Title: Prenatal exposure to dioxin-like compounds is associated with decreased cord blood IgE and increased risk of wheezing in children aged up to 7 years: The Hokkaido Study.

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Abbreviations: AHR, aromatic hydrocarbon receptor; CI, confidence interval; DLC, dioxin-like compound; Ig, immunoglobulin; HRGC/HRMS, high-resolution gas chromatography/high-resolution mass spectrometry; β, partial regression coefficient; PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated dibenzofurans; PCB, polychlorinated biphenyls; RSV, respiratory syncytial virus; TEF, toxic equivalent

factor; TEQ, toxic equivalent.

Abstract

【Introduction】In utero exposure to dioxin-like compounds (DLCs) may cause imbalance of immune development in early infancy. However, there are few epidemiological studies into the effects of in utero exposure to DLCs on allergies and infections during childhood. This study evaluates associations between concentrations of maternal DLCs and cord blood immunoglobulin (Ig) E, as well as allergies and infections during childhood.【Method】We recruited 514 pregnant women in a maternity hospital in Sapporo, Japan, and measured concentrations of DLCs in 426 maternal blood samples using high-resolution gas chromatography/high-resolution mass spectrometry. We examined the relationship between concentrations of maternal DLCs and cord blood IgE at birth (n=239), as well as for allergies and infections in children at 3.5 (n = 327) and 7 (n = 264) years, using regression analysis adjusted for confounding variables. 【Results】We found a positive association between maternal DLC concentrations and frequency of wheezing in children aged up to 7 years [odds ratio (OR); 7.81 (95% confidence interval (CI), 1.42 to 42.9)]. At 3.5 years, boys showed inverse associations between maternal DLC concentrations and cord blood IgE [partial regression coefficient; -0.87 (95% CI), -1.68 to -0.06], and frequency of wheezing [OR; 0.03 (95% CI), 0.00 to 0.94] but girls did not. 【Discussion】As one reason for the significant association observed at 7 but absent at 3.5 years, we suggest that allergic symptoms are more obvious in older children due to matured immune function.【Conclusion】The findings suggest that prenatal exposure

to DLCs may modify offspring immune responses and result in increased risk of allergy among children of school age.

1. Introduction

The general population experiences widespread exposure to persistent organic pollutants, including dioxin-like compounds (DLCs) from environmental sources and daily food intake (Todaka et al., 2008). Seventeen polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs) and 12 polychlorinated biphenyls (PCBs) have been categorized as DLCs (Van den Berg et al., 2006). In utero exposure to DLCs can cause various toxicities, including carcinogenicity, teratogenicity; endocrine, immune, and reproductive disruption; and neurobehavioral effects (WHO, 2012). DLCs are aromatic hydrocarbon receptor (AHR) agonists that disrupt normal fetal development by binding to AHRs (Van den Berg et al., 2006).

Animal studies have demonstrated that fetal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure inhibits cellular differentiation and maturation, particularly of T lymphocytes; causes thymic atrophy; and leads to immunosuppression in offspring (Yoshizawa et al., 2007). In humans, a systematic review by Gascon et al. (2013) included several studies reporting higher risk of respiratory infections, including acute otitis media, among infants (Cao et al., 1997; Dallaire et al., 2004; Glynn et al., 2008; Miyashita et al., 2011; Stolevik et al., 2011) in relation to in utero exposure to PCBs and/or dioxins. In the Rotterdam study of the Netherlands cohort, dioxins in breast milk were significantly associated with a higher prevalence of infections as well as a lower prevalence of

shortness of breath with asthma at 42 months. In addition, these indicators were related to a reduction in antibody responses to vaccinations and an increased number of T-lymphocytes at 42 months (Weisglas-Kuperus et al., 2000). In Japan, increased concentrations of DLCs were significantly associated with increased lymphocyte subset ratio in offspring (Nagayama et al., 2007). Most of the current knowledge about in utero exposure to DLCs relates to reduced immune response, and higher incidences of infectious symptoms in early infancy have been relatively consistent.

However, human studies focusing on possible long-term effects of in utero exposure to DLCs on outcomes for allergic and infectious symptoms among school age children and young adults are scarce. In the Rotterdam study, higher PCB levels, but not dioxins in breast milk, were associated with less shortness of breath with wheezing at 7 years (Weisglas-Kuperus et al., 2004). In the Amsterdam study of the Netherlands cohort, a decrease in allergies at 8 years (ten Tusscher et al., 2003) and decreased lung function among offspring aged 7–12 years were associated with an increasing concentration of DLCs in the mother's breast milk (ten Tusscher et al., 2001). On one hand, in a Danish cohort of 965 pregnant women, there was a positive association between maternal concentration of dioxin-like PCBs and offspring risk of using asthma medication during a 20-year follow-up period (Hansen et al., 2014). The same cohort study indicated that in utero exposure to dioxin-like PCBs appeared to be associated with airway obstruction but not allergic sensitization among the 421 offspring at age 20 (Hansen et al., 2016). The findings of such studies on humans

regarding the association between in utero exposure to DLCs and allergies or infections during childhood and adolescence have been inconsistent due to the small number of previous studies on long-term effects of DLCs.

Only one cross-sectional study previously reported a positive correlation for a dioxin-like PCB congener: PCB118 in maternal placental tissue and cord blood IgE using the Spearman correlation (Reichortova et al., 1999). We have previously reported increased incidence of infections, otitis media at 18 months of age, but not allergy, associated with increasing concentration of DLCs in maternal blood (Miyashita et al., 2011). While that study indicated that in utero exposure to DLCs might affect immune function immediately after birth, we did not evaluate immune response at birth. The present study evaluates associations between concentrations of maternal DLCs and cord blood IgE, as well as allergies and infections in children aged up to 3.5 and 7 years.

2. Materials and Methods

2.1. Study participants and collection of baseline questionnaire data and medical records at birth

The participants in this study were enrolled in the Hokkaido Study on Environment and Children's Health. A total of 514 pregnant Japanese women were recruited at the Sapporo Toho Hospital in Hokkaido, Japan, from July 2002 to September 2005 (Kishi et al., 2013). Details regarding the study participants and the collection of baseline questionnaires and medical records at birth have been described previously (Kishi et al., 2013). An overview of this study is shown in Figure 1. Among the 514 women, 10 were excluded due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study until birth. Medical records were obtained from 504 participants.

2.2. Data collection from follow-up questionnaires at age 3.5 and 7 years

At 3.5 years post-delivery, participants completed another self-administered questionnaire. Of the 443 women to whom the questionnaire was mailed, 345 (77.8%) responded. Thirty-five participants were excluded due to death of the infant, relocation, or voluntary withdrawal for the follow-up period. At 7 years post-delivery, participants completed another self-administered questionnaire, with a response rate of 71.0% (281/396). The follow-up questionnaires included

information related to breast-feeding, environmental exposure to tobacco smoke, keeping pets in the home, living environment, daycare attendance, infant vaccination, and previous or current medical history of infant allergies and infectious symptoms aged up to 3.5 and 7 years.

For this study, all participating women provided written informed consent, and the study protocol was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Center for Environmental and Health Sciences.

2.3. Assessment of infant allergies and infections

Outcomes of allergies and infections during childhood were assessed based on mothers' self-administered questionnaire responses concerning children aged 3.5 and 7 years. Food allergy was defined as a positive response to the following question: “Has your child ever had symptoms such as hives, swelling of the lips, emesis, diarrhea, or respiratory distress when they ate food allergens including milk, egg rice gruel, egg-drop, shrimp, or other foods?” Eczema was defined, using a modified part of the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) phase-I questionnaire (ISAAC Steering Committee, 1998), as a positive response to the following questions: “1) Has your child ever had an itchy rash which was coming and going for at least 6 months? If yes: 2) Has your child ever had an itchy rash at least one time during 12 months? If yes: 3) Has this itchy rash at any time affected any of the following places: the

folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?" Wheezing was defined, using a modified part of the Japanese version of the American Thoracic Society-Division of Lung Diseases (ATS-DLD) questionnaire (Nishima et al., 2009), as a positive response to at least one of the following questions: 1) "Has your child ever had an attack of wheezing and/or shortness of breath in the past? 2) Has your child ever had a doctor's diagnosis for possible bronchial asthma, asthmatic, or pediatric asthma in the past?" Any allergies of outcome were defined as children who had at least one allergic symptom, including food allergy, eczema, and wheezing in the follow-up questionnaires at ages 3.5 or 7 years.

To estimate the proportion of infectious diseases, we defined an outcome based on the following criteria: If children had a positive response to the following medical question: "Has your child ever had a doctor's diagnosis, hospitalization, or medical treatment for the following diseases: febrile convulsion, otitis media, pneumonia, bronchitis, RSV diseases, chicken pox, and other infections?" Infections of outcome were defined as children who had at least one infectious symptom (as listed in the above question) indicated in the follow-up questionnaires at ages 3.5 or 7 years. Respiratory infections of outcome were defined as children who had at least one infectious symptom, including pneumonia and bronchitis.

2.4. Measurement of DLC concentrations in maternal blood

A 40-ml blood sample was taken from the maternal peripheral vein after the 2nd trimester to the 3rd trimester of pregnancy. Doctors did not allow collection of blood samples from pregnant women with poor physical conditions. In such cases, we obtained maternal blood samples during hospitalization immediately after delivery. All samples were stored at –80°C until analysis.

The concentrations of DLCs in maternal blood were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large-volume injection system at Fukuoka Institute of Health and Environmental Sciences. Details of the measurement method have been described in previous reports (Todaka et al., 2003; 2008).

2.5. Measurement of IgE in cord blood

At the time of delivery, a blood sample (10–30 mL) was collected from the umbilical cord. All samples were stored at –80°C until analysis. Concentrations of total IgE in cord blood were measured using an enzyme-linked immunosorbent assay (IMx analyzer, Abbott Japan Co., Ltd., Tokyo, Japan). IgE concentrations were measured in 268 cord blood samples at SRL, Inc. (Tokyo, Japan).

2.6. Statistical analysis

Fig. 1 shows an overview of follow-up participants in this study. We ultimately included

239 mother–child pairs in the analysis at birth for whom DLCs and IgE had been measured, 327 pairs who subsequently provided follow-up responses at 3.5 years post-delivery, and 264 pairs in the follow-up questionnaire at 7 years post-delivery.

We used the Spearman correlation test, the Mann–Whitney U-test, and the Kruskal–Wallis test to investigate possible associations between concentrations of maternal DLCs, cord blood IgE, characteristics of participants, and children's health outcomes. Crude and adjusted linear regression analyses were performed to evaluate associations between concentrations of maternal DLCs and cord blood IgE among boys only, girls only, and among all children. Concentrations of DLCs and IgE were converted to a log10 scale to fit a normal distribution. In linear regression models, we evaluated partial regression coefficients (β) for log10-transformed concentrations of cord blood IgE with maternal DLCs. Multivariate analyses were adjusted for confounding variables that influenced cord blood IgE in binominal analyses, possible risk factors reported in previous studies such as maternal age, BMI, parity, education, smoking during pregnancy, blood lipid, allergic history, infant gender, birth season, distance from home to highway and household income (Hansen et al., 2016, Okada et al., 2012), and the blood sampling period. Multivariate linear regression analyses were adjusted for confounding variables, including maternal factors (age (continuous), parity (primiparous/multiparous), smoking during pregnancy (yes/no), pelagic fish intake during pregnancy (<once/week or ≥once/week), allergic history (yes/no)), and paternal allergic history (yes/no), annual

household income (≤5 million yen or >5 million yen), blood sampling period (<28 weeks, 28 to <36 weeks, ≥36 weeks, or after delivery), and infant gender (Table 4). In an additional analysis, we included potential covariates such as maternal education, distance from home to highway and birth season.

Crude and adjusted logistic regression analyses were performed to evaluate associations between DLC concentrations and the risk of allergies and infections among boys, girls, and among all children. In the logistic models, we evaluated odds ratios (ORs) for the risk of allergies and infection using log10-transformed maternal DLCs levels. Multivariate logistic regression analyses were adjusted for confounding variables that influenced allergies or infections in binominal analyses, possible risk factors reported in previous studies, such as maternal age, pre-pregnancy BMI, education level, parity, parental allergic history, infant gender, duration of breast-feeding, environmental tobacco smoke exposure, and day care attendance (Miyashita et al., 2011; ten Tusscher et al., 2003; Weisglas-Kuperus et al., 2000; 2004), and the blood sampling period. We employed adjusted modeling in two stages (1 and 2) to confirm changed ORs of allergies caused by controlling for potentially confounding variables. In the first step, adjusted model 1 included confounding variables of maternal factors (pre-pregnancy body mass index (BMI) (continuous), parity, educational level (≤12 years and ≥13 years), allergic history), paternal allergic history, and infant gender. In the second step, adjusted model 2 included

environmental factors after birth (breast-feeding period (<4 months or ≥4 months), environmental tobacco smoke exposure (yes/no), daycare attendance (yes/no)) in addition to confounding variables of model 1. We conducted two steps of adjusted models 1 and 2 to confirm changes in the ORs of infections resulting from controlling for potentially confounding variables as following allergic adjusted models, excluding variables describing maternal and paternal allergy history. In regression analysis stratified by infant gender, a variable of infant gender was excluded from controlling for confounding variables. All confounding factors for controlling each model are shown as footnotes in Tables 4, 5, and 6. Statistical analyses used SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Results were considered statistically significant at $p < 0.05$.

3. Results

Median DLC concentrations among the three participant groups (at birth, n=239; age 3.5, n=327; age 7, n=264) were: 14.0 TEQ pg/g lipid (interquartile range, IQR 10.1–18.0) at birth: 14.2 TEQ pg/g lipid (IQR 10.3–18.9) in children at age 3.5 years; and 15.0 TEQ pg/g lipid (IQR 11.0–20.0) in children at age 7 years. DLC concentration ranges were not significantly different between the three participant groups. The median IgE concentration of the group at birth was 0.21 IU/mL (IQR 0.08–0.55).

Possible associations between maternal DLC concentrations, cord blood IgE concentrations, and various characteristics of parents and infants were analyzed (Tables 1 and 2). The concentrations of DLCs in maternal blood were significantly associated with maternal factors, including age, parity, educational level, and blood sampling period (Tables 1 and 2). The concentrations of cord blood IgE were significantly associated with maternal factors, including pelagic fish intake and allergic history; and with infant gender (Table 1). No associations were observed between cord blood IgE concentration and allergies or infections in children aged up to 3.5 and 7 years (data not shown).

The numbers and frequency of children who developed allergies (including food allergy, eczema, and wheezing), and infectious (including otitis media and respiratory infections) aged up to 3.5 and 7 years were also determined (Table 3). Children who had any infections up to age 7 showed

higher DLC concentrations compared with non-infected children aged up to 7 years (Table 3). However, there were no differences in DLC concentrations between other allergies and infections (Table 3). Thus, we did not analyze individual outcomes of chicken pox, bronchitis, RSV diseases, rhinitis, pneumonia, skin infection, and other viral infections in subsequent analyses because the numbers of cases of infection were very low except for otitis media and respiratory infections, and because sufficient statistical power could not be ensured in the multivariate analysis.

Possible associations were analyzed between participant characteristics and allergies/infectious in children aged up to 3.5 and 7 years (supplemental Tables 1 and 2). The frequencies of allergies and infections among children were significantly associated with maternal factors (including parity, BMI before pregnancy, educational level, pelagic fish intake, tobacco smoking and alcohol consumption during pregnancy, allergic history, and blood sampling period), paternal allergic history, living environment after birth (including environmental tobacco exposure and keeping pets), and child factors (including gender, duration of breast-feeding and daycare attendance) ($p < 0.05$).

Table 4 shows β and 95% confidence intervals (CI) between log10-transformed concentrations of maternal DLCs and cord blood IgE at birth (n = 239). IgE concentration in cord blood decreased significantly with high maternal dioxin concentration among boys, but not among girls or both sexes combined, as shown by regression analysis adjusted for confounding factors

[adjusted β; -0.87 (95%CI, -1.68 to -0.06) among boys] (Table 4).

Tables 5 and 6 show the results of logistic regression analyses between maternal DLC concentrations and allergies and infections in children aged up to 3.5 and 7 years. No significant associations were observed between maternal DLC concentrations and food allergies, eczema, wheezing, and infections at aged 3.5 years in either the crude or adjusted models among all (Tables 5 and 6). Significant positive associations were observed between maternal DLC concentrations and wheezing in the adjusted models (Table 5), as well as infections in the crude model at age 7 years among all (Table 6) [adjusted 2 OR; wheezing = 7.81 (95% CI, 1.42 to 42.9), crude OR; infections = 4.70 (95% CI, 1.03 to 21.5)]. No significant associations were observed between maternal DLC concentrations and food allergy, eczema, otitis media, and respiratory at age 7 years in both the crude and adjusted models among all.

In stratified analysis by gender, Tables 5 and 6 show the results of logistic regression analyses between maternal DLC concentrations and allergies and infections in children up to 3.5 and 7 years among boys and girls. Among boys, significant inverse associations were observed between maternal DLC concentrations and wheezing in the adjusted models at 3.5 years (Table 5) [adjusted 2 OR; wheezing = 0.03 (95% CI, 0.00 to 0.94)]. Regarding other outcomes of allergies and infections in children aged up to 3.5 and 7 years, no significant associations were observed for maternal DLC concentrations in either the crude or adjusted models among boys or girls.

4. Discussion

4.1. Allergies and infections during childhood

In the logistic regression analysis adjusted for confounding factors, we found positive association between maternal DLC concentration and frequency of wheezing at 7 years of age among all. Our observations suggest that prenatal exposure to DLCs may modify offspring immune responses, resulting in increased risk of allergy among school-age children. We performed an additional sensitivity analysis excluding or including potential covariates associated with maternal DLCs, such as maternal age, parity, smoking during pregnancy, allergic history, and household income. The analysis was conducted considering the abovementioned covariates both individually and collectively. When maternal parity was excluded, the positive correlation between maternal DLCs and wheezing children aged up to 7 years was not significant. This suggests that maternal parity may be a controlling factor affecting the elimination of DLCs from the maternal body (Milbrath et al., 2009), and maternal parity is associated with the existence of siblings of children, which has been reported as an associated factor for the wheezing symptom (Rossi et al., 2017). The results remained essentially the same with the inclusion or exclusion of the other covariates. The additional sensitivity analysis complimented the validity of our observations. Our observation is inconsistent with that of the Amsterdam study, which reported inverse association between

concentrations of DLCs maternal breast milk and allergic symptoms at 8 years of age (ten Tusscher et al., 2003). The number of participants in the Amsterdam study was relatively small (n=27), and the study included only infants who were breast-fed for at least two months. These different parameters may have resulted in the inconsistent findings between our findings and those of the Amsterdam study. In a Danish study, there was a positive association between maternal concentration of dioxin-like PCBs and offspring risk of using asthma meditation (Hansen et al., 2014), but not allergic sensitization among the offspring at 20 years of age (Hansen et al., 2016). We cannot directly compare the results for children in this study with those for young adults in the Danish study, due to substantial changes in allergic sensitization and hormonal balance pre- versus post-puberty. However, this study may provide complementary evidence regarding the long-term effects of in utero exposure to DLC from early infancy and young adults on the balance of the immune response.

We found no clear associations between allergies and infections at 3.5 years of age among all, which was consist with the results at 18 months of age in the same cohort of the present study (Miyashita et al., 2011). In contrast, we found positive association between maternal DLC concentration and frequency of wheezing at 7 years. As one reason for the significant association in children aged 7 but not 18 months and 3.5 years, we consider viral bronchitis (RSV) damage to the bronchial development and mucous membrane, which has been reported as a risk factor for

wheezing (Rossi et al., 2017). However, in our study, no association between bronchitis and DLCs was observed. Immune suppression at early infancy from prenatal exposure to DLCs may potentially result in decreased resistance to infections, including that in the bronchial tube, and may be a risk factor of wheezing in children aged up to 7 years. During infancy, allergies are sometimes confused with infectious or others symptoms because they often share symptoms. For example, food allergies share symptoms with lactose intolerance (Assa'ad, 2006), and asthmatic conditions share symptoms with respiratory infections (Custovic et al., 2005). Therefore, the clear results at age 7 might derive from more certain diagnosis of allergy symptoms, which were distinct from infections.

Regarding comparison with levels of DLCs exposure, median concentrations of DLCs in breast milk in the same cohort of the present study (10 TEQ pg/lipid) (Todaka et al., 2010) were lower than those reported from other regions: Netherlands (35.8 TEQ pg/p lipid), Italy (30.0 TEQ pg/g lipid), Germany (26.3 TEQ pg/g lipid); the USA (11.8 TEQ pg/g lipid), and Osaka, Japan (24.5 TEQ pg/g lipid) (Nakatani et al., 2005). Inconsistent with our results, two prospective cohort studies in the Netherlands showed that exposure to DLCs might be correlated with a lower prevalence of allergic diseases at age 3.5 years, due to postnatal increase of infectious diseases caused by DLCs (Weisglas-Kuperus et al., 2000; 2004). Therefore, one of the reasons for the inconsistent results may be the difference in DLC exposure level.

4.2. Cord blood IgE at birth.

Linear regression analysis adjusted for confounding factors showed no clear association between maternal DLC concentrations and cord blood IgE at birth among all. Our results are partly consistent with a study from Slovakia, which reported no association between concentrations of placental DLCs and cord IgE, excluding PCB-118 (Reichrtova et al., 1999). Jusko et al. (2011) expressed uncertainty about this positive association between PCB-118 and IgE, because it may disappear after controlling for potential confounders, and reveal no evidence with more toxic properties of AhR-mediated immunotoxicity about PCB-118, compared to other DLCs (Hogaboam et al., 2008). Therefore, cord blood total IgE may be insufficiently sensitive to detect subtle perturbations in immune responses caused by prenatal exposure to DLCs.

4.3. Gender differences in effects of DLC exposure

Among boys, we found inverse associations between maternal DLCs and both cord blood IgE at birth and also wheezing aged up to 3.5 years, but these were not observed for girls. In the same cohort, a previous evaluation of offspring birth weight, neurodevelopment, and risk of infection at 18 months of age (Miyashita et al., 2011), suggested that male infants are more susceptible than female infants to DLCs (Kishi et al., 2013). This finding is in accordance with the present study and previous studies regarding sex ration (Mocarelli et al., 1996), lymphocyte subset

rate (Nagayama et al., 2007), and birth weight (Sonneborn et al., 2008). It appears that male infants are more susceptible to exposure to DLCs, which might be due to gender-specific endocrine activities. In animal studies, male rat offspring may be more sensitive than females to TCDD-mediated suppression of T-cell activity because the lowest-observed-adverse-effect-level for immunosuppression was lower in male (median 0.1 μg/kg) than in female (median 0.3 μg/kg) offspring (Luebke et al., 2006). In addition, before adolescence, boys may be more susceptible to infections and allergies than girls because both innate and acquired immunity are influenced by reproductive hormones (Eshima et al., 2012; Wright et al., 2006). Sex- and age-related differences in the morbidity of infections and allergy might reflect differences in the relative physiological development of immune, endocrine, and reproductive systems between boys and girls as they grow (Eshima et al., 2012; Wright et al., 2006). Therefore, we suggest that, for boys, who appear to be more vulnerable to DLCs, prenatal DLC exposure may continually affect immune responses at birth, in infancy, and among pre-school children, resulting in increased frequency of allergies in children at school age. However, in this study, whereas CIs from small sample sizes trend be wide, may produce less precise results; we therefore need more evidence from larger studies.

4.4. Strengths and limitations

We prospectively assessed cord IgE at birth, and the frequency of allergies and infections

among offspring up to 3.5 and 7 years, thereby achieving continuous evaluation of the effects among offspring of prenatal DLC exposure. Secondly, we ensure the outcome validity of allergy data by using a modified ISAAC phase-I (ISAAC Steering Committee, 1998) or ATS-DLD questionnaire (Nishima et al., 2009), which are internationally standardized procedures. Finally, the present study measured the concentrations of 29 DLC congeners in maternal blood during pregnancy, but not breast milk, using HRGC/HRMS, thereby utilizing reliable data for direct assessment of prenatal DLC exposure levels.

The principal limitation of our study is its small sample size. A larger sample might reveal more clearly any gender difference in susceptibility to in utero DLC exposure as a factor for allergic and infectious symptoms. However, potential bias may occur because we did not evaluate the effects of postnatal or early childhood exposure through breast-feeding and food intake, which may determine children's body burden of DLCs (Milbrath et al., 2009). Participants including in the present study had different characteristics as compared with those who could not follow up (supplemental Table S3). However, DLC concentration ranges were not significantly different between the three participant groups. Moreover, questionnaire recovery rates were >70% (Fig. 1); therefore selection bias may become minimized in the present study. In future studies, the effects of postnatal DLC exposure via breast milk or foods, and the relationship to allergies and infections, should also be evaluated.

5. Conclusion

Our participating parents showed low exposure to DLCs compared with levels reported in other areas in Japan, the USA, and Europe. Nevertheless, prenatal exposure to DLCs may modify offspring immune responses and result in increased risk of allergy among children at school age. Moreover, male infants may be more susceptibility to maternal exposure to DLCs.

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Table 1 Characteristics of study participants related to concentrations of maternal dioxin-like compounds (DLCs) at birth (n=239)

				DLCs (TEQ pg/g lipid)		cord IgE	
		No. (%)	Mean ± SD	Median (IQR)	r	Median (IQR)	r
Mother							
Age at delivery (years)		239	30.3 ± 4.9		0.32**		-0.08
Pre-pregnancy BMI (kg/m^2)		239	20.9 ± 3.0		0.01		0.02
Parity	0	127 (53.1)		14.9 (12.2–19.5)**		0.21 (0.08–0.49)	
	≥1	112 (46.9)		12.6 (9.10–17.1)		0.20 (0.09–0.57)	
Educational level	≤12 years	103 (43.1)		13.0 (9.34–17.8)		0.19 (0.07–0.48)	
	>12 years	136 (56.9)		14.5 (10.7–18.9)		0.24 (0.09–0.58)	
Tobacco smoking during pregnancy	No	197 (82.4)		14.2 (10.6–18.4)*		0.22 (0.09–0.56)	
	Yes	42 (17.6)		12.2 (8.88–17.1)		0.20 (0.06–0.49)	
Alcohol consumption during pregnancy	No	159 (66.5)		14.0 (9.48–18.0)		0.20 (0.08–0.47)	
	Yes	80 (33.5)		14.1 (10.8–18.6)		0.24 (0.11–0.61)	
Shoreline fish	<once/week	128 (53.6)		13.7 (9.9–17.7)		0.20 (0.09–0.38)	
	≥once/week	111 (46.4)		14.5 (10.4–18.5)		0.26 (0.07–0.90)	
Pelagic fish	<once/week	108 (45.2)		13.9 (10.3–17.8)		0.18 (0.08–0.37)*	
	≥once/week	131 (54.8)		14.1 (10.1–18.8)		0.26 (0.09–0.86)	
Allergic history	No	173 (72.4)		13.8 (9.71–17.8)*		0.19 (0.07–0.45)*	
	Yes	66 (27.6)		14.6 (11.0–20.2)		0.30 (0.17–0.66)	
Blood sampling period	<28 weeks	11 (4.6)		18.8 (9.48–26.9)		0.33 (0.16–1.90)	
	28 to <36 weeks	90 (37.7)		14.4 (11.0–18.2)		0.22 (0.08–0.62)	
	≥36 weeks	64 (26.8)		13.9 (9.38–17.8)		0.19 (0.07–0.38)	
	After delivery	74 (31.0)		13.7 (9.72–17.8)		0.20 (0.10–0.65)	
Father							
Allergic history	No	198 (82.8)		13.9 (10.0–17.9)		0.20 (0.08–0.55)	
	Yes	41 (17.2)		14.7 (10.8–19.2)		0.24 (0.14–0.60)	
Living environment							
Annual household income during pregnancy	≤5 million yen	168 (70.3)		13.6 (9.76–17.0)**		0.19 (0.08–0.45)	
	>5 million yen	71 (29.7)		16.3 (11.1–20.8)		0.30 (0.11–0.69)	
Child							
Gender	Boy	112 (46.9)		14.1 (10.4–17.9)		0.29 (0.10–0.71)*	
	Girl	127 (53.1)		13.8 (9.85–18.7)		0.19 (0.07–0.42)	
Gestational age (wks)			39.3 ± 1.0		0.05		0.07
Birth weight (g)			3146.4 ± 335.9		-0.03		0.04

BMI: body mass index. r: Spearman's rank correlation coefficient. *p<0.05, **p<0.01 by Mann–Whitney U-test and Spearman's rank correlation test.

Table 2 Characteristics of study participants related to concentrations of maternal dioxin-like compounds (DLCs) in childhood

		3.5 years of age (n=327)				7 years of age (n=264)			
				DLCs (TEQ pg/g lipid)				DLCs (TEQ pg/g lipid)	
		No. (%)	Mean ± SD	Median (IQR)	r	No. (%)	Mean ± SD	Median (IQR)	r
Mother									
Age at delivery (years)		327	31.0 ± 4.4		0.24**	264	31.3 ± 4.4		0.22**
Pre-pregnancy BMI (kg/m^2)		327	21.2 ± 3.2		0.03	264	21.1 ± 3.0		0.03
Parity	0	164 (50.3)		15.4 (11.6–20.8)**		129 (49.0)		16.6 (12.4–21.1)**	
	≥1	162 (49.7)		13.8 (9.8–17.7)		134 (51.0)		14.1 (10.1–18.3)	
Educational level	≤12 years	127 (38.8)		13.6 (9.3–18.2)*		91 (34.5)		14.0 (9.4–18.8)	
	>12 years	200 (61.2)		15.1 (11.3–19.6)		173 (65.5)		15.3 (11.5–20.2)	
Tobacco smoking during pregnancy	No	289 (88.4)		14.4 (10.6–19.2)		236 (89.4)		15.2 (11.3–19.9)	
	Yes	38 (11.6)		12.8 (9.0–18.6)		28 (10.6)		13.7 (8.9–21.1)	
Alcohol consumption during pregnancy	No	228 (69.7)		14.0 (9.7–18.1)		180 (68.2)		14.7 (10.4–19.6)	
	Yes	99 (30.3)		14.9 (12.0–20.0)		84 (31.8)		15.6 (12.0–20.2)	
Shoreline fish	<once/week	179 (54.7)		13.8 (9.9–18.1)		147 (55.7)		14.0 (10.2–19.5)	
	≥once/week	148 (45.3)		15.3 (10.9–19.4)		117 (44.3)		16.3 (11.4–20.4)	
Pelagic fish	<once/week	150 (45.9)		13.8 (9.8–18.0)		115 (43.6)		14.0 (10.3–18.9)	
	≥once/week	177 (54.1)		15.0 (10.7–19.7)		149 (56.4)		15.8 (11.0–20.4)	
Allergic history	No	234 (71.6)		14.1 (9.9–18.7)		184 (69.7)		15.1 (10.3–19.4)	
	Yes	93 (28.4)		14.2 (11.0–20.2)		80 (30.3)		15.0 (11.4–20.8)	
Blood sampling period	<28 weeks	20 (6.1)		18.4 (14.4–25.7)*		17 (6.4)		18.8 (14.6–26.6)	
	28 to <36 weeks	146 (44.6)		15.0 (10.4–19.6)		119 (45.1)		15.2 (11.0–20.0)	
	≥36 weeks	67 (20.5)		13.4 (9.3–17.2)		52 (19.7)		14.2 (9.6–18.7)	
	After delivery	94 (28.7)		13.9 (10.5–18.5)		76 (28.8)		14.3 (11.3–19.4)	
Father									
Allergic history	No	266 (81.3)		14.2 (10.2–19.0)		215 (81.4)		15.1 (10.7–20.0)	
	Yes	61 (18.7)		14.0 (11.2–18.9)		49 (18.6)		14.9 (11.5–19.9)	
Living environment									
Environmental tobacco exposure	No	138 (42.2)		15.3 (11.9–20.0)*		133 (50.4)		15.0 (11.3–20.1)	
	Yes	189 (57.8)		13.7 (9.4–18.2)		131 (49.6)		15.3 (10.4–19.7)	
Keeping pets	No	275 (84.1)		14.6 (11.0–19.1)*		219 (83.3)		15.1 (11.5–20.0)	
	Yea	52 (15.9)		12.6 (8.7–17.4)		44 (16.7)		14.7 (8.7–21.5)	
Annual household income during pregnancy	≤5 million yen	212 (64.8)		13.6 (10.0–17.9)**		161 (61.0)		13.8 (10.5–18.9)*	
	>5 million yen	115 (35.2)		16.3 (11.0–20.8)		103 (39.0)		16.8 (11.4–21.0)	
Child									
Gender	Boy	160 (48.9)		14.2 (10.3–18.8)		134 (50.8)		14.5 (11.0–19.6)	
	Girl	167 (51.1)		14.2 (10.2–19.0)		130 (49.2)		15.2 (10.9–20.1)	
Birth weight (g)		—				—			
Duration of breast-feeding	<4 months	52 (16.0)		15.6 (9.9–19.6)		40 (15.8)		16.2 (10.5–20.7)	
	≥4 months	272 (84.0)		14.1 (10.3–18.9)		213 (84.2)		14.4 (11.0–19.7)	
Daycare attendance	No	153 (46.8)		14.1 (10.1–19.2)		—			
	Yes	174 (53.2)		14.3 (10.8–18.9)		—			

BMI; body mass index. r: Spearman's rank correlation coefficient. *p<0.05, **p<0.01 by Mann–Whitney U-test and Spearman's rank correlation test.

Table 3 Outcomes of allergy and infection in childhood related to concentrations of maternal dioxin-like compounds (DLCs).

		3.5 years of age (n=327)			7 years of age (n=264)		
			DLCs (TEQ pg/g lipid)			DLCs (TEQ pg/g lipid)	
		No. (%)	median (IQR)	p	No. (%)	median (IQR)	p
Allergy	No	191 (58.4)	14.4 (10.0–19.4)	0.77	116 (43.9)	15.1 (9.8–20.1)	0.44
	Yes	136 (41.6)	13.8 (10.8–18.3)		148 (56.1)	15.0 (11.5–19.9)	
Food allergy	No	255 (78.0)	14.1 (10.0–18.9)	0.40	206 (78.0)	14.4 (10.3–20.0)	0.30
	Yes	72 (22.0)	15.3 (11.6–18.8)		58 (22.0)	15.8 (12.5–19.7)	
Eczema	No	258 (78.9)	14.5 (10.3–19.2)	0.64	183 (69.3)	15.3 (10.3–20.0)	0.92
	Yes	69 (21.1)	13.6 (10.7–18.6)		81 (30.7)	14.1 (11.4–19.8)	
Wheezing	No	285 (87.2)	14.2 (10.4–19.0)	0.70	175 (66.3)	14.2 (10.4–19.4)	0.07
	Yes	42 (12.8)	13.6 (9.7–18.7)		89 (33.7)	16.2 (11.8–20.9)	
Infections	No	108 (33.0)	14.5 (11.6–19.0)	0.35	60 (22.7)	13.6 (9.6–17.2)	0.03
	Yes	219 (67.0)	14.1 (10.0–18.9)		204 (77.3)	15.7 (11.5–20.5)	
Otitis media	No	191 (58.4)	14.3 (11.5–19.0)	0.26	149 (56.4)	14.3 (11.3–19.4)	0.35
	Yes	136 (41.6)	13.9 (9.8–18.8)		115 (43.6)	16.1 (10.6–20.6)	
Respiratory infection	No	261 (79.8)	14.2 (10.3–18.9)	0.96	216 (81.8)	15.2 (11.4–19.9)	0.49
	Yes	66 (20.2)	13.8 (10.3–19.6)		48 (18.2)	13.9 (9.6–20.5)	

P values were calculated by the Mann–Whitney U-test.

Table 4 Concentrations of cord blood IgE related to concentrations of maternal dioxin-like compounds (DLCs).

	cord IgE	
	Crude	Adjusted
	β (95%CI)	β (95%CI)
All[a]	-0.11（-0.53, 0.32)	-0.14（-0.63, 0.35)
Boy[b]	-0.54（-1.18, 0.11)	-0.87（-1.68, -0.06)*
Girl[b]	0.25（-0.31, 0.81)	0.27（-0.38, 0.91)

Adjusted[a] (ALL): adjusted for maternal factors (age, parity, smoking during pregnancy, pelagic fish intake during pregnancy, allergic history), and paternal allergic history, annual household income, blood sampling period, and infant gender.

Adjusted[b] (boy and girl): adjusted for potential confounding factors excluding infant gender from adjusted[a] (ALL) in stratified analysis by infant gender.

* Statistically significant (p-value ＜0.05).

Table 5 Odds ratio (95% CI) between concentrations of dioxin-like compounds (DLCs) and allergy risk

		3.5 years of age			7 years of age		
		Crude	Adjusted 1	Adjusted 2	Crude	Adjusted 1	Adjusted 2
		OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
All[a]	allergy	0.83（0.26, 2.69)	0.60 (0.16, 2.21)	0.61 (0.16, 2.30)	2.19（0.60, 7.96)	2.62 (0.62, 11.03)	2.17 (0.49, 9.59)
	food allergy	1.49（0.37, 6.05)	0.97 (0.20, 4.59)	0.99 (0.21, 4.79)	2.08（0.43, 10.02)	1.71 (0.31, 9.61)	1.39 (0.24, 8.05)
	eczema	0.95（0.23, 3.91)	0.87 (0.19, 4.06)	0.82 (0.17, 3.96)	1.47（0.36, 5.91)	1.42 (0.31, 6.47)	1.02 (0.21, 4.88)
	wheezing	0.64（0.11, 3.55)	0.33 (0.04, 2.62)	0.44 (0.05, 3.59)	4.06（1.00, 16.53)	8.09 (1.57, 41.68)*	7.81 (1.42, 42.94)*
Boy[b]	allergy	1.06（0.19, 5.78)	0.76 (0.11, 5.05)	0.85 (0.13, 5.71)	3.20（0.53, 19.40)	4.42 (0.52, 37.34)	5.29 (0.58, 48.45)
	food allergy	3.42（0.45, 26.12)	2.38 (0.24, 23.22)	2.68 (0.26, 27.99)	7.06（0.78, 63.72)	5.57 (0.40, 76.75)	3.91 (0.28, 54.75)
	eczema	0.55（0.07, 4.52)	0.47 (0.05, 4.64)	0.54 (0.05, 5.66)	0.57（0.08, 3.90)	0.56 (0.06, 5.21)	0.60 (0.06, 6.30)
	wheezing	0.20（0.02, 2.38)	0.02 (0.00, 0.64)*	0.03 (0.00, 0.94)*	5.97（0.88, 40.50)	10.06 (0.95, 106.38)	12.05 (0.99, 146.37)
Girl[b]	allergy	0.66（0.13, 3.36)	0.40 (0.06, 2.71)	0.42 (0.06, 3.15)	1.50（0.23, 9.76)	1.35 (0.16, 11.01)	0.95 (0.10, 8.73)
	food allergy	0.66（0.09, 4.70)	0.32 (0.03, 3.22)	0.36 (0.03, 3.98)	0.50（0.05, 5.03)	0.38 (0.03, 5.39)	0.35 (0.02, 6.11)
	eczema	1.50（0.22, 10.28)	2.22 (0.22, 22.34)	1.84 (0.16, 20.57)	4.14（0.52, 32.90)	6.93 (0.66, 73.28)	4.49 (0.37, 53.87)
	wheezing	1.89（0.17, 21.36)	1.77 (0.09, 36.22)	2.52 (0.10, 60.34)	2.69（0.33, 21.70)	4.77 (0.39, 58.78)	4.49 (0.33, 60.44)

The odds ratios (OR) and 95% confidence intervals (95% CI) for allergy risk were calculated using the log10 translated concentrations of Dioxin-like compounds. 3.5Y Adjusted 1[a] (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, smoking and drinking during pregnancy, pelagic fish intake during pregnancy, allergic history), and paternal allergic history, blood sampling period, and infant gender. 3.5Y Adjusted 1[b] (boy and girl): adjusted for potential confounding factors excluding of infant gender from adjusted 1[a] (ALL) in stratified analysis by infant gender. 3.5Y Adjusted 2[a] (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, drinking during pregnancy, pelagic fish intake during pregnancy, allergic history), and paternal allergic history, blood sampling period, infant gender, environmental tobacco exposure, keeping pets,

duration of breast-feeding, and daycare attendance. 3.5Y Adjusted 2[b] (boy and girl): adjusted for potential confounding factors excluding infant gender from adjusted 2[a] (ALL) in stratified analysis by infant gender. 7Y Adjusted 1[a] (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, smoking and drinking during pregnancy, pelagic fish intake during pregnancy, allergic history), and paternal allergic history, blood sampling period, and infant gender. 7Y Adjusted 1[b] (boy and girl): adjusted for potential confounding factors excluding of infant gender from adjusted 1[a] (ALL) in stratified analysis by infant gender. 7Y Adjusted 2[a] (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, drinking during pregnancy, pelagic fish intake during pregnancy, allergic history), and paternal allergic history, blood sampling period, infant gender, environmental tobacco exposure, duration of breast-feeding, and keeping pets. 7Y Adjusted 2[b] (boy and girl): adjusted for potential confounding factors excluding infant gender from adjusted 2[a] (ALL) in stratified analysis by infant gender. * Statistically significant (p-value ＜0.05)

Table 6 Odds ratio (95% CI) between dioxins concentrations of dioxin-like compounds (DLCs) and infection risk

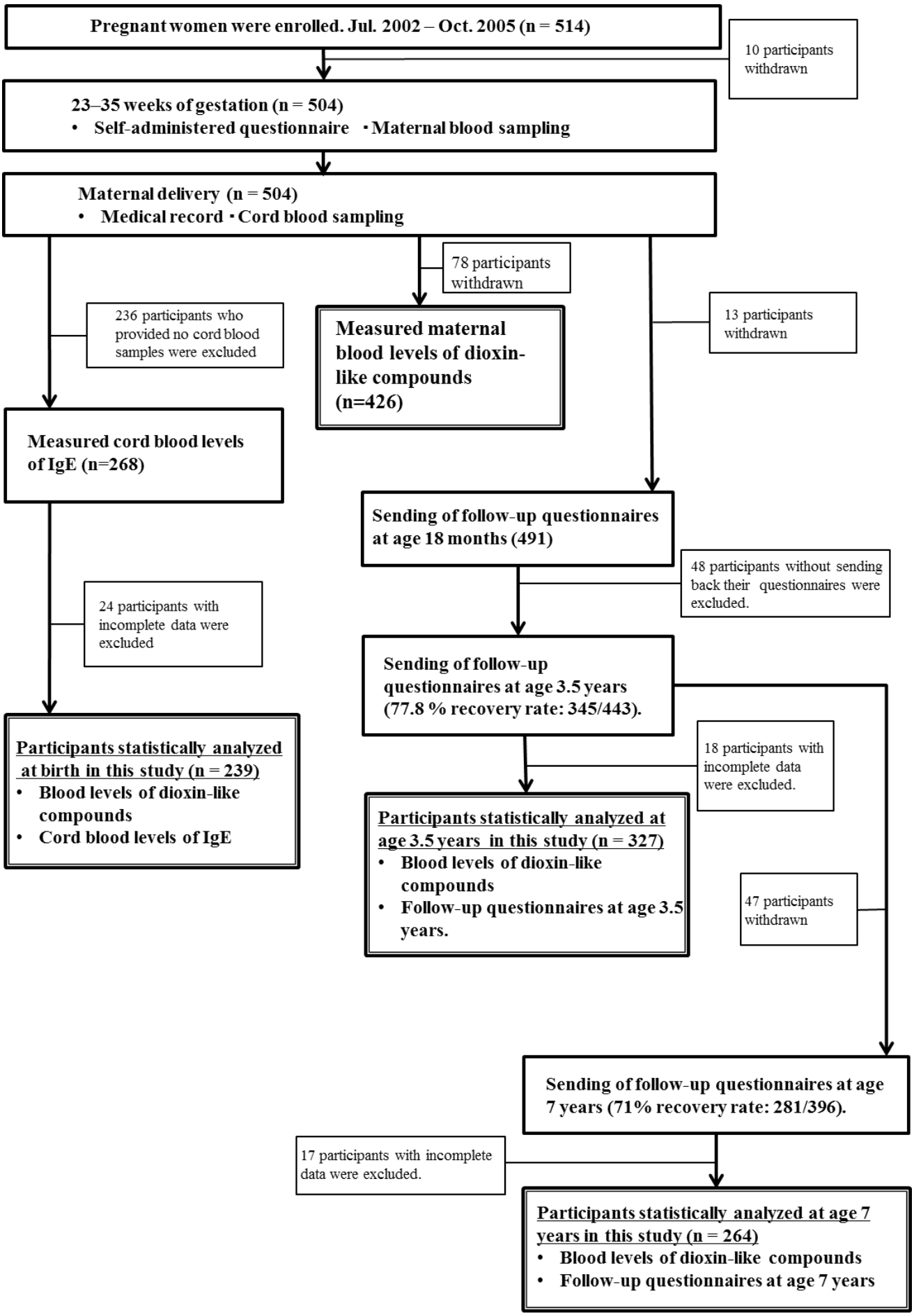
		3.5 years of age			7 years of age		
		Crude	Adjusted 1	Adjusted 2	Crude	Adjusted 1	Adjusted 2
		OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
All	Infections	0.49（0.14, 1.71)	0.70 (0.18, 2.71)	0.85 (0.21, 3.43)	4.70（1.03, 21.45)*	4.57 (0.92, 22.81)	3.80 (0.70, 20.61)
	Otitis media	0.44（0.14, 1.45)	0.43 (0.12, 1.56)	0.51 (0.14, 1.93)	1.64（0.45, 5.98)	1.11 (0.27, 4.58)	0.78 (0.17, 3.46)
	Respiratory infection	1.06（0.25, 4.46)	0.78 (0.16, 3.75)	1.03 (0.21, 5.13)	0.62（0.12, 3.19)	0.42 (0.07, 2.47)	0.51 (0.09, 3.11)
Boy	Infections	1.07（0.18, 6.40)	0.98 (0.14, 6.91)	1.33 (0.18, 9.78)	3.83（0.47, 31.04)	2.90 (0.28, 30.21)	3.07 (0.26, 35.59)
	Otitis media	0.36（0.06, 2.01)	0.46 (0.07, 3.15)	0.61 (0.08, 4.44)	1.26（0.22, 7.28)	1.13 (0.16, 8.15)	0.91 (0.11, 7.28)
	Respiratory infection	2.74（0.26, 29.29)	2.00 (0.15, 27.57)	2.50 (0.17, 36.28)	1.03（0.09, 12.00)	1.40 (0.09, 21.67)	1.91 (0.11, 31.61)
Girl	Infections	0.24（0.04, 1.38)	0.41 (0.05, 3.09)	0.32 (0.04, 2.70)	5.96（0.65, 54.34)	6.71 (0.60, 74.95)	5.82 (0.45, 75.08)
	Otitis media	0.53（0.10, 2.68)	0.44 (0.07, 2.87)	0.47 (0.07, 3.41)	2.39（0.34, 16.62)	1.31 (0.15, 11.60)	0.69 (0.07, 7.18)
	Respiratory infection	0.61（0.10, 3.80)	0.59 (0.08, 4.54)	0.82 (0.09, 7.26)	0.38（0.04, 3.56)	0.12 (0.01, 1.74)	0.15 (0.01, 2.45)

The odds ratios (OR) and 95% confidence intervals (95% CI) for allergy risk were calculated using log10 translated concentrations of Dioxin-like compounds. 3.5Y Adjusted 1^a (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, smoking and drinking during pregnancy, pelagic fish intake during pregnancy), and blood sampling period, and infant gender. 3.5Y Adjusted 1^b (boy and girl): adjusted for potential confounding factors excluding of infant gender from adjusted 1^a (ALL) in stratified analysis by infant gender. 3.5Y Adjusted 2^a (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, drinking during pregnancy, and pelagic fish intake during pregnancy) blood sampling period, infant gender, environmental tobacco exposure, keeping pets, duration of breast-feeding, and daycare attendance. 3.5Y Adjusted 2^b (boy and girl): adjusted for potential confounding factors excluding of infant gender from adjusted 2^a (ALL) in stratified analysis by infant gender. 7Y Adjusted 1^a (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, smoking and drinking during pregnancy, pelagic fish intake during pregnancy), and blood sampling period, and infant gender. 7Y Adjusted 1^b (boy and girl): adjusted for potential confounding factors excluding of infant gender from adjusted 1^a (ALL) in stratified analysis by infant gender. 7Y

Adjusted 2[a] (ALL): adjusted for maternal factors (BMI before pregnancy, parity, educational level, drinking during pregnancy, and pelagic fish intake during pregnancy) blood sampling period, infant gender, environmental tobacco exposure, duration of breast-feeding, and keeping pets. 7Y Adjusted 2[b] (boy and girl): adjusted for potential confounding factors excluding of infant gender from adjusted 2[a] (ALL) in stratified analysis by infant gender. * Statistically significant (p-value 0.05).

Figure legends

Figure 1 Research overview.


Pregnant women were enrolled. Jul. 2002 – Oct. 2005 (n = 514)
10 participants withdrawn
23–35 weeks of gestation (n = 504)
• Self-administered questionnaire ・Maternal blood sampling
Maternal delivery (n = 504)
• Medical record ・Cord blood sampling
78 participants withdrawn
236 participants who provided no cord blood samples were excluded
Measured maternal blood levels of dioxin-like compounds (n=426)
13 participants withdrawn
Measured cord blood levels of IgE (n=268)
Sending of follow-up questionnaires at age 18 months (491)
48 participants without sending back their questionnaires were excluded.
24 participants with incomplete data were excluded
Sending of follow-up questionnaires at age 3.5 years (77.8 % recovery rate: 345/443).
18 participants with incomplete data were excluded.
Participants statistically analyzed at birth in this study (n = 239)
• Blood levels of dioxin-like compounds
• Cord blood levels of IgE
Participants statistically analyzed at age 3.5 years in this study (n = 327)
• Blood levels of dioxin-like compounds
• Follow-up questionnaires at age 3.5 years.
47 participants withdrawn
Sending of follow-up questionnaires at age 7 years (71% recovery rate: 281/396).
17 participants with incomplete data were excluded.
Participants statistically analyzed at age 7 years in this study (n = 264)
• Blood levels of dioxin-like compounds
• Follow-up questionnaires at age 7 years