ELSEVIER

Journal of Hazardous Materials A121 (2005) 1–10

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in primipara breast milk from Taiwan: estimation of dioxins and furans intake for breastfed infants☆

How-Ran Chao[a, b], Shu-Li Wang[a, c, *], Pen-Hua Su[d, f], Hui-Yen Yu[a], Sheng-Tsung Yu[a], Olaf Päpke[e]

[a] *Division of Environmental Health and Occupational Medicine, National Health Research Institute, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan*
[b] *Department of Environmental and Safety Engineering, Chung Hwa College of Medical Technology, Rende, Tainan County, Taiwan*
[c] *Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung, Taiwan*
[d] *Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan*
[e] *ERGO Research Laboratory, Hamburg, Germany*
[f] *Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan*

Received 20 April 2004; received in revised form 22 September 2004; accepted 29 September 2004
Available online 26 February 2005

Abstract

Postnatal exposure to dioxins in breastfed infants occurs mainly during breast-feeding. The exposure to a substantial amount of endocrine disruptors in the early days of life may cause long-term health effects. Test subjects were healthy and primiparous mothers with a mean age of 28 (S.D. = 3.8) in 2001. The PCDD/F congeners were analyzed in the breast milk using gas chromatograph/high resolution mass spectrometry. The mean level of PCDD/Fs was 7.4 pg-WHO-TEQ/g lipid, which is significantly lower than the level found in individuals from other countries. The total PCDD WHO-TEQ levels in breast milk had a significant positive association with maternal age and a slightly negative association with perinatal BMI (body mass index of the period before and after the delivery). The estimated daily intake of 10.5 pg-WHO-TEQ/kg/day from individual breast milk was predicted for a breastfed infant at 6 months of age with proper assumption of 8 kg body weight, 854 g milk per day of consumption, 95% of dioxin absorption rate, and linear decline of dioxin during lactation. Based on the lower WHO-TEQ levels in the breast milk, breast-feeding should still be encouraged and continued in Taiwan.

Keywords: Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Breast milk; Breast-feeding; Infant exposure

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are lipophilic endocrine disruptors [1] with high persistence to biodegradation in the environment [2]. PCDD/Fs are undesirable by-products and are inadvertently released into the environment during a variety of industrial and thermal processes, such as emission of waste incineration, smelting of metals, production of organochlorine pesticides, and bleaching of pulp [3]. These dioxin compounds persist in the environment, enter the food chain, and eventually accumulate in the fatty tissues of animals including humans.

The major source of PCDD/Fs is food. Postnatal exposure to dioxin compounds in breastfed infants has occurred mainly through breast-feeding [4–6]. Although it was demonstrated that the obviously lower levels of WHO-TEQs in cord blood compared to maternal blood at birth, the levels in infants' blood were found to be 1.5–3.6 times higher than those

☆ The Scientific content of this manuscript has been reviewed and approved for publication by the Division of Environmental Health and Occupational Medicine of the National Health Research Institutes. Approval for publication does not necessarily signify that the content reflects the view and policies of the DEHOM/NHRI, or condemnation or endorsement and recommendation for use on this issue presented.

* Corresponding author. Tel.: +886 7 312 6772x4015/4014; fax: +886 7 322 1912/3595.
E-mail address: slwang@nhri.org.tw (S.-L. Wang).

0304-3894/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2004.09.021

in maternal blood at the end of the first lactation year [6]. Elevated levels of dioxin were reported to alter the thyroid hormone status of breastfed infants [7]. Therefore, maternal breast milk containing significant levels of PCDD/Fs may not only pose risks to the mother, but can also pose potential serious health risks in infants, such as altering the pattern of differentiation of cells of the immune system, as well as risk to cognitive development, such as spatial learning/memory and motor deficits [4].

High levels of PCDD/Fs were found in samples of breast milk from Kazakhstan, Russia, Belgium, and The Netherlands [8–11]. In our previous study [12], we reported the levels of dioxin-like and indicator PCBs in breast milk from Taiwan. Data on the dioxin levels of human milk and epidemiological factors in the Taiwanese population have yet to be established.

Breast-feeding has several significant advantages. The immunological benefits, it confers on infants, help them experience less respiratory and ear infections. Children who were breastfed for a longer duration were reported to have higher levels of parental attachment and tended to perceive their mothers as more care and less overprotection toward them compared with formula-fed children [13]. The Health Department of Taiwan and of other countries follow the World Health Organization (WHO) guidelines to encourage breastfeeding.

Based on the fact that PCDD/Fs toxic equivalent (TEQ) levels in human milk from primipara mothers were significantly higher than those from multipara mothers [14–16], our present study focused on primipara mothers in central Taiwan to determine the PCDD/Fs TEQ levels in human breast milk. We examined the associated factors with PCDD/Fs TEQ levels and estimated the infant's daily intake of dioxins.

2. Materials and methods

2.1. Study population

From December 2000 to November 2001, we surveyed pregnant women who had infants without clinical complications. The women were recruited from a medical center in central Taiwan that serves local residents [17]. The participants answered detailed questionnaires in the obstetric clinic, including their age, parity, health status, dietary habit before and after pregnancy, and possible exposure through occupational and non-occupational contact, such as smoking and pesticide use. General physical parameters, including height and weight, were measured by trained nurses. If the women agreed to participate in our study and follow our collection instructions, particularly for human milk collection, we carefully checked that they had never breastfed an infant. When the total volume of collected milk they donated was more than 90 ml, we considered them to be subjects in the study. Finally, 30 samples of primipara human milk were selected for the analyses of dioxins, based on sufficient milk samples, first parity, and collection of other three specimens, placenta, cord blood, and maternal venous blood.

This study was reviewed by the Human Ethical Committee of National Health Research Institutes in Taiwan. The ethical standards formulated in the Helsinki Declaration of 1964 (revised in 2000) were followed. Each of the participants provided their informed consent after hearing detailed explanations of the study and any possible consequences.

2.2. Sample collection and analysis

Milk samples were collected approximately 2 weeks after delivery. Breast milk collection protocol followed the WHO third round procedure [18]. In brief, mothers were given three carefully decontaminated bottles (Pyrex glass) with Teflon lining caps to collect the milk samples. The women were asked to keep the bottles with collected milk in their home freezer until 90–120 ml human milk were collected. The sample was taken to our laboratory and stored at −20 °C. Milk samples of 50 ml were packaged in chemically clean containers and shipped frozen to ERGO Laboratory in Germany for the analysis.

The analytical methods used in the present study were well established and previously published [19]. Briefly, $^{13}C_{12}$-PCDD/Fs were used as an internal standard and were added to 25 g of these samples prior to the extraction. After performing the gravimetric lipid determination, the clean up was done on a multicolumn system (carbon-glass fiber, combination, and alumina-column). The 17 congener-specific 2,3,7,8-substituted PCDD/Fs were analyzed and quantified by high resolution gas chromatograph equipped with high resolution mass spectrometry (HP GC5890 Series II/ VG-AutoSpec) after clean up and capillary column (DB-5) separation. Two isotope masses were measured for each component. The quantification was carried out using internal/external standard mixtures by the isotope dilution method.

The coefficient of variation (CV) of concentrations in the blind samples was less than 15% for 90% of all analyzed congeners. Higher CV values were found for OCDD (24%) and 1,2,3,4,6,7,8-HpCDF (22%). There were larger variations, higher concentrations, and lower toxicity equivalency factors (TEFs) for these two congeners. CV for PCDD/F WHO-TEQ levels was less than 1% because of smaller variations of the congeners with higher TEFs. A control sample, which included a standard, blank, and milk pool samples, was added into every 10 samples of human milk in each batch of samples to verify the accuracy and precision of each measurement. Blank results averaged at 0.06 pg-WHO-TEQ/g lipid. Quality assurance measurements in this study included regular checks of instrument blanks and quality control samples (e.g., milk pools), daily calibration verification tests, and method performance checks by analyzing control samples of known PCDD/F concentrations. Limit of detection (LOD) was calculated according to PCDD/F concentration ranged from 0.01 pg/g lipid to 10 pg/g lipid, which belonged to lower scales (<0.1 pg/g lipid, i.e., 1,2,3,7,8-PeCDD), mid-

Table 1
General demographic and lifestyle characteristics of pregnant women and their infants

Subject characteristics	Mean (S.D.) and percentage	Range
Mothers ($n = 30$)		
Age (years)	27.8 (3.8)	20–35
Pre-pregnant BMI (kg/m^2)	20.4 (2.67)	17.6–25.8
Perinatal BMI at 36–38th week[a] (kg/m^2)	26.4 (2.71)	22.4–33.3
ΔBMI[b] (kg/m^2)	5.97 (1.47)	3.58–10.9
Giving birth a male infant (%)	50.0	–
Change of dietary habit after pregnancy (%)	30.0	–
Smoker (%)	6.67	–
Daily passive smoker (%)	43.3	
Potential dioxin-exposed occupation		
Farm (%)	3.3	–
Incinerator (%)	6.7	–
Chemical or other factories (%)	0.0	–
Non-occupational exposure (%)	90.0	–
Living near factories (<5 km) (%)	40.0	–
Use of pesticide more than once a week (%)	73.3	–
Infants ($n = 30$)		
Gestational age (weeks)	39 (1.47)	35–42
Birth weight (g)	3140 (278)	2400–3700
Birth length (cm)	51.3 (2.13)	48.0–54.0
Head circumference (cm)	33.1 (1.19)	30.5–35.0
Chest circumference (cm)	33.0 (1.10)	29.0–35.0
Milk feeding in perinatal period		
Breast-feeding (%)	23.3	–
Partial breast-feeding (%)	76.7	–
Lipid content of the milk (%)	2.92 (1.27)	0.890–5.79

[a] Perinatal BMI: body mass index of the period before and after the delivery.
[b] ΔBMI = perinatal BMI − pre-pregnant BMI.

dle scales (0.1–1.0 pg/g lipid, i.e., 2,3,7,8-TCDD), and larger scales (>1.0 pg/g lipid, OCDF) depending on the specific PCDD/F congener. We make sure that such LOD did not affect the total TEQ value at one decimal level. The recovery of the ^{13}C-labelled internal standards was in the range of 70–130%. The original concentrations were corrected by the recovery rate of the internal standards. ERGO laboratory has regularly and successfully participated in inter-laboratory studies, e.g., PCDD/Fs in chicken, butter, and fish (e.g., National Institute of Public Health, Norway in 2000).

2.3. Estimation of dioxins intake

The dioxin daily intake was calculated for the breastfed infants from birth until 6 months of age based on infant's body weight, breast-milk's weight per day of consumption, dioxin absorption rate, and decline of dioxin duration lactation. The decline of dioxin levels in human milk, dioxin absorption rate, birth weight, and infant body weight were taken into consideration. The prediction of the decline in dioxin levels from maternal milk was based on a one-compartment first-order pharmacokinetic model, the dioxin-TEQ levels decreased linearly by 50% after 6 months [20]. According to the infant's growth curve from the Department of Health in Taiwan, we assumed an infant weighing 8 kg was approximately 6 months of age [21]. The dioxin absorption rate in the intestinal tract was 95% [22]. Exposure to dioxins in a breastfed infant was estimated using the WHO-suggested nursing duration of 6 months and 854 g/day of milk consumption [23].

2.4. Statistical analysis

Measurements below LOD were set to zero in this study. WHOs toxicity equivalency factors were used to calculate PCDD/F TEQ levels [24]. Pearson correlation coefficients were calculated based on normal distributions of the data. Stepwise linear regression analysis was utilized to examine significant predictors of mothers' and infants' factors for total dioxin TEQ levels. Student's *t*-test was used for comparisons between groups (i.e., living near or away from the associated factories). The statistical analyses were performed using the Statistical Package for Social Science (SPSS) 10.0 Version.

3. Results

3.1. Demographic characteristics

The general characteristics of mothers and infants are summarized in Table 1. Maternal age ranged from 20 to 35 years (mean = 27.8 years, S.D. = 3.8). The increment of body mass index (ΔBMI) was from 3.6 to 10.9 kg/m^2 (mean = 6.0 kg/m^2) comparing perinatal BMI (mean = 26.4 kg/m^2) to pre-pregnant BMI

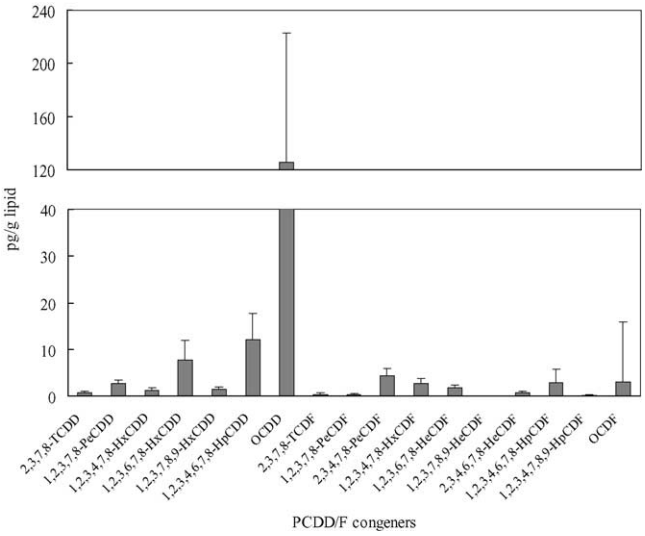

Fig. 1. The distribution of 2,3,7,8-substituted PCDD/F congeners in human milk (pg/g lipid).

(mean = 20.4 kg/m^2). The lipid contents of 30 primiparous milk samples ranged between 0.9% and 5.8% and their mean value was 2.9% (S.D. = 1.3). Data on maternal lifestyles, infant's gender, and milk feeding pattern are also summarized in Table 1. Smokers (n = 2) and passive smokers (n = 13) comprised 50% of our subjects. Twelve mothers lived near factories (40%) and most of our participants used pesticide mostly at home more than once a week for killing mosquitoes (n = 22, 73.3%). Breastfed and partially breastfed (mix of breast feeding and formula-feeding) infants were 23.3% and 76.7%, respectively. None of the subjects were vegetarians nor drank alcohol.

3.2. PCDD/Fs WHO-TEQ levels in breast milk and comparisons with other populations

Most of the PCDD/F concentrations were above LOD except for 2,3,4,6,7,8-HxCDF (0% >LOD), 1,2,3,4,7,8,9-HpCDF (23.3% >LOD), and OCDF (30% >LOD) (Fig. 1). The mean WHO-TEQ level of PCDD/F congeners for human milk samples was 7.4 pg-WHO-TEQ/g lipid (S.D. = 2.4) in Table 2. The predominant congeners were 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF comprising 36% and 30% of the total PCDD/F WHO-TEQ levels, respectively. The total WHO-TEQs of PCDDs (4.6 pg-WHO-TEQ/g lipid) was approximately 1.6 times of those of PCDFs (2.8 pg-WHO-TEQ/g lipid) in our study. For specific PCDDs and PCDFs WHO-TEQ levels, 1,2,3,7,8-PeCDD contributed 58% of the total PCDDs and 2,3,4,7,8-PeCDF contributed most (78%) of the total PCDFs. The WHO-TEQ level of the most toxic compound, 2,3,7,8-TCDD, was only 0.73 pg-WHO-TEQ/g lipid contributing 10% of the total PCDD/F WHO-TEQs.

Recent data on breast milk have been reported in other studies from Asian and European countries, including Korea, Japan, Sweden, Spain, Kazakhstan, Belgium, Samara Region, Russia, and Russian Siberia [8–10,16,25–28]. Those data are summarized in Table 3 using WHOs TEFs [24]. The WHO-TEQ levels of PCDDs and PCDFs in primiparous mother breast milk investigated from central Taiwan were significantly lower than those reported from Korea,

Table 2
Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans levels (pg-WHO-TEQ/g lipid) in 30 primipara human milk samples

Congener	TEFs	>LOD[a] (%)	Mean (S.D.)	Range
PCDDs				
2,3,7,8-TCDD	1.0	96.7	0.732 (0.314)	<0.001[b]–1.53
1,2,3,7,8-PeCDD	1.0	100	2.65 (0.851)	1.53–4.83
1,2,3,4,7,8-HxCDD	0.1	100	0.128 (0.051)	0.060–0.295
1,2,3,6,7,8-HxCDD	0.1	100	0.779 (0.407)	0.251–1.85
1,2,3,7,8,9-HxCDD	0.1	100	0.138 (0.064)	0.061–0.341
1,2,3,4,6,7,8-HpCDD	0.01	100	0.121 (0.057)	0.039–0.289
OCDD	0.0001	100	0.013 (0.010)	0.003–0.037
PCDFs				
2,3,7,8-TCDF	0.1	70.0	0.042 (0.032)	<0.001–0.110
1,2,3,7,8-PeCDF	0.05	96.7	0.021 (0.009)	<0.001–0.045
2,3,4,7,8-PeCDF	0.5	100	2.18 (0.773)	1.13–4.59
1,2,3,4,7,8-HxCDF	0.1	100	0.277 (0.094)	0.126–0.470
1,2,3,6,7,8-HxCDF	0.1	100	0.179 ± 0.054	0.101–0.281
2,3,4,6,7,8-HxCDF	0.1	0.0	<0.001	<0.001
1,2,3,7,8,9-HxCDF	0.1	96.7	0.074 (0.029)	<0.001–0.129
1,2,3,4,6,7,8-HpCDF	0.01	100	0.030 (0.028)	0.010–0.167
1,2,3,4,7,8,9-HpCDF	0.01	23.3	0.001 (0.003)	<0.001–0.008
OCDF	0.0001	30.0	0.0003 (0.0012)	<0.001–0.007
Total WHO-TEQs for PCDDs		99.5	4.56 (1.59)	2.35–8.86
Total WHO-TEQs for PCDFs		71.7	2.81 (0.952)	1.54–5.71
Total WHO-TEQs		86.3	7.37 (2.40)	4.03–13.4

[a] Limit of detection.
[b] <0.001 = lower than LOD.

Table 3
Comparisons of WHO-TEQ levels in human milk in the recent studies (pg-WHO-TEQ/g lipid)

	Primipara						Primipara and multipara		
	Taiwan/30/2001–2002/20–36[a]	Korea/8/1997/24–38	Japan/299/1998–2000/21–40	Sweden/29/1997–1999/21–41	Spain/15/1996/25–35	Kazak[b]/41/1997/23 ± 5[c]	Belgium/20/2000–2001/26–38	Samara/40/1997–1998/22[d]	Siberia/18/1998/NS[e]
PCDDs (%)									
2,3,7,8	0.732	0.12	1.54	0.92	1.04	34.5	2.26	23.2	3.48
1,2,3,7,8	2.65	0.92	6.3	2.55	4.02	9.27	8.34	7.88	2.54
1,2,3,4,7,8	0.128	0.42	0.20	0.13	0.28	0.16	0.80	0.28	<0.001
1,2,3,6,7,8	0.779	2.79	1.9	1.01	2.79	0.077	2.91	2.65	0.44
1,2,3,7,8,9	0.138	0.6	0.36	0.22	0.455	NS	0.47	0.43	0.10
1,2,3,4,6,7,8	0.121	0.54	0.11	0.18	0.383	0.13	0.27	0.10	0.039
OCDD	0.013	0.7	0.007	0.009	0.015	0.003	0.02	0.04	<0.001
PCDFs (%)									
2,3,7,8	0.042	1.42	0.008	0.04	0.068	0.014	0.13	0.19	0.32
1,2,3,7,8	0.021	0.08	0.003	0.01	0.016	0.005	0.04	0.45	0.06
2,3,4,7,8	2.18	6.58	6.8	3.37	3.98	2.91	12.58	3.37	13.1
1,2,3,4,7,8	0.277	1.35	0.46	0.15	0.306	0.212	0.54	1.7	<0.001
1,2,3,6,7,8	0.179	1.65	0.58	0.12	0.249	0.236	0.65	1.13	3.58
1,2,3,7,8,9	<0.001	2.12	NS	<0.01	0.004	0.037	0.01	0.79	<0.001
2,3,4,6,7,8	0.074	0.63	0.34	0.06	0.1	0.12	0.32	0.03	0.10
1,2,3,4,6,7,8	0.03	0.75	0.023	0.02	0.02	NS	0.05	0.02	<0.001
1,2,3,4,7,8,9	0.001	0.12	NS	<0.01	0.001	NS	NS	<0.001	<0.001
OCDF	0.0003	0.03	NS	0.009	<0.01	NS	NS	<0.001	<0.001
PCDDs (%)	4.56	6.09	10.4	5.02	8.98	44.1	15.1	34.6	6.61
PCDFs (%)	2.81	14.7	8.25	3.78	4.74	3.53	14.3	7.68	17.2
Total WHO-TEQs	7.37	20.8	18.7	8.80	13.7	47.6	29.4	42.3	23.8
PCDD/PCDF	1.62	0.41	1.26	1.33	1.89	12.5	1.05	4.51	0.38

[a] Component/number/sampling year/age.
[b] Kazakhstan rural area.
[c] Mean ± S.D.
[d] Mean.
[e] Data not shown.

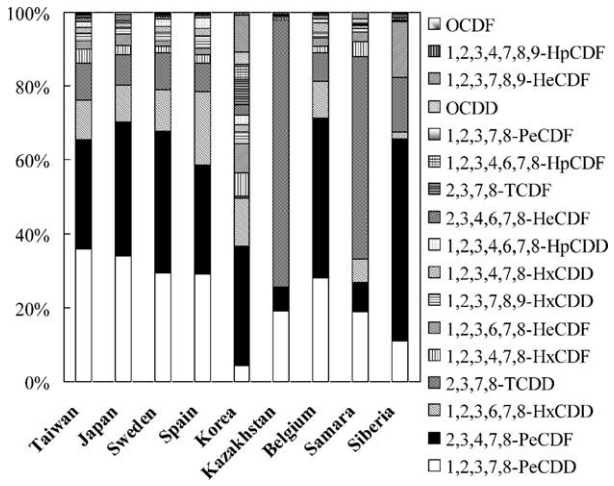

Fig. 2. The congener distributions of PCDD/F TEQs in breast milk from different countries.

Japan, Sweden, Spain, and Kazakhstan. Our result was also lower than the level of dioxin-TEQs in primiparous and multiparous milk from Belgium, Samara Region, and Siberia. The congener distributions of PCDD/F-TEQs in breast milk of the different populations were shown in Fig. 2.

3.3. The associated factors with PCDD/F levels

Highly significant Pearson correlation coefficients among PCDD/F congeners were found in breast milk in Table 4. The highest coefficient in WHO-TEQ levels was found between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF ($r = 0.93$, $P < 0.001$). This finding of high correlation between TCDD and the other PCDD/Fs levels is consistent with those reported by Hooper et al. [29]. For the congeners of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, and 2,3,4,7,8-PeCDF with the highest WHO-TEQ levels, high correlations ($r = 0.52$–0.82) were found. Furthermore, the congener of 1,2,3,7,8-PeCDD being the highest WHO-TEQ level was correlated the best ($r = 0.57$–0.86) with the individual PCDD/F congener. We found that the WHO-TEQ levels of 2,3,7,8-substituted pentachlorinated PCDD/F congeners had a higher association with other PCDD/F WHO-TEQ levels in human milk from Taiwan.

The WHO-TEQ levels of 1,2,3,6,7,8-HxCDD ($r = 0.51$, $P = 0.008$), 1,2,3,7,8,9-HxCDD ($r = 0.43$, $P = 0.019$), and 1,2,3,4,6,7,8-HpCDD ($r = 0.047$, $P = 0.011$) were found to be positively and significantly correlated with mother's age by Pearson correlation analyses. The levels of 1,2,3,7,8-PeCDD had slightly negative correlations with pre-pregnant ($r = -0.39$, $P = 0.035$) and perinatal ($r = -0.41$, $P = 0.024$) BMI. The PCDD WHO-TEQ level was only negatively associated with perinatal BMI ($r = -0.37$, $P = 0.045$). No correlation was found between any PCDF congener WHO-TEQ level and mother's age, mother's BMI, and lipid content. In breast milk of individuals in Kazakhstan, one PCDD and five PCDFs levels were positively correlated with mother's

Table 5
Significant predictors for PCDDs, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD by stepwise linear regression analysis

	β	Standard error	R^2 change	P-value	Adjusted R^2
PCDDs					
Age	0.145	0.070	0.118	0.048	0.199
Perinatal BMI	−0.213	0.098	0.136	0.038	
1,2,3,6,7,8-HxCDD					
Age	0.058	0.016	0.258	0.001	0.340
Pre-pregnant BMI	−0.055	0.023	0.127	0.025	
1,2,3,7,8,9-HxCDD					
Age	0.008	0.003	0.181	0.008	0.243
Lipid content	−0.018	0.008	0.114	0.047	

Table 4
Significant Pearson correlation coefficients ($P < 0.05$) between WHO-TEQ levels of PCDD/F congeners in human milk ($n = 30$)

	PCDD						Total PCDDs	PCDF	
	2,3,7,8	1,2,3,7,8	1,2,3,4,7,8	1,2,3,6,7,8	1,2,3,7,8,9	1,2,3,4,6,7,8		2,3,4,7,8	1,2,3,4,7,8
PCDDs									
2,3,7,8									
1,2,3,7,8	0.797***								
1,2,3,4,7,8	0.764***	0.860***							
1,2,3,6,7,8	0.601***	0.774***	0.771***						
1,2,3,7,8,9	0.606***	0.767***	0.835***	0.886***					
1,2,3,4,6,7,8	0.408*	0.570**	0.639***	0.737***	0.748***				
PCDF									
2,3,4,7,8	0.663***	0.823***	0.664***	0.519**	0.496**	0.434*	0.762***		
1,2,3,4,7,8	0.574**	0.725***	0.662***	0.545**	0.562**	0.634**	0.709***	0.860***	
1,2,3,6,7,8	0.680***	0.830***	0.722***	0.600***	0.631***	0.629***	0.805***	0.894***	0.930***
Total PCDFs	0.676***	0.822***	0.672***	0.517**	0.510**	0.460**	0.765***		

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

age [29]. There was no relationship between the lipid content of human milk and the birth weight of infant to PCDD/Fs WHO-TEQs in breast milk of Taiwanese individuals.

It is noteworthy that the WHO-TEQ levels of 1,2,3,4,6,7,8-HpCDD ($r = -0.49$, $P = 0.002$) and 1,2,3,7,8,9-HxCDD ($r = -0.34$, $P = 0.047$) were negatively correlated with lipid content after adjustment of mother's age. Total PCDDs ($r = -0.37$, $P = 0.038$ for perinatal BMI) and 1,2,3,6,7,8-HxCDD ($r = -0.36$, $P = 0.025$ for pre-pregnant BMI) adjusted with the maternal age were slightly associated with mother's BMI.

The significant results of the WHO-TEQ levels of total PCDDs, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD by the analysis of stepwise linear regression are shown in Table 5. Mother's perinatal BMI and age were slightly associated with total PCDDs, which explained 19.9% of the total variances. Mother's age and pre-pregnant BMI were significantly predictive for 1,2,3,6,7,8-HxCDD WHO-TEQs with the overall model adjusted $R^2 = 0.34$. Mother's age explained the more variance indicated by R^2 change = 0.26. Mother's age and lipid content in breast milk were significant regression predictors of the WHO-TEQ levels of 1,2,3,7,8,9-HxCDD and 1,2,3,4,6,7,8-HpCDD explaining 24.3% and 42.3% of the variance, respectively.

There was no significant difference in the WHO-TEQ levels of human milk from women with potential occupational exposure (6.2 pg-WHO-TEQ/g lipid) and in those without occupational exposure (7.5 pg-WHO-TEQ/g lipid, $P = 0.55$). The number of potentially exposed workers was small: one working in the farm and the other two working in the small incinerators. We also found no significant differences between mothers living near factories (<5 km) (7.3 pg-WHO-TEQ/g lipid) and away from factories (≥5 km) (7.4 pg-WHO-TEQ/g lipid, $P = 0.96$), using pesticide to kill insects at home more than once a week (7.0 pg-WHO-TEQ/g lipid) and never using pesticide (8.3 pg-WHO-TEQ/g lipid, $P = 0.56$). No significant difference in WHO-TEQ levels of PCDD/Fs from breast milk between nonsmokers (7.39 pg-WHO-TEQ/g lipid) and smokers or passive smokers (7.35 pg-WHO-TEQ/g lipid, $P = 0.76$) was observed in our study.

3.4. Daily intake of PCDD/Fs for infants

Within 2 weeks of birth, the measured PCDD/F daily intake of a newborn would be 31.8 pg-WHO-TEQ/kg/day, calculating from the birth weight of an infant, the milk consumption of 150 ml/kg weight, PCDD/F WHO-TEQ levels of individual maternal milk, and 95% dioxin absorption rate. The estimated PCDD/Fs dietary intake of a breastfed infant should be 10.5 pg-WHO-TEQ/kg/day (range 3.21–24.7 pg-WHO-TEQ/kg/day) at 6 months of age. The daily dioxin intake for a breastfed infant would decrease to 67% during a lactation period of 6 months.

4. Discussion

The dioxin-WHO-TEQ levels of breast milk in the present study were lower than those listed in Table 3. This may be due to the lower levels of chlorinated congeners with the highest TEFs including 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF in the present study. Congener distribution of PCDD/Fs in Taiwan was similar to those in Japan, Sweden, and Spain (Fig. 2). However, the distribution differed from those in Kazakhstan, Samara Region, and Siberia where the highest TCDD WHO-TEQ levels were found. There were also studies showing that the former Soviet Union elevated TCDD levels in breast milk [30,31]. The WHO-TEQ levels of the total PCDDs contributed 61.9% of the total PCDD/Fs WHO-TEQ levels in our study. According to a previous study [32], the WHO-TEQ levels of the total PCDDs in most countries varied greatly, ranging from 50.0% to 66.7% of the total PCDD/Fs WHO-TEQ levels. Our value of 61.9% was within this normal range.

Comparing dioxin levels in human milk among East Asian countries, the PCDD/F WHO-TEQ levels in Korean and Japanese studies were 2.82 and 2.54 times higher than those found in Taiwan. There were less published reports for congener-specific data from Korea. The general profiles of congener of WHO-TEQ levels in the Japanese [15,25,33] were found to be quite similar to those in our study. This may be because of the similarity between the Taiwanese and Japanese diet. Fish, shellfish, and dairy products are more polluted with PCDD/Fs than other foodstuffs in Japan and Taiwan [34,35]. The consumption of fish and shellfish by Japanese is relatively higher than that by the Europeans, American, and other Asians. In addition, Japanese people consume dairy products more than Taiwanese people. The daily intake of dietary fat among Japanese people was approximately 85 g, and Taiwanese daily intake level of dietary fat was 80 g for man and 61 g for women, respectively [36,37]. The mean daily dietary intake of PCDD/Fs in Taiwanese was assessed to be 0.45 pg-WHO-TEQ/kg/day as compared to the Japanese daily PCDD/Fs intake of 0.90 pg-WHO-TEQ/kg/day from the foods of animal origin, mainly fish, shellfish, dairy products, meat, and eggs [34,35]. The lower dioxin-WHO-TEQ levels in Taiwan may be due to lower intake rate of fish, shellfish, and dairy products and a lower level of these contaminants in food compared to Japan.

Among the industrialized countries listed in Table 3, Taiwan had the lowest PCDD/F WHO-TEQ level in human milk with comparable sampling year and subjects' age. This is probably due to the lower animal fat and small amounts of dairy products in the Taiwanese diet. This may also be due to the fact that lower WHO-TEQ levels in breast milk compared to European and East Asian countries is because of low bioaccumulative levels in the environment probably from the shortened duration of the use of incinerators and severe emission standard of PCDD/Fs in Taiwan.

Age was positively correlated with body burden of PCDD/F WHO-TEQ levels by means of linear regression analyses [38]. The age-dependency of PCDD/Fs WHO-TEQs was not obvious in our subjects. The slight correlation of maternal age and PCDD WHO-TEQ levels in human milk was found due to the small variation of maternal ages (20–35 years old), the shorter periods of bioaccumulation, and the smaller sampling size ($n = 30$). In a Finnish study [14], the PCDD/Fs I-TEQs in primiparous milk were modeled by an equation where slight effects, such as lipid content and pre-pregnant BMI, and stronger effects, such as maternal age and the consumption of fish, were taken into account. We believe that maternal age, lipid content, and BMI would affect the TEQ levels of total PCDDs, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD.

The WHO-TEQ levels of dioxins in human milk among incinerator workers and residents near chemical factories were found to be not higher than those of the general public in our present study. This may be explained by the fact that food, mainly fish, meat, milk and dairy products, is the main source of PCDD/Fs in human [5]. Air exposure may not affect human accumulation levels to a significant amount. In the present study, the subjects who often sprayed pesticide without organochlorinated compounds at home did not directly come in contact with organochlorinated pesticides and, therefore, it is not probable that they were exposed to dioxins from using pesticide. There was no significant difference in dioxin-WHO-TEQs from breast milk between nonsmokers and smokers or passive smokers in our study. This observation was consistent with those in another study [14]. It mainly resulted from only two women smoking less than 4 cigarettes/day and most of passive smokers being nonsmokers.

The daily intake of dioxins for an infant was evaluated at birth (31.8 pg-WHO-TEQ/kg/day) and at 6 month of age (10.5 pg-WHO-TEQ/kg/day), respectively. These two values were obviously lower than those in Japanese (121 pg I-TEQ/kg/day for a newborn) [15], Spanish (43.3 pg I-TEQ/kg/day for 5 kg weight of infant) [27], Belgian (76 pg-WHO-TEQ/kg/day for a 7 kg infant) [10], and Kazakhstan (50.1 pg I-TEQ/kg/day for 6 kg weight of infant) reports [31]. The estimated daily intake for breastfed infants in four studies were overestimated because the absorption rate of dioxin in a breastfed infant and the decline in dioxin exposure levels in maternal milk duration was not taken into account. Our estimated dioxin intake at the age of 6 months was obviously low compared to the result of Korean study calculating at the first year (85 pg-WHO-TEQ/kg/day) [16]. The decline of daily dioxin intake for a breastfed infant is due to the decline of dioxin concentration in maternal milk and increase of infant body weight [16,20]. However, our estimated level was still higher than the tolerable daily intake (1–4 pg I-TEQ/kg/day) for adults according to the WHO [39].

The proportion of exclusively breastfed infants after birth is approximately 50.9%, but decreases to 25.3% after 1 month of age, according to figures from the Bureau of Health Promotion, Department of Health in Taiwan. Based on the WHO recommendation, breast-feeding is still the best food source for an infant even though high contamination of PCDD/Fs has been found in various countries [40]. Our study has shown relatively much lower levels compared to other countries. Consequently, breast-feeding can help infants receive essential elements and immunological antibodies for their health and development. We also have recommended promotion and continuation of breast-feeding based on the lower daily dioxin intake for infants in Taiwan.

Our present study is valuable and important because it is the first detailed report of PCDD/Fs levels in human milk in individuals in Taiwan. Based on the results of this study, the regression model will probably be better if the frequency of fish consumption is considered and a larger sampling size is used. The TEF of dioxins is mainly based on the aryl hydrocarbon (Ah) receptor reactivity, however, not all health effects are related to the Ah receptor. In Taiwanese individuals' breast milk, some congeners with lower TEFs have elevated levels, and their health impacts are yet to be investigated and followed. Continuous surveillance of PCDD/Fs, PCBs, and organochlorinated pesticide levels in human milk is necessary. According to the lower levels of PCB-WHO-TEQ in the previous study [12] and dioxin-WHO-TEQ in our present study from Taiwanese human milk, the promotion and continuation of breast-feeding by Taiwanese mothers should be recommended. Persistent organic pollutants contaminated in human milk were also related to maternal body burden, which might transfer to fetus via placenta during the crucial stage of differentiation. Our future attention will focus on infant exposure to other persistent organic pollutants, such as organochlorinated pesticides and non-dioxin-like polychlorinated biphenyls, via human milk.

5. Conclusions

First, we have established the WHO-TEQ levels of PCDD/Fs in human milk from Taiwan which were significantly lower compared to the results from Asian and European studies. The reason was probably due to low dioxin daily intake in Taiwanese diet with consideration of decreasing trend of dioxin levels over time. Second, the WHO-TEQ levels of PCDDs in breast milk were significantly and positively associated with maternal age, but slightly and negatively associated with maternal perinatal BMI. Third, there were no significant differences in dioxin-WHO-TEQ levels of human milk among potential occupational and non-occupational exposure, the residents living near factories and away from factories, spraying pesticide at home and never using pesticide, and smokers/passive smokers and nonsmokers. Fourth, the estimated PCDD/Fs dietary intake of a breastfed infant in our study was obviously lower than those in other studies. This value was still higher than the tolerable daily intake for adults based on the WHOs guideline. Our findings could suppose

the promotion and encourage of breast-feeding in the policy of Taiwanese Department of Health.

Acknowledgments

This research was supported by a grant (EO-092-pp-05 and EO-093-pp-01) from the National Health Research Institutes (NHRI), Taiwan. The authors also acknowledge ERGO Forschungsgesellschaft mbH in Germany for their assistance in the PCDD/Fs analysis.

References

[1] L.S. Birnbaum, Developmental effects of dioxins and related endocrine disrupting chemicals, Toxicol. Lett. 82–83 (1995) 743–750.
[2] A.G. Smith, S.D. Gangolli, Organochlorine chemicals in seafood: occurrence and health concerns, Food Chem. Toxicol. 40 (2002) 767–779.
[3] R.E. Alcock, R. Gemmill, K.C. Jones, Improvements to UK PCDD/PCDF and PCB atmospheric emission inventory following an emission measurement programme, Chemosphere 38 (1999) 759–770.
[4] G. Lindström, K. Hooper, M. Petreas, R. Stephens, A. Gilman, Workshop on perinatal exposure to dioxin-like compounds. I. Summary, Environ. Health Perspect. 103 (1995) 135–142.
[5] M. Huisman, S.E.J. Eerenstein, C. Koopman-Esseboom, M. Brouwer, V. Fidler, F.A.J. Muskiet, P.J.J. Sauer, E.R. Boersma, Perinatal exposure to polychlorinated biphenyls and dioxins through dietary intake, Chemosphere 31 (1995) 4273–4287.
[6] K. Abraham, O. Päpke, A. Gross, O. Kordonouri, S. Wiegand, U. Wahn, H. Helge, Time course of PCDD/PCDF/PCB concentrations in breast-feeding mothers and their infants, Chemosphere 37 (1998) 1731–1741.
[7] C. Koopman-Essebom, D.C. Morse, N. Weisglas-Kuperus, I.J. Lutkeschipholt, C.G. Van Der Paauw, L.G.M.T. Tuinstra, A. Brouwer, P.J.J. Sauer, Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants, Pediatr. Res. 36 (1994) 468–473.
[8] K. Hooper, T. Chuvakova, G. Kazbekova, D. Hayward, A. Tulenova, M.X. Petreas, T.J. Wade, K. Benedict, Y.-Y. Cheng, J. Grassman, Analysis of breast milk to assess exposure to chlorinated contaminants in Kazakhstan: sources of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposures in an agricultural region of southern Kazakhstan, Environ. Health Perspect. 107 (1999) 447–457.
[9] B. Revich, E. Aksel, T. Ushakova, I. Ivanova, N. Zhuchenko, N. Klyuev, B. Brodsky, Y. Sotskov, Dioxin exposure and public health in Chapaevsk, Russ. Chemosph. 43 (2001) 951–966.
[10] J.-F. Focant, C. Pirard, C. Thielen, E. De Pauw, Levels and profiles of PCDDs, PCDFs and cPCBs in Belgian breast milk. Estimation of infant intake, Chemosphere 48 (2002) 763–770.
[11] S.D. Soechitram, S.M. Chan, E.A.S. Nelson, A. Brouwer, P.J.J. Sauer, Comparison of dioxin and PCB concentrations in human breast milk samples from Hong Kong and The Netherlands, Food Addit. Contam. 20 (2003) 65–69.
[12] H.R. Chao, S.L. Wang, L.Y. Lin, H.Y. Yu, Y.K. Lu, W.L. Chou, Y.L. Guo, L.W. Chang, Polychlorinated biphenyls in Taiwanese primipara human milk and associated factors, Bull. Environ. Contam. Toxicol. 70 (2003) 1097–1103.
[13] D.M. Fergusson, L.J. Woodward, Breast-feeding and later psychosocial adjustment, Paediatr. Perinat. Epidemiol. 13 (1999) 144–157.
[14] T. Vartiainen, J.J.K. Jaakkola, S. Saarikoski, J. Tuomisto, Birth weight and sex of children and correlation to the body burden of PCDDs/PCDFs and PCBs of Mother, Environ. Health Perspect. 106 (1998) 61–66.
[15] T. Iida, H. Hirakawa, T. Matsueda, S. Takenaka, J. Nagayama, Polychlorinated dibenzo-*p*-dioxins and related compounds in breast milk of Japanese primiparas and multiparas, Chemosphere 38 (1999) 2461–2466.
[16] J. Yang, D. Shin, S. Park, Y. Chang, D. Kim, M.G. Ikonomou, PCDDs, PCDFs, and PCBs concentrations in breast milk from two areas in Korea: body burden of mothers and implications for feeding infants, Chemosphere 46 (2002) 419–428.
[17] S.-L. Wang, Y.L. Guo, C.-Y. Lin, H.-Y. Yu, W.-L. Chou, Y.-K. Lu, L.-Y. Lin, L.W. Chang, Body burden of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls in pregnant women and their infants—correlation between prenatal and postnatal exposure to infants, Organohalogen Compd. 55 (2002) 255–258.
[18] World Health Organization, PCDDs and PCDFs in Human Milk: Protocol for Third Round of Exposure Studies, World Health Organization European Centre for Environment and Health, Geneva, 2000, pp. 5–18.
[19] O. Päpke, M. Ball, Z.A. Lis, K. Scheunert, PCDD/PCDF in whole blood samples of unexposured persons, Chemosphere 19 (1989) 941–948.
[20] M. Lorber, L. Phillips, Infant exposure to dioxin-like compounds in breast milk, Environ. Health Perspect. 110 (2002) 325–332.
[21] Department of Health, Handbook of Nutrition and Care in Infancy and Childhood, Department of Health, Taiwan, 2001.
[22] P. Dahl, G. Lindström, K. Wiberg, C. Pappe, Absorption of polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans by breast-fed infants, Chemosphere 30 (1995) 2297–2306.
[23] N.F. Butte, M.G. Lopez-Alarcon, C. Garza, Nutrient Adequacy of Exclusive Breastfeeding for the Team Infant during the First 6 Months of Life, World Health Organization, France, 2002, pp. 8–14.
[24] M. Van den Berg, L. Birnbaum, A.T.C. Bosveld, et al., Toxic equivalency factors (TEFs) for PCBs, PCDDs and PCDFs for humans and wildlife, Environ. Health Perspect. 106 (1998) 775–792.
[25] M. Takekuma, K. Saito, M. Ogawa, R. Matumoto, S. Kobayashi, Levels of PCDDs, PCDFs and Co-PCBs in human milk in Saitama, Japan, and epidemiological research, Chemosphere 54 (2004) 127–135.
[26] A.W. Glynn, S. Atuma, M. Aune, P.O. Darnerud, S. Cnattingius, Polychlorinated biphenyl congeners as markers of toxic equivalents of polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans in breast milk, Environ. Res. 86 (2001) 217–228.
[27] M. Schuhmacher, J.L. Domingo, J.M. Llobet, H. Kiviranta, T. Vartiainen, PCDD/F concentrations in milk of nonoccupationally exposed women living in southern Catalonia, Spain, Chemosphere 38 (1999) 995–1004.
[28] A. Schecter, P. Fürst, C. Fürst, W. Groebel, S. Kolesnikov, S. Michail, A. Beim, A. Boldnov, E. Trubitsun, B. Vlasov, Levels of dioxins, dibenzofurans and other chlorinated xenobiotics in human milk from the Soviet Union, Chemosphere 20 (1990) 927–934.
[29] K. Hooper, M.X. Petreas, T. Chuvakova, et al., Analysis of breast milk to assess exposure to chlorinated contaminants in Kazakhstan high levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in agricultural villages of southern Kazakhstan, Environ. Health Perspect. 106 (1998) 797–806.
[30] A. Schecter, A.L. Piskac, E.I. Grosheva, N.I. Matorova, J.J. Ryan, P. Fürst, O. Päpke, J. Adibi, M. Pavuk, A. Sliver, S. Ghaffar, Levels of dioxins and dibenzofurans in breast milk of women residing in two cities in the Irkutsk region of Russian Siberia compared with American levels, Chemosphere 47 (2002) 157–164.
[31] C. Lutter, V. Iyengar, R. Barnes, T. Chuvakova, G. Kazbekova, T. Sharmanov, Breast milk contamination in Kazakhstan: implications for infant feeding, Chemosphere 37 (1998) 1761–1772.
[32] B.C. Gladen, A. Schecter, O. Päpke, Z.A. Shkyryak-Nyzhnyk, D.O. Hryhorczuk, R.E. Little, Polychlorinated dibenzo-*p*-dioxins, poly-

chlorinated dibenzofurans, and coplanar polychlorinated biphenyls in breast milk from two cities in Ukraine, J. Toxicol. Environ. Health A 58 (1999) 119–127.
[33] S. Horii, T. Sasamoto, H. Otaka, Improved analytical method for residual dioxins in human milk, Bull. Environ. Contam. Toxicol. 70 (2003) 1121–1127.
[34] T. Tsutsumi, T. Tanagi, M. Nakamura, et al., Update of daily intake of PCDDs, PCDFs, and dioxin-like PCBs from food in Japan, Chemosphere 45 (2001) 1129–1137.
[35] M.-S. Hsu, P.-S. Cheng, E. Ma, et al., A preliminary total diet study of PCDD/Fs-intake from food in Taiwan, Organohalogen Compd. 55 (2002) 231–234.
[36] Y. Matsumura, Nutrition trend in Japan, Asia Pacific J. Clin. Nutr. 10 (2001) 40–47.
[37] S.-J. Wu, Y.-H. Chang, C.-W. Fang, W.-H. Pan, Food sources of weight, calories, and three macronutrients—NAHSIT 1993–1996, Nutr. Sci. J. 24 (1999) 41–58.
[38] C. Falk, L. Hanrahan, H.A. Anderson, M.S. Kanarek, L. Draheim, L. Needham, D. Patterson Jr., The Great Lakes Consortium. Body burden levels of dioxin, furans, and PCBs among frequent consumers of great lakes sport fish, Environ. Res. 80 (1999) 19–25.
[39] F.X.R. Van Leeuwen, M. Feeley, D. Schrenk, J.C. Larsen, W. Farland, M. Younes, Dioxins: WHOs tolerable daily intake (TDI) revisited, Chemosphere 40 (2000) 1095–1101.
[40] A. Brouwer, U.G. Ahlborg, F.X. Van Leeuwen, M.M. Feeley, Report of the WHO working group on the assessment of health risks for human infants from exposure to PCDDs, PCDFs and PCBs, Chemosphere 37 (1998) 1627–1643.