Effects of prenatal exposure to dioxin-like compounds on allergies and infections during infancy

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Abstract

Dioxin-like compounds are endocrine disruptors. The effects of prenatal exposure to environmental levels of dioxins on immune function during infancy have not been clarified, although dioxins induce immunosuppression in offspring of animals. Moreover, human studies have not assessed the effects of gender- or congener-specific differences. The purpose of this study was to investigate the association between dioxin levels in maternal blood and the risk of infection and allergies in infancy. We examined 364 mothers and their infants enrolled in a Hokkaido Study on Environment and Children's Health between 2002 and 2005 in Sapporo, Japan. Relevant information was collected from a baseline questionnaire during pregnancy, medical records at delivery, and a follow-up questionnaire when the child was 18 months of age that assessed development of allergies and infections in infancy. Dioxin-like compound levels in maternal blood were measured with high-resolution gas chromatography/high-resolution mass spectrometry. Relatively higher levels of polychlorinated dibenzofuran were associated with a significantly increased risk of otitis media, especially among male infants (odds ratio = 2.5, 95% confidence interval = 1.1-5.9). Relatively higher levels of 2,3,4,7,8-pentachlorodibenzofuran were also associated with a significantly increased risk of otitis media (odds ratio = 5.3, 95% confidence interval = 1.5-19). However, we observed a weak association between dioxin-like compound levels and allergic symptoms in infancy. At environmental levels, prenatal exposure to dioxin-like compounds may alter immune function and increase the risk of infections in infancy, especially among males. The compound 2,3,4,7,8-pentachlorodibenzofuran may be responsible for this.

Key words: Dioxin-like compounds, infant, prenatal exposure, allergy, infection.

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Abbreviations:

AhR: aryl hydrocarbon receptor, ATS-DLD: American Thoracic Society-Division of Lung Diseases, BMI: body mass index, CI: confidence interval, DL: detection limit, DLC: dioxin-like compound, DL PCB: dioxin-like polychlorinated biphenyl, HxCB: hexachlorobiphenyl, Ig: immunoglobulin, ISAAC: International Study of Asthma and Allergies in Childhood, ND: not detectable, NDL PCB: non-dioxin-like PCB, OR: odds ratio, PCDD: polychlorinated dibenzo-*p*-dioxin, PCDF: polychlorinated dibenzofuran, PeCB: pentachlorobiphenyl, PeCDF: pentachlorodibenzofuran, TCB: tetrachlorobiphenyl, TCDD: tetrachlorodibenzo-*p*-dioxin, TEQ: toxic equivalent, TEF: toxic equivalency factor

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL PCBs) are endocrine disruptors that persistently exist in the food chain and environment. These compounds are classified as dioxin-like compounds (DLCs) because of their similarities in structure and mechanism of toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Yoshizawa et al., 2007). Humans are mainly exposed to DLCs through intake of contaminated animal products. DLCs are reported to accumulate mostly in adipose tissue over multiple years due to their high lipophilicity and resistance to biodegradation (Schecter and Gsiewicz, 2003). In humans, DLCs cross the placenta of pregnant women and are transferred to the fetal tissue and cord blood (Todaka et al., 2010).

Animal studies have demonstrated that fetal TCDD exposure inhibits cellular differentiation and maturation, particularly of T lymphocytes, causes thymic atrophy, and leads to immunosuppression in offspring (Yoshizawa et al., 2007). Offspring of maternal rats treated with TCDD during the third trimester have a greater sensitivity to immune toxicity induced by TCDD than adults, and the adverse effects that occur at critical windows of maturation persist later in life. In addition, male rat offspring may be more sensitive than females to TCDD-mediated suppression of T cell activity (Luebke et al., 2006). At comparable environmental levels, exposure to complex mixtures of DLCs may induce immunosuppression in both mice and humans (Smialowicz et al., 2008).

In the Taiwan Yucheng accident, children born to mothers who had accidentally ingested high levels of contaminated rice oil had higher frequencies of bronchitis, reduced serum levels of

immunoglobulin (Ig) A, IgG, and IgM at 6 months (Yu et al., 1998), and a higher incidence of influenza and otitis media at 6 years of age than unexposed controls (Chao et al., 1997; Rogan et al., 1988). PCDFs, rather than PCBs, may be primarily responsible for the immunotoxicity related to Yucheng symptoms (Masuda, 2001). In Japan, infants born to mothers occupationally exposed to high levels of PCBs have a higher frequency of colds and gastrointestinal complaints (Hara, 1985). In Inuit infants born to mothers who had ingested high levels of contaminated marine mammals, higher prenatal PCB exposure led to a significantly elevated incidence of infections such as acute otitis and respiratory problems (Dallaire et al., 2004, 2006). On the Faroe Islands, PCB levels in maternal serum were inversely associated with an antibody response to diphtheria toxoid at 18 months of age and tetanus toxoid at 7 years of age (Heilmann et al., 2006). In an eastern Slovakia study, higher PCB levels in maternal serum were associated with newborns who had a smaller thymus, the organ responsible for lymphocyte maturation (Park et al., 2008).

A few human studies have addressed prenatal exposure to environmental levels of PCBs/dioxins, although several of these studies were conducted in populations exposed to high levels. In the Rotterdam study, PCBs in maternal blood and dioxins in breast milk were significantly associated with a higher prevalence of otitis media and chicken pox, as well as a lower prevalence of shortness of breath with asthma. In addition, these indicators were related to a reduction in measles, mumps, and rubella reactivity after primary vaccination and an increased number of T lymphocytes at 42 months (Weisglas-Kuperus et al., 2000, 2004). On the other hand, in the Amsterdam study, dioxin levels in breast milk were associated with decreased allergies but not with any infections at 8 years of age (ten Tusscher et al., 2003). In Spain, PCB levels in cord blood were not related to the prevalence of asthma at

4 years of age (Sunyer et al., 2005). In Japan, DLC levels in breast milk were significantly associated with an increased lymphocyte subset ratio in the peripheral blood of breast-fed infants at 10 months (Nagayama et al., 2007), but no association was observed in another cohort of Japanese infants at 12 months of age (Kaneko et al., 2006).

In environmentally exposed populations, data for associations between prenatal exposure to DLCs (with consequent immunosuppression) and increased incidence of infectious diseases are relatively consistent, although causality has never been established. In contrast, only a few studies have addressed allergies or asthma, and these findings appear controversial. In addition, human studies have yet to assess gender- or congener-specific differences regarding the effects of prenatal exposure to DLCs on allergies and infections in infancy, and have not used DLC levels in maternal blood as indicators of prenatal exposure.

The subjects of this study were recruited in the Hokkaido Study on Environment and Children's Health, which previously reported that environmental pollution levels in Sapporo were relatively lower than in other areas of Japan, Europe, and the USA (Konishi et al., 2009). Furthermore, it also reported that maternal DLC levels were inversely correlated with IgE levels in cord blood (Washino et al., 2007). These findings suggested that prenatal exposure to low levels of DLCs may affect immune function immediately after birth. The purpose of this study was to investigate the effects of prenatal exposure to DLCs on allergies and infections during the first 18 months of life.

2. Materials and Methods

2.1. Study population.

Details of the population and data collection until delivery have been reported previously (Kishi et al., In press). In brief, a prospective cohort study was performed from July 2002 to September 2005 at the Sapporo Toho Hospital in Hokkaido, Japan (Hokkaido Study on Environment and Children's Health). We contacted 1796 pregnant women in their second or third trimester during regular antenatal visits. Of these, 514 (28.6%) native Japanese residents of Sapporo or surrounding areas agreed to participate.

In their last trimester, the patients completed a self-administered questionnaire regarding information on dietary habits, smoking status, alcohol intake, caffeine intake, household income, educational level, and medical history. Maternal smoking status during pregnancy was classified into two categories: Non-smokers who had never smoked or had quit smoking during the first trimester, and smokers who smoked after their first trimester. The information at delivery was obtained from medical records and included pre-pregnancy body mass index (BMI), pregnancy complications, gestational age, infant gender, parity, congenital anomalies, and infant physical size.

From recruitment to 18 months after delivery, 23 mothers were excluded for reasons of miscarriage (4), stillbirth (2), relocation (8), infant mortality (1), or voluntary withdrawal (8). At 18 months after delivery, follow-up questionnaires were mailed to 491 subjects, of whom 390 (79.4%) responded. From the questionnaires, we obtained information about potential confounding factors such as early feeding type (breast-feeding, bottle feeding, or both), breast-feeding duration (weeks), age of starting solid foods, parental smoking status, living with a smoker excluding mother, living environment,

day care attendance, vaccination history during the first 18 months of life, and infant height and weight at 18 months of age. We defined infants exposed to environmental tobacco smoke as those living with a smoker including their mother.

2.2. Assessment of infant allergies and infections.

From the follow-up questionnaire, we collected information about hospitalization or medical treatment of infants for asthma, eczema, other allergic diseases, otitis media, febrile seizures, respiratory syncytial virus infection, and other diseases from birth until 18 months of age. In addition, we used a modified version of the International Study of Asthma and Allergies in Childhood (ISAAC) phase-I questionnaire (ISAAC Steering Committee, 1998) and the American Thoracic Society-Division of Lung Diseases (ATS-DLD) questionnaire (Nishima et al., 2009). We defined development of allergies or infections if infants had a doctor’s diagnosis, hospitalization, or medical treatment between birth and 18 months of age. In addition, we expanded the definition of asthma to include cases in which the mother had positive responses to all questions on the modified ATS-DLD (Nishima et al., 2009). We expanded the definition of food allergy to include cases in which the infant had an adverse reaction such as hives, swollen lips, emesis, diarrhea, or respiratory distress after ingestion of potential allergens included in milk, egg products, shrimp, or other foods. This study was conducted with written informed consent from all patients and was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.3. Exposure assessment.

PCDD/PCDF levels and DL PCB levels were measured using previously published methods (Todaka et al., 2003). A 40-ml blood sample was taken from the maternal peripheral vein in the last trimester. When we were unable to withdraw blood due to pregnancy-related anemia, we obtained the sample during hospitalization immediately after delivery. All samples were stored at −80°C until analysis. DLC concentrations were measured with high-resolution gas chromatography/high-resolution mass spectrometry at Fukuoka Institute of Health and Environmental Sciences. Sample values below the detection limit (DL) were assigned a value of one-half the DL to estimate each total level. Toxic equivalent (TEQ) values, which were used to express the toxic potency of a mixture of DLCs, were calculated by multiplying the concentration of each individual congener by its specific toxic equivalency factor (TEF) value as defined by the World Health Organization in 2006 (Van den Berg et al., 2006). DLC levels were measured in 426 samples, of which 356 were taken during pregnancy and 148 were taken after delivery. The remaining samples were not analyzed due to unavailable or insufficient sample volumes for measurement. One sample was excluded from the study because it contained extremely high levels of PCDFs.

2.4. Statistical analysis.

We analyzed correlations between DLC levels and characteristics of mothers and infants with the Spearman correlation test, Mann-Whitney U-test, Kruskal-Wallis test, and Univariate regression analysis. To assess risk or protective factors on infant illnesses, the characteristics of parents and infants were introduced as explanatory variables in binominal logistic regression analyses. Crude and adjusted logistic regression analyses were performed to evaluate associations between DLC levels and the risk of

allergy and infection among all infants, male infants, and female infants. DLC levels were lipid adjusted (pg/g lipid) and categorized as quartile distributions of each level. In logistic models, we evaluated odds ratios (ORs) for the risk of allergies and infection with DLC levels in the second to fourth quartiles compared with those in the first quartile (reference). As another model, to assess the dose-response relationship, trend p values were obtained using the quartile of DLC levels as an ordinal variable. Multivariate analyses were adjusted for confounding variables that influenced the development of allergies or infections in binominal analyses ($p < 0.05$), possible risk factors reported in previous studies, and the sampling period. Multiple analyses of the development of allergies were adjusted for maternal age (continuous), pre-pregnancy BMI (continuous), maternal education level (under or over 12 years), parity (first child, or two or more children), parental allergic history (ever/never), infant gender, duration of breast-feeding (less or more than 4 months), environmental tobacco exposure (yes/no), day care attendance (yes/no), and sampling period (pre- or post-delivery). Multivariate analyses of the development of infections were adjusted for maternal education level, parity, infant gender, duration of breast-feeding, environmental tobacco exposure, day care attendance, and sampling period. Statistical analyses were performed using the Statistics Package for Social Sciences (SPSS, Inc., USA) software for Windows version 15.0J.

3. Results

Table 1 presents total maternal dioxin TEQs in relation to characteristics of the mothers and infants. Our study included 364 mother-infant pairs from whom both DLC levels and follow-up questionnaires were obtained. Based on the questionnaires, only 15 infants (4%) were fed on formula alone, and 210 infants (58%) were exposed to environmental tobacco smoke. We found no significant difference between male and female infant characteristics except for birth weight (males; 3109 g, females; 3011 g, $p = 0.01$). The total dioxin TEQ was positively correlated with maternal age ($\beta = 0.265$, $p < 0.001$). The median total dioxin TEQ was significantly different ($p < 0.05$) according to parity, smoking status, maternal educational level, early feeding type, environmental tobacco exposure, and annual household income (Table 1). The frequency of maternal dietary intake of fish and meat during pregnancy, which are the main sources of human exposure to DLCs in Japan (Todaka et al., 2010), was not related to total dioxin TEQs.

Table 2 shows the concentrations of each congener and the combined TEQs of the seven PCDDs, ten PCDFs, four non-*ortho* PCBs, eight mono-*ortho* PCBs, and total dioxins. There were no significant differences between male and female infants regarding maternal DLC TEQ levels, DLC concentrations, or number of samples in which compounds were not detectable (ND).

The numbers (%) of infants who developed allergies or infections during the first 18 months of life were as follows: food allergies: 62 (17.0) of whom 32 were male and 30 were female; eczema: 41 (11.3) of whom 22 were male and 19 were female; asthma: 32 (8.8) of whom 15 were male and 17 were female; otitis media: 68 (18.7) of whom 40 were male and 28 were female; rhinitis: 7 (1.9); pharyngitis:

3 (0.8); bronchitis: 4 (1.1); pneumonia: 8 (2.2); respiratory syncytial virus infection: 8 (2.2); chicken pox: 17 (4.7); other virus infections (rotavirus, cytomegalovirus, adenovirus, and herpes virus): 20 (5.5); and skin infection: 3 (0.8). There was no significant difference in the rate of developing illnesses by gender. Binominal analysis showed that positive allergic history in parents, day care attendance, long duration of breast-feeding (≥4 months), increasing maternal BMI, and multiparity were risk factors for developing allergies or infections (Table 3).

Table 4 shows ORs and 95% confidence intervals (CIs) for DLC TEQ levels as quartiles for the development of food allergies, eczema, asthma, and otitis media following crude and adjusted logistic regression analyses. The adjusted OR of PCDFs for development of otitis media was significantly increased in the highest quartile compared with the lowest quartile (OR = 2.5, 95% CI = 1.1-5.9), with a significant dose-response relationship (p for trend = 0.027). The adjusted ORs of non-*ortho* PCB TEQs and mono-*ortho* PCB TEQs for the development of food allergies significantly increased in the second and third quartile compared with the first quartile without a dose-response relationship.

Table 5 presents adjusted ORs (95% CI) of DLC levels as quartiles for the development of otitis media in the fully adjusted model among male and female infants. Among male infants, independently significant trends were observed for adjusted ORs of PCDDs, PCDFs, non-*ortho* PCBs, and total dioxins except mono-*ortho* PCBs (p for trend = 0.032, 0.012, 0.050, and 0.032, respectively). Significant increases were observed for adjusted ORs of the highest quartile of PCDFs, the third quartile of mono-*ortho* PCBs, and the highest quartile of total dioxin TEQs compared with the reference. However, among female infants, a significant increase was only observed for the adjusted OR of the second quartile of PCDFs compared with the reference (Table 5).

Congener-specific analyses were performed only for congeners detected in over 60% of the samples. Table 6 shows that adjusted ORs of congeners had significant associations only between each quartile of congener and otitis media among all infants. Significant positive trends were observed for congeners of 2,3,4,7,8-PeCDF and 33'44'-tetrachlorobiphenyl (TCB) (#77) (p for trend = 0.015, and 0.006, respectively). Significant increases were observed for adjusted ORs of the second to highest quartiles of OCDD, the highest quartile of 2,3,4,7,8-PeCDF, the highest quartile of TCB-77, and the second and highest quartiles of HxCB-157 compared with the reference. Trends for a decrease were observed for adjusted ORs from the second to fourth quartiles of OCDD. No significant decrease was observed for adjusted ORs of the third quartile of TCB-77 and HxCB-157 compared with the second or fourth quartile.

4. Discussion

Our results indicate that prenatal exposure to environmental levels of DLCs increases the risk of developing infections such as otitis media during the first 18 months of life, especially in males. In the environmentally exposed population in Rotterdam, DLC levels in breast milk were significantly associated with a higher prevalence of infections in 175 of the 207 children. The median DLC level in breast milk was 35.8 TEQ pg/lipid, which was higher than the level in the breast milk of our cohort, which had a median of 10 TEQ pg/lipid (Weisglas-Kuperus et al., 2000; Todaka et al., 2010). Therefore, this study indicates that prenatal DLC exposure at relatively low environmental levels leads to increased infections during infancy. Our observation was inconsistent with that in an Amsterdam study (ten Tusscher et al., 2003). The number of participants in the Amsterdam study was relatively small, and the study included only infants who were breast-fed for at least 2 months. These different parameters may have resulted in the inconsistent findings between the Amsterdam study and this study.

For all infants, both PCDFs and 2,3,4,7,8-penta-CDF showed significant positive trends for a risk of otitis media. In Yucheng patients who were exposed to high levels of DLCs, 2,3,4,7,8-penta-CDF was described as the primary contributor to the toxic effects because this compound accounted for 70% of the total dioxin TEQ levels in maternal blood (Masuda, 2001). Matsueda (2007) indicated that both toxicity kinetics and the half-life for elimination of DLCs vary depending on the exposure source. Therefore, 2,3,4,7,8-penta-CDF may affect infant health more strongly than other DLC congeners, regardless of the exposure source.

In analyses stratified by gender, significant positive trends were observed for PCDDs, PCDFs,

and total dioxins in male infants. Among female infants, however, a significant association was observed between the second quartile of PCDFs and the risk of otitis media. In rats treated with TCDD on gestational day 14, the maternal lowest-observed-averse-effect-level for immunosuppression in male offspring (median; 0.1 μg/kg) was lower than that in female offspring (median; 0.3 μg/kg) (Luebke et al., 2006). A few human studies have shown gender-specific differences in the effects of prenatal exposure to DLCs with respect to gender ratio (Hertz-Picciotto et al., 2008; Mocarelli et al., 1996), lymphocyte subset rate (Nagayama et al., 2007), and birth weight (Sonneborn et al., 2008; Konishi et al., 2009). Similar to previous findings, our results show that male offspring may be more susceptible to DLCs than female offspring.

Significant positive associations were observed for PCDDs for the risk of otitis media among all infants and male infants. Borderline significant trends were observed for non-*ortho* PCBs among male infants. Uncertain associations were observed for mono-*ortho* PCBs. Each congener of OCDD, TCB-77, and HxCB-157 may be a partial cause of each association. However, these associations were independent of the magnitude for toxic potency such as half-lives, which are 3.7, 0.5, and 18 years for OCDD, TCB-77, and HxCB-157, respectively (Nakai et al., 2009). In addition, each contribution rate to the total dioxin TEQ is low. The rates (%) of OCDD, TCB-77, and HxCB-157 are 1.0, 0.01, and 0.11, respectively. These effects of each congener on otitis media may represent effects of other congeners because complex mixtures of DLCs in mammals may have multiple effects (Smialowicz et al., 2008). Our results indicate that not only PCDD/Fs but also DL-PCBs may contribute to infections in infancy.

We found no relationship between DLC levels and infections except for otitis media. This finding may be due to unknown confounding factors that potentially influence the development of

infections. For a few months after birth, the sustained effects of multiple environmental factors may play a larger role in the onset of infections than effects that occur before birth (Dallaire et al., 2004). This is the first study to report gender-specific differences in immune health following prenatal exposure to DLCs during infancy according to not only total TEQ levels but also specific congener levels. Thus, more studies are needed to address the toxic potency of DLCs.

Although no significant trend was observed for DLCs with respect to any particular allergy, the risk of food allergies was significantly increased with exposure to DL PCBs in the second and/or third quartiles compared with references. The interrelationship between food allergy and asthma and eczema is known as the atopic march (Schroeder et al., 2009). The onset of infections, especially those with respiratory symptoms, may be a risk factor for developing allergies (Aberg et al., 1996). Moreover, a marginally positive significant trend was observed for PCDFs in the risk of asthma. However, these observations may be partly explained by misclassification of food allergies, because mothers may not be able to distinguish food allergies from other symptoms such as food intolerance. Therefore, from our results, we speculate that prenatal DLC exposure may help to provoke allergic symptoms after birth.

The Rotterdam study suggested that increased infections in early life may stimulate maturation of the immune system, resulting in decreased development of allergies (Weisglas-Kuperus et al., 2000, 2004). However, the current study found a significant association between DLC levels and otitis media but a weak association between DLC levels and allergic symptoms. Therefore, the results of the current study suggest that DLC exposure may impair immune function, resulting in decreased resistance to infections after birth. This idea is consistent with a previous study in which DLC levels correlated inversely with IgE levels (Washino et al., 2007). However, the current study may not provide sufficient

evidence for this hypothesis because biological markers such as lymphocyte immunophenotypic distributions and Ig levels at 18 months of age were not measured.

Based on maternal parity, infants that were the second born or later were considered to have a sibling. Multivariate analyses were adjusted for risk factors commonly reported to influence infections or allergies (ten Tusscher et al., 2003; Weisglas-Kuperus et al., 2000, 2004). In an additional model, adjusting for the frequency of fish and meat intake, season of birth, distance of home from a highway, or alcohol intake did not change the relationships in this study. This prospective cohort study led to minimal recall bias. We defined the development of allergies with ISSAC or ATC-DLD questionnaires, which are internationally standardized procedures. Infants that had been diagnosed or treated by a doctor were defined as having developed infections. These facts provided validity for the criteria for developing illness. However, we could not exclude the possibility that we underestimated the onset of infant illness because we did not use medical records or a diary for collecting data.

Our results have several limitations. First, our sample size was small for evaluating the low frequency of infant illnesses. Second, despite the small sample size in our study, it was still possible to observe significant relationships although there was a trend towards larger CIs. Third, selection bias may have occurred because this cohort was derived from a single area maternity hospital. Fourth, we could not obtain postnatal information for newborns who had birth anomalies because those newborns were transferred to other facilities. Finally, the participation rate was low (29%), partially because we excluded pregnant women who had decided to enroll in the Japanese cord blood bank (22% of those of approached) or who delivered their baby at another hospital (3% of those of approached). These exclusions may limit the extrapolation of our results to the general population.

In further studies, follow-up into later childhood will be needed, because increased reliability of allergy diagnosis due to immune maturation occurs. A larger population study may allow us to evaluate low-frequency infant health events. Furthermore, the effects of postnatal DLC exposure via breast milk or foods and the relationship to allergies and infections should also be evaluated. In conclusion, prenatal exposure to environmental levels of DLCs may alter immune function and increase the risk of infections in infancy, especially among males. The compound 2,3,4,7,8-PeCDF may be responsible for this.

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Table 1. Maternal total dioxin TEQs in relation to characteristics of parents and infants (n = 364)

					Total dioxin TEQs		
		No.	(%)	β	Median	(25th, 75th)	p value
Mother							
Age at delivery (years)		31 ± 4.5[a]		0.265			<0.001
Pre-pregnancy BMI (kg/m^2)		21 ± 3.2[a]		0.070			1.342
Parity	0	175	(48)		15.11	(11.52, 20.28)	<0.001
	≥1	189	(52)		13.38	(9.44, 16.90)	
Allergic history	No	270	(74)		13.73	(9.84, 18.00)	0.131
	Yes	94	(26)		14.31	(11.00, 20.34)	
Smoker during pregnancy	No	313	(86)		14.11	(10.38, 18.49)	0.046
	Yes	51	(14)		12.17	(8.86, 17.06)	
Smoking	No	286	(79)		14.50	(10.70, 18.93)	0.001
	Yes	78	(21)		12.27	(8.75, 14.75)	
Educational level	≤12 years	149	(41)		12.77	(8.81, 17.47)	0.004
	>12 years	215	(59)		14.43	(11.22, 19.43)	
Blood sampling period	During pregnancy	251	(69)		14.07	(10.11, 18.68)	0.493
	After delivery	113	(31)		13.67	(10.19, 18.05)	
Inshore fish intake during pregnancy	≤1–2 times/month	201	(55)		13.40	(9.88, 17.82)	0.139
	≥1–2 times/week	163	(45)		14.84	(10.40, 18.91)	
Deep-sea fish intake during pregnancy	≤1–2 times/month	169	(46)		13.62	(9.70, 17.49)	0.090
	≥1–2 times/week	195	(54)		14.43	(10.40, 19.43)	
Beef intake during pregnancy	≤1–2 times/month	270	(74)		13.64	(9.76, 17.98)	0.055
	≥1–2 times/week	91	(25)		15.32	(11.37, 19.29)	
Pork intake during pregnancy	≤1–2 times/month	29	(8)		12.74	(8.68, 15.66)	0.102
	≥1–2 times/week	335	(92)		14.02	(10.33, 18.49)	
Chicken intake during pregnancy	≤1–2 times/month	52	(14)		13.50	(9.53, 18.44)	0.808
	≥1–2 times/week	312	(86)		13.94	(10.31, 18.18)	
Father							
Allergic history	No	298	(82)		13.84	(9.98, 18.24)	0.489
	Yes	65	(18)		14.42	(11.21, 18.89)	
Smoking	No	164	(46)		14.88	(11.23, 19.42)	0.01
	Yes	193	(54)		13.04	(9.37, 17.88)	
Infant							
Gender	Male	182	(50)		13.89	(9.88, 18.16)	0.979
	Female	182	(50)		13.88	(10.32, 18.85)	
Gestational age (weeks)		39 ± 1.5[a]		0.055[b]			0.299
Birth weight (g)		3060 ± 373[a]		-0.008[b]			0.875
Duration of breast-feeding	<4 months	91	(25)		14.01	(10.82, 19.61)	0.235
	≥4 months	273	(75)		13.84	(9.93, 17.98)	
Early feeding type	Breast-feeding	143	(39)		13.67	(9.48, 17.55)	0.005
	Combined feeding	206	(57)		13.96	(10.40, 18.85)	
	Bottle-feeding	15	(4)		19.43	(13.91, 31.37)	
Day care attendance	No	288	(79)		13.89	(10.07, 18.27)	0.863
	Yes	76	(21)		13.96	(10.31, 19.28)	
Birth season	Spring	105	(29)		14.11	(10.43, 20.47)	0.187
	Summer	89	(24)		13.72	(9.76, 17.57)	
	Autumn	66	(18)		14.51	(11.68, 20.42)	
	Winter	104	(29)		13.25	(9.54, 17.55)	
Living environment							
Environmental tobacco exposure	No	154	(42)		14.89	(11.35, 19.41)	0.009
	Yes	210	(58)		13.23	(9.24, 17.93)	
Possessed pets	No	302	(83)		14.08	(10.34, 18.72)	0.097
	Yes	62	(17)		12.60	(8.76, 16.33)	
Annual household income	≤5 million yen	235	(65)		13.34	(9.48, 17.54)	0.001
	>5 million yen	128	(35)		15.70	(11.05, 20.72)	
Distance from highway to home	≤100 m	191	(53)		14.02	(10.29, 19.40)	0.599
	>100 m	172	(47)		13.80	(10.00, 18.07)	

[a]Mean ± S.D. BMI; body mass index

[b]r was calculated with the Spearman's correlation test.

Unknown smoking data for seven fathers (1.9%), beef intake during pregnancy for three mothers (1%), annual household income for one participant (0.3%), and distance from highway to home for one participant (0.3%).

$p < 0.05$, $p < 0.01$; statistically significant differences following the Spearman's correlation test, Mann-Whitney U-test, Kruskal-Wallis test, and Univariate regression analysis for total dioxin TEQs.

Table 2. Concentrations (pg/g lipid) and TEQs (TEQ pg/g lipid) for PCDDs, PCDFs, DL PCBs, and total dioxins in maternal blood (n = 364)

	DL	ND	(%)	Minimum	25th	Median	75th	Maximum
2,3,7,8-TCDD	1	198	(54.4)	0.50	0.50	0.50	1.28	3.44
1,2,3,7,8-PeCDD	1	8	(2.2)	0.50	2.88	3.93	5.15	12.90
1,2,3,4,7,8-HxCDD	2	227	(62.4)	1.00	1.00	1.00	2.28	13.60
1,2,3,6,7,8-HxCDD	2	0	(0.0)	2.37	9.60	13.27	17.51	113.84
1,2,3,7,8,9-HxCDD	2	160	(44.0)	1.00	1.00	2.18	3.00	25.10
1,2,3,4,6,7,8-HpCDD	2	0	(0.0)	8.35	18.31	23.31	30.99	85.38
OCDD	4	0	(0.0)	75.50	325.78	412.16	556.47	1491.50
Total PCDDs				92.69	365.51	460.90	608.97	1602.40
2,3,7,8-TCDF	1	290	(79.7)	0.50	0.50	0.50	0.50	8.41
1,2,3,7,8-PeCDF	1	335	(92.0)	0.50	0.50	0.50	0.50	4.60
2,3,4,7,8-PeCDF	1	2	(0.5)	0.50	4.08	5.54	7.16	19.93
1,2,3,4,7,8-HxCDF	2	146	(40.1)	1.00	1.00	2.22	2.88	12.47
1,2,3,6,7,8-HxCDF	2	109	(29.9)	1.00	1.00	2.49	3.26	10.09
2,3,4,6,7,8-HxCDF	2	345	(94.8)	1.00	1.00	1.00	1.00	3.86
1,2,3,7,8,9-HxCDF	2	ND						
1,2,3,4,6,7,8-HpCDF	2	149	(40.9)	1.00	1.00	2.22	3.07	19.53
1,2,3,4,7,8,9-HpCDF	2	ND						
[OCDD OR OCDF]	4	360	(98.9)	2.00	2.00	2.00	2.00	11.35
Total PCDFs				9.50	14.39	18.04	22.60	52.88
344'5-TCB (#81)	10	363	(99.7)	5.00	5.00	5.00	5.00	10.08
33'44'-TCB (#77)	10	132	(36.3)	5.00	5.00	11.09	14.20	41.54
33'44'5-PenCB (#126)	10	13	(3.6)	5.00	21.62	34.25	48.35	218.79
33'44'55'-HxCB (#169)	10	19	(5.2)	5.00	16.82	24.52	32.56	85.92
Total non-*ortho* PCBs				20.00	53.42	76.02	99.52	281.74
2'344'5-PeCB (#123)	10	6	(1.6)	5.00	68.64	112.71	156.26	941.94
23'44'5-PeCB (#118)	10	0	(0.0)	635.80	3875.39	5860.12	8454.33	25243.31
2344'5-PeCB (#114)	10	6	(1.6)	5.00	230.99	343.92	475.61	1695.19
233'44'-PeCB (#105)	10	0	(0.0)	256.11	992.24	1479.96	2069.13	5991.69
23'44'55'-HxCB (#167)	10	1	(0.3)	5.00	482.22	713.17	1002.73	3430.73
233'44'5-HxCB (#156)	10	0	(0.0)	282.13	1345.64	1978.72	2726.27	9421.77
233'44'5'-HxCB (#157)	10	1	(0.3)	5.00	333.09	489.52	670.48	2712.74
233'44'55'-HpCB (#189)	10	3	(0.8)	5.00	168.63	238.74	337.06	950.16
Total mono-*ortho* PCBs				1724.33	7747.11	11471.65	15641.75	49632.02
Total Dioxins				1847.56	8149.66	11968.14	16432.17	50477.45
PCDDs-TEQ				1.65	5.09	6.92	9.20	29.32
PCDFs-TEQ				0.64	1.79	2.38	3.06	7.77
Non-*ortho* PCBs-TEQ				0.65	2.75	4.22	5.86	23.17
Mono-*ortho* PCBs-TEQ				0.05	0.23	0.34	0.47	1.49
Total Dioxins-TEQ				3.17	10.14	13.89	18.35	43.35

ND, not detectable

DL, detection limit

TEQs were calculated with toxic equivalency factor values (Van den Berg et al. 2006)

Table 3. Characteristics of risk factors for food allergies, eczema, asthma, and otitis media following binominal logistic regression analyses

Characteristic	object/reference	OR (95% CI)
Food allergy: 62 (17)[a]		
Paternal allergic history	yes/no	2.21 (1.18-4.15)*
Duration of breast-feeding (months)	≥4/<4	2.19 (1.04-4.65)*
Eczema: 41 (11.3)[a]		
Paternal allergic history	yes/no	3.28 (1.61-6.65)**
Asthma: 31 (8.8)[a]		
Pre-pregnancy BMI (kg/m^2)		1.11[b] (1.01-1.22)*
Maternal allergic history	yes/no	2.12 (1.00-4.48)*
Paternal allergic history	yes/no	3.67 (1.71-7.89)**
Day care attendance	yes/no	2.51 (1.17-5.40)*
Otitis media: 68 (18.7)[a]		
Parity	≥1/0	1.77 (1.03-3.04)*
Day care attendance	yes/no	4.67 (2.63-8.29)**

[a]number (%) of infants who developed allergies or infections

[b]per increasing unit of BMI

*, $p < 0.05$

**, $p < 0.01$

Table 4. Unadjusted and Adjusted ORs (95% CI) versus quartile 1 of total dioxin levels as quartiles for otitis media

	Crude				Adjusted			
	Quartile 2	Quartile 3	Quartile 4	p value for trend[c]	Quartile 2	Quartile 3	Quartile 4	p value for trend[c]
	OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)	OR (95% CI)	
TEQs								
Food allergy[a]								
PCDDs	1.45 (0.68-3.10)	1.22 (0.56-2.68)	0.93 (0.41-2.10)	0.757	1.54 (0.68-3.44)	1.30 (0.56-3.04)	1.09 (0.44-2.72)	0.958
PCDFs	1.50 (0.69-3.27)	1.21 (0.54-2.72)	1.30 (0.58-2.88)	0.678	1.57 (0.70-3.53)	1.36 (0.57-3.26)	1.50 (0.62-3.61)	0.379
Non-*ortho* PCBs	2.25 (0.99-5.12)	2.58 (1.14-5.83)*	1.01 (0.40-2.56)	0.867	2.11 (0.89-4.99)	3.17 (1.30-7.74)*	1.09 (0.40-3.00)	0.575
Mono-*ortho* PCBs	2.16 (0.94-4.94)	2.69 (1.19-6.06)*	1.17 (0.47-2.91)	0.602	2.49 (1.04-5.98)*	3.14 (1.29-7.60)*	1.34 (0.49-3.70)	0.420
Total Dioxins	1.40 (0.62-3.16)	2.10 (0.97-4.55)	1.00 (0.42-2.36)	0.709	1.52 (0.65-3.56)	2.21 (0.97-5.03)	1.18 (0.45-3.08)	0.435
Eczema[a]								
PCDDs	0.68 (0.25-1.86)	1.40 (0.58-3.39)	1.13 (0.45-2.80)	0.475	0.57 (0.19-1.72)	1.34 (0.50-3.57)	1.22 (0.42-3.56)	0.389
PCDFs	0.71 (0.27-1.86)	1.03 (0.42-2.50)	1.01 (0.42-2.47)	0.799	0.53 (0.19-1.50)	0.97 (0.37-2.53)	0.94 (0.35-2.57)	0.841
Non-*ortho* PCBs	1.45 (0.61-3.47)	0.89 (0.34-2.30)	0.79 (0.30-2.10)	0.434	1.32 (0.51-3.38)	0.88 (0.31-2.51)	0.63 (0.21-1.92)	0.306
Mono-*ortho* PCBs	1.37 (0.57-3.29)	1.04 (0.41-2.63)	0.82 (0.31-2.18)	0.582	1.29 (0.50-3.33)	1.09 (0.39-3.03)	0.71 (0.23-2.17)	0.553
Total Dioxins	0.70 (0.27-1.83)	1.10 (0.46-2.65)	0.90 (0.36-2.23)	0.941	0.63 (0.22-1.76)	1.10 (0.43-2.85)	0.82 (0.28-2.37)	0.976
Asthma[a]								
PCDDs	1.16 (0.40-3.33)	1.20 (0.42-3.46)	1.33 (0.47-3.74)	0.590	1.05 (0.34-3.26)	0.96 (0.30-3.12)	1.56 (0.46-5.32)	0.444
PCDFs	1.19 (0.39-3.70)	1.21 (0.39-3.75)	2.18 (0.78-6.07)	0.133	1.33 (0.40-4.38)	1.27 (0.37-4.37)	2.82 (0.87-9.15)	0.059
Non-*ortho* PCBs	1.30 (0.46-3.66)	1.00 (0.34-2.98)	1.33 (0.47-3.75)	0.718	1.04 (0.34-3.15)	1.10 (0.33-3.72)	1.03 (0.30-3.50)	0.747
Mono-*ortho* PCBs	1.33 (0.47-3.74)	1.71 (0.63-4.63)	0.73 (0.22-2.40)	0.819	1.02 (0.33-3.18)	1.84 (0.60-5.69)	0.57 (0.14-2.32)	0.803
Total Dioxins	0.85 (0.27-2.63)	1.48 (0.54-4.08)	1.32 (0.47-3.70)	0.409	0.79 (0.24-2.63)	1.30 (0.43-3.93)	1.32 (0.38-4.59)	0.327
Otitis media[b]								
PCDDs	1.00 (0.47-2.11)	1.04 (0.49-2.20)	1.01 (0.48-2.13)	0.946	1.20 (0.53-2.71)	1.14 (0.50-2.56)	1.51 (0.65-3.51)	0.393
PCDFs	1.30 (0.58-2.88)	1.63 (0.75-3.53)	1.71 (0.79-3.69)	0.139	1.60 (0.68-3.76)	2.19 (0.93-5.14)	2.50 (1.07-5.88)*	0.027
Non-*ortho* PCBs	1.56 (0.72-3.39)	1.80 (0.84-3.86)	1.20 (0.54-2.69)	0.598	1.82 (0.79-4.16)	2.52 (1.07-5.96)*	1.51 (0.62-3.63)	0.293
Mono-*ortho* PCBs	1.57 (0.74-3.33)	1.61 (0.76-3.43)	1.05 (0.47-2.36)	0.875	1.73 (0.76-3.95)	2.00 (0.87-4.60)	1.13 (0.47-2.75)	0.705
Total Dioxins	1.80 (0.84-3.86)	1.48 (0.68-3.23)	1.28 (0.58-2.84)	0.718	2.10 (0.92-4.79)	1.66 (0.71-3.86)	1.69 (0.70-4.07)	0.376

[a]adjusted for maternal age, pre-pregnancy BMI, parental allergic history, maternal educational level, parity, infant gender, duration of breast-feeding, environmental tobacco exposure, day care attendance, and blood sampling period

[b]adjusted for maternal educational level, parity, infant gender, duration of breast-feeding, environmental tobacco exposure, day care attendance, and blood sampling period

[c]quartiles applied to ordinal variables in the model

* $p < 0.05$

Table 5. Adjusted ORs (95% CI) versus quartile 1 of total dioxin levels as quartiles for otitis media

	Adjusted						
	Quartile 2		Quartile 3		Quartile 4		p value for trend[a]
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	
TEQs							
Males							
PCDDs	0.47	(0.13-1.78)	2.00	(0.65-6.19)	2.89	(0.83-10.10)	0.032
PCDFs	0.97	(0.28-3.29)	2.92	(0.87-9.83)	3.80	(1.09-13.18)*	0.012
Non-*ortho* PCBs	2.40	(0.70-8.27)	2.89	(0.86-9.67)	3.61	(0.98-13.29)	0.050
Mono-*ortho* PCBs	2.26	(0.63-8.11)	3.83	(1.18-12.41)*	1.88	(0.50-7.05)	0.179
Total Dioxins	2.07	(0.61-6.99)	2.19	(0.67-7.14)	4.44	(1.20-16.45)*	0.032
Females							
PCDDs	2.32	(0.71-7.57)	0.47	(0.11-1.99)	1.10	(0.30-4.11)	0.443
PCDFs	4.03	(1.10-14.74)*	1.23	(0.30-5.14)	1.28	(0.29-5.77)	0.411
Non-*ortho* PCBs	1.32	(0.41-4.31)	1.90	(0.51-7.07)	0.83	(0.22-3.07)	0.856
Mono-*ortho* PCBs	1.11	(0.37-3.40)	0.73	(0.18-2.96)	0.73	(0.21-2.59)	0.500
Total Dioxins	2.60	(0.78-8.60)	1.01	(0.25-3.99)	1.04	(0.27-4.06)	0.571

adjusted for maternal educational level, parity, duration of breast-feeding, environmental tobacco exposure, day care attendance, and blood sampling period in the logistic regression model

[a]quartiles applied to ordinal variables in the model

* $p < 0.05$

Table 6. Adjusted ORs (95% CIs) versus quartile 1 of congener levels as quartiles for otitis media

		Adjusted						
		Quartile 2		Quartile 3		Quartile 4		
		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	p value for trend[a]
Concentrations								
All								
PCDDs	OCDD	3.42	(1.38-8.47)*	2.77	(1.09-7.03)*	2.63	(1.01-6.87)*	0.120
PCDFs	2,3,4,7,8-PeCDF	1.62	(0.68-3.88)	2.04	(0.88-4.77)	2.81	(1.20-6.59)*	0.015
Non-*ortho* PCBs	33'44'-TCB (#77)	2.40	(0.99-5.85)	1.42	(0.61-3.29)	3.38	(1.57-7.29)*	0.006
Mono-*ortho* PCBs	233'44'5'-HxCB (#157)	2.39	(1.04-5.51)*	1.08	(0.43-2.73)	2.51	(1.07-5.89)*	0.157

adjusted for maternal educational level, parity, duration of breast-feeding, infant gender, environmental tobacco exposure, day care attendance, and blood sampling period in the logistic regression model

[a]quartiles applied to ordinal variables in the model

* $p < 0.05$

1