INVESTIGATION ON MAIN SOURCE OF DIOXIN ANALOGUES IN HUMAN BREAST MILK

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Introduction

In Japan, more than 90% of dioxin analogues including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar PCBs (Co-PCBs) are released through the flue gas from several thousands municipal solid waste and industrial waste incinerators. The total annual emission in the fiscal year of 1998 was estimated to be ca. 3,000 gTEQ. The amount was remarkably larger than those of foreign countries such as Germany, Sweden and Netherlands etc. Consequently, the air pollution level in Japan was more than ten times greater compared to many foreign countries. Therefore, in recent years in Japan, there is a much concern to human exposure level to dioxin analogues.

In many countries, breast milk and blood samples have been used as a suitable indicator in order to examine human exposure level to dioxin analogues. For example, as a representative, there was a report[1)] of "Levels of PCBs, PCDDs, and PCDFs in breast milk" by WHO in 1989, in which a comparison of international human exposure level had been carried out using the data of dioxin analogues in human breast milk samples from various countries. In general, the milk level is considered to be reflecting to accumulation level in the body. In addition, it is well known that ca. 60% of the accumulation amount of dioxin analogues is excreted to the baby through breast milk by nursing for a year. If this is true, some questions are brewing. In 1989, Frust et al.[2)] reported a time course of concentrations of dioxin analogues in breast milk of one German during a period of 1 to 60 weeks after delivery. In the case of PCDFs, the level of 10 to 13 weeks after delivery was remarkably higher than that of 5 weeks. In addition, the PCBs level was also higher on the 10 to 13 weeks than on the 1 week. Thus, the pollution levels of chlorinated pollutants did not always decrease with a passing of time after childbirth. This suggests that all pollutants in breast milk might be not always derived from their storage in the body.

Therefore, in this study, we tried to investigate the main source of pollutants in human breast milk.

Materials and Methods

1) Breast milk samples

Breast milk samples were obtained from nine mothers in 1998 in Shizuoka prefecture, central Japan. The mothers were divided into two groups of Group 1 and Group 2. Group 1 and Group 2 included five persons of No. 1 to No. 5 with an average age of 23.0 years (see Table 2), and six of Nos. 1, 2, 5, 6, 7, 8 and 9 with an average age of 26.6 years (see Table 3), showing Nos. 1 and 2 to belong to both groups. About 100 ml of breast milk samples were separately gained from five individuals of Group 1 on 5 and 30 days, and from six ones of Group 2 on 30, 90 and 90 days after childbirth. On the other hand, the states of growth and daily intake of milk concerning infants were examined in details.

2) Analytical method:

2-1) Fat content

After addition of potassium oxalate (0.25 g), 10 g of sample was shaked with 25 ml of ethanol, 50 ml of diethyl ether and 50 ml of petroleum ether. After separation of the organic phase, the aqueous phase was extracted with 50 ml of diethyl ether/ petroleum ether (1:1). The combination of the first and second extracts was washed twice with 250 ml of 2% saline solution, followed by washing twice with 100 ml of water. After dryness over anhydrous sodium sulfate, the washed extract was concentrated and the rest solvent was completely evaporated. The fat content of milk was calculated on the basis of the gained fat weight.

2-2) Analysis of dioxin analogues

After addition of $^{13}C_{12}$-laveled internal standards of PCDDs and PCDFs, 30 g of sample was decomposed in 42 ml of 0.5 M ethanol solution for 30 min. under shaking at room temperature. After addition of 42 ml of 10% saline solution, the treated solution was extracted twice with 30 ml of hexane, followed by washing twice with 30 ml of water. The extract was concentrated and cleaned up on a multi-layer silica gel column and an alumina column according to our previous method[3)]. The cleaned up extract was analyzed for PCDDs and PCDFs in EI-SIM mode at a resolution of 10,000 using a Hewlett Packard 5890J gas chromatograph-JEOL M700 mass spectrometer. A calculation of 2,3,7,8-TCDD toxicity equivalency quantity (TEQ) of the dioxin analogues in analyzed samples was carried out on the basis of TEFs by WHO[4)].

Results and discussion

In this study, breast milk samples from nine mothers were analyzed for dioxin analogues. All infants from these mothers showed normal growth conditions, indicating their average height and body weight to be almost equal to Japanese average values during a period of 1 to 180 days after birth (Table 1). As shown in Table 2, an average concentration of PCDDs and PCDFs (abbreviated as DDs/DFs) of breast milk in Group 1 decreased 23.0 pgTEQ/g lipid on 5 days to 19.1 pgTEQ/g lipid on 30 days after delivery, whereas averages of milk fat content and daily milk intake by infant increased noticeably 2.89% to 3.5% and 484 ml/day to 744 ml/day, respectively. The daily intake of DDs/DFs by infant was calculated on the bases of these data. The amount was in a range of 69 to 583 pgTEQ/day with the average of 318 pgTEQ/day on 5 days and in a range of 86 to 892 pgTEQ/day with the average of 509 pgTEQ/day on 30 days.

As shown in Table 3, in a case of Group 2, the average level of DDs/DFs in milk had a decline tendency with a time progress after delivery, showing to be 14.5 pgTEQ/g lipid on 30 days, 13.1 on 90 days and 12.2 on 180 days. In the same way of Group 1, the daily intake of DDs/DFs was also computed on 30, 90 and 180 days in Group 2. Consequently, the average amounts were 467 pgTEQ/day on 30 days, 466 on 90 days and 351 on 180 days, respectively.

From results of both groups, it was revealed that the average intake of DDs/DFs roughly constant at the level of 318 pgTEQ/day to 509 pgTEQ/day through 5 to 180 days after delivery. If pollutants in breast milk are all derived from their body storage, their levels in milk must decrease in a linear course with an increase of time passing after puerperal. However, as shown in Table 4, there was a great difference in a time alteration of daily decrease rate of DDs/DFs in breast milk. The decrease rate of 5 to 30 days in Group 1 was in a wide range of –0.070 to 0.334 pgTEQ/day with the average of 0.154 pgTEQ/day. The rate of –0.070 in mother No. 4 means that the concentration of DDs/DFs in milk increased on 30 days than 5 days (see Table 2). The similarity was observed in the cases of Nos. 2 and 9 on 30 to 90 days, and Nos. 2, 6, 7 and 8 on 90 to 180 days in Group 2 (Table 4). In other words, the contamination level of DDs/DFs in breast milk does not always decrease with time passing after delivery. The average decline rates during a period of 5 to 30 days, 30 to 90 days and 90 to 180 days were respectively 0.154, 0.022 and 0.010 pgTEQ/day. The levels of the second (30 to 90 days) and third stages (90 to 180 days) were only a seventh and a fifteenth of the first stage (5 to 30 days). Therefore, it was revealed that the decline rate did not evidently show a linear course. This indicates that most pollutants in milk might be delivered from other sources except for their storage in the body.

Taking all results into consideration, we can conclude that DDs/DFs in the diet are the main sources for breast milk. This is the first discovery in the world. In addition, we also insist that the breast milk is not a suitable sample as an indicator for assessment of human exposure to dioxin analogues.

References

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Table 1. Growth states of tested infants

Age (Day)	Height (cm)				Weight (kg)			
	Tested infants		Japanese infants*		Tested infants		Japanese infants*	
	Male	Female	Male	Female	Male	Female	Male	Female
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
1	49.8 (1.4)	48.9 (1.1)	49.9	49.0	3.25 (0.32)	3.03 (0.07)	3.16	3.05
30	55.0 (1.7)	53.8 (0.8)	54.3	53.2	4.63 (0.48)	4.30 (0.18)	4.39	4.09
60	59.1 (1.6)	57.2 (1.4)	58.4	57.0	5.83 (0.46)	5.10 (0.26)	5.64	5.20
90	61.0 (0.9)	59.0 (1.7)	61.7	60.0	6.46 (0.62)	6.17 (0.33)	6.49	5.98
120	62.8 (0.7)	63.4 (1.3)	64.2	62.5	7.00 (0.80)	6.69 (0.33)	7.11	6.57
150	64.6 (1.8)	63.4 (2.4)	66.3	64.5	7.44 (0.90)	7.17 (0.41)	7.54	7.00
180	68.0 (0.9)	66.1 (1.4)	67.9	66.1	8.09 (1.05)	7.65 (0.58)	7.89	7.33

* : Data published in 1990 by Ministry of Welfare of Japan.

Figures in parentheses show S.D.

Table 2. Concentrations of PCDDs and PCDFs in breast milk of mothers and intake their infants' daily on 5 days and 30 days after deliver in Group 1

Mother		Infant	Con. of DDs/DFs (pgTEQ/g lipid)		Fat content of milk (%)		Intake of milk (ml/day)		Intake of DDs/DFs (pgTEQ/day)	
No.	Age	Sex	5days	30days	5days	30days	5days	30days	5days	30days
1	22	F	22.1	19.6	4.14	3.74	462	823	422	604
2	21	M	19.3	11.0	3.53	5.19	400	910	276	517
3	30	M	40.8	35.3	1.73	3.06	825	825	583	892
4	31	F	21.2	22.9	2.21	2.76	518	705	243	446
5	26	F	11.6	6.8	2.80	2.76	214	455	69	86
Av.	23.0		23.0	19.1	2.89	3.50	484	744	318	509
S.D	10.8		10.8	11.1	0.70	1.02	222	172	185	286

Table 3. Concentrations of PCDDs and PCDFs in breast milk of mothers and their infants' daily intake on 30, 90 and 180 days after deliver in Group 2

Mother		Infant	Conc. of DDs/DFs (pgTEQ/g lipid)			Daily intake of milk (ml/days)			Daily intake of DDs/DFs (pgTEQ/day)		
No.	Age	Sex	30 days	90 days	180 days	30 days	90 days	180 days	30 days	90 days	180 days
1	22	F	19.6	16.9	8.7	823	875	845	604	610	303
2	21	M	11.0	11.1	11.4	910	735	750	517	377	304
6	26	F	15.5	11.9	14.2	905	808	705	420	424	350
7	30	M	15.0	13.6	14.8	651	700	810	224	543	60
8	26	M	15.8	13.6	14.0	820	645	780	764	446	677
9	30	M	9.9	11.8	10.2	525	655	754	271	393	408
Av.	26.6		14.5	13.1	12.2	772	736	774	467	466	351
S.D	2.1		2.5	1.1	1.8	151	60	35	193	60	196

Table 4. Time alteration of daily decrease rate of dioxin analogues Group w in breast milk in Group 1 and after delivery

Mother	Daily decrease rate (pgTEQ/day)		
	Group 1	Group 2	Group 2
NO.	From 5 to 30 days	From 30 to 90 days	From 90 to 180 days
1	0.096	0.0466	0.0910
2	0.334	-0.0021	-0.0032
3	0.220		
4	-0.070		
5	0.190		
6		0.0592	-0.0252
7		0.0223	-0.0129
8		0.0372	-0.0049
9		-0.0303	0.0173
Av.	0.154	0.022	0.010
S.D	0.151	0.033	0.042