to be established. Using cotinine as the gold standard, the current study contributes to existing knowledge by establishing cut-off points and validating the self-reported smoking and SHS statuses of Japanese pregnant women.

2. Methods

2.1. Study, subjects and data collection

From April 2003 to December 2007, 9000 pregnant women were invited to participate in a prospective cohort study entitled "The Hokkaido Study on Environment and Children's Health". The inclusion criteria were enrollment for antenatal care during the first trimester of pregnancy, Japanese ethnicity; and residence and attendance at antenatal clinic within Hokkaido in Northern Japan. Informed consent was obtained from all the participants, and a participation rate of 95% (n = 8532) was achieved. During the first trimester of pregnancy and 4 months after delivery, self-administered questionnaires were used to obtain information on lifestyle behaviors including smoking habits and SHS exposure.

2.2. Cotinine analysis

Blood samples collected from the women during the third trimester of gestation were frozen at −80 °C until assayed. Cotinine measurement was carried out using the highly-sensitive enzyme-linked immunosorbent assay (ELISA) technique with 0.12 ng/mL as limit of detection (LOD) (Cosmic Corporation, Japan). Briefly, the ELISA 96-well plates coated with a rabbit anti-cotinine-4-bovine-γ-globulin polyclonal antibody were first incubated with 1% bovine serum albumin (BSA) after which 25 μL of blood plasma samples and 100 μL horseradish peroxidase-labeled (HRP) cotinine were added. The mixture was left to incubate at 20–25 °C for 1 h. Subsequent to three washes with 1% BSA, peroxidase substrate, tetramethylbenzidine, and H_2O_2 were added (Kirkegaad & Perry Laboratories, Gaithersburg, MD). The mixture was re-incubated for 30 min in the dark at the same temperature and 100 μL phosphoric acid was added to the wells to stop enzyme activity. The absorbance was read at a wavelength of 450 nm using an ELISA reader (E_{max}; Molecular Devices, Sunnyvale, CA). In their study, Matsumoto et al. (2010) made use of a similar technique for cotinine analysis.

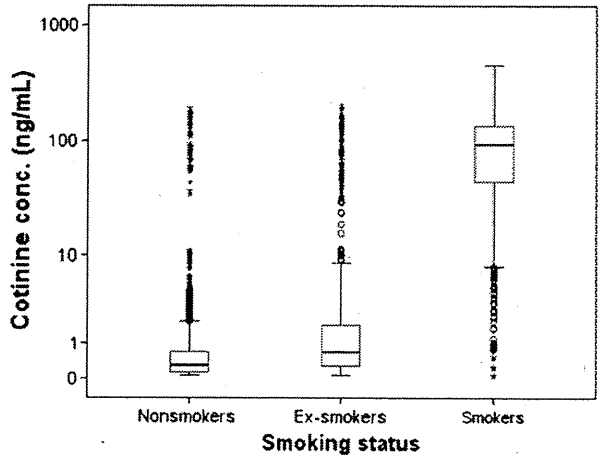

Fig. 1. Box and whisker plot of $\log_{10}$-transformed plasma cotinine concentrations in maternal blood samples based on self-reported exposure to tobacco smoke during pregnancy. Horizontal lines inside boxes signify the median, boxes indicate the interquartile range, whiskers represent the most extreme points, and circles indicate outliers.

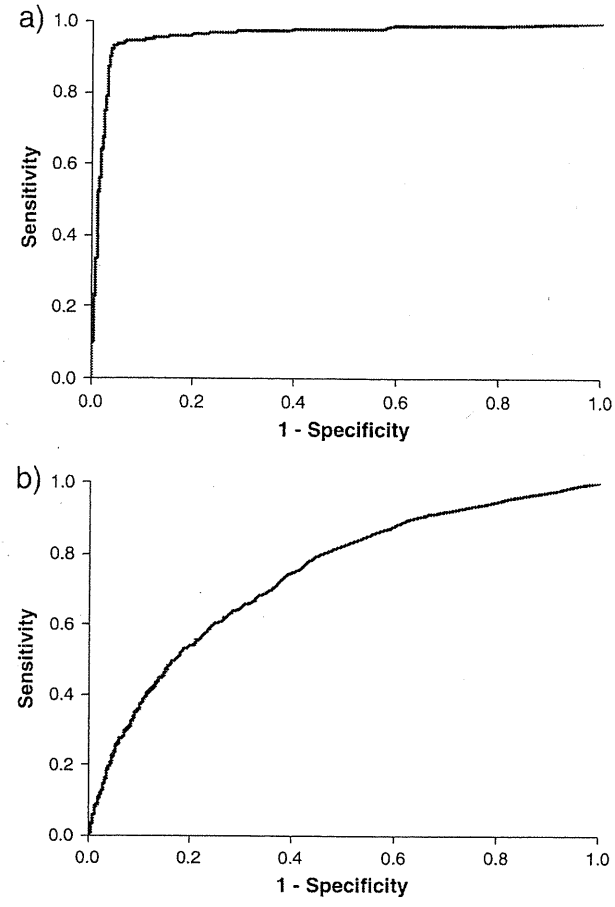

Fig. 2. Receiver operating characteristics curves to separate (a) smokers from nonsmokers and (b) exposed from unexposed nonsmokers.

2.3. Statistical analyses

Non-detectable cotinine concentrations were assigned a value half the detection limit, (0.06 ng/mL) before the statistical analysis. We determined the association between self-reported smoking status and cotinine biochemical analysis using the Spearman rank correlation coefficient. Second, the sensitivity and specificity of plasma cotinine were calculated using the receiver operating characteristic (ROC) curve analysis. The optimal cut-off values to separate smokers from nonsmokers; and exposed from unexposed group, were obtained by locating the points with maximum sensitivity and specificity on the curve.

Last, agreement between self-reported smoking status or SHS exposure and cotinine-classified groups were assessed using the kappa coefficient and 4 other measures of association: the positive predictive value (PV^+), the negative predictive value (PV^-), the likelihood ratio for a positive test (LR^+) and the likelihood ratio for a negative

Table 1
Comparison of frequency of self-reported smoking status with plasma cotinine measurements.

Smoking status	Plasma cotinine (ng/mL)		Total	Likelihood ratio
	>11.48	≤11.48		
Yes	575	119	694	27
No	134	4300	4434	0.2
Total	709	4419	5128	

Sensitivity = 81%, specificity = 97%, positive predictive value (PV^+) = 83%, negative predictive value (PV^-) = 97% ; kappa = 0.79.

Table 2
Comparison of self-reported exposure to SHS with plasma cotinine measurements.

SHS exposure	Plasma cotinine (ng/mL)		Total	Likelihood ratio
	0.22–11.48	≤0.21		
Yes	1837	591	2428	1.8
No	853	1019	1872	0.5
Total	2690	1610	4300	

Sensitivity = 68%, specificity = 63%, positive predictive value (PV^+) = 76%, negative predictive value (PV^-) = 54%; kappa = 0.31.

test (LR^-) (Chien and Khan 2001, Weinstein et al., 2005). The positive predictive value or precision rate is the proportion of correctly identified smokers or exposed nonsmokers with positive test results, calculated as $PV^+ = truepositives/(truepositives + falsepositives)$. The negative predictive value is the proportion of correctly identified nonsmokers or unexposed nonsmokers with negative test results, calculated from $PV^- = truenegatives/(truenegatives + falsenegatives)$. The likelihood ratio determines whether cotinine analysis valuably changes the probability that a subject is correctly classified as smoker or as exposed. A likelihood ratio greater than 1 indicates the cotinine test result is associated with self-reported smoking status or SHS, whereas a likelihood ratio less than 1 indicates otherwise. The likelihood ratio for a positive test was calculated as $LR^+ = sensitivity/(1 - specificity)$, while that for a negative test was calculated as $LR^- = (1 - sensitivity)/specificity$.

3. Results

The number of eligible women that filled the first trimester questionnaire was 8532. At 4 months after delivery, however, the response rate to the questionnaire information was 80%. Hence, 1707 pregnant women were excluded from the study. Of those remaining 6825 women, 844 were also not included due to incomplete data on tobacco smoke exposure during pregnancy. As a result, only 5128 women who met the inclusion criteria and had their blood plasma cotinine levels during pregnancy measured were involved in the analysis. Detectable cotinine concentrations were found in 83% while the remaining 17% had cotinine levels below the LOD. Fig. 1 shows box and whisker plots of plasma cotinine concentrations among the smoking groups. Based on self-reported smoking status, the prevalence of smoking amongst the subjects was 13%. The proportion of ex-smokers or "quitters" was slightly higher (14%) whereas 73% were nonsmokers. Cotinine concentrations ranged from 0.06 to 191.15 ng/mL for nonsmokers, from 0.06 to 202.43 ng/mL for ex-smokers and from 0.06 to 453.95 ng/mL for smokers. The median cotinine concentrations for nonsmokers, ex-smokers and smokers are 0.30, 0.69 and 94.4 ng/mL respectively. The rank correlation coefficient between self-reported smoking status and plasma cotinine concentration was 0.54 ($P<0.01$).

Receiver operating characteristic (ROC) curves to validate smoking status and SHS exposure are shown in Fig. 2. Before performing the ROC analysis, the first and second trimester quitters were reclassified as nonsmokers, but those who reported quitting during their third trimester of pregnancy were regarded as smokers. This is plausible as the collection of blood samples for cotinine measurements was done during the third trimester when nicotine in the blood could still be detected. Apart from that, studies have also found that the negative effect of tobacco smoke exposure in the third trimester of pregnancy on fetal growth is greater than exposure in early pregnancy (Lieberman et al., 1994, Windham et al., 2000, Ohmi et al., 2002). The cotinine cut-off concentration for differentiating smokers from non-smokers was 11.48 ng/mL (specificity = 97%, sensitivity = 81%, kappa = 0.79), whereas a concentration of 0.21 ng/mL separates unexposed from exposed nonsmokers (specificity = 63%, sensitivity = 68%, kappa = 0.31). Optimal cut-off values selected on the ROC curves are usually those that simultaneously maximize sensitivity and specificity. In their study, Lin et al. also made use of ROC to find the optimal cut-off values of body mass index, waist circumference, waist-to-hip ratio and waist-to-height ratio as

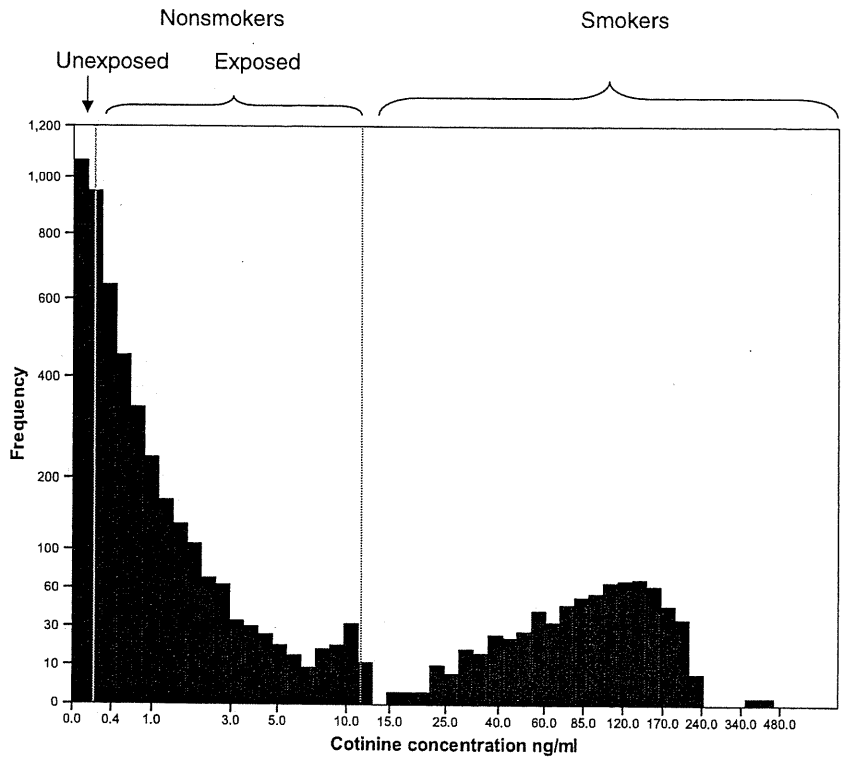

Fig. 3. Frequency distribution of plasma cotinine concentrations showing the cut-off points for unexposed and exposed non smokers and nonsmokers from smokers (N = 5128).

predictors of the prevalence of hypertension, diabetes and dyslipidemia (Lin et al., 2002).

Based on the cotinine measurements, 14% (n = 709) of the women were smokers (cotinine > 11.48 ng/mL), whereas 86% (n = 4419) were nonsmokers (Table 1). The mean and median plasma cotinine levels of cotinine-classified smokers were respectively 90.5 ng/mL and 90.3 ng/mL. The PV^+ (83%) and PV^- (97%) of self-report were relatively high. The percentage of the subjects who underreported active smoking was only 2.6% (that is, 134/5128; Table 1). The LR^+ of 27 led us to conclude that agreement of self-reporting with cotinine measurement was high (Akobeng, 2006).

Table 2 shows the validation of SHS where nonsmokers are classified into SHS-exposed (0.22–11.48 ng/mL) and unexposed (≤0.21 ng/mL). The proportion of SHS-exposed nonsmokers is 63% with mean plasma cotinine concentrations of 0.9 ng/mL. Among the 2690 cotinine-classified SHS-exposed women, 1837 (68%) reported exposure to SHS during pregnancy. Of the total subjects, 16.6% (that is, 853/5128 underreported exposure (Tables 1 and 2). The PV^+ of self-reports was fairly high (76%), whereas the LR^+ for SHS is much lower than that of smokers, signifying that exposure to SHS during pregnancy through self-reports is less reliable.

Fig. 3 presents the frequency distribution of cotinine concentration in the population (N = 5128). The first vertical line indicates the cut-off point (0.21 ng/mL) between the nonsmoking groups (unexposed and exposed), whereas the second line denotes the cut-off point (11.48 ng/mL) for distinguishing nonsmokers from the smokers.

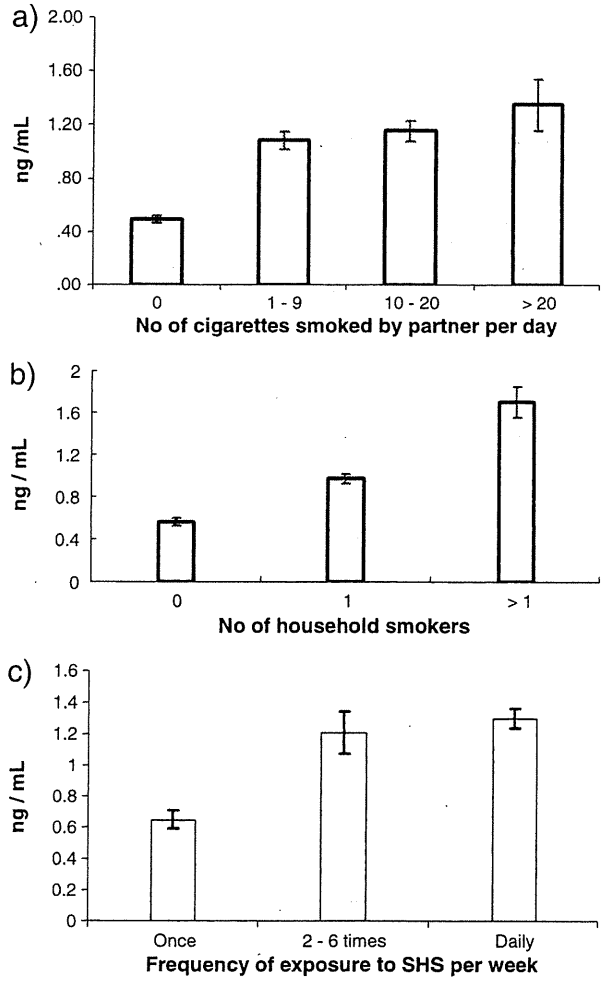

Fig. 4. Plasma cotinine concentrations and SHS exposure: relationship of plasma cotinine levels with (a) number of cigarettes smoked by partner daily; (b) number of household smokers; and (c) frequency of SHS exposure per week. All are significant at $P<0.01$. The standard errors are shown as vertical lines.

The relationships of plasma cotinine concentrations with SHS exposure parameters are shown in Fig. 4. The number of cigarettes smoked by partner per day, number of household smokers and frequency of SHS exposure per week are significantly related with prenatal exposure to SHS ($P<0.01$).

4. Discussion

In this study, data obtained from questionnaires and biochemical analysis of plasma cotinine provided information about smoking behavior and exposure to SHS. Until now, there has been no fixed cut-off value for distinguishing between smokers and non-smokers. We found a cut-off value of 11.48 ng/mL which is within the range of 10–20 ng/mL previously reported in other studies (Bernert et al., 2000; Vartiainen et al., 2002; Twardella et al., 2004; Hanke et al., 2004). In our study, the plasma cotinine cut-off value to differentiate the exposed from unexposed nonsmokers was 0.21 ng/mL, whereas similar studies reported 4.0 and 10 ng/mL (Bavazzano et al., 2007; Man et al., 2009). The lower cut-off values found in our study may be explained by the individual variations in cotinine metabolic rates. The levels of plasma or saliva cotinine were found to be lower in pregnant smokers than in non-pregnant ones (Rebagliato et al., 1998). Also, cotinine metabolic clearance increases during pregnancy leading to a shorter half-life of about 8 h between 16 and 40 weeks of gestation (Dempsey et al., 2002). As suggested by Dempsey et al. and based on our findings, the cut-off points that are used for classifying smokers and non-smokers among pregnant women should be lower compared to the non-pregnant ones. Furthermore, the intake of food and drinks containing nicotine (such as tomatoes, potatoes, eggplants, cauliflower, green tea, black tea and coffee) might falsely indicate exposure to SHS (Castro and Monji, 1986; Davis et al., 1991; Domino et al., 1993; Idle, 1990). A study on the impact of tea drinking on serum cotinine levels of nonsmokers in Scotland found that nicotine in tea seems to have a little but insignificant contribution to cotinine levels in most people, compared with nicotine SHS exposure (Tunstall-Pedoe et al., 1991). Although, this study is not designed to investigate into the dietary habits of pregnant women in relation to their blood cotinine level, it is worth considering in subsequent research.

Our study observed an increase in misclassification among nonsmoking groups compared to the smokers/nonsmokers. This result agrees with the studies on Swedish pregnant women (George et al., 2006; Lindqvist et al., 2002) and dental patients in Japan (Yamamoto et al., 2005). In their study, George et al. (2006) reported that most SHS-exposed women were misclassified as unexposed based on the self-reported information in early and late pregnancy. Other studies found self-reported smoking to be less reliable (Webb et al., 2003; Man et al., 2009). The method used in estimating exposure also has the tendency to affect validity. Significant misclassifications, for example, occurred when exposure was measured on the basis of hours per day (DeLorenze et al., 2002). Based on the cotinine measurements, we observed that 17% of 694 women misreported active smoking, whereas George et al. (2006) observed an underreporting of active smoking. Recall bias may be responsible for the relatively lower validity between the nonsmoking categories compared to smoking/nonsmoking discrimination (Tsutsumi et al., 2002).

The prevalence of smoking among Japanese men is one of the highest among industrialized countries. The institution of tobacco control policies against smoking in the workplace and in public, however, led to a gradual decline in the smoking prevalence in Japan (Sato et al., 2000). In our study, information about SHS exposure during pregnancy was limited to the home environment, where there may be higher possibility and more regular pattern of exposure for

nonsmoking pregnant women living with household smoker(s). We observed a significant association of plasma cotinine with the number of cigarettes smoked by partner per day, the number of household smokers and the frequency of exposure to SHS per week. In previous studies, having a husband or household members who smoker significantly affects SHS exposure (Loke et al., 2000; DeLorenze et al., 2002; Kaufman et al., 2002). Also, one study found higher plasma cotinine levels among non-smokers exposed at home than those exposed in the workplace (Chiu et al., 2008). Although the duration of exposure was only measured in terms of days and not hours in our study, a more detailed questionnaire could have improved the authenticity of self-reports.

Our findings suggest that, in addition to smoking control in the workplace and in public, a need exists for creating a conducive and healthy atmosphere for pregnant woman and her fetus in the home as well. Public awareness and a proper enlightenment campaign could help in this regard.

5. Conclusion

Levels of cotinine in the blood plasma of pregnant women are very useful in confirming the self-reported information on smoking and exposure to SHS.

Acknowledgment

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COHORT PROFILE

Cohort Profile: The Hokkaido Study on Environment and Children's Health in Japan

Reiko Kishi,[1]* Seiko Sasaki,[1] Eiji Yoshioka,[1] Motoyuki Yuasa,[2] Fumihiro Sata,[3] Yasuaki Saijo,[4] Norie Kurahashi,[5] Junko Tamaki,[6] Toshiaki Endo,[7] Kazuo Sengoku,[8] Katsuya Nonomura[9] and Hisanori Minakami,[10] for the Hokkaido Study on Environment and Children's Health[†]

[1]Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan, [2]Department of Public Health, School of Medicine, Juntendo University, Tokyo, Japan, [3]Department of Epidemiology, National Institute of Public Health, Wako, Japan, [4]Department of Health Science, School of Medicine, Asahikawa Medical College, Asahikawa, Japan, [5]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan, [6]Department of Public Health, School of Medicine, Kinki University, Osaka, Japan, [7]Department of Obstetrics and Gynecology, School of Medicine, Sapporo Medical University, Sapporo, Japan, [8]Department of Obstetrics and Gynecology, School of Medicine, Asahikawa Medical College, Asahikawa, Japan, [9]Department of Renal and Genitourinary Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan and [10]Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

*Corresponding author. Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan. E-mail: rkishi@med.hokudai.ac.jp
[†]The members of the Hokkaido Study on Environment and Children's Health are listed in Appendix 1

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How did the study come about?

The Hokkaido study of Environment and Children's Health is an ongoing cohort study that began in 2002. The study consists of two prospective birth cohorts: the Toho hospital cohort with one obstetric hospital in Sapporo City, and the Hokkaido large-scale cohort with 37 hospitals and clinics in Hokkaido prefecture. Hokkaido is the northernmost and the second largest island of Japan: it has an area of ~78 417 km^2, equivalent to that of Austria. Its population is 5 546 559, which is similar to that of Finland.

Persistent organic pollutants (POPs) including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), as well as perfluorooctanoate sulphonate (PFOS) and perfluorooctanoate (PFOA), the final metabolites of fluorinated organic compounds (FOCs), may accumulate in the human body. These environmental chemicals may contribute to numerous adverse health effects including growth retardation of fetuses and infants, disturbances of the neurodevelopment, thyroid, immune and reproductive systems and may exert genetic or epigenetic effects when metabolized. In the USA, lower level PCB exposure during pregnancy was associated with decreased birth weight.[1–3] In Finland, total toxic equivalent (TEQ) levels of PCDDs/PCDFs in breast milk negatively correlated with birth weight, especially of boys.[4] In a Dutch report, negative effects of prenatal dioxin and PCB exposure on cognitive and motor development were found in school-age children.[5] An assessment of childhood play behaviour at 9 years of age correlated higher prenatal PCB levels with less masculinized play in boys and with more masculinized play in girls. Higher prenatal dioxin levels were associated with more feminized play in both boys and girls.[6] Concerning the immunological effects of environmental exposure, prenatal PCB exposure was associated with less shortness of breath with wheezing; postnatal PCB exposure was associated with a higher prevalence of recurrent otitis media.[7,8] Two studies reported correlations between prenatal PFOS/PFOA exposure and reduced birth weight.[9,10]

Congenital malformations such as hypospadias are speculated to be related to genetic variations in the synthesis and metabolism of steroids combined with environmental factors. Hypospadias are a common congenital malformation caused by incomplete fusion of the urethral folds. Recently, a number of reports indicate an increased prevalence of hypospadias in various countries including Japan; this trend is speculated to be related to endocrine-disrupting chemicals.[11] Previous studies were limited to assess either genetic or environmental contributions to its etiology; for example, case–control studies reported genetic polymorphisms associated with hypospadias and nested case–control studies reported an association of hypospadias with agricultural exposures.[12–15]

Several birth cohorts have been established worldwide in recent years; however, few reports have been published on the relationship between low-level environmental exposures and adverse birth outcomes. In the field of infant development, variations in the human genome and their modifications on the effect of hazardous environmental exposures (gene–environment interaction) have not been thoroughly investigated.

The study was established to investigate the effects of environmental exposure combined with genetic predisposition in the development and health in prenatal period, infancy and early childhood.[16]

What does the study cover?

The aims of the study are the following: (i) to examine possible negative effects of perinatal environmental factors on birth outcomes including congenital anomalies and growth retardation; (ii) to follow the prevalence of allergic diseases or neurodevelopmental disorders, and perform longitudinal observation of child development; (iii) to identify a high-risk group classified by genetic susceptibility to environmental chemicals; and (iv) to identify additive effects of various chemicals encountered in the daily environment.

In particular, the Toho hospital cohort has focused on the association between child growth, neurodevelopment and allergy, and low-level exposure to environmental chemicals during pregnancy and infancy. The Hokkaido large-scale cohort was established primarily to assess the prevalence of congenital anomalies including cleft lip and plate, congenital heart defects, hypospadias and cryptorchidism, and has explored the possible causes of these malformations. This cohort also follows the prevalence of childhood allergies and neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD).

Who are in the sample?

The Institutional Ethical Board for Human Gene and Genome studies at Hokkaido University Graduate School of Medicine approved the study protocol. The study was conducted with the informed consent of all subjects. The enrolment of the Toho hospital cohort was conducted from July 2002 to October 2005. Of 1796 potentially eligible women, 514 agreed to participate. The subjects were women who enrolled at 23–35 weeks of gestation and delivered at the Toho hospital. All the subjects were resident in Sapporo City or surrounding areas. Since February 2003, the Hokkaido large-scale cohort has conducted enrolment of women in early pregnancy (<13 weeks of gestational age) that visited one of associated hospitals or clinics in the study area for prenatal health care at the maternity unit. The total number of infants whose congenital anomalies were surveyed in the cohort was 9335 until 2006. This cohort will consist of 20 000 children. Each cohort will follow its participants up to school age.

What has been measured?

The protocol for study design and exposure measurement items is presented in Figures 1 and 2, and Table 1.

In the Toho hospital cohort, a self-administered questionnaire was completed at the time of enrolment to obtain baseline information including parental demographic characteristics, dietary habits including the amount and species of fish consumed, exposure to chemical compounds in their daily life, smoking history, alcohol consumption, caffeine intake and household income. Information on pregnancy complications, gestational age at birth, infant gender and birth size was obtained from maternal and infant medical records. Maternal blood samples were

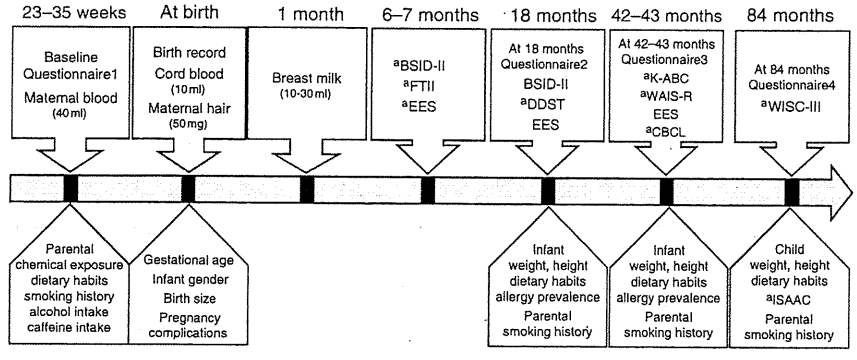

Figure 1 Study design of the Toho hospital cohort. BSID-II: The Bayley Scales of Infant Development second edition; FTII: The Fagan Test of Infant Intelligence; K-ABC: The Kaufman Assessment Battery for Children; WAIS-R: The Wechsler Adult Intelligence Scale-Revised; WISC-III: The Wechsler Intelligence Scale for Children third edition; EES: The Evaluation of Environmental Stimulation; DDST: The Denver Developmental Screening Tests; CBCL, the Child Behaviour Checklist; ISAAC: International Study of Asthma and Allergies in Childhood

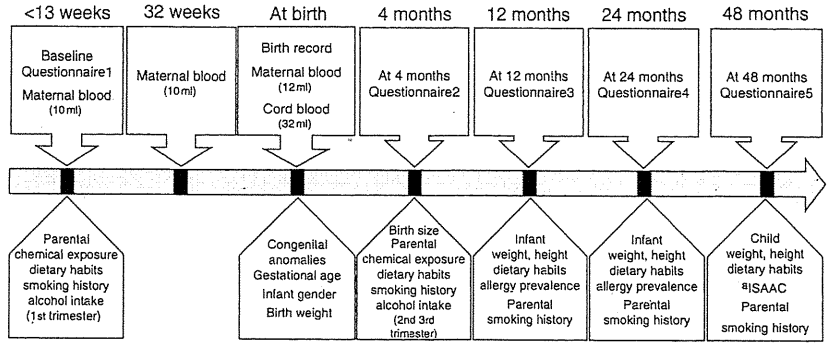

Figure 2 Study design of the Hokkaido large-scale cohort. ISAAC: International Study of Asthma and Allergies in Childhood

Table 1 Items measured in the Hokkaido study of Environment and Children's Health

Measurement	Description
Exposure measurement	
PCDDs, PCDFs	Maternal blood, cord blood and breast milk
PCBs	Maternal blood, cord blood and breast milk
OH-PCBs	Maternal blood, cord blood and breast milk
PFOS, PFOA	Maternal blood, cord blood and breast milk
BPA, NP	Maternal blood, cord blood and breast milk
DEHP	Maternal blood, cord blood, and breast milk
Pesticides	Maternal blood, cord blood and breast milk
Heavy metals	Maternal blood, cord blood and breast milk
MeHg	Maternal blood, cord blood and maternal hair
Cotinine	Maternal blood, cord blood and maternal hair
Other biochemical measurements	
TSH, FT4	Maternal blood and infant blood
Folic acid	Maternal blood and cord blood
IgE, IgA	Cord blood

OH-PCBs: hydroxylated polychlorinated biphenyls; BPA: bisphenol A; NP: nonylphenol; DEHP: Di(2-ethylhexyl)phthalate; MeHg: methylmercury; TSH: thyroid stimulating hormone; FT4: free thyroxine.

collected in late pregnancy, usually after the 30th week of gestation. Cord blood and placenta were taken immediately after birth. Maternal hair samples were also collected within 5 days after delivery, and breast milk from nursing mothers was collected within 4 weeks following birth.

The levels of PCDDs/PCDFs and PCBs in maternal blood and breast milk were measured using a high-resolution gas chromatography/high-resolution mass spectrometre (HRGC/HRMS) at Fukuoka Institute of Health and Environmental Sciences.[17–20] PFOS and PFOA levels in maternal blood, cord blood and breast milk were analysed by liquid chromatography-tandem mass spectrometry (LC/MS/MS) at Hoshi University.[21,22] Total mercury levels in maternal hair samples were measured by an oxygen combustion–gold amalgamation method using an atomic absorption detector at National Institute for Minamata Disease.[23] Cord serum immunoglobulin E (IgE) and immunoglobulin A (IgA) were also determined. In order to avoid the possibility of maternal blood contamination, we regarded any cord serum IgA > 10 mg/dl sample as inappropriate. Thyroid stimulating hormone (TSH) and FT4 levels of mother and newborn were measured as a mass screening programme in Sapporo City. For these examinations, a maternal blood sample was collected in the first trimester and a neonate blood sample on filter paper was collected between 4 and 7 days of age. Maternal serum cotinine concentration in maternal blood was

measured using an enzyme-linked immunosorbent assay (ELISA) kit to evaluate smoking exposure levels. Genetic polymorphisms were determined by means of the TaqMan (Applied Biosystems, Inc., Foster City, CA, USA) polymerase chain reaction (PCR) method, using minor groove binder (MGB) probes.

A follow-up questionnaire was also used at 18, 42 and 84 months of age to obtain relevant information including allergy prevalence, dietary habits and smoking history of mother and the partner. In addition, the Toho hospital cohort has assessed the influence of low-level intrauterine exposure of toxic chemicals on childhood neurodevelopment. The Bayley Scales of Infant Development second edition (BSID-II) was used to at 6–7 and 18 months of age. The Fagan Test of Infant Intelligence (FTII) was performed to measure visual recognition memory and cognitive ability on infants aged 6–7 months. To examine developmental progress, the Japanese version of the Denver Developmental Screening Tests (DDST) was used at age 18 months. At 42 months of age, child and maternal intelligence were measured by Japanese version of the Kaufman Assessment Battery for Children (K-ABC) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R), respectively. The Wechsler Intelligence Scale for Children third edition (WISC-III) was used to assess the attention and motor function of children at 84 months of age. The questionnaire of home environment, the Evaluation of Environmental Stimulation (EES), was used to investigate the environmental conditions of children at 6, 18 and 43 months of age. The Japanese version of the Child Behavior Checklist (CBCL) was used to collect information on child behaviour at age 43 months.

In the Hokkaido large-scale cohort, a baseline questionnaire survey was conducted at the time of enrolment during the first trimester to obtain parental information such as demographic characteristics, medical and obstetric history, dietary supplement intake during pregnancy and chemical exposure at work. Perinatal data such as birth weight, infant gender, mode of delivery, multiple births and prevalence of congenital anomalies were obtained from birth records completed by an obstetrician. The congenital anomalies were classified into 55 markers according to the criteria. The first follow-up questionnaire was used on infants at 4 months of age to obtain relevant data including birth size, gestational age at birth and parental smoking history in the second and third trimester. The follow-up questionnaire was administered at age 12, 24 and 48 months to obtain relevant information such as child weight and height measured at regular health checkups, vaccination history, dietary habits, parental smoking history and allergy prevalence using the International Study of Asthma and Allergies in Childhood (ISAAC). The CBCL was used to investigate association between perinatal exposure and developmental disorder. Maternal blood was collected three times: between 6 and 14 weeks of gestational age as an organogenetic period; during the third trimester; and at delivery. Cord blood was taken immediately after birth. Maternal serum was used to measure folic acid level and plasma cotinine concentration.

What has the study found?

A total of 514 mothers were registered in the Toho hospital cohort and 16306 mothers were registered in the Hokkaido large-scale cohort up to the end of November 2009. Table 2 shows characteristics of mothers and infants in the Toho hospital cohort, and Table 3 shows those of the Hokkaido large-scale cohort. These two cohorts did not show significant difference in characteristics of mothers and infants.

Effects of PCDD/PCDF and dioxin-like PCB exposure on birth weight

We measured 29 congener levels of PCDDs/PCDFs and dioxin-like PCBs in maternal blood to examine an association between these concentrations and infant

Table 2 Characteristics of mothers and infants in the Toho hospital cohort

	$N=484$ n (%) or mean ± SD
Maternal characteristics	
Age (years)	30.7 ± 4.9
Parity	
0	230 (47.5)
⩾ 1	254 (52.5)
Educational level (years)	
<10	14 (2.9)
10–13	201 (41.5)
13–17	261 (53.9)
⩾ 17	8 (1.7)
Household income (million yen)	
<3	93 (19.2)
3–5	241 (49.8)
⩾ 5	150 (31.0)
Smoking status during pregnancy	
Non-smoker	289 (59.7)
Quitter	105 (21.7)
Smoker	90 (18.6)
Infant characteristics	
Birth weight (g)	3065 ± 375
Gestational age (weeks)	39.0 ± 1.4

SD: standard deviation.

Table 3 Characteristics of mothers and infants in the Hokkaido large-scale cohort

	$N=2777$ n (%) or mean ± SD
Maternal characteristics	
Age (years)	29.9 ± 4.6
Parity ($n=2515$)	
0	992 (39.4)
⩾ 1	1523 (60.6)
Educational level (years, $n=2752$)	
<10	137 (5.0)
10–13	1294 (47.0)
13–17	1089 (39.6)
⩾ 17	232 (8.4)
Household income (million yen, $n=2297$)	
<3	487 (21.2)
3–5	1055 (45.9)
⩾ 5	755 (32.9)
Smoking status during pregnancy	
Non-smoker	625 (61.5)
Quitter	265 (26.1)
Smoker	126 (12.4)
Infant characteristics	
Birth weight (g)	3041 ± 411
Gestational age (weeks)	38.9 ± 1.5

birth weight in the Toho hospital cohort. The mean TEQ level was 17.5 TEQ pg/g lipid in maternal blood. We found a 272.7-g decrease in birth weight with a 10-fold increase in total PCDF levels [95% confidence interval (CI) −505.8 to −39.5] after adjustment for potential covariates such as infant gender, gestational age, parity, maternal age and maternal smoking status during pregnancy. Total PCDD TEQ (−231.5 g, 95% CI −417.4 to −45.6), total PCDF TEQ (−258.8 g, 95% CI −445.7 to −71.8), total PCDD/PCDF TEQ (−256.4 g, 95% CI −448.6 to −64.2) and total TEQ (−220.5 g, 95% CI −399.2 to −41.9) levels were significantly negatively associated with birth weight among all infants. Among male infants, significant adverse associations with birth weight were found for total PCDD TEQ level, total PCDD/PCDF TEQ level and total TEQ level; however, these significant adverse associations were not found among female infants. Moreover, we found significantly negative association with the levels of 2,3,4,7,8-PeCDF (−24.5 g, 95% CI −387.4 to −61.5). Our findings suggest that prenatal low-level exposure to PCDDs and PCDFs may result in lower birth weight, which may accumulate in the placenta and retard important placental functions.[24]

Effects of PFOS and PFOA exposure on birth weight

We examined a correlation between maternal serum PFOS and PFOA concentrations and infant birth weight in the Toho hospital cohort. Concentrations ranged from 1.3 to 16.2 ng/ml for PFOS and from below the detection limit to 5.3 ng/ml for PFOA (both detection limits were 0.5 ng/ml). A $\log_{10}$-unit increase in PFOS levels correlated with a decrease in birth weight of 148.8 g (95% CI 297.0 to 0.5) after adjusting for confounders; however, no correlation was observed between PFOA levels and birth weight. Our results indicate that *in utero* exposure to relatively low levels of PFOS negatively correlates with birth weight.[25]

Low birth size in relation to maternal smoking and genetic polymorphisms

The effects of maternal smoking and genetic polymorphisms on infant birth size were examined in the Toho hospital cohort. Birth weight and length were significantly lower among infants born to smokers with the aryl hydrocarbon receptor (*AhR*) GG genotype, the cytochrome P-450 1A1 (*CYP1A1*) TC/CC genotype or the glutathione *S*-transferase T1 (*GSTM1*) null genotype. When combinations of these genotypes were considered, birth weight and length were significantly lower for infants of continuously smoking women with the *AhR* GG genotype and *CYP1A1* TC/CC genotype (−315 g and −1.7 cm, respectively) and with the *CYP1A1* TC/CC genotype and *GSTM1* null genotype (−237 g and −1.3 cm, respectively). For polymorphisms in gene-encoding *N*-nitrosamine-metabolizing enzymes -NAD(P)H: quinone oxidoreductase 1 (*NQO1*)- birth weight, birth length and birth head circumference were significantly reduced (−199 g, −0.8 cm and −0.7 cm, respectively) among infants born to smokers with the *NQO1* CC genotype. This genotype did not confer adverse effects among women who had never smoked or who quit smoking during the first trimester. Our results suggest an important modulating role for polymorphisms in metabolizing enzyme genes in concert with adverse effects of maternal smoking on infant birth size.[26,27]

Effects of PCDD/PCDF and dioxin-like PCB exposure on neurodevelopment

We used the BSID-II to evaluate a correlation between maternal PCDD/PCDF and dioxin-like PCB levels and the mental and motor development of their 6-month-old infants in the Toho hospital cohort. The mean mental development index (MDI) and psychomotor development index (PDI) scores were 91.9 and 89.3, respectively. After adjustment for potential confounding variables, total PCDDs, total PCDDs/PCDFs and 1,2,3,4,6,7,8-HpCDD were significantly negatively associated with MDI. Total 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD,

2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 1,2,3,6,7,8-HxCDF were significantly negatively associated with PDI. However, the total levels of PCDDs/PCDFs and dioxin-like PCBs were not significantly associated with PDI, and the TEQ values were not significantly associated with MDI or PDI. Our results suggest that the low-level exposure of several congeners of PCDDs/PCDFs during pregnancy affect the neurodevelopment of 6-month-old infants.[28]

The prevalence of congenital anomalies

We estimated the prevalence of congenital anomalies in Hokkaido prefecture. Among 9335 infants included in the Hokkaido large-scale cohort between 2003 and 2006, there were 215 infants with congenital anomalies. The most frequent congenital anomaly was congenital heart defects, followed by Down syndrome, hydronephrosis, polydactyly and cryptorchidism (Table 4). The total prevalence of congenital anomalies was similar to nationwide data reported by the Japan Association of Obstetricians & Gynecologists (JAOG); however, the number of serious cases was less than that of the JAOG. Members of JAOG consist of university and tertiary hospitals, whereas those of the Hokkaido Study on Environment and Children's Health are general hospitals and clinics.

What are the main strengths and weaknesses of the study?

The design of our study is a prospective cohort study intended to collect data on environmental exposures during the fetal period and to control for potential confounders. The detailed measurements are adequate to detect various effects of perinatal environmental and genetic determinant on outcomes in childhood. The Toho hospital cohort study has conducted face-to-face examinations for neurodevelopment assessment. The Hokkaido large-scale cohort is the largest birth cohort in Japan. Potential problems with both cohorts include the fact that they are hospital-based studies, which may result in selection bias. Some levels of attrition related to individuals moving outside the study area has occurred, though we send participants periodical newsletters to maintain the profile of study.

Can I get hold of the data?

Additional information about the Hokkaido study is available at the study website: http://www.med.hokudai.ac.jp~pubmed-w/EnglishHP/e%20research%20groups%202.htm. All collected source data are maintained and stored at the Department of Public Health Sciences, Hokkaido University Graduate School of Medicine. Initial approaches or enquiries

Table 4 Prevalence of congenital anomalies in the Hokkaido large-scale cohort (2003–06)

	Hokkaido large-scale cohort (in 10 000 persons)	JAOG (2006) (in 10 000 persons)
Head		
Anencephaly	2.8	–
Microcephaly	0.7	–
Hydrocephaly	2.1	6.5
Holoprosencephaly	1.4	–
Ear		
Microtia	1.4	–
Meatal atresia	1.4	–
Cryptotia	2.1	–
Low-set ears	1.4	10.8
Orofacial		
Cleft lip	4.2	8.4
Cleft plate	4.9	6.4
Cleft lip and cleft plate	7.0	15.1
Upper limb		
Polydactyly	7.7	6.5
Syndactyly	2.8	5.1[b]
Trunk		
Myelomeningocele (spina bifida)	2.1	5.0
Omphalocele	2.1	4.3
Gastroschisis	2.8	–
Other abdominal wall defects	7.7	–
Heart		
Congenital heart disease	26.4	–
Digestive organ		
Esophageal atresia	1.4	–
Anorectal anomaly	3.5	–
Intestinal atresia	2.8	6.8
Doudenal atresia	2.1	–
Urinary and genital organs		
Hydronephrosis	9.0	–
Renal dysplasia	2.8	–
Hypospadias[a]	8.3	3.7[b]
Cryptorchidism[a]	12.5	–
Ambiguous genitalia	5.6	–
Leg		
Polydactyly	2.8	4.7[b]
Syndactyly	2.8	–
Cleft foot	0.7	–
Syndromes or chromosomal aberration		
Down syndrome	9.7	9.7
Trisomy 18 syndrome	2.1	–

Dashes represent data not surveyed.
[a]Male infants only, the prevalence rate shown in the Table is per 10 000 male infants.
[b]Data from 1997 to 2005.

regarding the study can be made to the principal investigator (rkishi@med.hokudai.ac.jp).

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Appendix 1: Members of the Hokkaido Study on Environment and Children's Health

Members of the Hokkaido Study are: Drs K. Cho, T. Yamada, S. Ban, A. Kanazawa, N. Washino, K. Konishi, S. Katoh, A. Uno, T. Baba, T. Yila, C. Miyashita, T. Braimoh, I. Kashino, E. Okada, S. Kobayashi, Y. Otake, and M. Limpar, Hokkaido University Graduate School of Medicine, Sapporo; S. Nakajima and T. Baba, Sapporo Medical University, Sapporo; T. Miyamoto, Asahikawa Medical College, Asahikawa; N. Maeda, Toho Obstetrics & Gynecology Hospital, Sapporo; and H. Haruyama and K. Okuyama, Sapporo City Hospital, Sapporo.

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

Chihiro Miyashita [a], Seiko Sasaki [a], Yasuaki Saijo [b], Noriaki Washino [a], Emiko Okada [a], Sumitaka Kobayashi [a], Kanae Konishi [a], Jumboku Kajiwara [c], Takashi Todaka [d], Reiko Kishi [a,*]

[a] Department of Public Health, Hokkaido University Graduate School of Medicine, North 15 West 7 Kita-ku, Sapporo, Hokkaido, Japan
[b] Department of Health Science, Asahikawa Medical College, Midorigaoka, E2-1-1-1, Asahikawa, Hokkaido, Japan
[c] Fukuoka Institute of Health and Environmental Sciences, Fukuoka, Japan
[d] Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

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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.

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 Gasiewicz, 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

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

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* Corresponding author at: Department of Public Health, Hokkaido University Graduate School of Medicine, North 15 West 7 Kita-ku, Sapporo 060-8638, Japan. Fax: +81 11 706 7805.

E-mail address: rkishi@med.hokudai.ac.jp (R. Kishi).

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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 number (%) 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

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

		No.	%		Total dioxin TEQs		p value
				β	Median	(25th, 75th)	
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)	

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.

[a] Mean ± SD; BMI, body mass index.
[b] r was calculated with the Spearman's correlation test.

because this compound accounted for 70% of the total dioxin TEQ levels in maternal blood (Masuda, 2001). Mastueda et al. (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

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	25,243.31
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	11,471.65	15,641.75	49,632.02
Total dioxins				1847.56	8149.66	11,968.14	16,432.17	50,477.45
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.
* $p<0.05$.
** $p<0.01$.

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., 2001). 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

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						p value for trend[a]
	Quartile 2		Quartile 3		Quartile 4		
	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.
* $p < 0.05$.

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

Table 6
Adjusted ORs (95% CIs) versus quartile 1 of congener 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)	
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.
* $p < 0.05$.

significantly increased with exposure to DL PCBs in the second and/or third quartiles compared with references. The inter-relationship 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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