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Dioxins and Fatty Acids in Breast Milk of Primiparas in Yonago District, Tottori Prefecture, Japan

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We analyzed the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in breast milk collected 1 week after childbirth from 8 primiparas in Yonago district, Tottori Prefecture, and investigated the relationship between PCDDs or PCDFs and fatty acids in concentration, and the relationship between dioxin concentration and kind of daily foods. The mean total content of dioxins (PCDDs and PCDFs) was 0.48 pg-toxic equivalent (TEQ)/g (range 0.12–1.04 pg-TEQ/g) in breast milk, and 16.7 pg-TEQ/g-fat (range 9.6–32.7 pg-TEQ/g-fat) in total lipids of breast milk. The 8 primiparas showed a low mean dioxin concentration: the levels were lower in 6 of them and higher in 2 of them than in primiparas living in other cities in Japan. For 1 of the 2 mothers, the reason for the high level was thought to be her poor intake of vegetables in the diet. The total dioxin content was well correlated with the total lipid content ranging from 1% to 3%. Fatty acids with $C_{16:0}$ and $C_{18:1}$ dominated those with $C_{12:0}$, $C_{14:0}$, $C_{16:1}$, $C_{18:2}$ and $C_{18:0}$, which were commonly detected. The amount of fatty acids with $C_{10:0}$, $C_{20:1}$, $C_{20:2}$, $C_{20:3}$, $C_{20:4}$, $C_{22:5}$ and $C_{22:6}$ was small. Gas chromatograms of these fatty acids generally showed similar distributions in breast milk of the 8 primiparas. The contents of fatty acids ranged from 17.1 to 31.3 mg/g (average 24.0 mg/g) in bulk breast milk. No clear correlation was found in concentration between PCDDs or PCDFs and specific fatty acids in breast milk.

Key words: breast milk; fatty acid; food life; lipid; polychlorinated dibenzo-*p*-dioxin; polychlorinated dibenzofuran; primipara

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), which are reported as "endocrine disruptors", have a severe toxicity which acts as internal secretions causing sexual disturbances (Schuler et al., 1997; Kayama, 1998; Matsuo, 1998). The presence of dioxins (PCDDs and PCDFs) in breast milk has increased the advocation of women's and children's health (Sakai, 1998; Watanabe et al., 1999; LaKind et al., 2001). In recent years, dioxins in breast milk have been detected many times higher than the normal concentration in breast milk, and the influence on babies and infants is a serious problem (Ministry of Health and Welfare, 1998). We wanted to clarify the qualitative and quantitative relationship between the concentrations of dioxins and fatty acids, and the habitual intake of daily food. The exposure to dioxins has been decreasing as shown by a downward trend of

Abbreviations: GC, gas chromatography; MS, mass spectrometry, PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; TEF, toxic equivalency factor; TEQ, toxic equivalent

Table 1. Primiparas' data, dietary life and living environments

Sample number	Age (year)	Height (cm)	Weight (kg)	Status of milk secretion	Lipid content in breast milk (%)	Meal*			Residence
						Vegetable	Meet	Fish	
1	30	155	60.0	Good	2.49	–	2+	2+	Yonago city
2	25	157	53.5	Normal	2.32	2+	2+	2+	Yonago city
3	27	160	71.3	Good	1.25	2+	2+	2+	Yonago city
4	31	158	61.0	Good	3.03	2+	2+	2+	Saihaku-county
5	33	161	56.2	Not so good	3.26	3+	2+	+	Yonago city
6	27	150	63.0	Normal	3.54	2+	2+	2+	Tohaku-county
7	27	152	64.5	Good	4.31	2+	3+	2+	Saihaku-county
8	30	150	59.8	Very good	2.47	3+	2+	2+	Tohaku-county

* 3+, often; 2+, common; +, rare; –, little.

dioxins both in human breast milk and serum lipids during the last 20 to 30 years (Kiviranta et al., 1999; Ministry of Health, Labour and Welfare of Japan, 2000; Przyrembel et al., 2000); however, the level of dioxin content has not been made clear in smaller cities, which we think should be made evident. In the San-in region of Japan, except for Shimane Prefecture, breast milk has still not been analyzed for dioxins. Little information is available for allowing a good assessment of breast milk in mothers especially in Tottori Prefecture. A solution to the actual situation in Yonago district has long been awaited in order to help expecting mothers who visit in Tottori University Hospital learn more about breastfeeding.

For this study, we analyzed and estimated the contents of PCDDs, PCDFs and lipids in breast milk of primiparas (mothers of firstborn children) who live in Yonago district, and discussed the quantitative relationships among them.

Materials and Methods

Breast milk samples

Breast milk was obtained from 8 primiparas in good health who were hospitalized at Tottori University Hospital and had a normal delivery. Samples were collected after suckling the baby twice a day between day 3 and day 7. The collected quantity was 10 to 20 mL each, totaling approximately 100 mL. The specimen was then stored in a refrigerator at –80˚C until analysis.

Age and occupation of the primiparas were not fixed and control of meals was not performed. However, they ate hospital food during their hospitalization. Table 1 shows the conditions of the primiparas and other information.

The 8 primiparas well understood the purpose of the study as we explained it to them, and their consent to publish the data was obtained.

GC/MS conditions for dioxin analysis

A high-resolution gas chromatograph/high-resolution mass spectrometer (double-focusing type) is normally used for dioxin analysis. Gas chromatography/mass spectrometry (GC/MS) operating conditions recommended for dioxin analysis by the guideline of the Ministry of Health, Labour and Welfare of Japan are i) the resolution must be above 10,000 and ii) every m/z set up must be less than 4 decimal points (Ministry of Health and Welfare of Japan, 1997; Environment Agency of Japan Dioxin Risk Evaluation Committee, 1998a). The analysis of dioxins was done at the Hiroshima Environment and Health Association.

Table 2. Concentration of PCDDs and PCDFs in breast milk

Sample number	PCDD/total milk*		PCDF/total milk*		PCDD/total lipids†		PCDF/total lipids†	
	(pg/g)	(pg-TEQ/g)	(pg/g)	(pg-TEQ/g)	(pg/g-fat)	(pg-TEQ/g-fat)	(pg/g-fat)	(pg-TEQ/g-fat)
1	9.40	0.68	0.30	0.15	380	26.7	0	6.0
2	3.40	0.17	0.10	0.05	150	7.60	4	2.0
3	2.50	0.12	0.00	0.00	200	10.1	0	0.0
4	2.50	0.18	0.20	0.10	83	6.60	7	3.5
5	11.0	0.73	1.10	0.31	330	22.7	32	9.6
6	3.00	0.28	0.22	0.11	86	7.60	6	3.0
7	6.10	0.43	0.38	0.13	140	9.30	9	3.3
8	5.50	0.27	0.24	0.12	230	10.9	10	5.0

PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; TEQ, toxic equivalent.
*Total breast milk.
†Total lipids of breast milk.

GC and GC/MS conditions for fatty acid analysis

The analysis of fatty acids was performed at the Tottori University Faculty of Medicine Mass Spectrometry Laboratory. Breast milk was methylesterized according to the method of Yamamoto (1962). Quantitative analysis was performed by a Hitachi G-5000 gas chromatograph. A JMS-DX 303 (JEOL, Tokyo, Japan) and a GC 5890 (Hewlett Packard, Avondale, PA) were used for qualitative analysis by GS/MS.

GC conditions for the analyses were as follows: Carrier gas was helium (35 mL/min). The initial temperature was held at 150˚C for 2 min, and then raised at a ratio of 5˚C/min to 270˚C and finally retained at 270˚C for 15 min. The temperature of the injector port was 250˚C, and detector, 300˚C. A capillary column DB–5 (J & W Scientific Co., Folsom, CA) (weak polarity; internal diameter 0.25 mm; film 0.25 μm; length 30 m) for GC fatty acid analysis was employed.

Mass spectra were measured by positive electron impact ionization at 70 eV and 300 μA. The capillary column from the CBP 1 (J & W Scientific Co.) (internal diameter 0.25 mm; length 25 m) was employed for GC/MS use. The fatty acid concentration was standardized by heptadecanoic acid ($C_{17:0}$) as the standard by an area ratio.

Separation before GC and GC/MS analyses

The extraction of dioxin types was performed based on guidelines set by the Environment Agency of Japan Dioxin Risk Evaluation Committee (1998a). The typical process has usually followed the guideline of the Environment Agency of Japan. Separation, extraction and methylesterification of fatty acids were based on the modification of the method of Yamamoto (1962) and Maeda et al. (1996).

Results

Dioxin content in breast milk

Tables 2 and 3 show the PCDD and PCDF contents in breast milk samples obtained from the 8 primiparas. The concentration of dioxin is reported by a quantity, that is, toxic equivalent (TEQ) of total dioxins (PCDDs + PCDFs). The TEQ is the sum of every chemical isomers. The toxicity of dioxin differs for every chemical isomer. Because the toxicity of 2,3,7,8-4 chloridation is the strongest among isomers, that of the other isomers is expressed by toxic equivalency factor (TEF), as relative toxicity for 2,3,7,8-4 chloridation (TEF = 1) (Miyata, 1998a). We calculated total dioxins as the sum of PCDDs and PCDFs in pg-TEQ, to exclude coplanar-polychlorinated biphenyl.

Table 3. Concentration and TEQ of total dioxins (PCDDs + PCDFs)

Sample number	PCDDs + PCDFs/total breast milk (pg/g)	(pg-TEQ/g)	PCDDs + PCDFs/total lipids of breast milk (pg/g-fat)	(pg-TEQ/g-fat)
1	9.7	0.83	380	32.7
2	3.5	0.22	154	9.6
3	2.5	0.12	200	10.1
4	2.7	0.28	90	10.1
5	12.1	1.04	362	32.3
6	3.2	0.39	92	10.6
7	6.5	0.56	149	12.6
8	5.7	0.39	240	15.9

PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; TEQ, toxic equivalent.

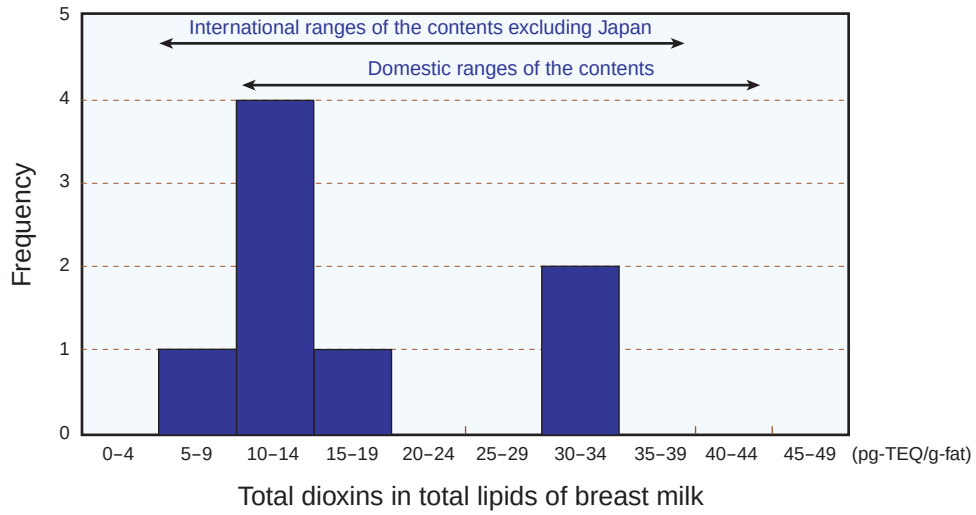

Fig. 1. Histogram of toxic equivalents (TEQ) for total dioxins (PCDDs + PCDFs) in the total lipids of breast milk.

Table 2 shows the PCDD content in total breast milk ranging from 0.12 to 0.73 pg-TEQ/g and the PCDF content ranging from 0 to 0.31 pg-TEQ/g. The total dioxin (PCDDs + PCDFs) content in total breast milk ranged from 0.12 to 1.04 pg-TEQ/g (average 0.48 pg-TEQ/g) (Table 3).

On the other hand, the PCDD content in the total lipid amount of breast milk ranged from 6.6 to 26.7 pg-TEQ/g-fat and the PCDF content, from 0 to 9.6 pg-TEQ/g-fat (Table 2). The total dioxin (PCDDs + PCDFs) content in the total lipid of breast milk ranged from 9.6 to 32.7 pg-TEQ/g-fat (average 16.7 pg-TEQ/g-fat). Figure 1 shows a histogram of the total dioxin content in the total lipids of breast milk. Primiparas 1 and 5 had abnormally high levels at 32.3 and 32.7 pg-TEQ/g-fat, but the other 6 primiparas had lower levels, 9.6 to 15.9 pg-TEQ/g-fat.

Their PCDD/PCDF ratio was about 2 times higher than that (42/30 = 1.4) analyzed from food intake in an adult population in the United States (Schecter et al., 2001).

Relationship between the dioxin and lipid contents

Figure 2 shows the relationship of the dioxin content in TEQ to the lipid content in breast milk. The lipid content was 1.25% to 4.31% in breast milk (Table 1). Exclusive of primiparas 1 and 5, the dioxin content in TEQ showed a high correlation ($r^2 = 0.819$) to the lipid content. Correlations were also high between TEQ-based contents of PCDDs and PCDFs in total breast milk and in total lipids of breast milk (Fig. 3).

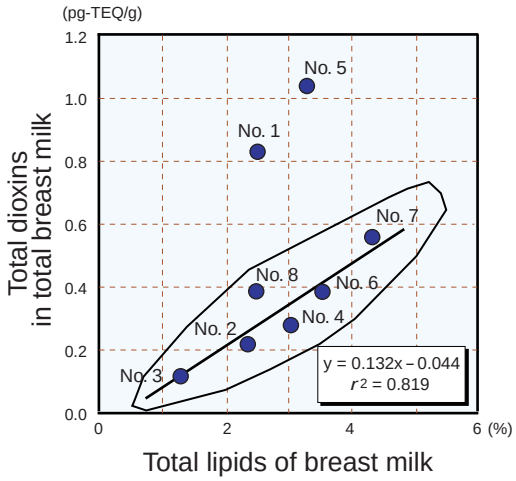

Fig. 2. Relationship between the total lipid and dioxin contents in breast milk. TEQ, toxic equivalent.

Fatty acid composition of breast milk

The representative GC tracings of isolated fatty acids and internal standard fatty acid are shown in Fig. 4 (methylesters). Identification of each fatty acid methylester was from a fragment pattern in the mass spectrum (Fig. 5). The most concentrated fatty acids in breast milk of the 8 primiparas were $C_{16:0}$ and $C_{18:1}$. The commonest fatty acids were $C_{12:0}$, $C_{14:0}$, $C_{16:1}$, $C_{18:2}$ and $C_{18:0}$. The fatty acids in trace amounts were $C_{10:0}$, $C_{20:1}$, $C_{20:2}$, $C_{20:3}$, $C_{20:4}$, $C_{22:5}$ and $C_{22:6}$. GC showed a similar tendency in the distribution patterns of these fatty acids throughout the 8 primiparas.

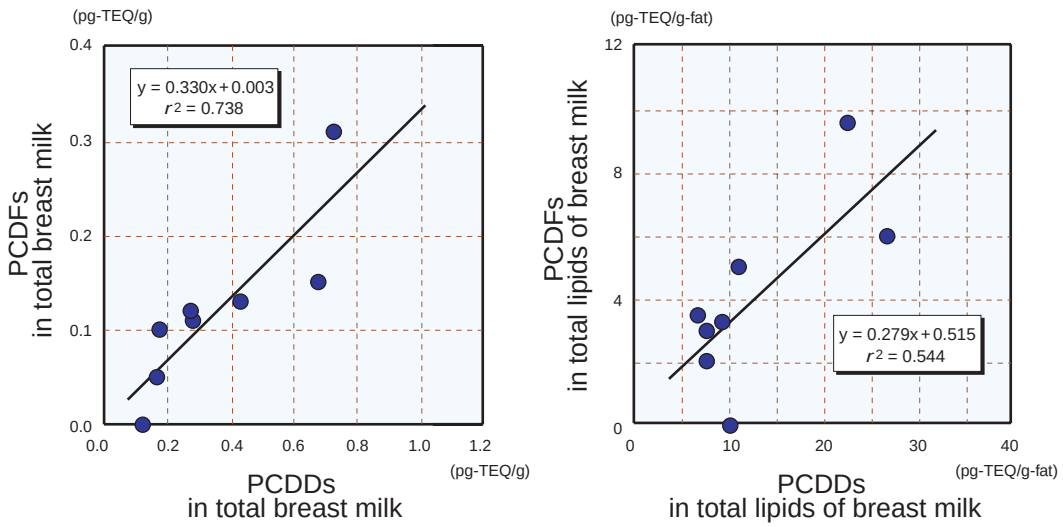

Fig. 3. Relationship between the PCDD and PCDF contents in total breast milk of 8 primiparas (***left***) and in total lipids of breast milk (***right***). PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; TEQ, toxic equivalent.

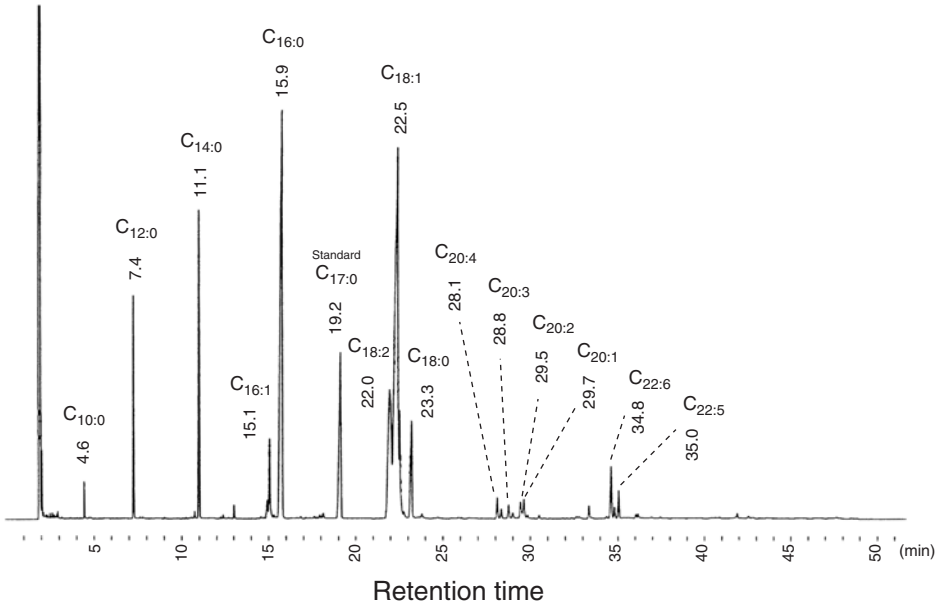

Fig. 4. Gas chromatogram of methylester of fatty acids from primipara 3.

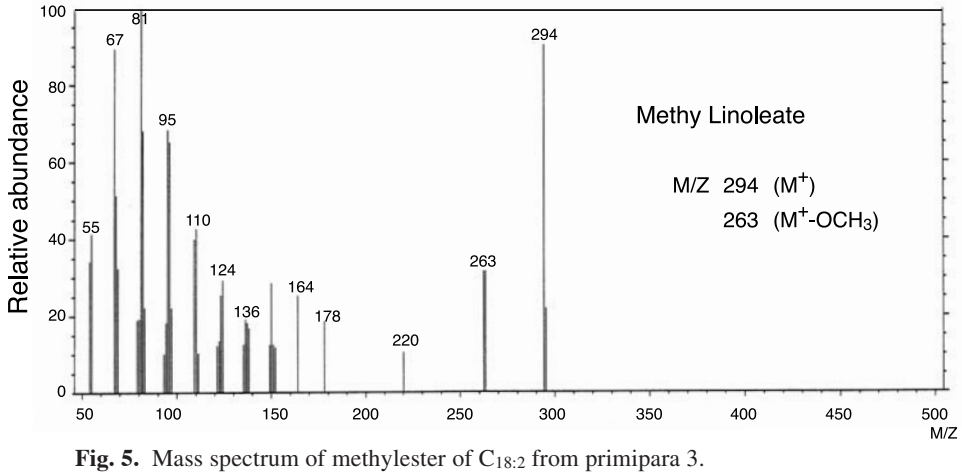

Fig. 5. Mass spectrum of methylester of $C_{18:2}$ from primipara 3.

Discussion

Factors controlling the dioxin content in breast milk

We measured the level of dioxins in breast milk of 8 primiparas in Yonago district. Only the breast milk of primiparas was used as samples, because PCDDs and PCDFs in the breast milk of primiparas are at significantly higher levels of concentration than in multiparas (Vartiainen, 1997; Iida et al., 1999). The total dioxin content we measured in total lipids of breast milk ranged from 9.6 to 32.7 pg-TEQ/g-fat (average 16.7 pg-TEQ/g-fat). The ranges of dioxin content reported in Europe and Asia were from 6 to 40 pg-TEQ/g-fat (average 21 pg-TEQ/g-fat) (Environment Agency of Japan Dioxin Risk Evaluation committee, 1998b). Domestic research

Table 4. Fatty acid compositions in breast milk 1 week after childbirth

Peak number	Retention time (min)	Sample number 2 (mg/g)	3 (mg/g)	4 (mg/g)	5 (mg/g)	6 (mg/g)	7 (mg/g)	8 (mg/g)
A ($C_{10:0}$)	4.6	0.38	0.16	0.55	0.63	1.10	0.67	0.25
1 ($C_{12:0}$)	7.4	2.71	1.29	3.71	2.96	5.29	3.28	1.76
2 ($C_{14:0}$)	11.1	4.75	2.47	6.17	3.45	6.70	4.41	3.27
3 ($C_{16:1}$)	15.1	1.64	0.92	2.93	1.41	2.95	2.91	1.82
4 ($C_{16:0}$)	15.9	11.65	7.60	18.35	11.66	17.48	19.47	12.25
6 (unknown)	18.4	0.38	0.08	0.21	0.13	0.25	0.28	0.14
7 ($C_{17:0}$)*	19.2	3.00	3.00	3.00	3.00	3.00	3.00	3.00
8 ($C_{18:2}$)	22.0	3.98	4.15	6.52	4.14	9.03	11.66	3.87
9 ($C_{18:1}$)	22.5	12.58	10.85	14.30	13.86	19.75	21.97	17.83
10 ($C_{18:0}$)	23.3	3.02	1.58	3.25	3.73	3.45	4.03	3.14
11 ($C_{20:4}$)	28.1	0.25	0.24	0.49	0.34	0.48	0.59	0.37
12 ($C_{20:3}$)	28.8	0.22	0.17	0.47	0.22	0.36	0.45	0.24
13 ($C_{20:2}$)	29.5	0.28	0.26	0.44	0.27	0.56	0.61	0.56
14 ($C_{20:1}$)	29.7	0.40	0.28	0.53	0.36	0.61	0.75	0.61
15 (unknown)	34.6	0.24	0.17	0.20	0.13	0.15	0.10	0.13
16 ($C_{22:6}$)	34.8	0.11	0.63	1.12	0.78	0.88	0.72	0.80
17 ($C_{22:5}$)	35.0	0.14	0.34	0.40	0.23	0.31	0.33	0.42
Total		45.74	34.18	62.63	47.30	72.34	74.56	50.47

* Standard.

has reported levels of 10.9 to 44.6 pg-TEQ/g-fat (average 23.9 pg-TEQ/g-fat) in total lipids of breast milk (Akita 10.9; Sendai 11.4; Niigata 18.3; Saitama 22.8; Hamamatsu 28.1; Osaka 44.6; Nara 40.5; Fukuoka 15.0; Okinawa 23.6 pg-TEQ/g-fat) (Miyata, 1998b; Saitama Prefecture Health and Welfare Division, 1998). The mean dioxin level we found in Yonago district was somewhat lower than these levels.

The total dioxin concentrations of the 8 primiparas were abnormally higher in 2 mothers (32.3 and 32.7 pg-TEQ/g-fat) than in the 6 others (9.6–15.9 pg-TEQ/g-fat). This does not seem to be due to their body size (height and weight in Table 1) or breast milk secreting states, but to differences in dietary habits. Table 1 shows daily food data obtained from the 8 primiparas during personal interviews. For primipara 1 with a high dioxin content in breast milk, her vegetable intake was extremely low, while the other primiparas ate a good amount of vegetables every day. This primipara normally ate fish and meat frequently, about 2 to 7 times a week.

According to the Fukuoka Prefecture Health Environment Research Laboratory (Miyata, 1998a), food fiber including cellulose absorbs dioxins in the small intestine, which are durable in the excretion of feces. Consequently, the reason why the dioxin content was high in primipara 1 might have been because of her low vegetable intake, although the number of breast milk samples analyzed may not have been necessarily adequate.

The 2nd-highest content of dioxins was in primipara 5 which requires another explanation. Although she had a high intake of vegetables, she had lived near a garbage incinerator in Osaka for a long time. Considering that approximately 80% of dioxins comes from trash burning facilities (Miyata, 1998a), the primary factor of her high content may be because of her residential environment. To uncover this as a possible cause, it should be further examined whether the primipara was a first born child or not, or how old she was when she delivered her first child.

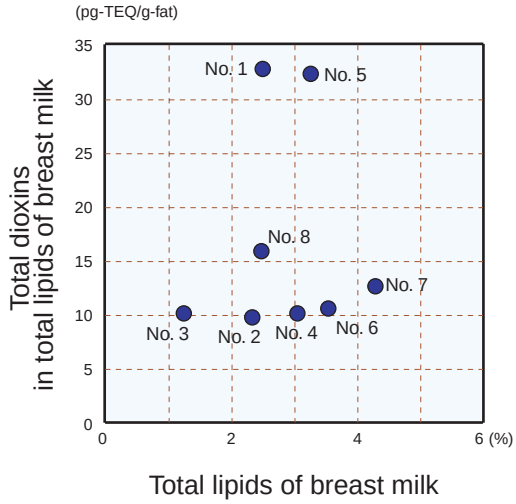

Fig. 6. Relationship between the lipid content in breast milk and the total dioxin content in total lipids of breast milk. TEQ, toxic equivalent.

The effects of smoking on the concentration of dioxins are not discussed in the present paper.

Relationship between the dioxin and lipid/ fatty acid contents

The GC patterns of distribution of common fatty acids were similar through out the 8 primiparas, as shown in Fig. 4. These results indicate that there was no clear relationship between the dioxin content and fatty acid composition. The most concentrated fatty acids were $C_{16:0}$ and $C_{18:1}$, in accordance with previous reports (Yamamoto, 1962; Maeda et al., 1996). The concentration of all fatty acids in breast milk ranged from 34.2 to 74.6 mg/g (average 55.3 mg/g) 1 week after childbirth (Table 4), and from 41.8 to 89.2 mg/g (average 69.7 mg/g) 1 month after childbirth (data not shown). The total lipid content in breast milk 1 month after was 2.4 times (average 1.43 times) higher than that 1 week after (Table 4). Fatty acid $C_{18:2}$ commonly increased 1 month after. $C_{18:2}$ is an important fatty acid for controlling the concentration of cholesterol in the plasma of the newborn (Potter and Nestel, 1976).

Our results suggest that the dioxin content in breast milk is not related to a specific fatty acid. However, a probable correlation was indicated between the dioxin and total lipid contents in breast milk, as shown in Fig. 2. PCDDs and PCDFs are dispersed equally among the lipid compartments of breast milk (Przyrembel et al., 2000). A higher lipid content (2–7%), on the contrary, is negatively correlated with the dioxin content in total lipids of breast milk (Saitama Prefecture Health Welfare Division, 1998). This could be due to a diluting effect of dioxins by lipids. However, our results did not show such a diluting effect, and we proved constant TEQ-based levels except for the 2 abnormally dioxin-abundant breast milk specimens of primiparas 1 and 5 (Fig. 6). In these cases, we must take notice that the total amount of dioxins in breast milk increased as the lipid content increased.

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