

# Maternal-fetal distribution and transfer of dioxins in pregnant women in Japan, and attempts to reduce maternal transfer with *Chlorella* (*Chlorella pyrenoidosa*) supplements

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## Abstract

Dioxins can be transferred from mother to fetus via the placenta, or to nursing infants via breast milk, potentially causing developmental health problems in children. To assess pediatric health risks from dioxins, exposure of mothers and children to dioxins must be clarified. Methods of reducing maternal transfer of dioxins should also be investigated. Concentrations of 28 dioxin (polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and co-planar polychlorinated biphenyls) congeners in blood, adipose tissue, breast milk, cord blood and placenta collected from 44 pregnant Japanese women were measured. In addition, to investigate potential reductions in maternal transfer of dioxins, 23 pregnant women were instructed to take *Chlorella pyrenoidosa* supplements during pregnancy.

Correlations were observed between dioxin total toxic equivalents (total TEQ) in blood and total TEQ in adipose tissue ( $r = 0.913$ ,  $P < 0.0001$ ), breast milk ( $r = 0.695$ ,  $P = 0.0007$ ), and cord blood ( $r = 0.759$ ,  $P < 0.0001$ ). Dioxin levels transferred to fetuses and nursing infants reflect cumulative maternal concentrations of dioxins. A linear regression equation was introduced to predict total TEQ in breast milk and cord blood from dioxin levels in maternal blood, which should prove useful in evaluating fetal and infant risk of dioxin exposure. Total TEQ in cord blood were approximately 26% lower than in maternal blood ( $P < 0.0001$ ). The results of this study suggest that transplacental transfer differs depending on the dioxin congener. Total TEQ in breast milk were approximately 30% lower in the *Chlorella* group than in controls ( $P = 0.0113$ ). This finding suggests that maternal transfer of dioxins can be reduced using dietary measures such as *Chlorella* supplements.

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## 1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and co-planar polychlorinated biphenyls (Co-PCBs) collectively represent environmental contaminants that exert a variety of harmful effects on the laboratory animals, including carcinogenicity (Dragan et al., 1992), reproductive toxicity (McNulty, 1984), and immunosuppression (Burleson et al., 1996). PCDDs, PCDFs and Co-PCBs (dioxins) continue to be released into the environment in Japan via industrial combustion and municipal garbage incineration processes. Dioxins that have been released into the environment can be present in the air, soil and water. As dioxins display extremely high chemical stability and are lipid soluble, these chemicals are primarily introduced into the body through food, as a result of biomagnification in the food-chain. Approximately 90% of dioxin intake in humans reportedly occurs through food, and 60–80% of this intake in Japan is through seafood (Tsutsumi et al., 2001). Conversely, in Europe and North America, the primary sources of dioxin are meat, eggs and dairy products (Schechter et al., 1994). Ingested dioxins are absorbed from the gastrointestinal tract, but are not readily catabolized by drug metabolizing enzyme system, and accumulate in the body over long periods of time. These accumulated dioxins can be transferred from mother to fetus via the placenta during pregnancy, and from mother to child via breast milk when nursing (Füerster et al., 1989; Schechter et al., 1990). Several cases of neurological developmental disorders in children such as hyperactivity and decreased intelligence quotient (IQ) have been reported following exposure to dioxins or polychlorinated biphenyls (PCBs) (Chen et al., 1994; Jacobson and Jacobson, 1996). In addition, experimental animal models reportedly show changes in brain function and postnatal behavioral dysfunction resulting from transplacental and lactational exposure to environmental contaminants such as dioxins (Bowman et al., 1989; Hany et al., 1999; Masuo et al., 2001; Maeda et al., 2003). Recent increases in attention deficit hyperactivity disorder (ADHD) and learning disabilities (LD) in school children have become major social problems in Japan and the West (Wolraich et al., 1996; Ohminami, 2003). The transplacental and lactational exposure to environmental contaminants such as dioxins may be regarded as one of cause of ADHD and LD in children.

In order to assess the health risks of dioxins on children, knowledge of the extent of dioxin exposure is crucial, including the mechanisms by which dioxins are

transferred from mother to child. Studies are also needed to investigate ways of reducing maternal transfer of dioxins.

The present study investigated the transfer of dioxins from mother to fetus or nursing infant, using pregnant women from the general population in Japan and their children as subjects. Moreover, in an attempt to mitigate maternal transfer of dioxins, we investigated the utility of *Chlorella pyrenoidosa* (*Chlorella*) supplements.

*Chlorella* is a unicellular green algae that grows in fresh water. Compared to other plants, *Chlorella* is much richer in proteins and chlorophylls and contains large quantities of vitamins, minerals, dietary fibers and nucleic acids. The proteins in *Chlorella* include all the essential amino acids for human growth and health. *Chlorella* and *Chlorella* extracts have been shown to lower cholesterol (Fujiwara et al., 1990), prevent stress-induced ulcers (Tanaka et al., 1977) and improve immunity against infection (Hasegawa et al., 1990), and antitumor effects have also been described (Tanaka et al., 1984; Konishi et al., 1985; Merchant et al., 1990). *Chlorella* has also been reported to exert a stimulatory effect on fecal excretion of dioxins in rats (Morita et al., 1999, 2001), and we have confirmed that *Chlorella* displays similar effects in mice (Takekoshi et al., 2005). At present, *Chlorella* is widely available in supplements and health foods in many regions of the world. The present clinical trial was conducted based on this information.

## 2. Materials and methods

### 2.1. Subjects

Subjects in this study comprised 151 healthy pregnant women (age range, 23–40 years; 120 primiparas, 31 multiparas) who were receiving prenatal care at Saiseikai Nara Hospital (Nara, Japan) and who provided written consent to participate in the study. Of these, 65 subjects (53 primiparas, 12 multiparas) agreed to take *Chlorella* tablets (*Chlorella* group). These subjects were asked to take *Chlorella* tablets for approximately 6 months, from gestational week 12–16 up until day of delivery. Dose was 6 g/day of *Chlorella* (30 tablets/day), in portions of 10 tablets after each main meal. Sun Chlorella A tablets (Sun Chlorella Corp., Kyoto, Japan) containing dried *Chlorella* powder as the active ingredient were used. Each tablet comprised the following (g/100 g): moisture, 4.7; chlorophyll, 2.4; dietary fiber, 9.8; protein ( $N \times 6.5$ ), 57.8; lipid, 10.4. No

restrictions were imposed on the remaining 86 participants (67 primiparas, 19 multiparas) who comprised the Control group, with the exception that they were prohibited from taking *Chlorella* tablets.

This study was conducted in accordance with the general principles of the Helsinki Declaration, and all study protocols were reviewed and approved by the Institutional Review Board of Saiseikai Nara Hospital.

## 2.2. Analysis of PCDDs, PCDFs, and Co-PCBs

Only participants for whom five samples comprising maternal peripheral blood (maternal blood), breast milk, maternal subcutaneous adipose tissue (adipose tissue), cord blood and placenta, or four samples except for maternal adipose tissue sample were available were selected for analysis of PCDDs, PCDFs, and Co-PCBs. Analysis was thus performed for 23 subjects in the *Chlorella* group and 21 participants in the Control group. Samples were collected between August 2001 and October 2002. Maternal blood samples (20 ml) were taken from each of the 44 participants on the day of delivery. Given that the participating subjects were pregnant, fasting was not imposed at the time blood samples were collected. Samples of placenta (20 g) which sufficiently removed blood were collected immediately postpartum. Samples of cord blood (20 ml) were collected immediately postpartum. Breast milk (10 ml each) samples were collected on one day during days 3–9 of puerperium, and were mixed. Samples of maternal adipose tissue (5–10 g) were collected during surgery from abdominal incisions in 33 subjects who underwent caesarean section deliveries. Samples were immediately frozen after collection, and sent to Obihiro University of Agriculture and Veterinary Medicine, where they were stored frozen at  $-20^{\circ}\text{C}$  until analysis. Concentrations of 6 PCDD congeners, 10 PCDF congeners, and 12 Co-PCB congeners (Table 2) were measured in each maternal sample.

Dioxin analysis was conducted in accordance with the analytical manual of the Japanese Ministry of Health, Labour, and Welfare (2000). Specifically, all samples were spiked using  $^{13}\text{C}$ -labelled internal standards (Wellington Laboratories, Guelph, Ontario, Canada) of PCDDs, PCDFs, and Co-PCBs. Then, for maternal blood, cord blood and breast milk samples, dioxin-containing lipid was separated from samples by combination with saturated ammonium sulfate, followed by ethanol, after which the lipid was extracted using *n*-hexane. For placenta and maternal adipose tissue samples, dioxin-containing lipid was extracted by sonication with a 2:1 (v/v) chloroform-methanol mixture. After measuring lipid content in each sample, dioxin-containing lipids were fed into a multilayer silica gel column comprising 2% (w/w) KOH-silica gel, 44% (w/w)  $\text{H}_2\text{SO}_4$ -silica gel, 22% (w/w)  $\text{H}_2\text{SO}_4$ -silica gel and 10%  $\text{AgNO}_3$ -silica gel, in that order, followed by passage

through an alumina (alumina oxide 90; Merck & Co., Inc., Darmstadt, Germany) column for sample clean-up. Analyses of PCDDs and PCDFs were performed using high-resolution gas chromatography-mass spectrometry (GC-MS) (HP-6890: Hewlett Packard, Palo Alto, CA, USA; AutoSpec-Ultima: Micromass, Manchester, England) equipped with a capillary column (SP-2331: SPERUCO, Bellefonte, PA, USA, DB-17HT: J&W Scientific, Folsom, CA, USA) employing the Electron Impact-Selected Ion Monitoring method at 10000 resolutions. Analysis of Co-PCBs was performed using GC-MS (HP-6890; AutoSpec-Ultima) with a DB-5MS fused silica capillary column (J&W Scientific). Quality control was performed by calculating recovery rates for PCDDs, PCDFs and Co-PCBs based on  $^{13}\text{C}$ -labelled 1,2,3,4-tetrachlorinated dibenzo-*p*-dioxin (tetraCDD) or  $^{13}\text{C}$ -labelled 2,3,3',5,5'-pentachlorinated biphenyls (pentaCB) as syringe spikes. Method blanks were analyzed parallel to samples. Detection limits were: tetraCDD/DF, 0.005 pg/g; pentaCDD/DF, 0.005 pg/g; hexaCDD/DF, 0.01 pg/g; heptaCDD/DF, 0.01 pg/g; octaCDD/DF, 0.02 pg/g; and Co-PCB, 0.01 pg/g.

## 2.3. Data analysis

Means and standard deviations (SDs) were calculated for the concentrations of each congener, and for total PCDD, total PCDF, total Co-PCB and total dioxin concentrations (PCDDs + PCDFs + Co-PCBs) for maternal blood, maternal adipose tissue, breast milk, cord blood and placenta. Total toxic equivalents (TEQ) were calculated using WHO toxic equivalency factor (WHO-TEF) values for each congener (Van den Berg et al., 1998). In these calculations, measured values of congeners with concentrations below the detection limit were regarded as 0.

Correlations between various samples were investigated to analyze maternal-fetal distributions for each dioxin. In addition, transplacental transmission of dioxins was assessed using concentration ratios for each congener in the following samples as indicators: ratio between placenta and maternal blood concentrations ( $C_p/C_{mb}$ , lipid-basis); ratio between cord blood and maternal blood concentrations ( $C_{cb}/C_{mb}$ , lipid-basis); and ratio between cord blood and placenta concentrations ( $C_{cb}/C_p$ , lipid-basis). Maternal-fetal distribution and transfer of dioxins was assessed solely on the basis of measured values in the Control group.

To analyze differences between primiparous and multiparous women, concentrations of each congener in each maternal sample, in addition to total concentrations of PCDDs, PCDFs, and Co-PCBs, total concentration of dioxins, and total TEQ were compared between Control group primiparas ( $n = 10$ ) and multiparas ( $n = 11$ ). To assess the effects of *Chlorella*

supplementation, the same parameters were compared between *Chlorella* and Control groups.

Mann–Whitney *U*-tests were used for comparisons between each group and between each maternal sample, while Spearman's rank correlation test was used to investigate correlations between maternal samples. Values of  $P < 0.05$  were considered statistically significant. All statistical procedures were performed using SPSS 11.0 for Windows statistical software (SPSS, Chicago, IL, USA).

### 3. Results

#### 3.1. Characteristics of participants

Characteristics of mothers and their children are shown in Table 1. No differences between *Chlorella* and Control groups were observed with regard to results of normal clinical examinations or interviews conducted at Saiseikai Nara Hospital during pregnancy (data not shown).

#### 3.2. Maternal-fetal distribution and maternal transfer of dioxins

The principal congeners found in maternal blood, maternal adipose tissue, breast milk and cord blood were 1,2,3,7,8-pentaCDD, 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF, 3,3',4,4',5-pentaCB (IUPAC #126) and 2,3,3',4,4',5-hexaCB (#156), with these 5 congeners representing approximately 80% of total TEQ. However, congeners 1,2,3,7,8-pentaCDD and 2,3,4,7,8-pentaCDF composed about 80% of total TEQ in the placenta (Tables 2 and 3). The profile for dioxin congeners present in the placenta differed substantially from that in maternal blood, maternal adipose tissue, breast milk and cord blood (Tables 2 and 3).

Correlations were observed between total TEQ (pg-TEQ/whole sample-g) in maternal blood and total TEQ in maternal adipose tissue, breast milk and cord

blood ( $r = 0.913$ ,  $P < 0.0001$ ;  $r = 0.695$ ,  $P = 0.0007$ ; and  $r = 0.759$ ,  $P < 0.0001$ , respectively) (Fig. 1). Similar correlations were observed on a lipid-basis.

No differences were observed between primipara and multipara subjects in measured dioxin values (Tables 2 and 3).

#### 3.3. Placental transfer of dioxins

Total concentrations of dioxins and total TEQ in cord blood were approximately 56% and 39% lower than corresponding values in maternal blood (calculated from total TEQ and measured values in Table 3; Mann–Whitney *U*-test:  $P < 0.0001$  for each).  $C_p/C_{mb}$  for the various congeners (lipid-basis) was 0.29–1.23 for PCDDs, 0.35–2.67 for PCDFs, and 0.33–0.90 for non-ortho-PCBs, and 0.24–0.33 for mono-ortho-PCBs, as accumulation in the placenta varied greatly depending on the specific congener (one-factor analysis of variance (ANOVA),  $P < 0.01$ ) (Fig. 2).  $C_p/C_{mb}$  for the major congeners was 1.23 for 2,3,7,8-tetraCDD (WHO-TEF:1), 2.68 for 1,2,3,7,8-pentaCDD (WHO-TEF:1), 1.33 for 1,2,3,7,8-pentaCDF (WHO-TEF:0.05), 2.67 for 2,3,4,7,8-pentaCDF (WHO-TEF:0.5). Moreover,  $C_{cb}/C_p$  (lipid-basis) was 0.09–4.79 for PCDDs, 0.00–1.19 for PCDFs, 0.48–3.61 for non-ortho-PCBs, and 0.90–2.12 for mono-ortho-PCBs, as transmission from placenta to cord blood differed depending on the congener (one-factor ANOVA,  $P < 0.01$ ) (Fig. 2). Observed ranges for  $C_{cb}/C_{mb}$  (lipid-basis) were 0.06–1.38 for PCDDs, 0.00–0.59 for PCDFs, 0.29–1.23 for non-ortho-PCBs, and 0.28–0.60 for mono-ortho-PCBs (one-factor ANOVA,  $P < 0.01$ ) (Fig. 2). Although placental transmission of several congeners was restricted, no restriction on the transfer of octaCDD or 3,3',4,4'-tetraCB (#77) into the cord blood was observed.

#### 3.4. Effect of *Chlorella* administration

The mean lipid contents ( $\pm$ SD) of maternal blood, cord blood and breast milk were significantly lower in

Table 1  
Characteristics of participants

Characteristics	Control group ( $n = 21$ )			<i>Chlorella</i> group ( $n = 23$ )			$P^a$
	Mean	SD	%	Mean	SD	%	
<i>Maternal</i>							
Age (years)	28.5	3.9	–	30.4	3.1	–	0.185
Weight before pregnancy (kg)	55.1	12.2	–	53.5	8.3	–	0.654
Parity (% primipara)	–	–	47.6	–	–	56.5	0.763
<i>Newborn</i>							
Male (%)	–	–	33.3	–	–	47.8	0.373
Weight (g)	2860.8	304.6	–	3064.5	335.9	–	0.040

<sup>a</sup> Mann–Whitney *U*-test or Fisher's exact test.

Table 2  
Concentrations (pg/whole sample-g) of PCDDs, PCDFs, Co-PCBs and total TEQs (pg-TEQ/whole sample-g) in maternal blood, adipose tissue, milk, cord blood, and placenta from the Primipara group and the Multipara group in the Control group

WHO-TEF	Blood		Adipose tissue		Milk		Cord blood		Placenta	
	Primipara	Multipara	Primipara	Multipara	Primipara	Multipara	Primipara	Multipara	Primipara	Multipara
	(n = 10)	(n = 11)	(n = 10)	(n = 11)	(n = 10)	(n = 10)	(n = 10)	(n = 11)	(n = 10)	(n = 11)
<b>PCDDs</b>										
2,3,7,8-TeCDD	0.005 (0.006) <sup>a</sup>	0.008 (0.008)	0.851 (0.304)	0.882 (0.433)	0.039 (0.019)	0.048 (0.021)	<LOD	<LOD	0.036 (0.016)	0.028 (0.016)
1,2,3,7,8-PeCDD	0.031 (0.010)	0.034 (0.021)	4.040 (1.290)	4.027 (1.968)	0.173 (0.070)	0.198 (0.083)	0.010 (0.002)	0.009 (0.005)	0.271 (0.106)	0.236 (0.098)
1,2,3,6,7,8-HxCDD	0.118 (0.055)	0.155 (0.094)	18.960 (7.676)	22.045 (9.814)	0.878 (0.408)	1.089 (0.482)	0.032 (0.012)	0.034 (0.015)	0.183 (0.062)	0.174 (0.065)
1,2,3,7,8,9-HxCDD	0.015 (0.010)	0.025 (0.019)	1.955 (1.041)	2.886 (1.979)	0.108 (0.056)	0.172 (0.106)	<LOD	<LOD	0.034 (0.024)	0.041 (0.027)
1,2,3,4,6,7,8-HpCDD	0.066 (0.018)	0.096 (0.044)	6.650 (3.290)	8.673 (4.551)	0.228 (0.171)	0.347 (0.194)	0.029 (0.007)	0.028 (0.006)	0.098 (0.036)	0.101 (0.035)
OCDD	15.900 (0.738)	16.182 (1.250)	504.000 (527.093)	371.818 (94.744)	16.400 (1.647)	18.000 (4.082)	15.300 (0.675)	15.364 (0.674)	14.300 (0.483)	14.000 (0.775)
<b>PCDFs</b>										
2,3,7,8-TeCDF	0.005 (0.004)	0.008 (0.006)	0.534 (0.225)	0.680 (0.525)	0.020 (0.011)	0.033 (0.018)	<LOD	<LOD	0.006 (0.006)	0.007 (0.008)
1,2,3,7,8-PeCDF	<LOD <sup>b</sup>	0.007 (0.005)	0.479 (0.197)	0.489 (0.424)	0.022 (0.012)	0.032 (0.018)	<LOD	<LOD	0.025 (0.013)	0.027 (0.020)
2,3,4,7,8-PeCDF	0.045 (0.015)	0.053 (0.036)	7.210 (2.175)	7.145 (3.334)	0.317 (0.127)	0.353 (0.149)	0.015 (0.003)	0.015 (0.004)	0.396 (0.131)	0.355 (0.147)
1,2,3,4,7,8-HxCDF	0.019 (0.005)	0.021 (0.019)	2.200 (0.648)	2.527 (1.411)	0.088 (0.035)	0.113 (0.056)	<LOD	<LOD	0.104 (0.036)	0.099 (0.045)
1,2,3,6,7,8-HxCDF	0.020 (0.006)	0.025 (0.016)	2.550 (0.774)	2.991 (1.576)	0.088 (0.034)	0.119 (0.054)	<LOD	<LOD	0.066 (0.022)	0.066 (0.037)
1,2,3,7,8,9-HxCDF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2,3,4,6,7,8-HxCDF	<LOD	0.011 (0.006)	1.151 (0.558)	1.545 (0.672)	0.047 (0.029)	0.070 (0.033)	<LOD	<LOD	0.011 (0.014)	0.015 (0.017)
1,2,3,4,6,7,8-HpCDF	0.011 (0.006)	0.011 (0.008)	1.466 (0.390)	1.482 (0.571)	0.037 (0.018)	0.047 (0.019)	<LOD	<LOD	0.025 (0.023)	0.030 (0.030)
1,2,3,4,7,8,9-HpCDF	<LOD	<LOD	0.263 (0.162)	0.097 (0.138)	<LOD	<LOD	<LOD	<LOD	0.016 (0.020)	0.020 (0.025)
OCDF	<LOD	<LOD	0.201 (0.325)	0.135 (0.308)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
<b>Non-ortho-Co-PCBs</b>										
3,4,4',5-TeCB (#81)	0.03 (0.01)	0.05 (0.03)	4.70 (3.48)	4.26 (2.54)	0.18 (0.07)	0.19 (0.11)	0.02 (0.01)	0.02 (0.01)	0.05 (0.06)	0.05 (0.07)
3,3',4,4'-TeCB (#77)	0.34 (0.06)	0.39 (0.13)	18.10 (5.22)	17.64 (7.33)	0.76 (0.24)	0.88 (0.22)	0.28 (0.03)	0.29 (0.02)	0.37 (0.11)	0.38 (0.21)
3,3',4,4',5-PeCB (#126)	0.19 (0.10)	0.32 (0.30)	36.30 (19.03)	48.91 (29.82)	1.71 (1.12)	2.34 (1.21)	0.05 (0.02)	0.07 (0.03)	0.29 (0.13)	0.32 (0.20)
3,3',4,4',5,5'-HxCB (#169)	0.15 (0.06)	0.19 (0.13)	37.20 (10.45)	34.73 (11.95)	1.24 (0.47)	1.41 (0.48)	0.03 (0.01)	0.03 (0.01)	0.42 (0.25)	0.40 (0.27)
<b>Mono-ortho-Co-PCBs</b>										
2,3,4,4',5-PeCB (#123)	0.70 (0.32)	1.07 (0.87)	105.20 (62.03)	139.73 (118.00)	5.04 (3.03)	7.31 (4.30)	0.28 (0.07)	0.32 (0.09)	0.65 (0.28)	0.76 (0.38)
2,3',4,4',5-PeCB (#118)	39.00 (22.17)	56.09 (50.89)	7040.00 (3993.11)	8563.64 (5891.40)	323.00 (208.38)	411.00 (234.87)	14.10 (4.20)	14.43 (4.58)	33.90 (13.45)	37.00 (18.86)
2,3,3',4,4'-PeCB (#105)	9.25 (4.39)	14.27 (13.56)	1671.00 (894.43)	2159.09 (1574.61)	73.90 (48.05)	102.60 (63.67)	3.92 (0.95)	4.18 (1.31)	8.78 (3.24)	10.14 (5.57)
2,3,4,4',5-PeCB (#114)	2.69 (1.38)	3.16 (2.65)	617.00 (325.75)	567.27 (348.26)	26.70 (18.11)	26.60 (15.07)	0.76 (0.28)	0.66 (0.28)	2.74 (1.01)	2.46 (1.19)
2,3',4,4',5,5'-HxCB (#167)	4.670 (2.497)	6.11 (5.11)	943.00 (492.46)	1002.73 (615.47)	37.30 (26.60)	43.80 (22.19)	1.11 (0.39)	1.03 (0.41)	3.51 (1.28)	3.61 (1.80)
2,3,3',4,4',5-HxCB (#156)	15.39 (7.56)	17.19 (12.70)	3740.00 (1709.58)	3354.55 (1797.42)	143.70 (100.18)	140.70 (76.57)	3.51 (1.07)	2.93 (1.17)	13.25 (5.29)	11.06 (5.48)
2,3,3',4,4',5'-HxCB (#157)	4.03 (2.09)	4.32 (3.20)	844.00 (386.01)	768.18 (393.49)	34.70 (23.34)	34.00 (16.86)	0.87 (0.31)	0.71 (0.31)	3.51 (1.39)	2.83 (1.29)
2,3,3',4,4',5,5'-HpCB (#189)	1.44 (0.66)	1.70 (1.27)	352.00 (123.36)	337.27 (156.59)	9.87 (4.67)	11.40 (4.50)	0.29 (0.07)	0.24 (0.08)	1.47 (0.53)	1.36 (0.64)
<b>Total PCDDs</b>										
Total PCDDs	16.14 (0.76)	16.50 (1.38)	536.46 (531.20)	410.33 (103.69)	17.83 (2.12)	19.85 (4.47)	15.37 (0.068)	15.44 (0.68)	14.92 (0.49)	14.58 (0.93)
<b>Total PCDFs</b>										
Total PCDFs	0.11 (0.03)	0.13 (0.01)	16.05 (4.54)	17.09 (8.06)	0.62 (0.25)	0.77 (0.31)	0.03 (0.01)	0.03 (0.02)	0.65 (0.22)	0.63 (0.30)
<b>Total Co-PCBs</b>										
Total Co-PCBs	77.88 (38.96)	104.85 (88.68)	15408.50 (7569.74)	16997.99 (10457.19)	658.10 (421.42)	782.23 (402.28)	25.22 (6.73)	24.89 (7.56)	68.94 (23.79)	70.38 (33.43)
<b>PCDDs/DFs/Co-PCBs</b>										
PCDDs/DFs/Co-PCBs	94.12 (38.94)	121.48 (89.84)	15961.01 (7938.21)	17425.41 (10537.30)	676.55 (423.23)	802.86 (405.59)	40.62 (6.74)	40.36 (7.73)	84.51 (23.83)	85.59 (34.35)
<b>Total TEQ levels</b>										
Total TEQ levels	0.116 (0.046)	0.150 (0.106)	18.920 (6.613)	20.629 (10.119)	0.827 (0.396)	0.991 (0.419)	0.033 (0.008)	0.036 (0.011)	0.597 (0.211)	0.535 (0.227)

<sup>a</sup> Mean (SD).

<sup>b</sup> Mean value below the limit of detection.

Table 3  
Concentrations (pg/lipid-g) of PCDDs, PCDFs, Co-PCBs and total TEQs (pg-TEQ/lipid-g) in maternal blood, adipose tissue, milk, cord blood, and placenta from the Primipara group and the Multipara group in the Control group

	Blood						Adipose tissue			Milk			Cord blood			Placenta																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
	Primipara (n = 10)		Multipara (n = 11)		Primipara (n = 10)		Multipara (n = 11)		Primipara (n = 10)		Multipara (n = 10)		Primipara (n = 10)		Multipara (n = 11)		Primipara (n = 10)		Multipara (n = 11)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
<b>PCDDs</b>																						2,3,7,8-TeCDD	0.95 (1.10) <sup>a</sup>	1.47 (1.37)	1.04 (0.36)	1.05 (0.50)	1.06 (0.41)	1.18 (0.48)	<LOD	<LOD	<LOD	<LOD	0.49 (0.87)	<LOD	2.17 (0.98)	1.69 (1.03)	1,2,3,7,8-PeCDD	5.88 (1.71)	5.84 (2.28)	4.97 (1.52)	4.78 (2.22)	4.67 (1.45)	4.96 (2.04)	0.65 (0.26)	0.80 (0.59)	0.54 (0.21)	0.85 (0.46)	0.48 (0.83)	0.92 (0.80)	0.36 (0.39)	0.40 (0.46)	1,2,3,6,7,8-HxCDD	22.40 (9.22)	27.36 (11.55)	23.30 (9.01)	26.09 (11.07)	23.40 (7.89)	26.80 (10.68)	0.60 (0.24)	0.57 (0.48)	0.58 (0.22)	0.84 (0.50)	0.36 (0.62)	0.15 (0.47)	1.48 (0.81)	1.66 (1.25)	1,2,3,7,8,9-HxCDD	2.87 (1.74)	4.27 (2.54)	2.36 (1.19)	3.44 (2.28)	2.85 (1.29)	4.26 (2.57)	8.84 (2.55)	8.59 (4.02)	8.47 (2.35)	8.86 (3.64)	4.07 (1.11)	3.81 (0.79)	23.50 (7.84)	21.93 (9.09)	1,2,3,4,6,7,8-HpCDD	12.70 (3.66)	17.74 (7.17)	8.19 (3.86)	10.31 (5.18)	5.92 (3.45)	8.75 (5.32)	2.70 (0.75)	3.00 (1.64)	2.37 (0.74)	2.84 (1.41)	0.96 (1.33)	0.75 (1.21)	6.16 (2.14)	6.15 (2.88)	OCDD	3040.00 (245.86)	3036.36 (621.73)	618.00 (627.90)	448.18 (143.79)	469.00 (99.27)	476.00 (153.78)	3.15 (0.86)	3.57 (1.87)	2.38 (0.71)	2.98 (1.37)	0.94 (1.30)	0.77 (1.24)	3.95 (1.42)	4.05 (2.36)								<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<b>PCDFs</b>																						2,3,7,8-TeCDF	0.90 (0.72)	1.34 (0.92)	0.65 (0.26)	0.80 (0.59)	0.54 (0.21)	0.85 (0.46)	1.43 (0.64)	1.82 (0.74)	1.73 (0.77)	1.17 (0.48)	0.47 (1.05)	<LOD	0.65 (0.89)	0.92 (1.07)	1,2,3,7,8-PeCDF	0.64 (0.68)	1.20 (0.74)	0.60 (0.24)	0.57 (0.48)	0.58 (0.22)	0.84 (0.50)	1.82 (0.43)	1.76 (0.65)	0.98 (0.37)	1.17 (0.48)	0.47 (1.05)	<LOD	1.49 (1.43)	1.84 (1.86)	2,3,4,7,8-PeCDF	8.41 (2.31)	9.07 (4.39)	8.84 (2.55)	8.59 (4.02)	8.47 (2.35)	8.86 (3.64)	0.32 (0.20)	0.11 (0.16)	<LOD	0.03 (0.08)	<LOD	0.99 (1.22)	0.99 (1.22)	1,2,3,4,7,8-HxCDF	3.63 (0.85)	3.43 (2.44)	2.70 (0.75)	3.00 (1.64)	2.37 (0.74)	2.84 (1.41)	0.24 (0.39)	0.16 (0.35)	0.04 (0.13)	0.16 (0.35)	<LOD	<LOD	0.32 (1.01)	0.73 (1.63)	1,2,3,6,7,8-HxCDF	3.83 (0.97)	4.24 (1.91)	3.15 (0.86)	3.57 (1.87)	2.38 (0.71)	2.98 (1.37)									1,2,3,7,8,9-HxCDF	<LOD <sup>b</sup>	<LOD	<LOD	<LOD	<LOD	<LOD									2,3,4,6,7,8-HxCDF	0.53 (1.16)	1.18 (1.45)	1.43 (0.64)	1.82 (0.74)	1.73 (0.77)	1.17 (0.48)									1,2,3,4,6,7,8-HpCDF	2.00 (1.16)	1.89 (1.30)	1.82 (0.43)	1.76 (0.65)	0.98 (0.37)	1.17 (0.48)									1,2,3,4,7,8,9-HpCDF	<LOD	<LOD	0.32 (0.20)	0.11 (0.16)	<LOD	0.03 (0.08)									OCDF	<LOD	<LOD	0.24 (0.39)	0.16 (0.35)	0.04 (0.13)	0.16 (0.35)									<b>Non-ortho-Co-PCBs</b>																						3,4,4',5-TeCB (#81)	6.39 (2.24)	8.23 (3.83)	5.84 (4.31)	5.05 (2.90)	4.91 (1.65)	4.88 (2.71)									3,3',4,4'-TeCB (#77)	64.60 (12.62)	69.46 (19.15)	44.50 (6.50)	20.91 (8.11)	21.30 (7.89)	22.90 (7.61)									3,3',4,4',5-PeCB (#126)	36.50 (17.49)	53.55 (34.99)	22.50 (22.29)	58.46 (34.89)	44.70 (21.67)	59.50 (31.92)									3,3',4,4',5,5'-HxCB (#169)	29.00 (10.44)	32.64 (16.04)	46.40 (13.81)	41.46 (14.26)	33.70 (9.21)	35.90 (11.87)									<b>Mono-ortho-Co-PCBs</b>																						2',3',4,4',5-PeCB (#123)	131.80 (54.59)	183.09 (105.22)	128.50 (73.68)	165.18 (132.64)	131.70 (62.90)	184.90 (110.10)									2',3',4,4',5-PeCB (#118)	7390.00 (3806.27)	9500.00 (6197.74)	8640.00 (4816.68)	10127.27 (6729.06)	8510.00 (4533.69)	10540.00 (6361.03)									2',3',3',4,4'-PeCB (#105)	1750.00 (732.201)	2400.00 (1638.29)	2046.00 (1070.33)	2563.64 (1801.82)	1935.00 (985.46)	2630.00 (1728.87)									2,3,4,4',5-PeCB (#114)	505.00 (241.07)	549.09 (390.37)	757.00 (383.18)	685.46 (438.46)	692.00 (355.71)	691.00 (412.67)									2,3',4,4',5,5'-HxCB (#167)	880.00 (436.40)	1035.46 (654.24)	1158.00 (588.84)	1208.18 (748.61)	970.00 (528.58)	1126.00 (604.98)									2,3,3',4,4',5-HxCB (#156)	2900.00 (1339.15)	2963.64 (1881.10)	4630.00 (2064.00)	4063.64 (2317.01)	3770.00 (1895.64)	3620.00 (2031.86)									2,3,3',4,4',5'-HxCB (#157)	757.00 (370.20)	749.09 (469.14)	1047.00 (464.93)	919.09 (498.37)	907.00 (428.25)	875.00 (448.83)									2,3,3',4,4',5,5'-HpCB (#189)	270.00 (107.60)	290.91 (162.14)	439.00 (167.50)	403.64 (193.92)	264.00 (92.28)	297.00 (124.19)									<b>Total PCDDs</b>	3084.8 (243.7)	3093.1 (627.2)	657.9 (632.3)	493.9 (153.5)	506.9 (97.6)	521.9 (161.6)									<b>Total PCDFs</b>	19.9 (5.3)	22.4 (11.3)	19.7 (5.0)	20.4 (9.3)	16.6 (4.5)	19.4 (7.7)									<b>Total Co-PCBs</b>	14720.3 (6673.7)	17835.1 (10992.1)	18964.3 (8976.2)	20262.0 (12259.4)	17284.3 (8359.6)	20087.1 (10995.7)									<b>PCDDs/DFs/Co-PCBs</b>	17825.0 (6568.1)	20950.5 (11004.7)	19642.3 (9386.6)	20776.2 (12372.8)	17807.8 (8349.2)	20628.5 (11104.4)									<b>Total TEQ levels</b>	21.93 (7.48)	25.67 (12.36)	23.25 (7.71)	24.63 (11.78)	21.97 (7.21)	24.98 (10.60)								
2,3,7,8-TeCDD	0.95 (1.10) <sup>a</sup>	1.47 (1.37)	1.04 (0.36)	1.05 (0.50)	1.06 (0.41)	1.18 (0.48)	<LOD	<LOD	<LOD	<LOD	0.49 (0.87)	<LOD	2.17 (0.98)	1.69 (1.03)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
1,2,3,7,8-PeCDD	5.88 (1.71)	5.84 (2.28)	4.97 (1.52)	4.78 (2.22)	4.67 (1.45)	4.96 (2.04)	0.65 (0.26)	0.80 (0.59)	0.54 (0.21)	0.85 (0.46)	0.48 (0.83)	0.92 (0.80)	0.36 (0.39)	0.40 (0.46)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
1,2,3,6,7,8-HxCDD	22.40 (9.22)	27.36 (11.55)	23.30 (9.01)	26.09 (11.07)	23.40 (7.89)	26.80 (10.68)	0.60 (0.24)	0.57 (0.48)	0.58 (0.22)	0.84 (0.50)	0.36 (0.62)	0.15 (0.47)	1.48 (0.81)	1.66 (1.25)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
1,2,3,7,8,9-HxCDD	2.87 (1.74)	4.27 (2.54)	2.36 (1.19)	3.44 (2.28)	2.85 (1.29)	4.26 (2.57)	8.84 (2.55)	8.59 (4.02)	8.47 (2.35)	8.86 (3.64)	4.07 (1.11)	3.81 (0.79)	23.50 (7.84)	21.93 (9.09)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
1,2,3,4,6,7,8-HpCDD	12.70 (3.66)	17.74 (7.17)	8.19 (3.86)	10.31 (5.18)	5.92 (3.45)	8.75 (5.32)	2.70 (0.75)	3.00 (1.64)	2.37 (0.74)	2.84 (1.41)	0.96 (1.33)	0.75 (1.21)	6.16 (2.14)	6.15 (2.88)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
OCDD	3040.00 (245.86)	3036.36 (621.73)	618.00 (627.90)	448.18 (143.79)	469.00 (99.27)	476.00 (153.78)	3.15 (0.86)	3.57 (1.87)	2.38 (0.71)	2.98 (1.37)	0.94 (1.30)	0.77 (1.24)	3.95 (1.42)	4.05 (2.36)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
							<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
<b>PCDFs</b>																						2,3,7,8-TeCDF	0.90 (0.72)	1.34 (0.92)	0.65 (0.26)	0.80 (0.59)	0.54 (0.21)	0.85 (0.46)	1.43 (0.64)	1.82 (0.74)	1.73 (0.77)	1.17 (0.48)	0.47 (1.05)	<LOD	0.65 (0.89)	0.92 (1.07)	1,2,3,7,8-PeCDF	0.64 (0.68)	1.20 (0.74)	0.60 (0.24)	0.57 (0.48)	0.58 (0.22)	0.84 (0.50)	1.82 (0.43)	1.76 (0.65)	0.98 (0.37)	1.17 (0.48)	0.47 (1.05)	<LOD	1.49 (1.43)	1.84 (1.86)	2,3,4,7,8-PeCDF	8.41 (2.31)	9.07 (4.39)	8.84 (2.55)	8.59 (4.02)	8.47 (2.35)	8.86 (3.64)	0.32 (0.20)	0.11 (0.16)	<LOD	0.03 (0.08)	<LOD	0.99 (1.22)	0.99 (1.22)	1,2,3,4,7,8-HxCDF	3.63 (0.85)	3.43 (2.44)	2.70 (0.75)	3.00 (1.64)	2.37 (0.74)	2.84 (1.41)	0.24 (0.39)	0.16 (0.35)	0.04 (0.13)	0.16 (0.35)	<LOD	<LOD	0.32 (1.01)	0.73 (1.63)	1,2,3,6,7,8-HxCDF	3.83 (0.97)	4.24 (1.91)	3.15 (0.86)	3.57 (1.87)	2.38 (0.71)	2.98 (1.37)									1,2,3,7,8,9-HxCDF	<LOD <sup>b</sup>	<LOD	<LOD	<LOD	<LOD	<LOD									2,3,4,6,7,8-HxCDF	0.53 (1.16)	1.18 (1.45)	1.43 (0.64)	1.82 (0.74)	1.73 (0.77)	1.17 (0.48)									1,2,3,4,6,7,8-HpCDF	2.00 (1.16)	1.89 (1.30)	1.82 (0.43)	1.76 (0.65)	0.98 (0.37)	1.17 (0.48)									1,2,3,4,7,8,9-HpCDF	<LOD	<LOD	0.32 (0.20)	0.11 (0.16)	<LOD	0.03 (0.08)									OCDF	<LOD	<LOD	0.24 (0.39)	0.16 (0.35)	0.04 (0.13)	0.16 (0.35)									<b>Non-ortho-Co-PCBs</b>																						3,4,4',5-TeCB (#81)	6.39 (2.24)	8.23 (3.83)	5.84 (4.31)	5.05 (2.90)	4.91 (1.65)	4.88 (2.71)									3,3',4,4'-TeCB (#77)	64.60 (12.62)	69.46 (19.15)	44.50 (6.50)	20.91 (8.11)	21.30 (7.89)	22.90 (7.61)									3,3',4,4',5-PeCB (#126)	36.50 (17.49)	53.55 (34.99)	22.50 (22.29)	58.46 (34.89)	44.70 (21.67)	59.50 (31.92)									3,3',4,4',5,5'-HxCB (#169)	29.00 (10.44)	32.64 (16.04)	46.40 (13.81)	41.46 (14.26)	33.70 (9.21)	35.90 (11.87)									<b>Mono-ortho-Co-PCBs</b>																						2',3',4,4',5-PeCB (#123)	131.80 (54.59)	183.09 (105.22)	128.50 (73.68)	165.18 (132.64)	131.70 (62.90)	184.90 (110.10)									2',3',4,4',5-PeCB (#118)	7390.00 (3806.27)	9500.00 (6197.74)	8640.00 (4816.68)	10127.27 (6729.06)	8510.00 (4533.69)	10540.00 (6361.03)									2',3',3',4,4'-PeCB (#105)	1750.00 (732.201)	2400.00 (1638.29)	2046.00 (1070.33)	2563.64 (1801.82)	1935.00 (985.46)	2630.00 (1728.87)									2,3,4,4',5-PeCB (#114)	505.00 (241.07)	549.09 (390.37)	757.00 (383.18)	685.46 (438.46)	692.00 (355.71)	691.00 (412.67)									2,3',4,4',5,5'-HxCB (#167)	880.00 (436.40)	1035.46 (654.24)	1158.00 (588.84)	1208.18 (748.61)	970.00 (528.58)	1126.00 (604.98)									2,3,3',4,4',5-HxCB (#156)	2900.00 (1339.15)	2963.64 (1881.10)	4630.00 (2064.00)	4063.64 (2317.01)	3770.00 (1895.64)	3620.00 (2031.86)									2,3,3',4,4',5'-HxCB (#157)	757.00 (370.20)	749.09 (469.14)	1047.00 (464.93)	919.09 (498.37)	907.00 (428.25)	875.00 (448.83)									2,3,3',4,4',5,5'-HpCB (#189)	270.00 (107.60)	290.91 (162.14)	439.00 (167.50)	403.64 (193.92)	264.00 (92.28)	297.00 (124.19)									<b>Total PCDDs</b>	3084.8 (243.7)	3093.1 (627.2)	657.9 (632.3)	493.9 (153.5)	506.9 (97.6)	521.9 (161.6)									<b>Total PCDFs</b>	19.9 (5.3)	22.4 (11.3)	19.7 (5.0)	20.4 (9.3)	16.6 (4.5)	19.4 (7.7)									<b>Total Co-PCBs</b>	14720.3 (6673.7)	17835.1 (10992.1)	18964.3 (8976.2)	20262.0 (12259.4)	17284.3 (8359.6)	20087.1 (10995.7)									<b>PCDDs/DFs/Co-PCBs</b>	17825.0 (6568.1)	20950.5 (11004.7)	19642.3 (9386.6)	20776.2 (12372.8)	17807.8 (8349.2)	20628.5 (11104.4)									<b>Total TEQ levels</b>	21.93 (7.48)	25.67 (12.36)	23.25 (7.71)	24.63 (11.78)	21.97 (7.21)	24.98 (10.60)																																																																																																																																							
2,3,7,8-TeCDF	0.90 (0.72)	1.34 (0.92)	0.65 (0.26)	0.80 (0.59)	0.54 (0.21)	0.85 (0.46)	1.43 (0.64)	1.82 (0.74)	1.73 (0.77)	1.17 (0.48)	0.47 (1.05)	<LOD	0.65 (0.89)	0.92 (1.07)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
1,2,3,7,8-PeCDF	0.64 (0.68)	1.20 (0.74)	0.60 (0.24)	0.57 (0.48)	0.58 (0.22)	0.84 (0.50)	1.82 (0.43)	1.76 (0.65)	0.98 (0.37)	1.17 (0.48)	0.47 (1.05)	<LOD	1.49 (1.43)	1.84 (1.86)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
2,3,4,7,8-PeCDF	8.41 (2.31)	9.07 (4.39)	8.84 (2.55)	8.59 (4.02)	8.47 (2.35)	8.86 (3.64)	0.32 (0.20)	0.11 (0.16)	<LOD	0.03 (0.08)	<LOD	0.99 (1.22)	0.99 (1.22)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
1,2,3,4,7,8-HxCDF	3.63 (0.85)	3.43 (2.44)	2.70 (0.75)	3.00 (1.64)	2.37 (0.74)	2.84 (1.41)	0.24 (0.39)	0.16 (0.35)	0.04 (0.13)	0.16 (0.35)	<LOD	<LOD	0.32 (1.01)	0.73 (1.63)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
1,2,3,6,7,8-HxCDF	3.83 (0.97)	4.24 (1.91)	3.15 (0.86)	3.57 (1.87)	2.38 (0.71)	2.98 (1.37)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
1,2,3,7,8,9-HxCDF	<LOD <sup>b</sup>	<LOD	<LOD	<LOD	<LOD	<LOD																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2,3,4,6,7,8-HxCDF	0.53 (1.16)	1.18 (1.45)	1.43 (0.64)	1.82 (0.74)	1.73 (0.77)	1.17 (0.48)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
1,2,3,4,6,7,8-HpCDF	2.00 (1.16)	1.89 (1.30)	1.82 (0.43)	1.76 (0.65)	0.98 (0.37)	1.17 (0.48)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
1,2,3,4,7,8,9-HpCDF	<LOD	<LOD	0.32 (0.20)	0.11 (0.16)	<LOD	0.03 (0.08)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
OCDF	<LOD	<LOD	0.24 (0.39)	0.16 (0.35)	0.04 (0.13)	0.16 (0.35)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>Non-ortho-Co-PCBs</b>																						3,4,4',5-TeCB (#81)	6.39 (2.24)	8.23 (3.83)	5.84 (4.31)	5.05 (2.90)	4.91 (1.65)	4.88 (2.71)									3,3',4,4'-TeCB (#77)	64.60 (12.62)	69.46 (19.15)	44.50 (6.50)	20.91 (8.11)	21.30 (7.89)	22.90 (7.61)									3,3',4,4',5-PeCB (#126)	36.50 (17.49)	53.55 (34.99)	22.50 (22.29)	58.46 (34.89)	44.70 (21.67)	59.50 (31.92)									3,3',4,4',5,5'-HxCB (#169)	29.00 (10.44)	32.64 (16.04)	46.40 (13.81)	41.46 (14.26)	33.70 (9.21)	35.90 (11.87)									<b>Mono-ortho-Co-PCBs</b>																						2',3',4,4',5-PeCB (#123)	131.80 (54.59)	183.09 (105.22)	128.50 (73.68)	165.18 (132.64)	131.70 (62.90)	184.90 (110.10)									2',3',4,4',5-PeCB (#118)	7390.00 (3806.27)	9500.00 (6197.74)	8640.00 (4816.68)	10127.27 (6729.06)	8510.00 (4533.69)	10540.00 (6361.03)									2',3',3',4,4'-PeCB (#105)	1750.00 (732.201)	2400.00 (1638.29)	2046.00 (1070.33)	2563.64 (1801.82)	1935.00 (985.46)	2630.00 (1728.87)									2,3,4,4',5-PeCB (#114)	505.00 (241.07)	549.09 (390.37)	757.00 (383.18)	685.46 (438.46)	692.00 (355.71)	691.00 (412.67)									2,3',4,4',5,5'-HxCB (#167)	880.00 (436.40)	1035.46 (654.24)	1158.00 (588.84)	1208.18 (748.61)	970.00 (528.58)	1126.00 (604.98)									2,3,3',4,4',5-HxCB (#156)	2900.00 (1339.15)	2963.64 (1881.10)	4630.00 (2064.00)	4063.64 (2317.01)	3770.00 (1895.64)	3620.00 (2031.86)									2,3,3',4,4',5'-HxCB (#157)	757.00 (370.20)	749.09 (469.14)	1047.00 (464.93)	919.09 (498.37)	907.00 (428.25)	875.00 (448.83)									2,3,3',4,4',5,5'-HpCB (#189)	270.00 (107.60)	290.91 (162.14)	439.00 (167.50)	403.64 (193.92)	264.00 (92.28)	297.00 (124.19)									<b>Total PCDDs</b>	3084.8 (243.7)	3093.1 (627.2)	657.9 (632.3)	493.9 (153.5)	506.9 (97.6)	521.9 (161.6)									<b>Total PCDFs</b>	19.9 (5.3)	22.4 (11.3)	19.7 (5.0)	20.4 (9.3)	16.6 (4.5)	19.4 (7.7)									<b>Total Co-PCBs</b>	14720.3 (6673.7)	17835.1 (10992.1)	18964.3 (8976.2)	20262.0 (12259.4)	17284.3 (8359.6)	20087.1 (10995.7)									<b>PCDDs/DFs/Co-PCBs</b>	17825.0 (6568.1)	20950.5 (11004.7)	19642.3 (9386.6)	20776.2 (12372.8)	17807.8 (8349.2)	20628.5 (11104.4)									<b>Total TEQ levels</b>	21.93 (7.48)	25.67 (12.36)	23.25 (7.71)	24.63 (11.78)	21.97 (7.21)	24.98 (10.60)																																																																																																																																																																																																																																																																																																																		
3,4,4',5-TeCB (#81)	6.39 (2.24)	8.23 (3.83)	5.84 (4.31)	5.05 (2.90)	4.91 (1.65)	4.88 (2.71)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
3,3',4,4'-TeCB (#77)	64.60 (12.62)	69.46 (19.15)	44.50 (6.50)	20.91 (8.11)	21.30 (7.89)	22.90 (7.61)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
3,3',4,4',5-PeCB (#126)	36.50 (17.49)	53.55 (34.99)	22.50 (22.29)	58.46 (34.89)	44.70 (21.67)	59.50 (31.92)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
3,3',4,4',5,5'-HxCB (#169)	29.00 (10.44)	32.64 (16.04)	46.40 (13.81)	41.46 (14.26)	33.70 (9.21)	35.90 (11.87)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>Mono-ortho-Co-PCBs</b>																						2',3',4,4',5-PeCB (#123)	131.80 (54.59)	183.09 (105.22)	128.50 (73.68)	165.18 (132.64)	131.70 (62.90)	184.90 (110.10)									2',3',4,4',5-PeCB (#118)	7390.00 (3806.27)	9500.00 (6197.74)	8640.00 (4816.68)	10127.27 (6729.06)	8510.00 (4533.69)	10540.00 (6361.03)									2',3',3',4,4'-PeCB (#105)	1750.00 (732.201)	2400.00 (1638.29)	2046.00 (1070.33)	2563.64 (1801.82)	1935.00 (985.46)	2630.00 (1728.87)									2,3,4,4',5-PeCB (#114)	505.00 (241.07)	549.09 (390.37)	757.00 (383.18)	685.46 (438.46)	692.00 (355.71)	691.00 (412.67)									2,3',4,4',5,5'-HxCB (#167)	880.00 (436.40)	1035.46 (654.24)	1158.00 (588.84)	1208.18 (748.61)	970.00 (528.58)	1126.00 (604.98)									2,3,3',4,4',5-HxCB (#156)	2900.00 (1339.15)	2963.64 (1881.10)	4630.00 (2064.00)	4063.64 (2317.01)	3770.00 (1895.64)	3620.00 (2031.86)									2,3,3',4,4',5'-HxCB (#157)	757.00 (370.20)	749.09 (469.14)	1047.00 (464.93)	919.09 (498.37)	907.00 (428.25)	875.00 (448.83)									2,3,3',4,4',5,5'-HpCB (#189)	270.00 (107.60)	290.91 (162.14)	439.00 (167.50)	403.64 (193.92)	264.00 (92.28)	297.00 (124.19)									<b>Total PCDDs</b>	3084.8 (243.7)	3093.1 (627.2)	657.9 (632.3)	493.9 (153.5)	506.9 (97.6)	521.9 (161.6)									<b>Total PCDFs</b>	19.9 (5.3)	22.4 (11.3)	19.7 (5.0)	20.4 (9.3)	16.6 (4.5)	19.4 (7.7)									<b>Total Co-PCBs</b>	14720.3 (6673.7)	17835.1 (10992.1)	18964.3 (8976.2)	20262.0 (12259.4)	17284.3 (8359.6)	20087.1 (10995.7)									<b>PCDDs/DFs/Co-PCBs</b>	17825.0 (6568.1)	20950.5 (11004.7)	19642.3 (9386.6)	20776.2 (12372.8)	17807.8 (8349.2)	20628.5 (11104.4)									<b>Total TEQ levels</b>	21.93 (7.48)	25.67 (12.36)	23.25 (7.71)	24.63 (11.78)	21.97 (7.21)	24.98 (10.60)																																																																																																																																																																																																																																																																																																																																																																																																				
2',3',4,4',5-PeCB (#123)	131.80 (54.59)	183.09 (105.22)	128.50 (73.68)	165.18 (132.64)	131.70 (62.90)	184.90 (110.10)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2',3',4,4',5-PeCB (#118)	7390.00 (3806.27)	9500.00 (6197.74)	8640.00 (4816.68)	10127.27 (6729.06)	8510.00 (4533.69)	10540.00 (6361.03)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2',3',3',4,4'-PeCB (#105)	1750.00 (732.201)	2400.00 (1638.29)	2046.00 (1070.33)	2563.64 (1801.82)	1935.00 (985.46)	2630.00 (1728.87)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2,3,4,4',5-PeCB (#114)	505.00 (241.07)	549.09 (390.37)	757.00 (383.18)	685.46 (438.46)	692.00 (355.71)	691.00 (412.67)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2,3',4,4',5,5'-HxCB (#167)	880.00 (436.40)	1035.46 (654.24)	1158.00 (588.84)	1208.18 (748.61)	970.00 (528.58)	1126.00 (604.98)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2,3,3',4,4',5-HxCB (#156)	2900.00 (1339.15)	2963.64 (1881.10)	4630.00 (2064.00)	4063.64 (2317.01)	3770.00 (1895.64)	3620.00 (2031.86)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2,3,3',4,4',5'-HxCB (#157)	757.00 (370.20)	749.09 (469.14)	1047.00 (464.93)	919.09 (498.37)	907.00 (428.25)	875.00 (448.83)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
2,3,3',4,4',5,5'-HpCB (#189)	270.00 (107.60)	290.91 (162.14)	439.00 (167.50)	403.64 (193.92)	264.00 (92.28)	297.00 (124.19)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>Total PCDDs</b>	3084.8 (243.7)	3093.1 (627.2)	657.9 (632.3)	493.9 (153.5)	506.9 (97.6)	521.9 (161.6)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>Total PCDFs</b>	19.9 (5.3)	22.4 (11.3)	19.7 (5.0)	20.4 (9.3)	16.6 (4.5)	19.4 (7.7)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>Total Co-PCBs</b>	14720.3 (6673.7)	17835.1 (10992.1)	18964.3 (8976.2)	20262.0 (12259.4)	17284.3 (8359.6)	20087.1 (10995.7)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>PCDDs/DFs/Co-PCBs</b>	17825.0 (6568.1)	20950.5 (11004.7)	19642.3 (9386.6)	20776.2 (12372.8)	17807.8 (8349.2)	20628.5 (11104.4)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
<b>Total TEQ levels</b>	21.93 (7.48)	25.67 (12.36)	23.25 (7.71)	24.63 (11.78)	21.97 (7.21)	24.98 (10.60)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														

<sup>a</sup> Mean (SD).<sup>b</sup> Mean value below the limit of detection.

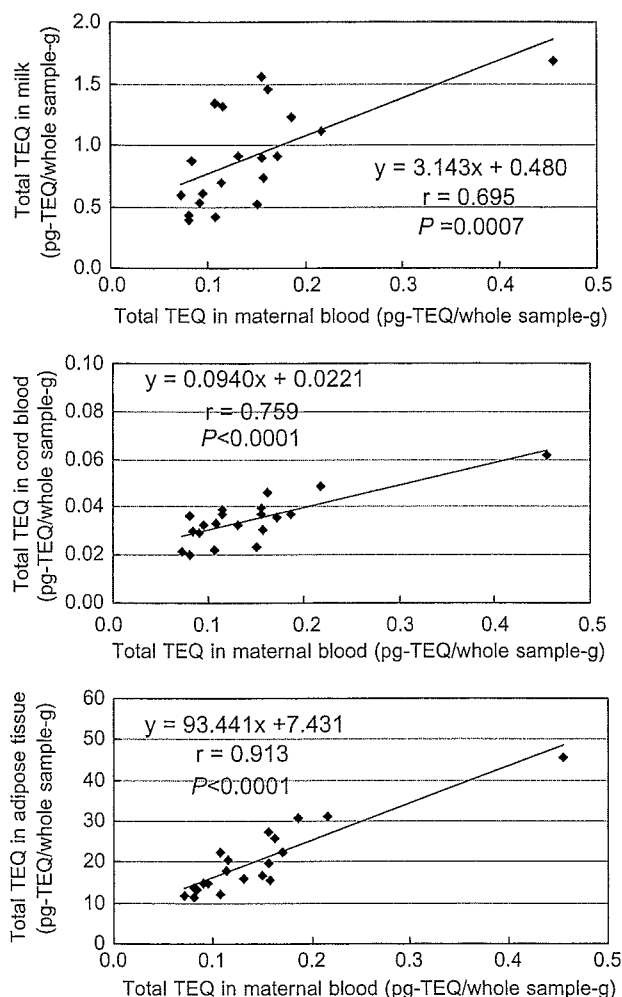


Fig. 1. Correlations between total TEQ in maternal blood and breast milk, maternal blood and adipose tissue, and maternal and cord blood, in a subset of the Control group ( $n = 20$ ).

the *Chlorella* group than in the Control group ( $0.43 \pm 0.07\%$  vs.  $0.54 \pm 0.09\%$ ;  $0.31 \pm 0.05\%$  vs.  $0.37 \pm 0.03\%$ ; and  $2.70 \pm 0.96\%$  vs.  $3.85 \pm 0.97\%$ , respectively; Mann–Whitney  $U$ -test,  $P < 0.05$  for all). In view of these reduced levels in lipid-containing samples, dioxin concentrations and TEQ in the *Chlorella* and control were compared on a whole sample-basis.

Total TEQ in breast milk was lower in the *Chlorella* group ( $0.643 \pm 0.387$  pg-TEQ/whole sample-g) than in the Control group ( $0.909 \pm 0.406$  pg-TEQ/whole sample-g; Mann–Whitney  $U$ -test,  $P = 0.0113$ ) (Table 4).

$C_p/C_{mb}$  (whole sample-basis) for PCDFs and PCDDs, excluding Co-PCBs, was higher in the *Chlorella* group than in the Control group (Mann–Whitney  $U$ -test,  $P = 0.0034$ ) (Fig. 3). Moreover,  $C_{cb}/C_p$  (whole sample-basis) for PCDDs and PCDFs tended to be lower in the *Chlorella* group than in the Control group (Mann–Whitney  $U$ -test,  $P = 0.0617$ ) (Fig. 3). No difference in  $C_{cb}/C_{mb}$  (whole sample-basis) was seen between groups (Mann–Whitney  $U$ -test,  $P = 0.2172$ ) (Fig. 3). In

addition, differences between the Control and *Chlorella* groups were observed in correlations between concentrations of PCDDs, PCDFs and Co-PCBs in cord blood and those in maternal blood (Table 5).

#### 4. Discussion

The first objective of this study was to survey the extent of exposure to PCDDs, PCDFs and Co-PCBs in ordinary mothers and their children in Japan, and to assess maternal-fetal distribution and transfer of dioxins.

Strong correlations were observed between total TEQ in maternal blood and total TEQ in maternal adipose tissue, reflecting the amount of dioxins that have accumulated in the bodies of mothers. Strong correlations were also seen between total TEQ in maternal blood and those in cord blood and breast milk. Concentrations of dioxins in maternal blood thus not only offer a predictor of dioxins levels that have accumulated in the body, but also seem to offer clues to understanding the amounts of dioxins transferred from the mother to the fetus or nursing infant. The linear regression equation introduced in this study should prove useful for predicting dioxin concentrations in breast milk and cord blood based on concentrations in maternal blood.

Various studies have reported measurements of dioxin levels in human tissues. In a study similar to the present, dioxin levels were measured in maternal blood, adipose tissue, milk, placenta and cord blood in five pregnant American women. In this study, mean TEQ in adipose tissue samples was 11.6 pg-TEQ/lipid-g (range: 8.17–15.62 pg-TEQ/lipid-g). In our study, mean TEQ in adipose tissue samples was 24.0 pg-TEQ/lipid-g (range: 13.29–51.87 pg-TEQ/lipid-g), slightly higher than reported by Schechter et al. (1998). Another study reported dioxin levels in the adipose tissue of two adults. In that study, 2,3,7,8-tetraCCD levels in abdominal adipose tissue samples were 5.7 and 7.4 pg/lipid-g. In our study, mean 2,3,7,8-tetraCCD level in adipose tissue samples was 1.0 pg/lipid-g (range: 0.55–2.30 pg/lipid-g), somewhat lower than the levels reported by Ryan et al. (1985).

The placenta serves to transfer nutrients, oxygen and endogenous bioactive substances from the mother to the fetus, and to excrete waste products produced by the fetus via the mother. An additional function is as a barrier to chemical and toxic substances; the so-called the placental barrier. The present results revealed that dioxin concentrations in cord blood (lipid-basis) were about 56% lower than in maternal blood. This was considered largely attributable to the placenta limiting the transport of lipids, although the placenta may not function as a complete barrier to dioxins. Transplacental transfer of dioxins was also investigated based on  $C_{cb}/C_{mb}$  ratio (lipid-basis). The results showed that  $C_{cb}/C_{mb}$  (lipid-basis) was 0.1 for

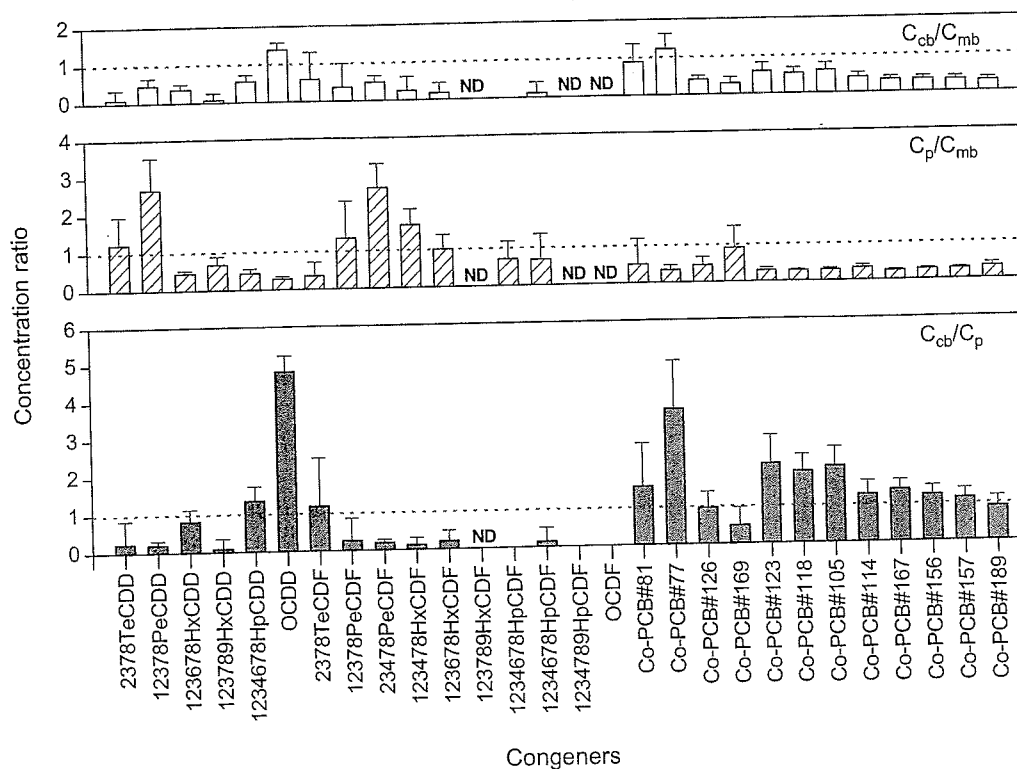


Fig. 2. Concentration ratios for dioxin congeners between maternal blood and placenta and cord blood in a subset of the Control group ( $n = 21$ ). Calculated for congeners detected in maternal blood or placenta samples. Mean (SD), ND: Congener not detected in maternal blood or placenta;  $C_p$ : concentration in placenta (pg/lipid-g);  $C_{mb}$ : concentration in maternal blood (pg/lipid-g);  $C_{cb}$ : Concentration in cord blood (pg/lipid-g).

2,3,7,8-tetraCDD, 0.06 for 1,2,3,7,8,9-hexaCDD, 1.38 for octaCDD, and 1.23 for 3,3',4,4'-tetraCB (IUPAC #77). These differences suggest that differences in the transplacental transfer of dioxins exist depend on the particular congener in question. The form in which a dioxin is present in the blood (such as binding to serum proteins, etc.) can differ depending on the congener (Piper et al., 1973; Patterson et al., 1989), and this is one possible cause of these differences. Research has also shown that 2,3,7,8-tetraCDD binds strongly to arylhydrocarbon (Ah) receptors, which are present in the placenta in addition to the liver and other tissues (Manchester et al., 1987; Nakai and Bunce, 1995). When WHO-TEF values were established for dioxin isomers, affinity to Ah receptors was established as one of the criteria (Van den Berg et al., 1998). Functioning of the placenta itself, including the expression of Ah receptors, may also represent a contributing factor. Moreover, as cytochrome P450 is also expressed in placenta, differences between isomers in receptivity to drug metabolizing enzymes may also affect transplacental transfer. Changes in placental function due to 2,3,7,8-tetraCDD exposure were reported in a study conducted in rats (Ishimura et al., 2002). Research relating to the transfer of environmental contaminants such as dioxins to the fetus, including effects on placental function, is expected to continue in the future. The results of

this study suggest the possibility that intake of *Chlorella* supplements may modify the transfer of dioxins. However, the physiological effects and mechanisms of action of *Chlorella* are as yet unclear, and warrant future investigation.

Studies of dioxin concentrations in breast milk are underway in many countries (Alder et al., 1994; González et al., 1996; Pöpke et al., 1996; Paumgarten et al., 2000). Recently, dioxin contamination in breast milk has decreased compared to past levels in Japan and other industrialized countries (survey of the Ministry of Health, Labour and Welfare Japan, 2001). The results of this study very closely approximate those of the 1998 and 1999 surveys conducted by the Japanese Ministry of Health, Labour and Welfare (2001), indicating that levels of dioxin contamination in breast milk have not changed greatly over the last few years. A survey of breast- and formula-fed infants (Abraham et al., 1996) found that at 1 month after birth, 2 breast-fed infants had ingested 82.2 and 106.2 g-TEQ/kg-body weight/day of PCDDs and PCDFs respectively, while 1 formula-fed infant had ingested 2.1 pg-TEQ/kg-body weight/day. Breast-fed infants also reportedly ingest about 50-fold greater quantities of PCDDs and PCDFs as formula-fed infants in the year after birth. Quantities of dioxins ingested by infants throughout nursing were

Table 4  
Total concentrations (pg/whole sample-g) of PCDDs, PCDFs, and Co-PCBs and total TEQ (pg-TEQ/whole sample-g) in maternal and cord blood, adipose tissue, breast milk and placenta in the Control and *Chlorella* groups

	Control group					<i>Chlorella</i> group				
	Blood (n = 21)	Adipose tissue (n = 21)	Milk (n = 20)	Cord blood (n = 21)	Placenta (n = 21)	Blood (n = 23)	Adipose tissue (n = 12)	Milk (n = 23)	Cord blood (n = 23)	Placenta (n = 23)
Total PCDDs	16.33 (1.11) <sup>a</sup>	470.39 (369.48)	18.84 (3.56)	15.41 (0.66)	14.74 (0.75)	14.91 (1.02) <sup>*</sup>	358.26 (51.25)	17.91 (2.99)	14.69 (0.78) <sup>*</sup>	14.65 (0.89)
Total PCDFs	0.12 (0.07)	16.60 (6.48)	0.70 (0.29)	0.03 (0.01)	0.64 (0.26)	0.10 (0.07)	13.30 (6.17)	0.59 (0.34)	0.03 (0.02)	0.54 (0.23)
Total Co-PCBs	91.17 (68.91)	16241.09 (9006.86)	720.17 (406.00)	25.05 (7.00)	69.69 (28.53)	82.86 (42.46)	12971.67 (5472.17)	525.66 (411.76) <sup>*</sup>	28.45 (7.67)	75.19 (26.43)
PCDDs/DFs/ Co-PCBs	108.45 (70.10)	16728.08 (9188.90)	739.70 (408.61)	40.49 (7.09)	85.08 (29.08)	97.88 (42.95)	13343.23 (5511.43)	544.16 (413.22) <sup>*</sup>	43.17 (7.67)	90.38 (26.78)
Total TEQ	0.134 (0.083)	19.815 (8.466)	0.909 (0.406)	0.035 (0.010)	0.565 (0.216)	0.129 (0.060)	16.295 (6.078)	0.643 (0.387) <sup>*</sup>	0.035 (0.011)	0.521 (0.225)

<sup>\*</sup> P < 0.05.

<sup>a</sup> Mean (SD).

calculated from total TEQ in the breast milk of study participants. Dioxin intake by nursing infants was estimated at approximately 107 pg-TEQ/kg body weight/day, assuming a mean breast milk intake of 120 ml/kg body weight/day (Matsueda et al., 1993). This value is more than 20-fold greater than the WHO recommended tolerable daily intake (TDI) of 1–4 pg-TEQ/kg body weight/day (Rolaf van Leeuwen et al., 2000). Although not restricted to the nursing period, the dioxin load in the bodies of infants nursing on breast milk is believed to be considerably higher than dioxin levels in adults.

The second objective of this study was to investigate the possibility of reducing maternal transfer of dioxins through maternal dietary improvement. In this study, total TEQ in puerperal breast milk were approximately 30% lower in mothers who took *Chlorella* tablets. With regard to promoting the excretion of lipophilic contaminants, several studies of dietary supplements such as cholestyramine, mineral oil, hexadecane, and dietary fiber have been reported using laboratory animals (Boylan et al., 1978; Rozman et al., 1981; Rozman et al., 1982; Morita et al., 1997). In addition, Moser et al. reported the enhancing effect of non-absorbable lipid substitute olestra on fecal excretion of PCDD/DFs and PCBs in the body in humans (Geusau et al., 1999; Moser and McLachlan, 1999). In these studies, the suppression of absorption and reabsorption due to the competitive sorption in the digestive tract are thought to represent mechanisms by which excretion of lipophilic contaminants such as dioxins is promoted. The primary mechanism by which *Chlorella* promotes excretion of dioxins is presumably through the formation of complexes between dioxins and the chlorophyll in *Chlorella* (Negishi et al., 1989). Dioxins are then absorbed by the dietary fiber also contained in *Chlorella* (Morita et al., 1995), thereby inhibiting the absorption and reabsorption of dioxins from the gastrointestinal tract. In addition, *Chlorella* supplements reportedly reduce serum cholesterol levels in hyperlipidemic patients (Fujiwara et al., 1990). As the reduction of lipid-containing quantities of breast milk is a direct cause of decreases in dioxin concentrations, intake of *Chlorella* may affect lipid dynamics, including the transfer of lipids to mammary gland.

The results of the present study confirm that the quantity of dioxins transferred to fetuses and nursing infants is closely correlated to dioxin concentrations in maternal adipose tissue, or, in other words, cumulative quantities of dioxins in the body. The latent risks of environmental contaminants such as dioxins may well be appearing in the form of symptoms such as ADHD and LD in children, who are more sensitive than adults to chemical substances and toxins. To thoroughly understand this causal relationship, the results of detailed long-term studies are awaited. At present, parallel measures should also be pursued that reduce, even if only slightly, the health risks

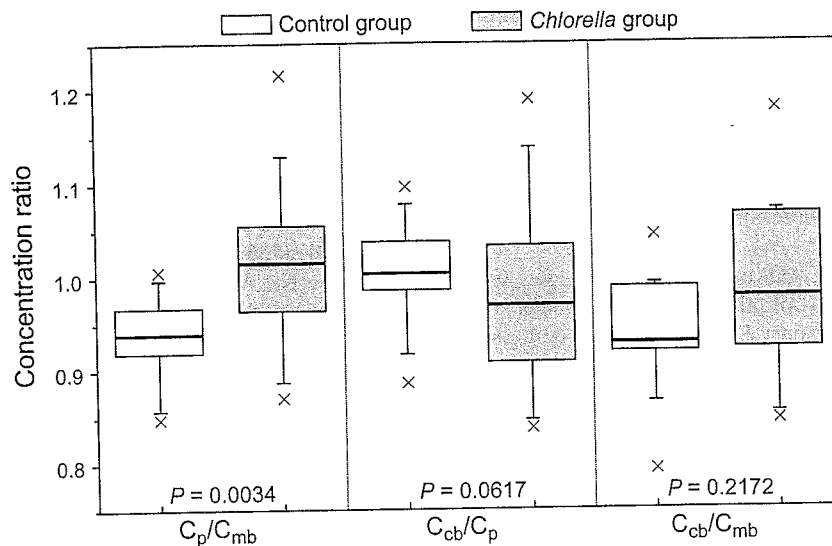


Fig. 3.  $C_p/C_{mb}$ ,  $C_{cb}/C_p$  and  $C_{cb}/C_{mp}$  of PCDD/DFs in subsets of the Control group ( $n = 21$ ) and *Chlorella* group ( $n = 23$ ).  $C_p$ : Concentration (pg/whole sample-g) in placenta of PCDD/DFs;  $C_{mb}$ : concentration (pg/whole sample-g) in maternal blood of PCDD/DFs;  $C_{cb}$ : concentration (pg/whole sample-g) in cord blood of PCDD/DFs;  $\times$  = outlier; dark line = median; box = interquartile range; bars = non-outlier range (5–95%).

Table 5

The correlation coefficients between the concentration of PCDDs, PCDFs, and Co-PCBs in cord blood and that in maternal blood from the Control and *Chlorella* groups

	PCDDs	PCDFs	Non-ortho-PCBs	Mono-ortho-PCBs
<i>Control (whole sample-basis)</i>				
Correlation coefficient	0.562	0.655	0.737	0.717
P-value	0.010	0.002	0.001	0.001
<i>Chlorella (whole sample-basis)</i>				
Correlation coefficient	0.255	0.308	-0.013	0.381
P-value	0.240	0.153	0.953	0.073

Spearman's correlation coefficient by rank test.

posed by environmental contaminants such as dioxins to fetuses and nursing infants. Dietary measures represent one of the most realistic ways to accomplish this. Our results suggest that *Chlorella* supplementation may be effective for reducing dioxin exposure. Studies that illuminate the actual health risks of environmental contaminants such as dioxins, and which also make effective use of the functionality of food for reducing exposure to these environmental contaminants, will become even more important in the future.

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