CONCENTRATIONS OF DIOXINS IN SETS OF PLACENTAL TISSUE, MATERNAL BLOOD AND UMBILICAL CORD BLOOD SAMPLES COLLECTED FROM JAPANESE PREGNANT WOMEN

Hori T[1], Takahashi K[1], Yasutake D[1], Morokuma S[2], Otera Y[2], Todaka T[2], Yamamoto T[3], Hirakawa H[1], Kajiwara J[1], Kawamoto T[4], Hirata T[1], Tsukimori K[5]

[1]Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano Dazaifu-shi, Fukuoka, Japan;
[2]Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka, Japan;
[3]Kitakyushu Life Science Center, 1-4 Nakabaru shinmachi Tobata-ku, Kitakyushu, Japan.
[4]University of Occupational and Environmental Health, 1-1 Iseigaoka Yahatanishi-Ku, Kitakyushu, Japan;
[5]Fukuoka Children's Hospital and Medical Center for Infectious Diseases, 2-5-1 Tojinmachi Chuo-ku, Fukuoka, Japan.

Introduction

The nationwide investigation of the mean dietary intake of dioxins started in 1998 using the total diet study method. As part of the 2009 investigation, nine sets of total diet study samples were prepared in seven different regions in Japan. Based on that investigation, the average intake was estimated to be 0.84 pg-TEQ/kg b.w./day, with a range of 0.28 – 1.49 pg-TEQ/kg b.w./day. The recent Japanese dietary intake of dioxins is estimated to be below the TDI, 4 pg-TEQ/kg b.w./day, and the estimated value has been gradually decreasing for the last decade[1),2)]. Therefore, the health risk caused by dioxins exposure from food is considered to be not so serious for Japanese adults at present, but there is little information regarding the risk during growing periods of small infants and fetuses. Dioxins that have accumulated in the maternal body as a result of long-term exposure to the chemicals can be transferred to the fetus via the placenta or to nursing infants via breast milk, potentially causing developmental health problems in children. In this study, we determined dioxin concentrations in human biological tissue samples collected to assess pediatric health risks from exposure of mothers and children to dioxins.

Materials and methods

We began recruiting pregnant women at Kyushu University Hospital in October 2009 to collect sets of tissue samples, that is, placenta, amniotic fluid, maternal blood, umbilical cord blood, umbilical cord, maternal fat, meconium, and other biological tissues. To date, we have accumulated 218 individual samples from 29 donors, 19 of whom had normal pregnancy deliveries and 10 of whom had fetal growth restriction (FGR) deliveries. All samples were frozen below －20 °C in glass containers until the chemical analysis. Among these collected samples, we decided to analyze three tissues first, that is, placenta, maternal blood, and umbilical cord blood, from the viewpoint of maternal-fetus transfer of dioxins.

Our analytical method of dioxins in biological samples consists of both efficient extraction using an accelerated solvent extractor instead of the usual liquid-liquid extraction and sensitive detection using HRGC/HRMS equipped with a large-volume

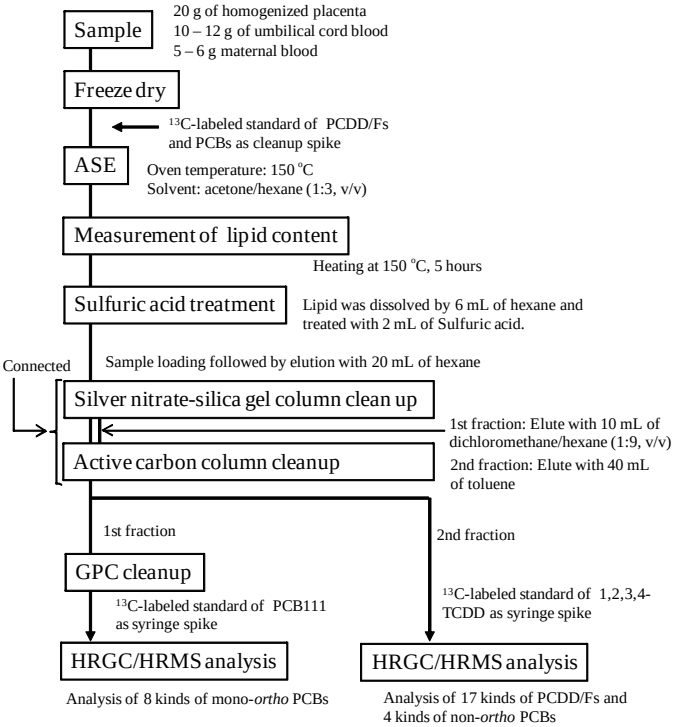

Fig.1 Flow chart of analysis of dioxins in biological samples

injection system, as shown in Fig. 1. Automated extraction was performed using an ASE-350 (Dionex) under the conditions of 150 °C, 1500 psi. Before lipid extraction, weighed biological samples were dehydrated in an AdVantage freeze-dryer (VirTis). As a result, homogeneous lipid solution was obtained without any emulsion formations, which often appear with the usual shaking extraction using saturated ammonium sulfate and ethanol/hexane. Dioxins were analyzed using a model 7890A gas chromatograph (Agilent Technologies) coupled to a model Autospec-Premier mass spectrometer (Waters). We employed a VF-5ms (0.25 mm x 30 m) capillary column (Agilent Technologies) on an LVI-S200 high-volume injection system (AiSTI SCIENCE) to determine PCDD/Fs and non-*ortho* PCBs. Detection limit values on a lipid weight basis were as follows: PCDD/Fs, 0.3-2 pg/g lipid for placenta and umbilical cord blood, 1-4 pg/g lipid for maternal blood; non-*ortho* PCBs, 0.3-0.6 pg/g lipid for placenta, 0.3-1 pg/g lipid for umbilical cord blood, 10 pg/g lipid for maternal blood. All TEQ values were calculated using WHO-TEF (2005), and concentration values below the detection limit were taken to be zero. Determination of 8 kinds of mono-*ortho* PCBs is underway for the entire collection of sets of samples, so these congeners were excluded from TEQ evaluations for the present.

Results and discussion

The lipid weight-based dioxin concentration was determined in 15 placenta tissue samples from normal pregnancy deliveries and 3 placenta samples from FGR deliveries. The total dioxin concentration calculated as the sum of 21 compounds was 5.2-36 pg-TEQ/g lipid with a mean of 13 pg-TEQ/g lipid in the former subjects, and 7.4-10 pg-TEQ/g lipid with a mean of 8.4 pg-TEQ/g lipid in the latter subjects. These values were slightly lower than those from another study on Japanese pregnant woman[3]. Our determined values were similar to those caused by exposure in a normal life environment, and there were no concentrated values as originated in specific environment (occupational exposure, etc.). The total TEQ concentrations in placenta increased as the maternal age increased, and decreased in relation to their parity; those from three donors of first delivery were 17-36 pg-TEQ/g lipid with a mean of 23 pg-TEQ/g lipid, while those from two donors of fourth delivery were 5.2 and 12 pg-TEQ/g lipid with a mean of 8.6 pg-TEQ/g lipid (Fig.2), respectively. It was suggested that delivery experience including breast-feeding was a significant means of elimination of dioxins from the maternal body.

Sets of total TEQ concentrations on a lipid weight basis in the placenta, maternal blood, and umbilical cord blood from 13 normal pregnancies were also determined. As shown in Table 1, the total dioxin concentration in maternal blood samples was 6.6-44 pg-TEQ/g lipid with a mean of 17 pg-TEQ/g lipid, while total dioxin concentration was 1.9-18 pg-TEQ/g lipid with a mean of 6.2 pg-TEQ/g lipid in umbilical cord blood samples. The mean total TEQ concentration in umbilical cord blood was about 60% lower than that in maternal blood samples, and this tendency was in agreement with that reported in other studies[4), 5)].

As shown in Fig. 3, there was a positive linear relationship between total TEQ concentrations in placenta and

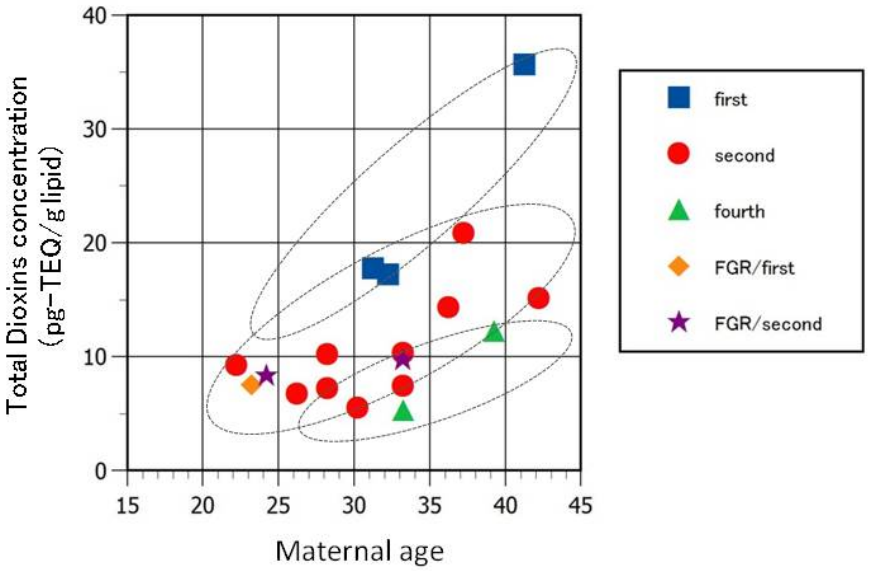

Fig.2 Relationship between total TEQ concentrations in placenta and maternal age at delivery

Table 1 Concentrations of dioxins in 13 sets of placenta, maternal blood and umbilical cord blood samples

Congener	LOD (pg/g lipid)	No.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
2,3,7,8-TCDD	0.3	0.93	1.7	0.91	0.60	0.42	1.2	0.32	0.40	0.69	0.42	0.66	1.0	2.2
1,2,3,7,8-PeCDD	0.3	8.3	11	6.9	3.8	3.8	11	3.0	2.8	5.6	4.0	5.7	8.0	20
1,2,3,4,7,8-HxCDD	0.7	1.4	2.1	1.5	ND	0.71	1.8	0.79	ND	1.0	0.76	1.2	2.0	3.7
1,2,3,6,7,8-HxCDD	0.7	3.2	4.4	2.6	1.1	1.0	3.0	1.2	1.3	2.7	3.7	2.0	6.9	11
1,2,3,7,8,9-HxCDD	0.7	ND	0.82	ND	ND	ND	0.9	ND	ND	ND	ND	ND	0.94	2.5
1,2,3,4,6,7,8-HpCDD	0.7	3.9	4.7	3.6	25	2.1	4.4	2.2	2.4	2.1	2.5	1.8	5.6	10
OCDD	2.0	68	30	60	48	43	43	38	31	39	76	34	94	110
2,3,7,8-TCDF	0.3	1.8	0.44	0.72	0.55	0.59	0.85	1.1	0.71	0.79	0.41	0.75	0.90	0.91
1,2,3,7,8-PeCDF	0.3	0.58	0.31	0.34	ND	ND	0.60	ND	0.36	ND	ND	0.40	ND	0.60
2,3,4,7,8-PeCDF	0.3	9.9	16	8.4	5.7	4.9	12	4.4	3.8	8.6	5.9	8.8	11	27
1,2,3,4,7,8-HxCDF	0.7	1.9	2.5	1.6	1.0	1.4	2.9	1.0	1.2	1.8	1.9	1.4	2.6	7.1
1,2,3,6,7,8-HxCDF	0.7	1.5	1.5	0.86	0.85	ND	1.5	ND	ND	1.0	1.1	0.9	1.6	3.7
2,3,4,6,7,8-HxCDF	0.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.0
1,2,3,7,8,9-HxCDF	0.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	0.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4,7,8,9-HpCDF	0.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
OCDF	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,4,4,'5-TCB(#81)	0.6	0.91	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.76
3,3',4',4'-TCB(#77)	1.0	1.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.2	2.7
3,3',4,4',5-PeCB(#126)	0.3	6.7	12	7.5	3.0	4.0	10	2.5	4.7	3.0	1.5	4.6	7.2	19
3,3',4,4',5,5'-HxCB(#169)	0.3	8.7	8.8	5.8	3.9	3.7	4.3	2.7	3.1	5.6	3.8	4.5	11	12
Total PCDDs (pg-TEQ/g)		10	14	8.3	4.8	4.5	12.5	3.6	3.3	6.7	5.0	6.7	10	24
Total PCDFs (pg-TEQ/g)		3.5	5	2.8	1.9	1.7	4.0	1.5	1.3	2.9	2.1	2.9	3.8	9
Total PCDD/Fs (pg-TEQ/g)		13	19	11	6.7	6.1	16.5	5.1	4.7	10	7.1	10	14	33
Total non-*ortho* PCBs (pg-TEQ/g)		0.93	1.5	0.92	0.42	0.51	1.2	0.33	0.57	0.47	0.26	0.6	1.1	2.3
Total dioxins (pg-TEQ/g)		14	21	12	7.1	6.6	18	5.4	5.2	10	7.3	10	15	36
Total dioxins in maternal blood (pg-TEQ/g)		25	24	14	6.6	6.8	15	6.9	8.2	12	11	15	26	44
Total dioxins in umbilical cord blood (pg-TEQ/g)		7.1	7.4	4.2	3.2	3.3	5.0	3.1	1.9	5.6	4.7	6.7	11	18
Maternal age at delivery		36	37	39	28	26	31	30	33	28	33	33	42	41
Parity		2	2	4	2	2	1	2	4	2	2	2	2	1

those in maternal blood (r^2=0.870). The individual total TEQ concentrations in maternal blood were higher than those in placenta, excepting two donors. In these two donors, small differences were observed in TEQ concentrations between the specimens. These results were similar to the results from the analysis of five sets of placenta and maternal blood collected in the United States, in which the dioxin concentrations in maternal blood were higher than those in placenta, excepting one donor[5]. In all 13 pregnant women, individual total TEQ concentrations in maternal blood were higher than those in cord blood. Fig. 4 shows the positive linear relationship (r^2=0.914) between the concentrations in maternal blood and in cord blood.

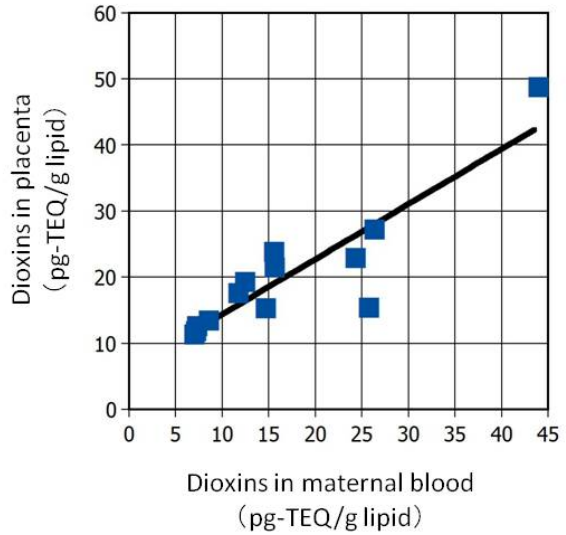

Fig.3 Relationship between total TEQ concentrations in maternal blood and in placenta

We found that the mean total TEQ concentrations for three kinds of specimen were in the order of maternal blood (1.0) > placenta (0.80) > cord blood (0.38); relative concentration ratios were shown in parentheses based on maternal blood. The downtrends in the lipid weight-based dioxin concentration as close to fetus suggest that the placental tissue suppresses the transfer of dioxin molecules to cord blood and fetuses.

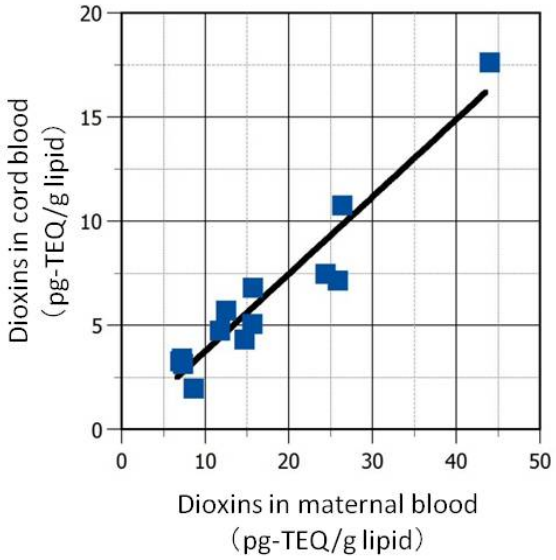

Fig.4 Relationship between total TEQ concentrations in maternal blood and in cord blood

Acknowledgements

This study was supported by the Environment Research and Technology Development Fund (ERTDF) of the Ministry of the Environment (MOE) of Japan (C-0903).

References

1. Tsutsumi T, Yanagi T, Nakamura M, Kono Y, Uchibe H, Iida T, Hori T, Nakagawa R, Tobiishi K, Matsuda R, Sasaki K, Toyoda M. (2001); *Chemosphere.* 45: 1129-1137
2. Ministry of the Environment, Japan. The Accumulation of Dioxins in the Japanese People (2008)
3. Nakano S, Nakai K, Noguchi T, Takekoshi H, Suzuki G, Nakano H. (2005); *Chemosphere.* 61: 1244-1255
4. Nakamura T, Nakai K, Matsumura T, Suzuki S, Saito Y, Satoh H. (2008); *Sci Total Environ.* 394: 39-51
5. Schecter A, Kassis I, Päpke O. (1998); *Chemosphere.* 37: 1817-1823