the fat content in breast milk hardly receives the influence by fast.

Table 2. Variation per day of the fat content in breast milk from eight mothers

Experimental day	Fat content (%) in breast milk							
	Mother A	Mother B	Mother C	Mother D	Mother E	Mother F	Mother G	Mother H
1st day	3.51	2.33	3.13	5.07	5.81	5.20	8.06	1.30
2nd day	2.83	1.97	3.09	4.93	5.67	3.49	5.00	3.02
3rd day	3.60	2.25	2.71	4.99	4.30	2.56	6.40	2.34
4th day	3.50	1.81	2.50	7.61	4.16	2.10	5.22	3.31
5th day	-	1.17	2.24	7.65	6.14	2.41	-	-
Average	3.36	1.91	2.73	6.05	5.22	3.15	6.17	2.49
S.D.	0.356	0.413	0.341	1.29	0.821	1.12	1.40	0.893
CV (%)	10.6	21.7	12.5	21.3	15.7	35.7	22.7	35.8
-: No data								

In order to investigate the influence by fast, a comparison examination of the experiment 3 - 5 day concentration was carried out with experiment 1-2 day average concentration. In Mother D, the Dioxins concentration in the 3rd experimental day (the fast start day) was a little lower than the average value, showing the level to be equivalent to 93% in PCDD/DFs, 80% in Co-PCBs and 88% in the total,

Table 3. Influence on the pollution level of Dioxin analogues in breast milk in five mothers by fasting

Experimental day	Mother D			Mother E			Mother F		
	PCDDs/DFs	Co-PCBs	Total	PCDDs/DFs	Co-PCBs	Total	PCDDs/DFs	Co-PCBs	Total
1st day	5.64	4.35	9.99	1.81	3.47	5.28	2.93	3.55	6.48
2nd day	7.99	4.77	12.8	2.32	3.49	5.81	3.01	4.11	7.12
Average	6.82	4.56	11.4	2.07	3.48	5.55	3.13	3.83	6.80
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
3rd day	6.31	3.65	9.96	3.65	3.46	7.11	8.42	4.46	12.9
	(93)	(80)	(88)	(177)	(99)	(128)	(269)	(116)	(189)
4th day	3.58	3.09	6.67	1.60	3.54	5.135	4.00	4.63	8.63
	(53)	(85)	(67)	(77)	(102)	(93)	(128)	(121)	(127)
5th day	2.57	2.69	5.26	1.69	3.95	5.641	3.98	3.94	7.92
	(38)	(59)	(46)	(82)	(114)	(102)	(127)	(103)	(116)

Average: An average of the concentrations on the 1st day and the 2nd day

Figure in parenthesis: A percentage to an average of the concentrations In the 1st day and 2nd day.

Experimental day	Mother G			Mother H		
	PCDDs/DFs	Co-PCBs	Total	PCDDs/DFs	Co-PCBs	Total
1st day	0.949	0.61	1.56	0.64	1.83	2.47
2nd day	2.733	1.63	4.36	0.88	3.22	4.10
Average	1.84	1.12	2.96	0.765	2.53	3.29
	(100)	(100)	(100)	(100)	(100)	(100)
3rd day	0.506	1.35	1.86	0.55	2.66	3.211
	(27)	(120)	(63)	(72)	(105)	(98)
4th day	0.588	1.09	1.68	0.28	3.45	3.727
	(32)	(97)	(57)	(36)	(137)	(113)

respectively (Table 3). The decrease tendency was observed clearly on the 4th day and 5th day, that is, the level of total Dioxins decreased to 67% on the 4th day and 46% on the 5th day of the average level of the 1st day and 2nd day. PCDDs/DFs decreased more greatly in comparison with Co-PCBs.

Such a downward tendency of PCDDs/DFs was also observed in the Mothers E, G and H, showing the relative concentration to be 77% on the 4th day and 82% on the 5th day in Mother E, 27% on the 3rd day and 32% on the 4th day in Mother G, and 72% on the 3rd day and 36% on the 4th day in Mother H. However, in the case of Co-PCBs, thus decrease tendency was not observed in Mothers E, F, G and H. In addition, only Mother F did not show reduction of PCDDs/DFs.

From above the above results, the concentration of PCDDs/DFs in breast milk became clear decreasing greatly by fast. However, in the case of Co-PCBs, such concentration reduction did not take place by fast, due to their high concentration accumulation in the body. If the PCDDs/DFs in breast milk follow the conventional idea of originating in what is being accumulated into a human body, the level will not decrease by fast for only a few days.

As a conclusion, our result emphasizes that the pollution of breast milk is greatly influenced not only with the accumulation contaminants in a human body but with the contaminants of meal origin.

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