INVESTIGATION ON MAIN SUORCE OF DIOXIN ANALOGUES IN HUMAN BREAST MILK (SECOND REPORT)

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Introduction

In many countries, the breast milk sample has been used as a suitable indicator in order to examine human exposure level to Dioxins. In general, the breast milk level is considered to be reflecting to their accumulation level in the body. In addition, it is considered that ca. 60% of the accumulation amount of Dioxins is excreted to the baby through breast milk by nursing for a year. However, are these things true? In 1989, Frust et al.[1] reported a time course of concentrations of Dioxins (abbreviated as Dioxins) in breast milk of one German during a period of 1 - 60 weeks after delivery. In the case of PCDFs, the level of 10 - 13 weeks after delivery was remarkably higher than that of 5 weeks. In addition, the PCBs level on the 10 to 13 weeks was also higher in comparison with on the 1 week. Thus, their pollution levels did not always decrease with a passing of time after childbirth. This suggests that all Dioxins in breast milk might be not derived from their storage in the body.

Therefore, in 2001, we investigated the time alteration on the pollution level of Dioxins in breast milk from nine mothers and on their infants' daily intake of Dioxins by nursing[2]. Consequently, it was revealed that the average daily intake of PCDD/DFs (PCDDs + PCDFs) was roughly constant during a period of 5 to 180 days after delivery. If all PCDD/DFs in breast milk are derived from only their body storage, the pollution level in milk must decrease in a linear course during a period of 5 to 180 days after delivery. However, thus linear decrease of pollution level was not observed in all tested mothers. These results indicated that PCDD/DFs in milk might be also delivered from other sources except for their storage in the body. Therefore, in this study, we tried to investigate the source of Dioxins in human breast milk.

Methods and Materials

1) Breast milk, blood and meal samples: Breast milk samples were obtained from nine mothers during 2002 in Osaka and Hyogo Prefectures of western Japan. The mothers were divided into three groups as follows: Group 1 (Mothers A, B and C), Group 2 (Mothers D and E) and Group 3 (Mothers F, G and I). Blood samples at hungry time were also obtained from all mothers. In addition, meal samples were obtained from 6 mothers (Mothers D – I). On the other hand, the intake amount of breast milk in each infant was examined in detail manners.

2) Analytical method:
2-1) Breast milk: About 50 g of sample was analyzed for Dioxins according to our previous methods [2,3].
2-2) Blood: About 30 ml of blood sample was analyzed for Dioxins according to our method[4].
2-3) Meal: About 1000 g of meal sample was dried in the decompression chamber for 3 days. After addition of $^{13}C_{12}$-labeled internal standards, the dried sample was extracted with 400 ml of toluene for 4 hrs. 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 and analyzed for Dioxins according to our previous methods [2,3].

Results and Discussion

1) Monthly alteration on pollution levels of Dioxins in breast milk

Breast milk samples were obtained 3 times at a rate of once a month at hungry time from Mothers A, B and C. Blood samples were collected at hungry time from these mothers. As shown in Table 1, the concentration of total Dioxins in breast milk from Mother A was almost constant during a period of the first to third months after delivery. In addition, the composition ratios of PCDDs, PCDFs and Co-PCBs to the total TEQ were also roughly constant, showing the ratios to be similar to those of blood sample. However, in the case of Mothers B and C, the pollution level was not stable at the range of 19.2 to 28.8 pgTEQ/g lipid and 7.81 to 9.95 pgTEQ/g lipid, respectively, showing the level to be higher in the 6th month than the 5th month after delivery. In addition, in the case of Mother B, the composition ratio changed fairly for 3 months. For example, the ratio of PCDDs was in the range of 38.7% (the 6th month) to 54.1% (the 5th month). Consequently, in all tested mothers, there was not observed a reliable decrease of the pollution level in breast milk during a period of 2 months after childbirth.

Table 1. Monthly alteration on the level of Dioxins in breast milk from Group 1 mothers.

Compound	Concentration (pgTEQ/g lipid)											
	Mother A				Mother B				Mother C			
	Blood	Breast milk			Blood	Breast milk			Blood	Breast milk		
	1st*	1st*	2nd*	3rd*	4th*	4th*	5th*	6th*	4th*	4th*	5th*	6th*
PCDDs	13.7	17.5	15.0	15.2	6.00	13.7	10.4	9.40	1.94	4.99	3.40	4.03
	(47.9)	(51.3)	(46.0)	(47.0)	(46.3)	(47.4)	(54.1)	(38.7)	(43.5)	(50.1)	(43.5)	(47.5)
PCDFs	6.27	8.13	7.86	8.21	3.07	8.48	5.01	9.00	0.800	2.28	1.92	2.32
	(21.9)	(23.8)	(24.1)	(25.4)	(23.7)	(29.5)	(26.1)	(37.1)	(18.0)	(22.9)	(24.5)	(27.4)
Co-PCBs	8.67	8.53	9.75	8.93	3.90	6.66	3.81	5.89	1.71	2.68	2.49	2.13
	(30.2)	(24.9)	(25.4)	(27.6)	(30.0)	(23.1)	(19.8)	(24.2)	(38.5)	(27.0)	(32.0)	(25.1)
Total	28.6	34.2	32.6	32.3	13.0	28.8	19.2	24.3	4.45	9.95	7.81	8.48
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)

Figure in parenthesis shows composition ratio (%) to the total. *: number of month after deliver

2) Daily alteration on pollution levels of Dioxins in breast milk

Breast milk samples were respectively obtained on consecutive 4 days from Mother D in the 9th month after delivery and from Mother E in the 12th month after delivery (Table 2). In Mother D, the total concentration of Dioxins in the breast milk was almost constant at a range of 5.21 to 6.57 pgTEQ/g lipid. However, the composition ratios of PCDDs, PCDFs and Co-PCBs in the total were in a wide range of 25.4 to 36.1%, 14.0 to 20% and 43.6 to 60.6%, respectively. In addition, especially, these ratios of PCDDs and PCDFs were remarkably different from those (2.91% and 36.3%, respectively) of the blood sample.

On the other hand, in the case of Mother E, the total pollution level of breast milk was roughly stable in the range of 2.98 to 4.60 pgTEQ/g lipid. However, compared to the total, the alteration of PCDDs and PCDFs were greater, showing the ratio of maximum versus minimum level to be 1.8 and 2.1, respectively. As well as the case of Mother D, all breast milks gave a quite difference in the composition ratio from the blood. The ratio (31.6–50.1%) of PCDDs in the breast milk was remarkably higher than that (24.8%) in the blood, whereas the ratio (25.9–38.8) of PCDFs in the former was lower than that (51.3%) in the latter.

From above results, it was confirmed that the composition ratio of Dioxins was fairly altered even within 4 days and that the ratio in breast milk was remarkably different from that in the blood. This insists that a part of Dioxins in the breast milk might be brought from meal. Therefore, we studied minutely in the next section.

Table 2. Daily alteration on the level of Dioxins in breast milk from Group 2 mothers.

Compound	Concentration (pgTEQ/g lipid)									
	Mother D					Mother E				
	Blood	Breast milk				Blood	Breast milk			
	1st*	1st*	2nd*	3rd*	4th*	1st*	1st*	2nd*	3rd*	4th*
PCDDs	0.0482	2.27	1.90	2.02	1.61	0.396	2.03	1.10	1.52	1.00
	(2.91)	(34.5)	(36.4)	(31.0)	(25.4)	(24.8)	(47.5)	(37.0)	(50.1)	(31.6)
PCDFs	0.0605	1.25	1.04	1.17	0.886	0.820	1.11	1.14	2.30	1.23
	(36.3)	(19.1)	(20.0)	(18.0)	(14.0)	(51.3)	(25.9)	(38.4)	(26.8)	(38.8)
Co-PCBs	1.01	3.04	2.27	3.32	3.84	0.381	1.13	0.73	1.23	0.941
	(60.8)	(46.4)	(43.6)	(51.0)	(60.6)	(23.9)	(26.6)	(24.5)	(23.1)	(29.6)
Total	1.66	6.57	5.21	6.51	6.33	1.60	4.27	2.98	4.60	3.18
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)

Figure in parenthesis shows composition ratio (%) to the total. *: Experimental day

3) Effect on the concentration and composition of Dioxins in breast milk by a meal

Breast milk, meal and blood samples were obtained from three mothers (Mothers F, G and H) at a period of the 8^{th}, 7^{th} and 7^{th} month after childbirth, respectively. The breast milk was essentially sampled at a rate of once every 3 hours for consecutive 4 days. The meal sample including between-meal snack was 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). The blood was sampled at the time of hungry on the 1^{st} experimental day.

As shown in Fig. 1, in the case of Mother F, there were few ingestion amounts of the dioxin via a meal on the 1^{st} and 2^{nd} experimental days with 7.55 – 21.5 pgTEQ/time zone and 5.63 – 12.3 pgTEQ/time zone, respectively. However, on the 3^{rd} day, the ingestion amount was elevated to 98.7 pgTEQ in the morning and 40.8 pgTEQ in the evening time zones. The composition ratios of Dioxins were 29.2% for PCDDs, 29.0% for PCDFs and 41.9% for Co-PCBs in the morning time zone, and 38.0% for PCDDs, 36.8% for PCDFs and 25.2% for Co-PCBs in the evening time

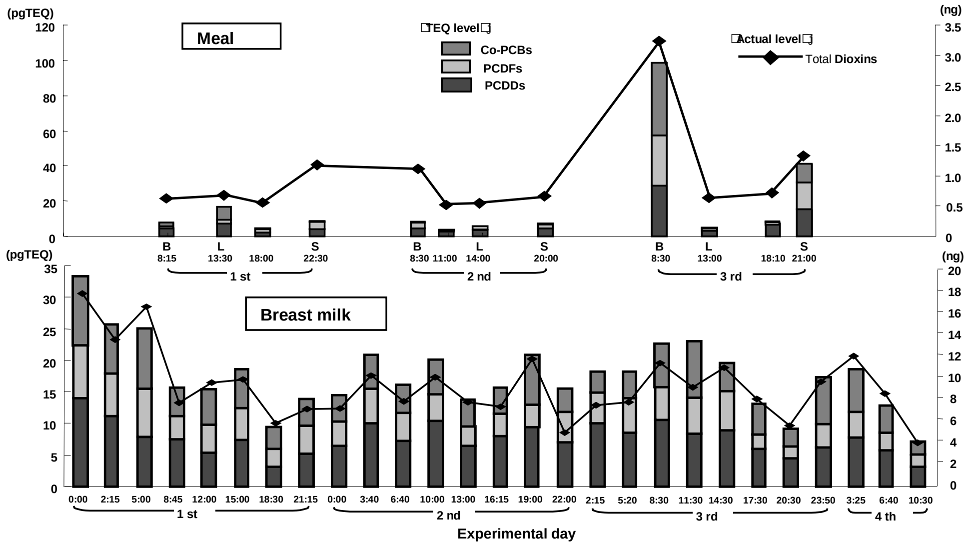

Figure 1. Ingestion amount of Dioxins via a meal and ingestion amount to infant by a suckling's nursing in Mother F.

zones, respectively. These large intakes gave an increase tendency in the ingestion of Dioxins by a suckling's nursing, showing the ingestion amount to increase to 22.7 pgTEQ at 8:30 and 23.0 pgTEQ at 11:30 from 18.2 pgTEQ at 5:20. In addition, compared with meal before, the composition ratio of Co-PCBs was also increased. After meal at 21:00, the by a suckling's nursing in Mother G infant's intake amounts at 23:50 and at 3:25 were elevated compared with 9.15 pgTEQ at 20:30 before the meal.

The same phenomenon was also seen in the case of Mother G (Fig. 2). The intake amount via meal was especially larger in the evening time zone on the 1st and 3rd experimental days, showing to be 70.7 pgTEQ and 59.5 pgTEQ, respectively. The major was Co-PCBs with the composition ratio of 53.0% in the 1st day and 62.2% in the 3rd day. After these meals, the excretion amount of pollutants via breast milk feeding seemed that the tendency which was increasing was accepted. However, the increase excretion was not reflecting the composition of Dioxins via the meal.

As shown in Fig. 3, in the case of Mother H, there were few ingestion amounts of the dioxin via a meal on the 1st and 3rd experimental days with 9.42–9.58 pgTEQ/meal and 6.82–23.2 pgTEQ/meal, respectively. However, on the 2nd day, the ingestion amount was elevated to 28.7 pgTEQ in the morning, 492 pgTEQ in the daytime and 230 pgTEQ in the evening time zones. The composition ratios of Dioxins in the daytime and evening time zones were respectively 21.7% of PCDDs, 26.4% of PCDFs and 51.9% of Co-PCBs, and 16.9% of PCDDs, 18.4% of PCDFs and 64.6% of Co-PCBs. Thus, more than 50% of Dioxins were attributable to Co-PCBs. Mother H ingested larger amount of Dioxins via the lunch at 13:30 and the supper at 20:00 on the 2nd day. In addition, the main component was Co-PCBs. Therefore, we expected that she excretion amount via breast milk increased and that the composition ratio of Co-PCBs increased. However, as shown in Fig. 3, such a phenomenon did not attributable to Co-PCBs. Mother H ingested larger amount of Dioxins via the lunch at 13:30 and the supper at 20:00 on the 2nd day. In addition,

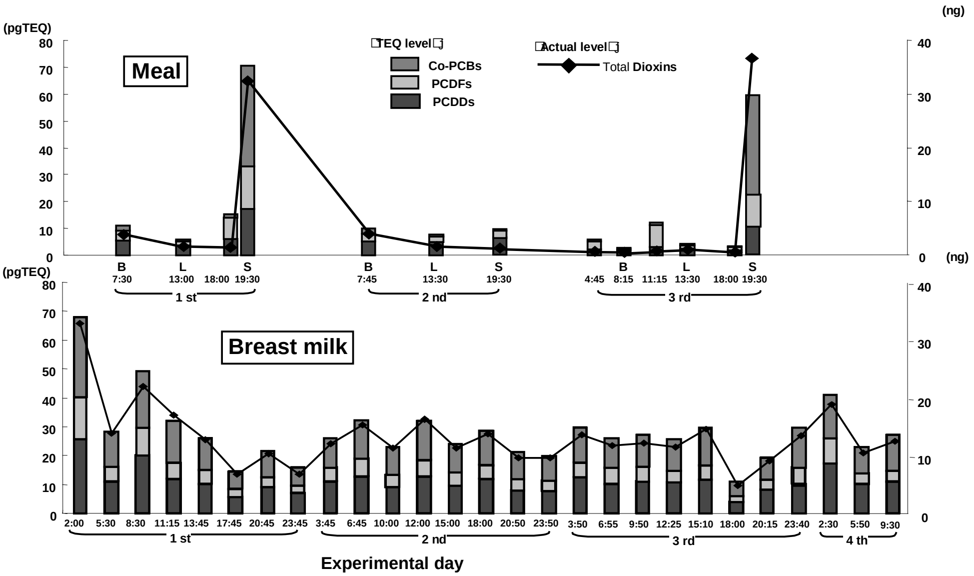

Figure 2. Ingestion amount of Dioxins via a meal and ingestion amount to infant by a suckling's nursing in Mother G.

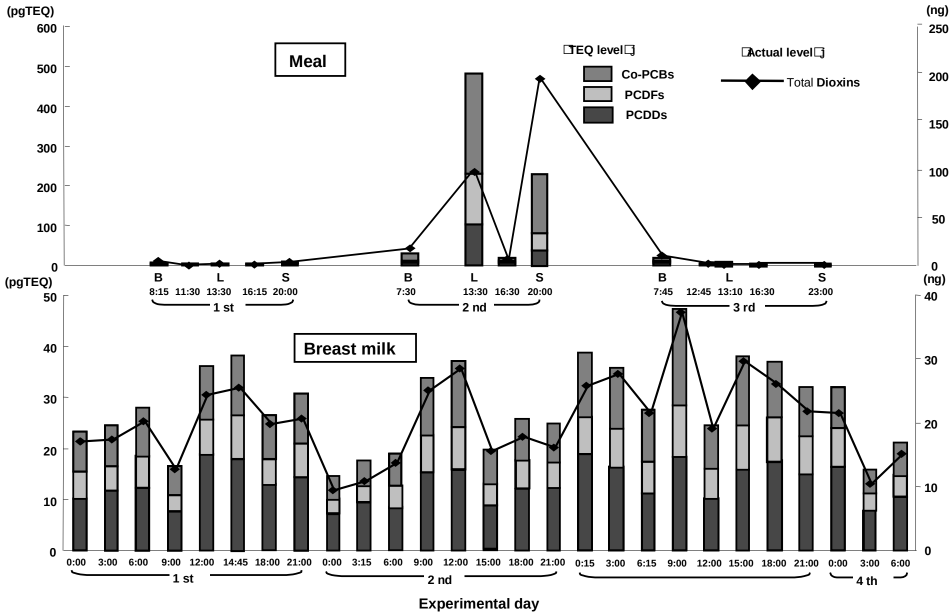

Figure 3. Ingestion amount of Dioxins via a meal and ingestion amount to infant by a suckling's nursing in Mother H.

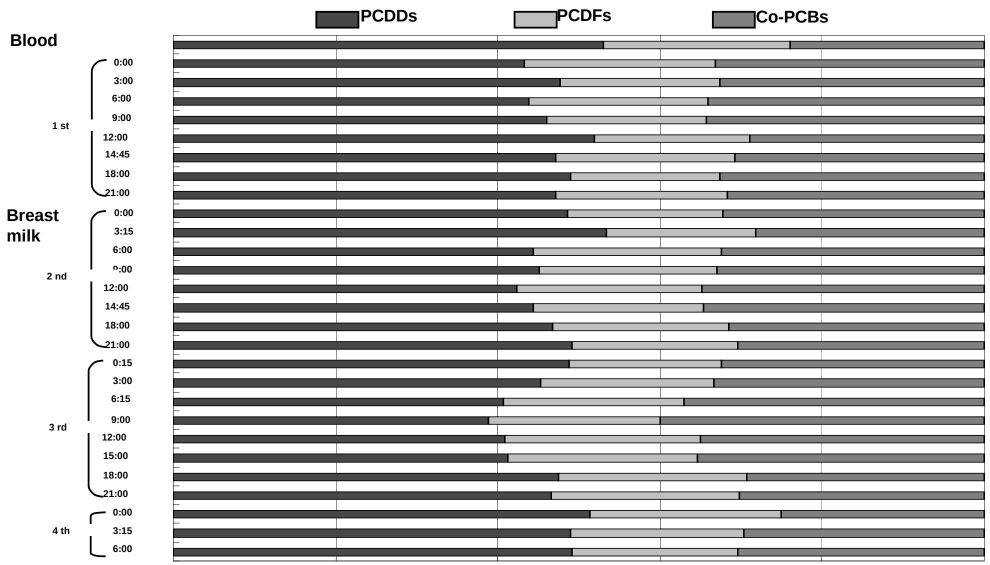

Figure 4. Composition ratios of Dioxins in breast milk and blood in Mother F.

the main component was Co-PCBs. Therefore, we expected that he excretion amount via breast milk increased and that the composition ratio of Co-PCBs increased. However, as shown in Fig. 3, such a phenomenon did not happened. The composition ratios of Dioxins in the blood were 53.0% for PCDDs, 23.0% for PCDFs and 24% for Co-PCBs (Fig. 4). Compared to the blood, the breast milk had smaller composition ratios of PCDDs with 46.3% on the average (range: 38.8 – 53.4%) and PCDFs with 21.4% (18.5–24.1%), and larger composition ratio of Co-PCBs with 32.3% (25.1–39.9%).

From the above results, it became clear that the concentration and composition ratio of Dioxins in the breast milk were partly influenced by a meal. However, those alterations seemed to be large more than the influence of a meal. At present, the cause is unknown. Hereafter, the cause is due to be considered in detail.

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