Low birth weight of Vietnamese infants is related to their mother’ s dioxin and glucocorticoid levels

Low birth weight of Vietnamese infants is related to their mother's dioxin and glucocorticoid levels

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ABSTRACT

We aimed to determine the relationship between dioxin congeners in maternal breast milk and maternal glucocorticoid levels with newborn birth weight after nearly 45 years of use of herbicides in the Vietnam War. The study subjects comprised 58 mother–infant pairs in a region with high dioxin levels in the soil (hotspot) and 62 pairs from a control region. Dioxin levels in maternal breast milk were measured by HRGC-HRMS. Salivary glucocorticoid levels were determined by LC-MS/MS. Dioxin congener levels in mothers from the hotspot were found to be 2–5-fold higher than those in mothers from the control region. Birth weight was inversely correlated with 2,3,7,8-TeCDD and 2,3,4,7,8-PeCDF congener levels. The rate of newborns whose birth weight was less than 2500 g was 3-fold higher in the hotspot (12%) than in the control region (4%). Salivary glucocorticoid levels in mothers with low birth weight infants were significantly higher than those in the normal birth weight group. Low birth weight of Vietnamese newborns in a hotspot for dioxin levels is related to some dioxin congener levels and high glucocorticoid levels in mothers. This finding in mother–infant pairs suggests that excess maternal glucocorticoid levels are related to dioxin burden and they result in low birth weight.

Keywords: Dioxin congener, Dioxin hotspot, Vietnam, Low birth weight, Growth parameter, Glucocorticoid

INTRODUCTION

Dioxins are a group of several compounds with similar structures, they greatly vary in toxicity, and are known as polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). These compounds of highly toxic dioxin or dioxin-like species are known to be persistent organic pollutants. The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic substances found in the environment (Stellman et al. 2003). These compounds enter the human body via the food chain or by respiration and accumulate in fatty tissue, where they have a half-life of 7–11 years because of their lipophilic nature (Milbrath et al. 2009). Dioxins are unwanted by-products of the manufacture of various chlorinated herbicides and pesticides. Dioxins are also released into the atmosphere when polychlorovinyl substances are incinerated. PCBs are a class of chemically stable compounds that were widely used for 50 years in industry as heat-transfer fluids in capacitors and transformers.

However, the situation in Vietnam differs from that described above. Between 1961 and 1971, the US Air Force sprayed over 80 million liters of the defoliant Agent Orange, which is one of several defoliants containing large amounts of a toxic dioxin, and other herbicides over more than 10% of the surface area of south Vietnam. This spaying was performed to defoliate tropical forests and destroy agricultural production (Stellman et al. 2003).

Similar to many other persistent chemicals that appear in breast milk, dioxin levels decrease with time. The highest levels of TCDD (1832 parts per trillion [ppt]) in breast milk were recorded in 1970 (Schecter et al. 1995) and declined to 11–20 ppt in samples collected from 2000 subjects during 1985–1988. This significant decrease in dioxin levels in the soil in sprayed regions is due to the effects of tropical rain, erosion, and chemical breakdown over the past 45 years. However, considerably higher levels of dioxins associated with Agent Orange use have been observed in the soil around former US airbases in Bien Hoa, Phu Cat, and Da Nang, which are known as hotspots (Dwernychuk, 2005).

We have reported that dioxin levels in blood or breast milk from hotspot residents are 3–5-fold higher than those in a control region in North Vietnam (Kido et al. 2014, Manh et al. 2014, Sun et al. 2014). A similar study in Seveso (Italy) showed TCDD levels in females to be 5-fold higher in an exposed region than in a control region after 30 years (Mocarelli et al. 2008). The results of residential research suggest that dioxins cause adverse effects on human health by increasing cancer, reproductive problems, immunodeficiency, and endocrine disruption.

Recently, low doses of dioxins and PCBs have been recognized as having adverse effects on human endocrine and neurogenic health. There are several reports concerning the adverse health effects of dioxins, such as neurodevelopmental dysfunction, thyroid deficiency, immunodeficiency, and growth retardation in fetuses (Brouwer et al. 1999, Rogan &Ragan 2007, Schantz et al. 2003). These chemical toxics, which include polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and PCBs, affect children's growth as a result of prenatal or postnatal exposure (Wang et al. 2004). Pregnant and nursing women may pass the dioxins and/or PCBs to their newborns via transplacental transfer and by breastfeeding mothers (Suzuki et al. 2005, Wang et al. 2004). Several studies on exposure to lower levels of dioxins or PCBs during pregnancy have shown a relationship with low birth weight and other growth parameters (Hertz-Picciotto et al. 2005, Patandin et al. 1998, Sonneborn et al. 2008).

However, in hotspot areas in Vietnam, the effect of low-level exposure to dioxins on birth size and growth of newborns has received little attention. Therefore, this study aimed to evaluate the effect of low dose levels of dioxin on birth weight and growth parameters of Vietnamese newborns in a hotspot and in an unexposed region. In addition, we investigated the correlation between individual congeneric dioxin levels in maternal breast milk and birth weight to identify the harmful congeners. Furthermore, we analyzed the relation between maternal glucocorticoid levels and low birth weight of newborns as a marker for an adverse intrauterine environment.

MATERIALS AND METHODS

Study region

- Agent Orange/dioxin hotspot

The research areas in this study were in or around Phu Cat airbase. This area was selected as a dioxin hotspot because it currently has the highest concentration of TCDD in the soil. This is one of three dioxin hotspots in South Vietnam where chemical herbicides were stored and spilled, and where aircrafts that sprayed Agent Orange/dioxin were washed, during the Vietnam War (Dwernychuk 2005).

- Control region

The comparison site was a reference region in Kim Bang district, located in Ha Nam province in north Vietnam. This site was not exposed to chemical defoliants during the war, which is why it was selected as the control region. The hotspot and control region are rural and have not been affected by industrial pollution.

Population

The study subjects consisted of 58 mother–infant pairs from the dioxin hotspot and 62 pairs from the control region. The age of lactating mothers was between 20 and 30 years, with infants aged between 7 and 16 weeks at the time of sampling. All lactating mothers were asked to complete a health status questionnaire to collect information concerning their family, age, family income, and residence period.

The body mass index (BMI) for lactating mothers and growth parameters (height, weight, and head and chest circumference) for their newborns were determined by medical staff. Breast milk (20 ml) and saliva samples (2–3 ml) were collected from lactating women from both regions in September 2008 by medical staff. Saliva samples were collected from the hotspot (n=41) and the non-exposed area (n=36) between 08:00 and 10:00 am. We did not collect saliva from lactating mothers who had samples collected in the afternoon since hormone levels were higher in the morning than in the afternoon.. These samples were stored in chemically cleaned cooling containers and frozen on dry ice for several days. They were then transported to Japan and stored at −30°C until analysis.

The temperature in both regions was approximately 30–34°C at the time of study. After local government officials and medical staff had explained the purpose of the study to 120 lactating women (58 from the dioxin hotspot and 62 from the control region), they all agreed to participate.

Analysis of dioxin in breast milk

In this study, dioxin was defined as only PCDDs/PCDFs, not including PCBs. Breast milk samples were analyzed according to a previously reported method (Tai et al. 2011). After extraction of fat from 10 g of breast milk, 40–80 pg of 17 $^{13}C_{12}$-labeled PCDD/PCDF congeners were added as an internal standard.

A series of purification steps involving alkali digestion and chromatography on a multi-layer silica gel column and an active carbon-dispersed silica gel column were applied to separate and collect the PCDDs/PCDFs. The final sample extract was dried under nitrogen steam and then re-dissolved by adding 20 ml of nonane containing 40 pg of $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,7,8-TCDF as external standards. Finally, dioxin/furan/fractions were determined using high resolution mass spectrometry (MS station-JMS-700; JEOL, Tokyo, Japan) coupled with gas chromatography (HP-6980; Hewlett-Packard, Palo Alto, CA, USA).

Dioxin analysis was carried out in a selected ion monitoring mode at a resolution of 10,000, and the obtained values were converted into toxic equivalents (TEQs) using the World Health Organization toxicity equivalency factors (Van den Berg et al. 2006). Quality control and quality assurance were ensured following the guidelines described in the Japanese Industrial Standard. Eligibilities for the analysis of dioxin were certified using the natural reference powder milk CRM607, which was provided by the European Commission.

The recovery rate was typically in the range of 60–95%, and the detection limits were determined at a signal-to-noise ratio of 3 on a lipid basis. Congener levels below the limits of detection were set to 50% of these limits.

Analysis of salivary steroids by LC-MS/MS

The analytical procedure for the salivary glucocorticoid hormone assay using LC-MS/MS was as reported previously in detail (Kido et al. 2014).

Estimation of the daily dioxin intake of infants from breast milk

The daily dioxin intake (DDI) was estimated as per previous publications by assuming that the infant (0–24 weeks) consumes 800 ml of milk per day (Schecter et al. 2001, Tai et al. 2011) and taking the weight of the infant at the time into account. The intake value was calculated using the following equation:

DDI = 800 × (% fat content of milk/100) × (PCDD/PCDF TEQ in breast milk in pg/g lipid)/(kg of infant weight).

Statistical analyses

The data are shown as mean and standard deviation (SD) for normal and non-normal distribution, as determined using the Shapiro–Wilk test. Student's t-test was used in the case of normal distribution, and the chi-squared test was used to compare two groups according to continuous or categorical variables. The Mann–Whitney U or Kruskal–Wallis tests were used in the case of non-normal distribution to compare two or more than two groups, respectively. Pearson's correlation coefficients were calculated. The significance level was set at $P<0.05$. All statistical analyses were carried out using SPSS 12.0 software and Microsoft Excel 2010.

Ethics

The Medical Ethics Committee of Kanazawa University approved this study. The permission number was Health 89 in 2008. All lactating mothers provided written informed consent.

RESULTS

Comparison of dioxin congener levels in breast milk of lactating mothers from the dioxin hotspot and unexposed region

The demographic characteristics of the mothers were not significantly different between the dioxin hotspot and the unexposed region in Vietnam, including mean values for residency, age, weight, height, BMI, and family income.

The TEQs of dioxin congener levels in the milk of lactating mothers in the hotspot and unexposed region are shown in Table 1. The levels of most dioxin congeners were significantly higher in the hotspot than in the unexposed region ($p<0.001$). The TEQ level of the most toxic congener, 2,3,7,8-TeCDD, was 3.3-fold higher in the hotspot than in the unexposed region. There was no significant difference in the TEQ of 2,3,7,8-TeCDF levels between the two regions. In addition, the TEQ of PCDD levels, TEQ of PCDF levels, and total TEQ of PCDD/PCDF levels in the breast milk of primiparae were significantly higher than those in multiparae.

Comparison of body size and DDI in infants from the hotspot and unexposed region

Comparison of the size of infants and DDI are shown in Table 2. The DDI from breast milk was 3–4-fold higher in the hotspot than in the unexposed region for infants aged 8–9 or 12–14 weeks. However, there were no significant differences in growth parameters (height, weight, head and chest circumference) between the hotspot and unexposed region.

Correlations between dioxin congener levels in maternal breast milk and birth weight or infant size in the hotspot and unexposed region

The rate of newborns with low birth weight (<2500 g) was higher in the hotspot (n=6, 12.0%) than in the unexposed region (n=2, 4.0%).

The correlations between dioxin congeners and birth weight are shown in Table 3. The TEQs of 2,3,7,8-TeCDD and 2,3,4,7,8-PeCDF levels showed a significant negative correlation with birth weight (Fig. 1). After birth, there was no significant correlation between infant size and dioxin isomer levels at either 8–9 or 12–14 weeks.

Comparison of salivary glucocorticoid levels and total TEQ of dioxin levels in breast milk of lactating mothers

Table 4 shows the dioxin levels in maternal breast milk and salivary cortisol and cortisone levels from lactating mothers (n=78) for the three subgroups based on their birth weight of babies. Salivary cortisol and cortisone levels in lactating mothers were significantly higher in the group of low birth weight infants (<2500 g) than in the normal weight group (>2800 g). Although the 2,3,7,8-TeCDD, TEQ 2,3,4,7,8-PeCDF and Total TEQ PCDD/Fs were higher in the group of low birth weight infants (<2500 g), the statistic were not significant. The reason for the statistic was not significant might be due to large difference in the number of low birth weight infant (3) and the number of normal birth weight infants (66).

DISCUSSION

Although the end of Operation Ranch Hand was 45 years ago, dioxin residue levels are still high in the soil and in the local residents. This study is important for assessing adverse effects on human health due to dioxin exposure in the second and third generations of the Vietnamese population after the war. We studied a dioxin hotspot and an unexposed region to evaluate the effect of dioxin congeners on growth parameters of Vietnamese newborns in a hotspot. Our study showed that almost 17 dioxin congeners in maternal breast milk (see Table 1) in the hotspot were higher than those in the unexposed region, and some congeners were related to low weight birth. We also evaluated the relation between maternal corticoids levels and low birth weight.

Recent studies have shown that levels of dioxin in humans are 3–5-fold higher in hotspots than in unexposed regions (Kido et al. 2014, Manh et al. 2014). Mothers who are exposed to dioxins could potentially expose their offspring to dioxin during pregnancy as a result of circulation of maternal blood through the placenta (Suzuki et al. 2005). A previous report estimated that an infant who is breastfed for 1 year accumulates a dose of dioxin that is 6-fold higher than that in an infant who is not breastfed (Lorber & Phillips 2002). Our study showed that DDI from breast milk in infants was approximately 3–4-fold higher in the hotspot than in the unexposed region at 8–9 weeks, decreasing to 3-fold at 12–14 weeks (Tables 1 and 2). Hue et al. (2014) studied a different hotspot (Da Nang) to our study and also showed a higher average DDI from breast milk than in the corresponding control region.

There have been several studies on dioxins and their adverse effects on human health, especially during the prenatal and early postnatal periods. The effects of maternal exposure to dioxins on the infant may not manifest themselves immediately and could affect developing organs and lead to disease onset later in life (Konishi et al. 2009, Tai et al. 2011). Our study showed that the rate of low birth weight infants (<2500 g) was 3-fold higher in the hotspot than in the unexposed region. Overall, our findings are in agreement with the report by Taylor et al. (1984) on the number of risk factors for infants born with low birth weight. These authors also reported an increased rate of low birth weight infants and shortened gestational age among women who were occupationally exposed to toxic chemicals, such as TCDD or PCBs (Taylor et al. 1989). Similarly, an epidemiological study that aimed to identify dioxin congeners that cause low fetal birth weight reported that 2,3,4,7,8-PeCDF was mainly responsible for this effect (Konishi et al. 2009). Our study showed that two of the 17 dioxin congeners studied in maternal breast milk, namely 2,3,7,8-TeCDD and 2,3,4,7,8-PeCDF, reduced birth weight (Table 3 and Fig. 1). This is the first such finding regarding the relationship between the frequency of low birth weight and dioxin congeners in Vietnam.

The mechanism by which dioxins induce low birth weight in pregnancy remains unknown. We found that salivary cortisol and cortisone levels in lactating mothers in the low birth weight group (<2500 g) were significantly

higher than those in the normal weight group (>2800 g). Based on our findings, we propose a mechanism whereby exposure to excess glucocorticoids inhibits fetal development, with cortisol likely causing low fetal birth weight. We previously reported that glucocorticoid levels in lactating mothers from a hotspot region were higher than those from non-exposed regions, with a strong correlation between glucocorticoids (cortisol and cortisone) and dioxin levels in maternal breast milk (Kido et al. 2014). Several reports have shown that fetal growth is retarded by excess glucocorticoids or pharmacological doses of dexamethasone (Goedhart et al. 2010, Hauser et al. 2007, Koppe et al. 1977, Thorp et al. 2002). Furthermore, a recent study in rats demonstrated that fetuses that were overexposed to high levels of maternal glucocorticoids had intrauterine growth retardation (Kou et al. 2014). However, little is known about the levels of cortisol and how they may affect fetal and postnatal growth.

The placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) is known to regulate cortisol levels in the fetus to protect against excess maternal cortisol (Welberg et al. 2000). This enzyme has two forms of 11β-HSD types 1 and 2. The role of type 2 (11β-HSD2) is to oxidize biologically active cortisol into inactive cortisone (Murphy et al. 1974). In humans, lower placental 11β-HSD2 activity is correlated with lower birth weight, and infants homozygous for deleterious mutations of the 11β-HSD2 gene have a lower birth weight (Reynolds 2013). Exposure of the developing fetus to excess cortisol leads to fetal hypothalamic–pituitary–adrenal axis activation, which is associated with low birth weight and long-term adverse outcomes, including metabolic and brain sequelae (Moisiadis & Matthews 2014). Another action of 11β-HSD may be to protect cartilage against extensive cortisol action, even in the presence of high cortisol levels. These results indicate that cortisol and its related activating enzyme contribute to the development of catabolism.

In our study, although the DDI from breast milk at 12–14 weeks or longer was higher in the hotspot than in the unexposed area (Table 2), infant body size was not affected (Table 3). This finding suggests that, although low dioxin levels do not continue to affect body size (weight, height, head or chest circumference) of children after birth, they may affect metabolism in children as a result of endocrine disruption. Further research focusing on pediatric endocrinology is necessary to determine any possible adverse health effects of dioxin.

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Table 1. Dioxin isomer levels in maternal breast milk in the hotspot and control region

	Dioxin Isomers (pg/g of lipid)	Control region			Hotspot region				
		N	Mean	SD	N	Mean	SD	H/C[a]	p value[b]
PCDDs	TEQ "2,3,7,8-TeCDD"	62	0.458	0.345	58	1.514	0.712	3.3	***
	TEQ "1,2,3,7,8-PeCDD"	62	1.314	0.594	58	4.434	1.816	3.4	***
	TEQ "1,2,3,4,7,8-HxCDD"	62	0.065	0.026	58	0.209	0.085	3.2	***
	TEQ "1,2,3,6,7,8-HxCDD"	62	0.138	0.061	58	0.741	0.349	5.4	***
	TEQ "1,2,3,7,8,9-HxCDD"	62	0.061	0.026	58	0.281	0.118	4.7	***
	TEQ "1,2,3,4,6,7,8-HpCDD"	62	0.025	0.009	58	0.141	0.062	5.7	***
	TEQ "OCDD"	62	0.004	0.002	58	0.021	0.009	5.9	***
PCDFs	TEQ "2,3,7,8-TeCDF"	62	0.067	0.029	58	0.070	0.033	1.1	
	TEQ "1,2,3,7,8-PeCDF"	62	0.014	0.008	58	0.065	0.037	4.6	***
	TEQ "2,3,4,7,8-PeCDF"	62	0.914	0.333	58	1.909	0.716	2.1	***
	TEQ "1,2,3,4,7,8-HxCDF"	62	0.196	0.072	58	1.592	0.845	8.1	***
	TEQ "1,2,3,6,7,8-HxCDF"	62	0.166	0.062	58	0.926	0.451	5.6	***
	TEQ "1,2,3,7,8,9-HxCDF"	62	0.014	0.008	58	0.041	0.029	2.9	***
	TEQ "2,3,4,6,7,8-HxCDF"	62	0.055	0.029	58	0.159	0.063	2.9	***
	TEQ "1,2,3,4,6,7,8-HpCDF"	62	0.015	0.008	58	0.183	0.120	12.4	***
	TEQ "1,2,3,4,7,8,9-HpCDF"	62	0.002	0.001	58	0.018	0.012	9.9	***
	TEQ "OCDF"	62	0.000	0.000	58	0.001	0.001	4.8	***
Total	TEQ "Total PCDDs"	62	2.064	0.931	58	7.342	2.865	3.6	***
	TEQ "Total PCDFs"	62	1.442	0.482	58	4.965	2.054	3.4	***
	TEQ "PCDDs + PCDFs"	62	3.506	1.354	58	12.307	4.431	3.5	***

Data are shown as mean ± SD and were analyzed using the Mann–Whitney U test.
Toxic equivalent: TEQ (pg/g of lipid).
[a] Ratios of mean dioxin levels in the hotspot and control region.
[b] ***$p<0.001$.

Table 2. Comparison of body size of infants and the DDI in the two age groups from the hotspot and control regions

Infant group		Control region			Hotspot region			p value
	index	N	Mean	SD	N	Mean	SD	
8-9 weeks	Age (weeks)	20	9.7	0.88	32	9.0	1.69	0.090
	Height (cm)	20	59.5	2.35	32	59.8	3.10	0.642
	Weight (g)	20	5433	481	32	5415	963	0.937
	Head (cm)	20	39.0	1.19	32	39.2	1.38	0.623
	Chest (cm)	20	40.0	1.49	32	39.6	2.60	0.543
	DDI (TEQ pg/kg/d)	20	18.0	8.97	32	54.2	28.20	0.000
12-14 weeks	Age (weeks)	41	14.6	2.02	23	13.9	1.84	0.139
	Height (cm)	41	62.2	2.42	23	62.4	2.52	0.767
	Weight (g)	41	6277	821	23	6108	807	0.430
	Head (cm)	41	40.7	1.74	23	40.9	1.57	0.672
	Chest (cm)	41	41.5	2.11	23	40.9	2.39	0.312
	DDI (TEQ pg/kg/d)	41	12.3	4.93	22	42.7	23.96	0.000

DDI: daily dioxin intake.
Data are shown as mean ± SD.
Statistical significance was tested using Student's t-test.

Table 3. Correlations between dioxin isomer levels in maternal breast milk and birth weight and infant size

		Birth	8-9 weeks old				12-14 weeks old			
		N = 120	N = 52				N = 64			
		Weight	Height	Weight	Head	Chest	Height	Weight	Head	Chest
PCDDs	TEQ "2,3,7,8-TeCDD"	−0.184*	−0.148	−0.038	0.022	−0.098	−0.037	−0.052	0.126	−0.052
	TEQ “1,2,3,7,8-PeCDD”	−0.147	−0.145	−0.068	−0.040	−0.095	0.098	−0.047	0.124	−0.104
	TEQ "1,2,3,4,7,8-HxCDD"	−0.158	−0.028	0.027	0.017	−0.005	0.069	−0.030	0.112	−0.034
	TEQ "1,2,3,6,7,8-HxCDD"	−0.127	−0.150	−0.097	−0.021	−0.110	0.063	−0.054	0.071	−0.068
	TEQ "1,2,3,7,8,9-HxCDD"	−0.145	−0.061	−0.047	0.027	−0.091	0.069	−0.039	0.092	−0.039
	TEQ "1,2,3,4,6,7,8-HpCDD"	−0.098	0.065	0.053	0.065	−0.018	0.133	−0.030	0.091	−0.058
	TEQ “OCDD”	−0.145	0.016	0.021	0.014	−0.025	0.178	−0.041	0.108	−0.110
PCDFs	TEQ "2,3,7,8-TeCDF"	−0.118	−0.230	−0.170	0.022	−0.083	−0.132	−0.033	−0.047	−0.016
	TEQ "1,2,3,7,8-PeCDF"	−0.156	0.025	−0.022	0.122	−0.014	0.018	0.026	0.040	0.050
	TEQ "2,3,4,7,8-PeCDF"	−0.206*	−0.238	−0.215	−0.125	−0.226	0.074	−0.019	0.195	−0.046
	TEQ "1,2,3,4,7,8-HxCDF"	−0.131	−0.034	−0.059	0.052	−0.068	0.086	−0.003	0.088	−0.014
	TEQ "1,2,3,6,7,8-HxCDF"	−0.141	−0.053	−0.086	0.036	−0.114	0.103	−0.006	0.105	−0.013
	TEQ "1,2,3,7,8,9-HxCDF"	−0.05	0.010	−0.032	−0.007	−0.021	0.081	0.008	0.026	−0.024
	TEQ "2,3,4,6,7,8-HxCDF"	−0.164	0.016	−0.023	0.084	−0.062	0.058	−0.006	0.080	−0.008
	TEQ "1,2,3,4,6,7,8-HpCDF"	−0.122	0.085	0.005	0.115	−0.030	0.095	0.015	0.061	0.003
	TEQ "1,2,3,4,7,8,9-HpCDF"	−0.093	0.099	0.064	0.112	0.032	0.136	0.017	0.085	−0.032
	TEQ “OCDF”	−0.048	0.060	−0.024	0.134	0.019	−0.015	−0.069	−0.072	−0.076
Total	TEQ "Total PCDDs"	−0.159	−0.142	−0.062	−0.019	−0.097	0.067	−0.049	0.121	−0.086
	TEQ "Total PCDFs"	−0.167	−0.110	−0.123	−0.011	−0.137	0.087	−0.007	0.121	−0.022
	TEQ "PCDDs + PCDFs"	−0.168	−0.135	−0.088	−0.017	−0.117	0.078	−0.032	0.125	−0.060

Head: head circumference, chest: chest circumference.
* $p<0.05$

Table 4. Salivary glucocorticoid and dioxin levels in breast milk in lactating mothers with division into three groups based on birth weight

	Baby group									
	Group 1 < 2500			Group 2 2500-2800			Group 3 >2800			p value
	N	Mean	SD	N	Mean	SD	N	Mean	SD	P^{1-3}
Cortisol (ng/ml)	3	6.52	4.39	9	3.2	3.4	66	2.19	1.53	*
Cortisone (ng/ml)	3	20.94	5.34	9	14.63	9.86	66	11.71	6.67	*
TEQ 2,3,7,8-TeCDD	3	1.00	0.89	9	1.50	0.80	66	1.01	0.76	ns
TEQ 2,3,4,7,8-PeCDF	3	1.56	1.32	9	1.76	0.88	66	1.43	0.78	ns
TEQ "PCDDs + PCDFs"	3	9.09	8.21	9	10.29	6.30	66	8.05	5.57	ns

Data are shown as mean ± SD, and were analyzed using the Kruskal–Wallis test for more than two groups and the Mann–Whitney U test for two groups.
Toxic equivalent: TEQ (pg/g of lipid).
* $p<0.05$.

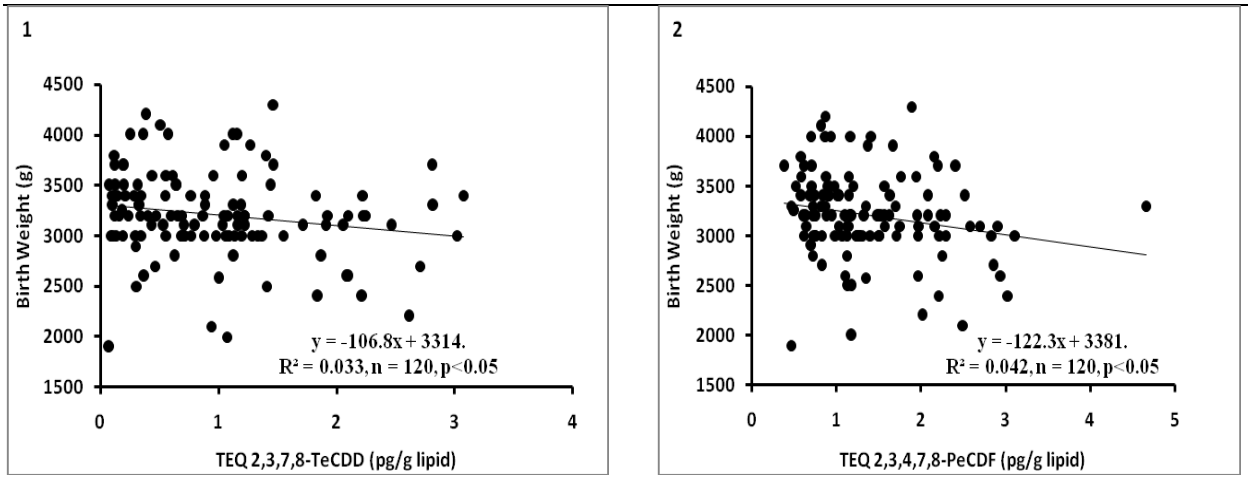

Fig. 1 Correlations between birth weight and dioxin isomers in maternal breast milk.