further research is needed to verify this observation and elucidate the biological mechanisms associated with this SNP.

### Effects of the maternal 5,10-*MTHFR* C677T polymorphism and tobacco smoke on infant birth weight

The 5,10-*MTHFR* 677T allele is associated with low folate and high homocysteine levels. In this study, the 677T allele was associated with lower birth weight in offspring of active smokers. The 677CT genotype was protective against low birth weight, especially among male offspring, but only in the absence of active or passive tobacco smoke. This might be due to the presence of higher mean serum folate levels among nonsmokers. In a Korean population, the 677T allele had a weak protective association with lung carcinoma.[56] The protective effect of adequate folate status might be mediated by the stabilization of flavin-adenine-dinucleotide (FAD) binding at the catalytic domain.[2] The birth weight of female infants of 677TT homozygous passive smokers was significantly lower than that of female infants born to passive smokers of similar genotypes. Male fetuses were favored, probably because pregnant mothers carrying male fetuses have a higher nutritional intake than those with female fetuses.[57] Poorly understood phenomena on fetal sex-specific signals have been implicated in fetal growth, especially in response to glucocorticoid activity that might modify the fetal response to stress.[58]

### Study strengths and limitations

The participants were indigenous Japanese; hence, we overcame issues of population stratification in genetic association studies. Overall, this study might be limited by selection bias, as we utilized integrated data from only 61% of the total recruitment during the study period. However, because we randomly selected the final study population based on known pooled population frequencies of the genetic factors and tobacco smoking among pregnant women, we believe that this study does not substantially differ from one with a higher participation rate (ie, ≥70%). The sample size of the study was adequate to detect gene-environment interactions; however, multiple comparisons and small subgroup sample sizes might have affected the study power. There could be misclassification bias from self-reported tobacco smoking; however, a previous study reported very low misclassification bias among Japanese women.[59] Therefore, the findings of the present study are likely to be reliable. Nevertheless, this does not diminish the importance of using biomarkers like cotinine. The findings might have been confounded by other B vitamins, which were not studied, or by other unidentified sources common to cohort study designs. Finally, this study was hospital-based; therefore, our findings should not be generalized. Further studies of other factors in the folate-homocysteine pathway should prove interesting.

### Public health implications

Analysis of the 5,10-*MTHFR* A1298C polymorphism shows that the frequency of the 1298AA genotype is greater than 60.0% in the Japanese population.[38] Its association with low folate status is thus a considerable public health concern. With the recent increasing prevalence of smoking among young Japanese women, particularly in Hokkaido,[60] maternofetal morbidity and mortality might also increase. Smoking cessation and targeted use of folic acid supplements could therefore prove to be very important public health tools in this population.

### Conclusions

Our findings suggest that the maternal 5,10-*MTHFR* C677T polymorphism is independently associated with higher infant birth weight, especially among nonsmokers, while the 5,10-*MTHFR* A1298C variant is not independently associated with birth weight. In addition, the 5,10-*MTHFR* 1298AA polymorphism might be associated with folate impairment and could interact with tobacco smoke to further decrease birth weight.

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Conflicts of interest: None declared.

## REFERENCES


1. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) associated with decreased enzyme activity. Mol Genet Metab. 1998;64:169–72.
2. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA. 2002;99:5606–11.
3. Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, Kubo T. Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J Med Genet. 1997;34:525–6.
4. Isotalo PA, Wells GA, Donnelly JG. Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet. 2000;67:986–90.
5. van der Molen EF, Arends GE, Nelen WL, van der Put NJ, Heil SG, Eskes TK, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene as a new risk factor for placental vasculopathy. Am J Obstet Gynecol. 2000;182:1258–63.
6. Murakami S, Matsubara N, Saitoh M, Miyakaw S, Shoji M, Kubo T. The relation between plasma homocysteine concentration and methylenetetrahydrofolate reductase gene polymorphism in pregnant women. J Obstet Gynaecol Res. 2001;27:349–52.
7. Hefler L, Jirecek S, Heim K, Grimm C, Antensteiner G, Zeillinger R, et al. Genetic polymorphisms associated with thrombophilia and vascular disease in women with unexplained late intrauterine fetal death: a multicenter study. J Soc Gynecol Investig. 2004;11:42–4.
8. Valdez LL, Quintero A, Garcia E, Olivares N, Celis A, Rivas F Jr, et al. Thrombophilic polymorphisms in preterm delivery. Blood Cells Mol Dis. 2004;33:51–6.
9. Johnson WG, Scholl TO, Spychala JR, Buyske S, Stenroos ES, Chen X. Common dihydrofolate reductase 19-base pair deletion allele: a novel risk factor for preterm delivery. Am J Clin Nutr. 2005;81:664–8.
10. Relton CL, Pearce MS, Burn J, Parker L. An investigation of folate-related genetic factors in the determination of birthweight. Paediatr Perinat Epidemiol. 2005;19:360–7.
11. Mtiraoui N, Zammiti W, Ghazouani L, Braham NJ, Saidi S, Finan RR, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses. Reproduction. 2006;131:395–401.
12. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr. 2006;83:993–1016.
13. Stonek F, Hafner E, Philipp K, Hefler LA, Bentz EK, Tempfer CB. Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. Obstet Gynecol. 2007;110:363–8.
14. Ozbek N, Ataç FB, Verdi H, Cetintaş S, Gürakan B, Haberal A. Relationship between small-for-gestational age births and maternal thrombophilic mutations. Thromb Res. 2008;122:175–8.
15. Pilsner JR, Hu H, Wright RO, Kordas K, Ettinger AS, Sánchez BN, et al. Maternal *MTHFR* genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. Am J Clin Nutr. 2010;92:226–34.
16. Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, et al. Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod. 2000;15:458–62.
17. Kobashi G, Yamada H, Asano T, Nagano S, Hata A, Kishi R, et al. Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and preeclampsia in Japanese women. Am J Med Genet. 2000;93:122–5.
18. Gebhardt GS, Scholtz CL, Hillermann R, Odendaal HJ. Combined heterozygosity for methylenetetrahydrofolate reductase (MTHFR) mutations C677T and A1298C is associated with abruptio placentae but not with intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol. 2001;97:174–7.
19. McCowan LM, Craigie S, Taylor RS, Ward C, McLintock C, North RA. Inherited thrombophilias are not increased in "idiopathic" small-for-gestational-age pregnancies. Am J Obstet Gynecol. 2003;188:981–5.
20. Chen DF, Hu YH, Yang F, Wu BY, Chen L, Fang ZA, et al. Mother's and child's methylenetetrahydrofolate reductase C677T polymorphism is associated with preterm delivery and low birth weight. Beijing Da Xue Xue Bao. 2004;36(3):248–53.
21. Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland Homocysteine Study. Am J Med. 2004;117:26–31.
22. Resch B, Gallistl S, Kutschera J, Mannhalter C, Muntean W, Mueller WD. Thrombophilic polymorphisms—factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations—and preterm birth. Wien Klin Wochenschr. 2004;116:622–6.
23. Also-Rallo E, Lopez-Quesada E, Urreizti R, Vilaseca MA, Lailla JM, Balcells S, et al. Polymorphisms of genes involved in homocysteine metabolism in preeclampsia and in uncomplicated pregnancies. Eur J Obstet Gynecol Reprod Biol. 2005;120:45–52.
24. Engel SM, Olshan AF, Siega-Riz AM, Savitz DA, Chanock SJ. Polymorphisms in folate metabolizing genes and risk for spontaneous preterm and small-for-gestational age birth. Am J Obstet Gynecol 2006;195:1231.e1–11.
25. Ananth CV, Peltier MR, De Marco C, Elsasser DA, Getahun D, Rozen R, et al; New Jersey-Placental Abruption Study Investigators. Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption. Am J Obstet Gynecol 2007;197:385.e1–7.
26. Richter B, Stegmann K, Röper B, Böddeker I, Ngo ET, Koch MC. Interaction of folate and homocysteine pathway genotypes evaluated in susceptibility to neural tube defects (NTD) in a German population. J Hum Genet. 2001;46(3):105–9.
27. Chango A, Fillon-Emery N, Mircher C, Bléhaut H, Lambert D, Herbeth B, et al. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br J Nutr.

2005;94:166–9.
28. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998; 62:1044–51.
29. Hayashi K, Matsuda Y, Kawamichi Y, Shiozaki A, Saito S. Smoking during pregnancy increases risks of various obstetric complications: a case-cohort study of the Japan Perinatal Registry Network database. J Epidemiol. 2011;21(1):61–6. Epub 2010 Nov 13.
30. Brown KS, Kluijtmans LA, Young IS, Murray L, McMaster D, Woodside JV, et al. The 5,10-methylenetetrahydrofolate reductase C677T polymorphism interacts with smoking to increase homocysteine. Atherosclerosis. 2004;174:315–22.
31. Ozerol E, Ozerol I, Gökdeniz R, Temel I, Akyol O. Effects of smoking on serum concentrations of total homocysteine, folate, Vitamain B12, and Nitric Oxide in Pregnancy: A preliminary study. Fetal Diagn Ther. 2004;19:145–8.
32. Jauniaux E, Johns J, Gulbis B, Spasic-Boskovic O, Burton GJ. Transfer of folic acid inside the first-trimester gestational sac and the effect of maternal smoking. Am J Obstet Gynecol 2007;197:58.e1–6.
33. Takamura N, Kondoh T, Ohgi S, Arisawa K, Mine M, Yamashita S, et al. Abnormal folic acid-homocysteine metabolism as maternal risk factors for Down syndrome in Japan. Eur J Nutr. 2004;43:285–7.
34. Kobashi G, Kato EH, Morikawa M, Shimada S, Ohta K, Fujimoto S, et al. *MTHFR* C677T Polymorphism and factor V Leiden mutation are not associated with recurrent spontaneous abortion of unexplained etiology in Japanese women. Semin Thromb Hemost. 2005;31:266–71.
35. Kobashi G. Genetic and environmental factors associated with the development of hypertension in pregnancy. J Epidemiol. 2006;16:1–8.
36. Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. Cohort profile: the Hokkaido study on environment and children's health in Japan. Int J Epidemiol. 2011;40:611–8. Epub 2010 May 26.
37. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151:862–77.
38. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol. 2003;157:571–82.
39. Sasaki S, Kondo T, Sata F, Saijo Y, Katoh S, Nakajima S, et al. Maternal smoking during pregnancy and genetic polymorphisms in the Ah receptor, CYP1A1 and GSTM1 affect infant birth size in Japanese subjects. Mol Hum Reprod. 2006;12(2):77–83.
40. Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies, 2006. Available from: http://hydra.usc.edu/gxe.
41. Bock JL. The New Era of Automated Immunoassay. Am J Clin Pathol. 2000;113:628–46.
42. Sujaku K, Ogiwara T, Kawasaki Y. Determination of vitamin B12 and folate by ADVIA Centaur. J Anal Bio-Sci. 2006; 29:235–42 (in Japanese).
43. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res. 2001;11:1262–8.
44. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet. 2005; 76:887–93.
45. Matsuo K, Suzuki R, Hamajima N, Ogura M, Kagami Y, Taji H, et al. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. Blood. 2001;97(10):3205–9.
46. Lwin H, Yokoyama T, Date C, Yoshiike N, Kokubo Y, Tanaka H. Are the associations between life-style related factors and plasma total homocysteine concentration different according to polymorphism of 5,10-methylenetetrahydrofolate reductase gene (C677T *MTHFR*)? A cross-sectional study in a Japanese rural population. J Epidemiol. 2002;12:126–35.
47. Yin G, Kono S, Toyomura K, Hagiwara T, Nagano J, Mizoue T, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. Cancer Sci. 2004;95:908–13.
48. Kim NK, Choi YK, Kang MS, Choi DH, Cha SH, An MO, et al. Influence of combined methylenetetrahydrofolate reductase (*MTHFR*) and thymidylate synthase enhancer region (TSER) polymorphisms to plasma homocysteine levels in Korean patients with recurrent spontaneous abortion. Thromb Res. 2006;117:653–8.
49. Castro R, Rivera I, Ravasco P, Jakobs C, Blom HJ, Camilo ME, et al. 5,10-Methylenetetrahydrofolate reductase 677C→T and 1298A→C mutations are genetic determinants of elevated homocysteine. QJM. 2003;96:297–303.
50. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr. 2000;72:324–32.
51. Jhaveri MS, Wagner C, Trepel JB. Impact of extracellular folate levels on global gene expression. Mol Pharmacol. 2001;60:1288–95.
52. Courtemanche C, Elson-Schwab I, Mashiyama ST, Kerry N, Ames BN. Folate deficiency inhibits the proliferation of primary human $CD8^+$ T lymphocytes in vitro. J Immunol. 2004;173: 3186–92.
53. Infante-Rivard C, Rivard GE, Yotov WV, Génin E, Guiguet M, Weinberg C, et al. Absence of association of thrombophilia polymorphisms with intrauterine growth restriction. N Engl J Med. 2002;347:19–25.
54. Moriyama Y, Okamura T, Kajinami K, Iso H, Inazu A, Kawashiri M, et al. Effects of serum B vitamins on elevated plasma homocysteine levels associated with the mutation of methylenetetrahydrofolate reductase gene in Japanese. Atherosclerosis. 2002;164:321–8.
55. Parle-McDermott A, Mills JL, Molloy AM, Carroll N, Kirke PN, Cox C, et al. The *MTHFR* 1298CC and 677TT genotypes have opposite associations with red cell folate levels. Mol Genet Metab. 2006;88:290–4.
56. Cui LH, Shin MH, Kim HN, Song HR, Piao JM, Kweon SS, et al. Methylenetetrahydrofolate reductase C677T polymorphism in patients with lung cancer in a Korean population. BMC Med Genet. 2011 Feb 22;12:28.
57. Tamimi RM, Lagiou P, Mucci LA, Hsieh CC, Adami HO,

Trichopoulos D. Average energy intake among pregnant women carrying a boy compared with a girl. BMJ. 2003;326:1245–6.
58. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta and fetus. Endocr Rev. 2006;27:141–69.
59. Tsutsumi A, Kagawa J, Yamano Y, Nakadate T, Shimizu S. Relation between Cotinine in the Urine and Indices Based on Self-Declared Smoking Habits. Environ Health Prev Med. 2002;6:240–7.
60. Smoking Rate Highest in Hokkaido: A survey by the Japan Tobacco Inc. 2009. Available from: http://e.nikkei.com/e/fr/tnks/Nni20100331D30HH717.htm.

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# Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants ☆

Emiko Okada [a], Seiko Sasaki [a], Yasuaki Saijo [b], Noriaki Washino [a], Chihiro Miyashita [a], Sumitaka Kobayashi [a], Kanae Konishi [a], Yoichi M. Ito [c], Rie Ito [d], Ayako Nakata [d], Yusuke Iwasaki [d], Koichi Saito [d], Hiroyuki Nakazawa [d], Reiko Kishi [e,*]

[a] Department of Public Health, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Department of Health Science, Asahikawa Medical University, Asahikawa, Japan
[c] Department of Biostatistics, Division of Advanced Medical Sciences, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[d] Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan
[e] Center for Environmental and Health Sciences, Hokkaido University, North 12 West 7 Kita-ku, Sapporo 060-0812, Japan

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ABSTRACT

*Background:* Recent studies have shown effects of prenatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) on infants in the general environmental levels. Laboratory animal studies have shown that exposure to PFOS and PFOA is associated with immunotoxic effects.
*Objectives:* To investigate the relationship between maternal PFOS and PFOA levels and infant allergies and infectious diseases during the first 18 months of life. Cord blood immunoglobulin (Ig) E levels were also evaluated.
*Methods:* We conducted a prospective cohort study of pregnant women from 2002 to 2005 in Sapporo, Japan. Maternal PFOS and PFOA levels were measured in relation to cord blood IgE concentrations ($n$=231) and infant allergies and infectious diseases ($n$=343). Characteristics of mothers and their infants were obtained from self-administered questionnaires and medical records. Development of infant allergies and infectious diseases was determined from self-administered questionnaires at 18 months of age. Concentrations of PFOS and PFOA in maternal serum and concentrations of IgE in umbilical cord serum at birth were measured.
*Results:* Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, there were no significant associations among maternal PFOS and PFOA levels and food allergy, eczema, wheezing, or otitis media in the 18 month-old infants (adjusted for confounders).
*Conclusions:* Although cord blood IgE level decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and infant allergies and infectious diseases at age in 18 months.



## 1. Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are end metabolites of perfluorinated chemicals (PFCs). PFOS and PFOA are persistent organic pollutants and are widely used in consumer products (e.g., surface-active agents, flame retardants, adhesives, and pesticides). PFOS and PFOA have recently been found to be widespread contaminants in the environment, wildlife, and humans (Lau et al., 2007). The half-life of PFOS in humans is 3.8 years, and that of PFOA is 5.4 years (Olsen et al., 2007). Possible health effects of PFOS and PFOA exposure in humans are a concern because of bioaccumulation and persistence in the environment, in animals, and in humans. It has been reported that PFC concentrations in human blood

*Abbreviations:* ATS—DLD, American Thoracic Society—Division of Lung Diseases; BMI, body mass index; CI, confidence interval; IgA, immunoglobulin A; ISAAC, International Study of Asthma and Allergies in Childhood; ND, non-detectable; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; PCBs, polychlorinated biphenyls; PFC, perfluorinated chemical; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate

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**Ethics approval**: 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.

* Corresponding author. Fax: +81 11 706 4725.
*E-mail address:* rkishi@med.hokudai.ac.jp (R. Kishi).

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increase with age (Harada et al., 2004, 2007). The main sources of human exposure are drinking water (Hölzer et al., 2008), foods such as red meat and animal fat (Halldorsson et al., 2008), contamination from food packaging (Begley et al., 2005; Tittlemier et al., 2007), and indoor dust (Björklund et al., 2009; Shoeib et al., 2005).

PFOS and PFOA pass the placental barrier and are transferred to the fetus in humans (Midasch et al., 2007; Monroy et al., 2008). Some studies have reported a negative association between prenatal PFOS and PFOA exposure and birth weight (Apelberg et al., 2007; Fei et al., 2007). We previously observed a strong positive correlation between PFOS concentrations in maternal blood and cord blood (Inoue et al., 2004a), and a negative correlation between relatively low levels of maternal blood PFOS concentration and birth weight among female infants (Washino et al., 2009).

The prevalence of allergies has recently increased in children (Zöllner et al., 2005). The main environmental risk factors associated with asthma and other wheezy disorders are genetics, viruses, bacteria, tobacco exposure, and allergic sensitization, as determined by long-term studies (Bisgaard and Bønnelykke, 2010). Certain oxidants, airborne particulate matter, diesel exhaust particles, and polycyclic aromatic hydrocarbons have also been suggested as risk factors for asthma (Ho, 2010). It has been suggested that susceptibility to the effects of environmental chemicals may be higher during prenatal development when many physiological systems are developing, including the immune system (Luster et al., 2003). In an epidemiological study, the Danish National Birth Cohort showed that prenatal exposure to PFOA or PFOS was not associated with risk of hospitalization for infectious diseases in early childhood (Fei et al., 2010). In the C8 Health Project that investigated residents in the vicinity of a PFOA plant, it was shown that immunoglobulin (Ig) A and C reactive protein levels had a significant decreasing trend with increasing PFOA levels in blood samples; this pattern was also indicated for IgE, but only in females (Fletcher et al., 2009). Effects of PFOS and PFOA exposure on immunity and allergy in laboratory animals include immunosuppression, suppression of IgM antibody production (Dewitt et al., 2008; Keil et al., 2008; Peden-Adams et al., 2007), and increased total IgE response in ovalbumin-sensitized mice (Fairley et al., 2007). However, reports that have examined effects of PFC on immunity and allergy in a prospective study are few. It is therefore necessary to epidemiologically evaluate the effects of PFC exposure on immunity and allergy in humans, and to determine relationships between PFC exposure and immunity and allergy in humans.

The aim of this study was to ascertain possible relationships between maternal PFOS and PFOA levels and allergies and infectious diseases in their infants during the first 18 months of life using a prospective cohort study. IgE concentrations in cord blood were also evaluated.

## 2. Materials and methods

### 2.1. Study population

This prospective birth cohort study was based on infants delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan, which is an obstetrics and gynecology hospital (Hokkaido Study on Environment and Children's Health). Details regarding the study population, data collection, and the content of the questionnaires have been previously described (Kishi et al., 2011). In brief, between July 2002 and October 2005, participants were native Japanese women who enrolled at 23–35 weeks of gestation and were residents of Sapporo City or surrounding areas. Of 1796 women asked to participate, 514 agreed (participation rate of 28.6%). Among the 514 women, 10 were excluded due to miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before follow-up. Thirteen women were excluded due to death of the infant, relocation, or voluntary withdrawal for the follow-up period from delivery to 18 months.

### 2.2. Data collection

Participants completed a self-administered questionnaire during the second trimester of pregnancy. The questionnaire included information related to previous medical history, educational level, household income, smoking status, alcohol intake, caffeine intake, and food intake frequency during pregnancy. Medical information including maternal age, maternal height, maternal pre-pregnancy weight, pregnancy complications, parity, gestational age, infant gender, birth weight, and birth length were obtained from medical records. At 18 months post-delivery, participants completed another self-administered questionnaire. Of the 491 women to whom the questionnaire was mailed, 390 responded (recovery rate of 79.4%). The questionnaire included information related to breast-feeding, infant weight and length at 18 months, smoking status of parents, environmental tobacco smoke exposure at 18 months, pets in the home, living environment, day care attendance at 18 months, infant vaccination, and previous or current medical history of infant allergies and infectious diseases (food allergy, eczema, asthma, febrile convulsion, respiratory syncytial virus (RSV) disease, otitis media, and other diseases). 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 Graduate School of Medicine.

### 2.3. Assessment of infant allergies and infections

Infant allergies and infectious diseases that developed during the first 18 months of life were assessed based on mothers' self-administered questionnaire at 18 months post-delivery. Food allergy was defined as a positive response to the following question: "Has your child ever had symptoms such as hives, swelling of the lip, 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). The part contained six questions: "(1) Has your child ever had an eczema in the past? If yes; (2) Has your child ever had an itchy rash, which was coming and going for at least 6 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?; (4) Has your child ever had dry skin in the past?; (5) Has your child ever had a doctor's diagnosis or diagnostic possibility for an eczema in the past?; (6) Does an itchy rash at any time affect 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 at present?" 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). The part contained five 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 twice or more attacks in the past?; (3) Has your child ever had a doctor's diagnosis possibility for a bronchial asthma, asthmatic, or pediatric asthma in the past?; (4) Could wheezing be heard during an attack?; (5) Has your child ever had shortness of breath and wheezing during an attack?" To estimate the proportion of allergies or infectious diseases, we defined an outcome based on the following criteria: if infants 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: asthma, eczema, other allergic diseases, otitis media, febrile convulsion, RSV diseases or other diseases, including chicken pox, bronchitis, rhinitis, pneumonia, skin infection and other viral infections?"

### 2.4. Measurement of PFOS and PFOA concentrations in maternal serum

Detailed sampling and laboratory methods for measuring PFOS and PFOA have been previously described (Washino et al., 2009). In brief, a 40 mL blood sample was taken from the maternal peripheral vein after the second trimester of pregnancy. When this was not possible because of anemia of the mother, a blood sample was taken after delivery. All samples were stored at −80 °C until analysis. The analytical detection method used was a variation of a method published previously (Nakata et al., 2005a, 2005b), and the methods for developing this variation of the analytical approach have also been described elsewhere (Inoue et al., 2004a, 2004b). Human serum samples (0.1 mL) were mixed with 0.2 mL internal standard solution containing acetonitrile, centrifuged at 1450g for 10 min, and the supernatant was transferred to a polypropylene tube. An aliquot of the filtered sample solution was subjected to column-switching liquid chromatography–tandem mass spectrometry. As a result of loss to follow-up, lack of serum specimen, and laboratory capacity, the concentrations of PFOS and PFOA were measured in 447 maternal serum samples. For participants with a concentration below the detection limit, a value equal to half of the detection limit was used for calculation purposes.

To examine possible effects of other environmental chemicals as confounding factors, we measured the concentrations of 7 polychlorinated dibenzo-*p*-dioxins

(PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 non-*ortho*-polychlorinated biphenyl (PCB) congeners, 8 mono-*ortho*-PCB congeners, and 58 non-dioxin-like PCB congeners in maternal blood ($n$=426) using high-resolution gas chromatography/high resolution mass spectrometry at the Fukuoka Institute of Health and Environmental Sciences (Todaka et al., 2007, 2008).

### 2.5. Measurement of IgE in cord serum

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 serum were measured using an enzyme-linked immunosorbent assay (IMx$^{(R)}$analyzer, ABBOTT JAPAN CO., LTD., Tokyo, Japan). IgE concentrations were measured in 268 cord serum samples at SRL, Inc. (Tokyo, Japan).

### 2.6. Statistical analysis

First, we analyzed a possible association between maternal PFOS and PFOA levels and cord blood IgE levels. For analysis of correlations between cord blood IgE levels and characteristics of mothers and infants, we used the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test. Because data did not fall into a normal distribution, PFOS, PFOA, and IgE concentrations were converted to a $\log_{10}$ scale. We calculated the residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels. Polynomial regression analysis was performed as the residual of potential confounding variables for dependent variables and $\log_{10}$-transformed maternal PFOS or PFOA levels for the independent variable, because the cubic polynomial regression model was better fitted than the linear regression model. Confounding variables were selected based on covariates that influenced cord blood IgE levels in univariate analyses and possible risk factors reported in previous studies, and also based on the change in estimate criterion. Variables considered in the analysis are: maternal age, maternal allergic history, infant gender, birth season, distance from home to highway, and sampling period. Parity was introduced into the final models because maternal PFOS and PFOA concentrations varied significantly primiparous and multiparous. Deep sea fish intake during pregnancy was also considered because IgE levels varied significantly, but since the results did not change, the variable was not introduced into the final models. For the fully adjusted model, polynomial regression analysis was applied adjusted for maternal age, maternal allergic history (yes/no), parity (primiparous/multiparous), infant gender, birth season, distance from home to highway ($<100$ m or $\geq 100$ m), and blood sampling period (during pregnancy/after delivery). We further performed stratified analysis by infant gender.

Second, we analyzed possible associations between maternal PFOS and PFOA concentrations and the development of infant allergies and infectious diseases during the first 18 months of life. For analysis of correlations between maternal PFOS and PFOA concentrations and characteristics of parents and infants, the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test were used. For analysis of correlations between allergies and infectious diseases during the first 18 months of life and characteristics of parents and infants, we used the Student's *t*-test and the Chi-square test. To assess risk factors or protective factors for 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 PFOS and PFOA concentrations and the risk of allergies and infections among all infants, male infants, and female infants. In logistic models, we evaluated odds ratios (ORs) for the risk of allergies and infection with $\log_{10}$-transformed maternal PFOS and PFOA levels. Multivariate analyses were adjusted for confounding variables that influenced allergies or infections in univariate analyses, possible risk factors reported in previous studies, and the sampling period. The fully adjusted model used logistic regression analysis of allergic disease adjusted for maternal age, maternal educational level ($\leq 9$ years, 10–12 years, 13–16 years, and $\geq 17$ years), pre-pregnancy body mass index (BMI), maternal allergic history (yes/no), paternal allergic history (yes/no), parity (primiparous/multiparous), infant gender, breast-feeding period ($<4$ months or $\geq 4$ months), environmental tobacco smoke exposure at 18 months (yes/no), day care attendance at 18 months (yes/no), and blood sampling period (during pregnancy/after delivery) and logistic regression analysis of infectious disease adjusted for maternal age, maternal educational level, parity, infant gender, breast-feeding period, environmental tobacco smoke exposure at 18 months, day care attendance at 18 months, and blood sampling period. Results were considered statistically significant when $p<0.05$.

## 3. Results

Concentrations of PFOS and PFOA (ng/mL) in maternal serum ($n$=343) and total IgE (IU/mL) in cord serum ($n$=231) were measured (Table 1). Detection limits for both PFOS and PFOA concentrations were 0.5 ng/mL. PFOS was detected in all samples, and PFOA was below the detection limit in 22 samples (6.4%); PFOS concentrations ranged from 1.3 to 16.2 ng/mL and the median value was 5.2 ng/mL. PFOA concentrations ranged from below the detection limit to 5.3 ng/mL and the median value was 1.3 ng/mL. The detection limit of IgE concentration was 0.05 IU/mL, and 39 samples (16.9%) were below the detection limit. Concentrations ranged from below the detection limit to 10.9 IU/mL and the median value was 0.21 IU/mL.

Possible associations between maternal serum PFOS and PFOA concentrations and various characteristics of parents and infants ($n$=343) were measured (Table 2). Univariate analyses indicated that increasing age of the mother was significantly associated with lower PFOS and PFOA concentrations. Concentrations in serum from multiparous women were significantly lower than from primipara women and concentrations in samples taken after delivery were significantly lower than in those taken during pregnancy.

Cord blood IgE concentrations were also measured in relation to several characteristics of the parents and infants ($n$=231). Statistically significant differences in IgE levels by maternal allergic history, infant gender, and deep sea fish intake during pregnancy ($p<0.05$) were observed (data not shown).

Infant allergies and infectious diseases during the first 18 months of life ($n$=343) were also determined (Table 3). The numbers of infants who developed allergies or infections up to age 18 months were as follows: food allergy, 57 (16.6%); eczema, 37 (10.8%); wheezing, 33 (9.6%); otitis media, 61 (17.8%); chicken pox, 16 (4.7%); bronchitis, 9 (2.6%); RSV diseases, 7 (2.0%); rhinitis, 6 (1.7%); pneumonia, 6 (1.7%); skin infection, 5 (1.5%); other virus infections (rotavirus, adenovirus, and cytomegalovirus), 15 (4.4%). Thus, we did not include 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 sufficient statistical power could not be ensured in the multivariate analysis. Possible associations between characteristics of participants and infant allergies and infectious diseases were analyzed. There was no significant difference between allergies and infectious diseases with regard to gender.

We observed statistically significant differences ($p<0.05$) for eczema by paternal allergic history, for wheezing by paternal

**Table 1**
Concentrations of PFOS and PFOA in maternal serum ($n$=343) and concentrations of IgE in cord serum ($n$=231).

| | Detection limit | ND[a], no. (%) | Mean | Minimum | 25th | Median | 75th | Maximum | Geometric mean |
|---|---|---|---|---|---|---|---|---|---|
| Maternal serum PFOS (ng/mL)[b] | 0.5 | 0 (0) | 5.6 | 1.3 | 3.4 | 5.2 | 7.2 | 16.2 | 5.0 |
| Maternal serum PFOA (ng/mL)[c] | 0.5 | 22 (6.4) | 1.4 | ND | 0.8 | 1.3 | 1.7 | 5.3 | 1.2 |
| Cord serum IgE (IU/mL)[d] | 0.05 | 39 (16.9) | 0.62 | ND | 0.08 | 0.21 | 0.58 | 10.9 | 0.22 |

[a] ND: not detected.
[b] PFOS: perfluorooctane sulfonate.
[c] PFOA: perfluorooctanoate.
[d] IgE: immunoglobulin E.

**Table 2**
Maternal PFOS and PFOA concentrations in relation to characteristics of parents and infants ($n$=343).

| | No. | (%) | PFOS (ng/ml)[a] | | PFOA (ng/ml)[b] | |
|---|---|---|---|---|---|---|
| | | | Median | (25th–75th) | Median | (25th–75th) |
| Parental characteristics | | | | | | |
| Maternal age (years) | 31.3 ± 4.4[c] | | $r$=−0.149[e] | | $r$=−0.114[e] | |
| Maternal pre-pregnancy BMI (kg/m$^2$)[d] | 21.2 ± 3.5[c] | | $r$=−0.077[e] | | $r$=−0.053[e] | |
| Annual household income (million yen)[f] | | | | | | |
| <5 | 224 | (65.3) | 5.2 | (3.4–7.1) | 1.3 | (0.8–1.7) |
| ≥5 | 118 | (34.4) | 5.5 | (3.3–7.2) | 1.3 | (0.9–1.8) |
| Maternal educational level (years) | | | | | | |
| ≤9 | 4 | (1.1) | 6.7 | (3.4–8.2) | 0.75 | (0.3–1.1) |
| 10–12 | 139 | (40.5) | 4.8 | (3.3–6.4) | 1.3 | (0.8–1.8) |
| 13–16 | 195 | (56.9) | 5.6 | (3.5–7.6) | 1.3 | (0.8–1.7) |
| ≥17 | 5 | (1.5) | 3.0 | (2.8–6.4) | 0.8 | (0.4–1.5) |
| Maternal smoking status during pregnancy | | | | | | |
| Nonsmoker | 292 | (85.1) | 5.3 | (3.5–7.4) | 1.3 | (0.8–1.8) |
| Smoker | 51 | (14.9) | 4.6 | (2.8–6.6) | 1.2 | (0.9–1.6) |
| Parity[f] | | | | | | |
| Primiparous | 163 | (47.5) | 5.7 | (3.9–8.0) | 1.5 | (1.2–2.2) |
| Multiparous | 179 | (52.2) | 4.8 | (3.0–6.7) | 0.9 | (0.6–1.4) |
| Blood sampling period | | | | | | |
| During pregnancy | 246 | (71.7) | 5.6 | (4.1–7.6) | 1.4 | (0.9–1.8) |
| After delivery | 97 | (28.3) | 3.6 | (2.5–6.1) | 1.1 | (0.7–1.6) |
| Maternal allergic history | | | | | | |
| No | 251 | (73.2) | 5.3 | (3.3–7.4) | 1.3 | (0.8–1.7) |
| Yes | 92 | (26.8) | 4.6 | (3.4–6.6) | 1.3 | (0.8–1.8) |
| Paternal allergic history | | | | | | |
| No | 280 | (81.6) | – | – | – | – |
| Yes | 63 | (18.4) | – | – | – | – |
| Infant characteristics | | | | | | |
| Gender | | | | | | |
| Male | 169 | (49.3) | – | – | – | – |
| Female | 174 | (50.7) | – | – | – | – |
| Birth season | | | | | | |
| Spring (March–May) | 103 | (30.0) | – | – | – | – |
| Summer (June–August) | 70 | (20.4) | – | – | – | – |
| Autumn (September–November) | 70 | (20.4) | – | – | – | – |
| Winter (December–February) | 100 | (29.2) | – | – | – | – |
| Breast-feeding period (months) | | | | | | |
| <4 | 70 | (16.5) | – | – | – | – |
| ≥4 | 273 | (83.5) | – | – | – | – |
| Environmental tobacco smoke exposure at 18 months | | | | | | |
| No | 210 | (61.0) | – | – | – | – |
| Yes | 133 | (39.0) | – | – | – | – |
| Day care attendance at 18 months[f] | | | | | | |
| No | 269 | (78.4) | – | – | – | – |
| Yes | 72 | (21.0) | – | – | – | – |
| Distance from home to highway[f] | | | | | | |
| <100 m | 183 | (53.3) | – | – | – | – |
| ≥100 m | 159 | (46.4) | – | – | – | – |

[a] PFOS: perfluorooctane sulfonate.
[b] PFOA: perfluorooctanoate.
[c] Mean ± SD.
[d] BMI: body mass index.
[e] $r$: Spearman's correlation coefficient.
[f] Missing data; annual household income (1), parity (1), day care attendance at 18 months (2), distance from home to highway (1).

allergic history, pre-pregnancy BMI, and day care attendance, and for otitis media by parity and by day care attendance (data not shown).

Next, possible associations between maternal serum PFOS and PFOA concentrations and immune system parameters were examined. Two samples with IgA levels >10 mg/dL were considered to be contaminated by maternal blood and were excluded. Ultimately, 231 mother-infant pairs were included in the analysis, for whom PFOS, PFOA, and IgE had been measured. Table 4 shows the results of cubic polynomial regression analysis between $\log_{10}$-transformed maternal serum PFOS or PFOA level and residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels ($n$=231). In analyses stratified by infant gender, cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, no significant associations were observed between maternal PFOS or PFOA level and cord blood IgE levels among male infants.

Fig. 1 shows scatterplots and the linear regression model, and the cubic polynomial regression model for the $\log_{10}$-transformed

**Table 3**
Number and proportion of infants who developed allergies or infections during the first 18 months of life ($n$=343).

| | No. | (%) |
|---|---|---|
| Allergic symptoms | | |
| Food allergy | 57 | (16.6) |
| Eczema | 37 | (10.8) |
| Wheezing | 33 | (9.6) |
| Infection | | |
| Otitis media | 61 | (17.8) |
| Chicken pox | 16 | (4.7) |
| Bronchitis | 9 | (2.6) |
| RSV disease[a] | 7 | (2.0) |
| Rhinitis | 6 | (1.7) |
| Pneumonia | 6 | (1.7) |
| Skin infection | 5 | (1.5) |
| Other viral infections[b] | 15 | (4.4) |

[a] RSV disease; respiratory syncytial virus disease.
[b] Rotavirus, adenovirus, or cytomegalovirus.

**Table 4**
Cubic polynomial regression between maternal PFOS or PFOA level (ng/mL) and residual of potential confounding variables to cord blood IgE levels (IU/mL).

| | Estimate | 95% CI |
|---|---|---|
| Overall ($n$=231)[c] | | |
| $\log_{10}$ PFOS[a] (linear) | −0.240 | (−0.891, 0.412) |
| $\log_{10}$ PFOS[a] (quadratic) | 0.145 | (−1.150, 1.440) |
| $\log_{10}$PFOS[a] (cubic) | 0.851 | (−3.729, 5.432) |
| $\log_{10}$ PFOA[b] (linear) | 0.282 | (−0.229, 0.792) |
| $\log_{10}$ PFOA[b] (quadratic) | −1.009 | (−1.918, −0.101) |
| $\log_{10}$ PFOA[b] (cubic) | −1.430 | (−3.384, 0.524) |
| Male infants ($n$=103)[d] | | |
| $\log_{10}$ PFOS[a] (linear) | −0.047 | (−1.051, 0.957) |
| $\log_{10}$ PFOS[a] (quadratic) | 0.911 | (−1.101, 2.922) |
| $\log_{10}$ PFOS[a] (cubic) | −0.101 | (−6.625, 6.422) |
| $\log_{10}$ PFOA[b] (linear) | −0.315 | (−1.114, 0.485) |
| $\log_{10}$ PFOA[b] (quadratic) | 0.227 | (−1.584, 2.037) |
| $\log_{10}$ PFOA[b] (cubic) | 1.277 | (−2.191, 4.744) |
| Female infants ($n$=128)[d] | | |
| $\log_{10}$ PFOS[a] (linear) | −0.342 | (−1.230, 0.546) |
| $\log_{10}$ PFOS[a] (quadratic) | −0.681 | (−2.500, 1.137) |
| $\log_{10}$ PFOS[a] (cubic) | 1.464 | (−5.354, 8.282) |
| $\log_{10}$ PFOA[b] (linear) | 0.766 | (0.104, 1.428) |
| $\log_{10}$ PFOA[b] (quadratic) | −1.429 | (−2.416, −0.422) |
| $\log_{10}$ PFOA[b] (cubic) | −3.078 | (−5.431, −0.726) |

[a] PFOS: perfluorooctane sulfonate.
[b] PFOA: perfluorooctanoate.
[c] Adjusted models included maternal age, maternal allergic history, distance from home to highway, infant gender, parity, birth season, and blood sampling period.
[d] Adjusted models included maternal age, maternal allergic history, distance from home to highway, parity, birth season, and blood sampling period.

maternal PFOS or PFOA level and residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels ($n$=231). We found a curvilinear relationship between maternal PFOA levels and cord blood IgE levels. In female infants, when $\log_{10}$-transformed maternal PFOA levels changed from −0.6 ng/mL to −0.4 ng/mL, $\log_{10}$-transformed cord blood IgE levels decreased by −0.150 IU/mL, and when $\log_{10}$-transformed maternal PFOA levels changed from −0.4 ng/mL to 0.3 ng/mL, $\log_{10}$-transformed cord blood IgE levels increased by 0.433 IU/mL, and when $\log_{10}$-transformed maternal PFOA levels changed from 0.3 ng/mL to 0.7 ng/mL, $\log_{10}$-transformed cord blood IgE levels greatly decreased by −0.863 IU/mL (Fig. 1F).

Table 5 shows the results of logistic regression analyses between $\log_{10}$-transformed maternal serum PFOS and PFOA levels and infant allergies and infectious diseases during the first 18 months of life. We excluded 4 women who did not answer the questions related to allergies and infections at 18 months post-delivery. Therefore, we included 343 mother-infant pairs in the analysis for whom both PFOS and PFOA had been measured and from which questionnaire data at 18 months post-delivery had been obtained. No significant associations were observed between maternal PFOS or PFOA levels and food allergy, eczema, wheezing, and otitis media in the infants in both the crude and adjusted models [adjusted ORs for a 10-fold increase in maternal PFOS or PFOA concentrations; food allergy PFOS=3.72 (95% CI, 0.81–17.10) and PFOA=1.67 (95% CI, 0.52–5.37); eczema PFOS=0.87 (95% CI, 0.15–5.08) and PFOA=0.96 (95% CI, 0.23–4.02); wheezing PFOS=2.68 (95% CI, 0.39–18.30) and PFOA=1.27 (95% CI, 0.27–6.05); otitis media PFOS=1.40 (95% CI, 0.33–6.00) and PFOA=1.51 (95% CI, 0.45–5.12)]. We performed stratified analysis by blood sampling period, but no significant associations were revealed (data not shown).

Possible associations between dioxin and PCB concentrations in maternal serum with PFOS and PFOA concentrations were evaluated. Dioxin concentrations, defined as the sum of 7 PCDDs, 10 PCDFs, and 12 dioxin-like PCBs (4 non-*ortho* PCBs and 8 mono-*ortho* PCBs) were positively correlated with PFOS and PFOA levels (Spearman rank correlation coefficients: 0.126, $p$=0.023; and 0.133, $p$=0.017, respectively). Furthermore, total PCB concentrations, defined as the sum of 70 PCB congeners (58 non-dioxin-like PCB congeners and 12 dioxin-like PCBs) were positively correlated with PFOA levels (Spearman rank correlation coefficient, 0.110; $p$=0.049) but not with PFOS levels (Spearman rank correlation coefficient, 0.062; $p$=0.267). When total dioxins and total PCBs were evaluated as potential confounders, no significant association remained between PFC concentrations and infant allergies and infectious diseases during the first 18 months of life (data not shown).

## 4. Discussion

In this study, cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, no association was observed between maternal serum PFOS and PFOA concentrations and occurrence of food allergy, eczema, wheezing, and otitis media in their infants during the first 18 months of life. To our knowledge, this is the first report that has examined possible effects of PFC prenatal exposure on infant allergy including cord blood IgE level as an immune system biomarker.

Inoue et al. (2004a) examined the correlation of PFC concentrations in maternal blood and cord blood in same sample population as this study. These PFOS in maternal and cord blood had a significant correlation. Midasch et al. (2007) and Monroy et al. (2008) reported a significant correlation of PFOS and PFOA levels in maternal and cord blood, and both studies suggested that PFOS and PFOA cross the placental barrier during pregnancy, resulting in fetal exposure to PFOS and PFOA. In this study we explored possible associations between maternal serum PFOS and PFOA concentrations with infant allergies and infectious diseases, and found no significant associations. Our results are thus consistent with results of the Danish National Birth Cohort (Fei et al., 2010), in which the mean concentrations of PFOS and PFOA were 35.3 ng/mL and 5.6 ng/mL, respectively; these levels were higher than those measured in our study. Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. The results of the C8 Health Project showed a significant decreasing trend in IgE levels with increasing PFOA levels in blood samples among females (Fletcher et al., 2009). Our results are consistent with those of that study, even though the

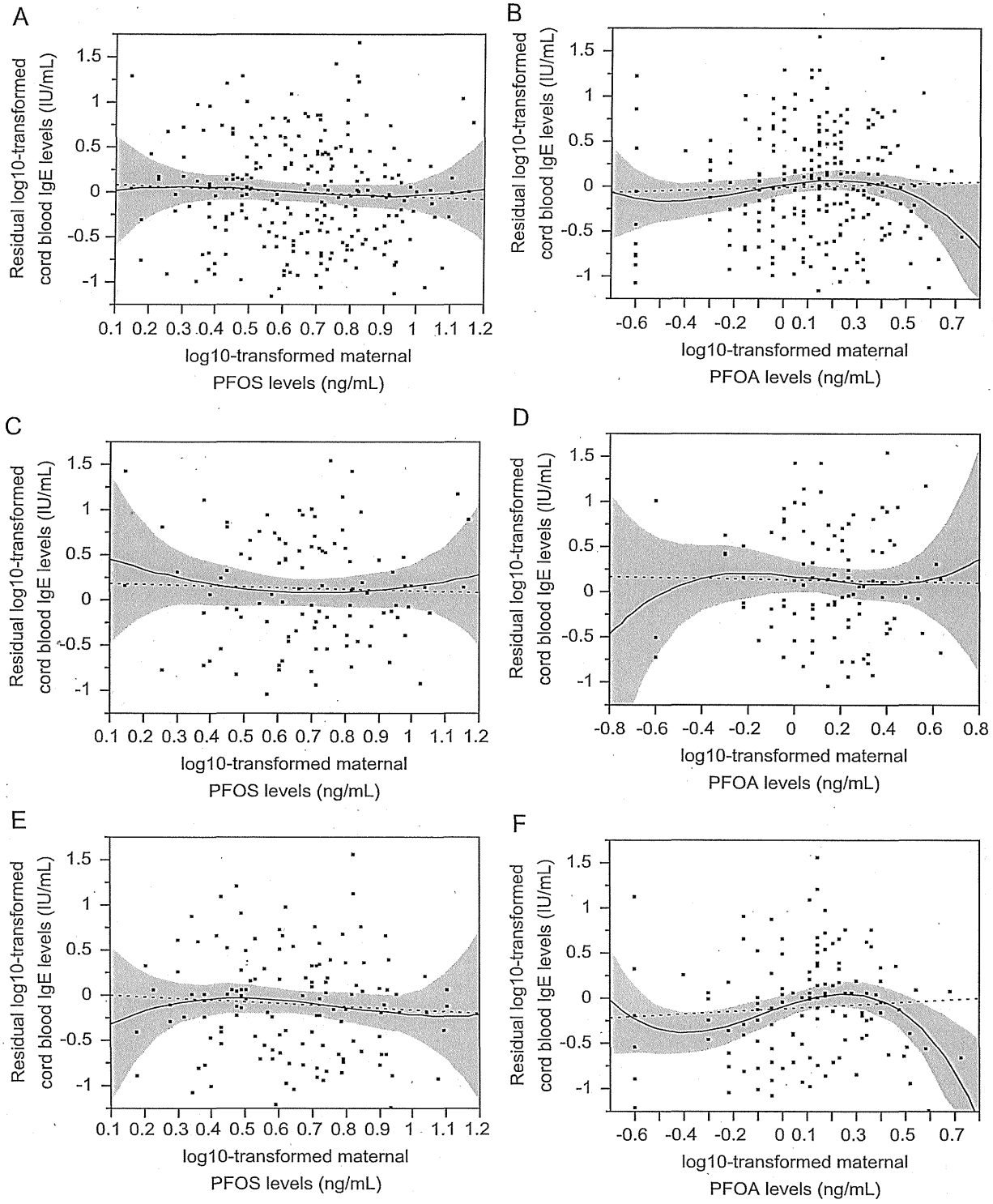


**Fig. 1.** Association between maternal $\log_{10}$-transformed PFOS and PFOA levels (ng/mL) and residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels (IU/mL). The dashed lines denote the predicted fit from a linear regression model. The solid lines denote the predicted fit from the cubic polynomial regression model and 95% confidence interval (CI). Corresponding estimates are presented in Table 4. (A, B) Among overall. (C, D) Among male infants. (E, F) Among female infants.

concentration of maternal PFOA was lower than that measured in other studies, including the C8 Health Project (Fletcher et al., 2009; Harada et al., 2007; Jensen and Leffers, 2008; Kannan et al., 2004); we note, however, that the PFOA level in any case was not associated with the development of allergies and infectious diseases up to age 18 months. It may be necessary to perform follow-up studies to investigate whether prenatal exposure to PFCs affects the immune system of offspring (and address

**Table 5**
Adjusted odds ratio (95% CI) between PFOS or PFOA concentrations in maternal serum and allergies and infectious diseases during the first 18 months of life.

| | Overall ($n$=343) | | | | Male infants ($n$=169) | | | | Female infants ($n$=174) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Crude | | Adjusted | | Crude | | Adjusted | | Crude | | Adjusted | |
| | OR[a] | (95% CI) | OR[a] | (95% CI) | OR[a] | (95% CI) | OR[a] | (95% CI) | OR[a] | (95% CI) | OR[a] | (95% CI) |
| $\log_{10}$ PFOS[b] | | | | | | | | | | | | |
| Food allergy[c] | 2.76 | (0.72, 10.54) | 3.72 | (0.81, 17.10) | 3.58 | (0.54, 23.64) | 5.42 | (0.62, 47.20) | 2.05 | (0.30, 13.82) | 2.75 | (0.31, 24.80) |
| Eczema[c] | 1.03 | (0.22, 4.91) | 0.87 | (0.15, 5.08) | 0.78 | (0.09, 6.63) | 0.62 | (0.06, 6.67) | 1.36 | (0.14, 13.55) | 1.24 | (0.08, 19.30) |
| Wheezing[c] | 1.81 | (0.34, 9.60) | 2.68 | (0.39, 18.30) | 6.32 | (0.51, 79.08) | 12.98 | (0.80, 212.00) | 0.62 | (0.06, 5.93) | 0.61 | (0.03, 11.50) |
| Otitis media[d] | 0.75 | (0.22, 2.65) | 1.40 | (0.33, 6.00) | 0.65 | (0.12, 3.53) | 1.38 | (0.18, 10.60) | 0.81 | (0.12, 5.55) | 1.43 | (0.17, 12.30) |
| $\log_{10}$ PFOA[e] | | | | | | | | | | | | |
| Food allergy[c] | 1.25 | (0.45, 3.52) | 1.67 | (0.52, 5.37) | 0.85 | (0.21, 3.49) | 0.87 | (0.16, 4.89) | 1.87 | (0.41, 8.40) | 2.37 | (0.50, 17.10) |
| Eczema[c] | 0.97 | (0.29, 3.30) | 0.96 | (0.23, 4.02) | 1.51 | (0.26, 8.67) | 1.12 | (0.15, 8.42) | 0.59 | (0.10, 3.40) | 0.88 | (0.09, 7.70) |
| Wheezing[c] | 0.85 | (0.24, 3.02) | 1.27 | (0.27, 6.05) | 2.43 | (0.32, 18.36) | 2.72 | (0.25, 29.90) | 0.36 | (0.06, 2.00) | 1.31 | (0.10, 18.00) |
| Otitis media[d] | 1.41 | (0.28, 2.02) | 1.51 | (0.45, 5.12) | 1.04 | (0.27, 3.97) | 1.92 | (0.35, 10.40) | 0.47 | (0.11, 2.05) | 0.95 | (0.16, 5.69) |

[a] OR for a 10-fold increase in maternal PFOS or PFOA concentrations.
[b] PFOS: perfluorooctane sulfonate.
[c] Logistic regression model adjusted for maternal age, maternal educational level, pre-pregnancy BMI, allergy of parents, parity, infant gender, breast-feeding period, environmental tobacco exposure, day care attendance and blood sampling period.
[d] Logistic regression model adjusted for maternal age, maternal educational level, parity, infant gender, breast-feeding period, environmental tobacco exposure, day care attendance and blood sampling period.
[e] PFOA: perfluorooctanoate.

potential gender-specific differences) from infancy to school-age, because it is difficult to obtain definitive diagnoses for infants.

Moreover, using univariate analyses of maternal PFOS and PFOA concentrations in relation to parent and infant characteristics, statistically significant differences with respect to maternal age, parity, and blood sampling period were identified in our study. In other studies, maternal PFOS and PFOA concentrations were significantly related to parity, with higher levels found in primipara (Fei et al., 2007), and significantly lower levels reported after delivery than during pregnancy (Monroy et al., 2008). In our study, we performed stratified analysis by blood sampling period, but the results of multiple analyses did not change.

In laboratory animals, immunosuppression and reduced IgM antibody production along with increased IgE levels were reported with high-dose PFOS and PFOA exposure (Dewitt et al., 2008; Fairley et al., 2007; Keil et al., 2008; Peden-Adams et al., 2007). Also, in these studies used the mouse strain known to be the most sensitive strain for immunomodulatory effects of PFOA and PFOS (Dewitt et al., 2009). On the other hand, sensitivity of the human fetus to PFCs may be higher than in animals because in our cohort study, maternal serum PFOS concentrations below those reported in the animal studies were negatively correlated with birth weight (Washino et al., 2009). It has been suggested that some effects of PFOS and PFOA on the immune system are independent of peroxisome proliferator-activated receptor alpha (PPARα) activation, but PPARα expression is lower in humans than in rodents (Dewitt et al., 2009). Because of these uncertainties, we cannot rule out the possibility that PFC exposure may be immunotoxic, and further experiments are necessary to clarify the possible effects of PFC exposure.

Sources of postnatal exposure to PFCs from birth to 18 months of age include breast milk, food, drinking water, indoor dust in living environments, and products in which PFCs are used (e.g., baby bottle and carpet). Previous studies have suggested that breast milk or indoor dust is a considerable source of PFC exposure for children (Björklund et al., 2009; Kärrman et al., 2007). In the same sample population as was used in our present study, we previously measured PFOS and PFOA concentrations in 40 breast milk samples and found that their concentrations were very low (Nakata et al., 2009). Therefore, we suggest that the effects of PFOS and PFOA in breast milk might be lower than the effects of these compounds in maternal blood. Postnatal exposure from intake of food and drinking water or from indoor dust from birth to 18 months of age was not investigated in our study. In future studies, it will be necessary to examine not only prenatal exposure but also postnatal exposure, because the latter may have affected the exposure assessment.

The present study has some limitations. First, the sample size was relatively small and was insufficient for identification of significant relationships between PFC exposure and allergies and infectious diseases, given the low number of cases in the cohort with these conditions. Second, selection bias may have occurred because the study population included only pregnant women that attended one local maternity hospital and the participation rate was low (29%), partly because we excluded pregnant women who had decided to enroll in the Japanese cord blood bank (22% of those approached) or who delivered their baby at another hospital (3% of those approached). These exclusions may limit the extrapolation of our results to the general population. Third, diagnosis of allergic disease such as wheezing in infants from the birth to 18 months might not have been accurate because of difficulties in obtaining diagnoses. The current prevalence of asthma in Japan is the highest it has been in six years, and tends to be decreasing with advancing age (Ministry of Education, Culture, Sports, Science and Technology, 2010). Because it is difficult to obtain a definitive diagnosis of allergy in infants, a follow-up study in which exposure is monitored from birth to school-age may be necessary to further examine possible effects of PFOS and PFOA on the immune system.

## 5. Conclusions

Although cord blood IgE levels decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and allergies and infectious diseases in infants at age 18 months. Future studies with a larger sample size are warranted to investigate the relationship between maternal PFC exposure and child allergies and infections from infancy to school-age.

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## References


Apelberg, B.J., Witter, F.R., Herbstman, J.B., Calafat, A.M., Halden, R.U., Needham, L.L., et al., 2007. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ. Health Perspect. 115, 1670–1676.

Begley, T.H., White, K., Honigfort, P., Twaroski, M.L., Neches, R., Walker, R.A., 2005. Perfluorochemicals: potential sources of and migration from food packaging. Food Addit. Contam. 22, 1023–1031.

Bisgaard, H., Bønnelykke, K., 2010. Long-term studies of the natural history of asthma in childhood. J. Allergy Clin. Immunol. 126, 187–197.

Björklund, J.A., Thuresson, K., De Wit, C.A., 2009. Perfluoroalkyl compounds (PFCs) in indoor dust: concentrations, human exposure estimates, and sources. Environ. Sci. Technol. 43, 2276–2281.

Dewitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W., 2008. Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6 J or C57BL/6 N female mice. Environ. Health Perspect. 116, 644–650.

Dewitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., et al., 2009. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit. Rev. Toxicol. 39, 76–94.

Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., Meade, B.J., 2007. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol. Sci. 97, 375–383.

Fei, C., McLaughlin, J.K., Tarone, R.E., Olsen, J., 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ. Health Perspect. 115, 1677–1682.

Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2010. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ. Res. 110, 773–777.

Fletcher, T., Steenland, K., Savitz, D., 2009. Status Report: PFOA and Immune Biomarkers in Adults Exposed to PFOA in Drinking Water in the Mid Ohio Valley: 〈http://www.c8sciencepanel.org/pdfs/Status_Report_C8_and_Immune_markers_March2009.pdf〉 (accessed 27 September 2011).

Halldorsson, T.I., Fei, C., Olsen, J., Lipworth, L., McLaughlin, J.K., Olsen, S.F., 2008. Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort. Environ. Sci. Technol. 42, 8971–8977.

Harada, K., Saito, N., Inoue, K., Yoshinaga, T., Watanabe, T., Sasaki, S., et al., 2004. The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. J. Occup. Health 46, 141–147.

Harada, K., Koizumi, A., Saito, N., Inoue, K., Yoshinaga, T., Date, C., et al., 2007. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere 66, 293–301.

Ho, S.-M., 2010. Environmental epigenetics of asthma: an update. J. Allergy Clin. Immunol. 126, 453–465.

Hölzer, J., Midasch, O., Rauchfuss, K., Kraft, M., Reupert, R., Angerer, J., et al., 2008. Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. Environ. Health Perspect. 116, 651–657.

Inoue, K., Okada, F., Ito, R., Kato, S., Sasaki, S., Nakajima, S., et al., 2004a. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ. Health Perspect. 112, 1204–1207.

Inoue, K., Okada, F., Ito, R., Kawaguchi, M., Okanouchi, N., Nakazawa, H., 2004b. Determination of perfluorooctane sulfonate, perfluorooctanoate and perfluorooctane sulfonylamide in human plasma by column-switching liquid chromatography–electrospray mass spectrometry coupled with solid-phase extraction. J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 810, 49–56.

ISAAC Steering Committee, 1998. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 351, 1225–1232.

Jensen, A.A., Leffers, H., 2008. Emerging endocrine disrupters: perfluoroalkylated substances. Int. J. Androl. 31, 161–169.

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., et al., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38, 4489–4495.

Kärrman, A., Ericson, I., van Bavel, B., Darnerud, P.O., Aune, M., Glynn, A., et al., 2007. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. Environ. Health Perspect. 115, 226–230.

Keil, D.E., Mehlmann, T., Butterworth, L., Peden-Adams, M.M., 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. Toxicol. Sci. 103, 77–85.

Kishi, R., Sasaki, S., Yoshioka, E., Yuasa, M., Sata, F., Saijo, Y., et al., 2011. Cohort profile: the Hokkaido study on environment and children's health in Japan. Int. J. Epidemiol. 40, 611–618.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.

Luster, M.I., Dean, J.H., Germolec, D.R., 2003. Consensus workshop on methods to evaluate developmental immunotoxicity. Environ. Health Perspect. 111, 579–583.

Midasch, O., Drexler, H., Hart, N., Beckmann, M.W., Angerer, J., 2007. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int. Arch. Occup. Environ. Health 80, 643–648.

Ministry of Education, Culture, Sports, Science and Technology, Japan. School Health Examination Survey 2010: 〈http://www.mext.go.jp/b_menu/toukei/chousa05/hoken/kekka/k_detail/__icsFiles/afieldfile/2011/03/25/1303380_2.pdf〉 (accessed 27 September 2011).

Monroy, R., Morrison, K., Teo, K., Atkinson, S., Kubwabo, C., Stewart, B., et al., 2008. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ. Res. 108, 56–62.

Nakata, H., Nakata, A., Kawaguchi, M., Iwasaki, Y., Ito, R., Saito, K., et al., 2005a. Development of an analytical method for perfluorochemicals in human plasma and blood by liquid chromatography-tandem mass spectrometry coupled with solid-phase extraction using a column-switching technique. Organohalogen Compd. 67, 219–221.

Nakata, H., Nakata, A., Okada, F., Ito, R., Inoue, K., Saito, K., et al., 2005b. Development of online solid-phase extraction-HPLC/MS/MS method for the determination of perfluorochemicals in human plasma. Bunseki Kagaku 9, 877–884 (in Japanese).

Nakata, A., Saito, K., Iwasaki, Y., Ito, R., Kishi, R., Nakazawa, H., 2009. Determination of perfluorinated compounds in human milk and evaluation of their transition from maternal plasma. Bunseki Kagaku 58, 653–659 (in Japanese).

Nishima, S., Chisaka, H., Fujiwara, T., Furusho, K., Hayashi, S., Hiraba, K., et al., 2009. Surveys on the prevalence of pediatric bronchial asthma in Japan: a comparison between the 1982, 1992, and 2002 surveys conducted in the same region using the same methodology. Allergol. Int. 58, 37–53.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., et al., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115, 1298–1305.

Peden-Adams, M.M., EuDaly, J.G., Dabra, S., EuDaly, A., Heesemann, L., Smythe, J., et al., 2007. Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. J. Toxicol. Environ. Health A 70, 1130–1141.

Shoeib, M., Harner, T., Wilford, B.H., Jones, K.C., Zhu, J., 2005. Perfluorinated sulfonamides in indoor and outdoor air and indoor dust: occurrence, partitioning, and human exposure. Environ. Sci. Technol. 39, 6599–6606.

Tittlemier, S.A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X.L., et al., 2007. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J. Agric. Food Chem. 55, 3203–3210.

Todaka, T., Hirakawa, H., Kajiwara, J., Hori, T., Tobiishi, K., Onozuka, D., et al., 2007. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere 69, 1228–1237.

Todaka, T., Hori, T., Hirakawa, H., Kajiwara, J., Yasutake, D., Onozuka, D., et al., 2008. Congener-specific analysis of non-dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere 73, 923–931.

Washino, N., Saijo, Y., Sasaki, S., Kato, S., Ban, S., Konishi, K., et al., 2009. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ. Health Perspect. 117, 660–667.

Zöllner, I.K., Weiland, S.K., Piechotowski, I., Gabrio, T., von Mutius, E., Link, B., et al., 2005. No increase in the prevalence of asthma, allergies, and atopic sensitisation among children in Germany: 1992–2001. Thorax 60, 545–548.

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# Blood persistent organochlorine pesticides in pregnant women in relation to physical and environmental variables in The Hokkaido Study on Environment and Children's Health

Ayako Kanazawa [a], Chihiro Miyasita [a], Emiko Okada [b], Sumitaka Kobayashi [b], Noriaki Washino [b], Seiko Sasaki [b], Eiji Yoshioka [b], Futoshi Mizutani [c], Youichi Chisaki [c], Yasuaki Saijo [d], Reiko Kishi [a,*]

[a] Center for Environmental Health and Sciences, Hokkaido University, Kita 12, Nish 7, Kita-ku, Sapporo 060-0812, Japan
[b] Department of Public Health Sciences, Graduate School of Medicine, Hokkaido University, Kita 15, Nish 7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan
[c] IDEA Consultants, Inc., Riemon 1334-5, Yaizu, Shizuoka 421-0212, Japan
[d] Department of Health Science, Asahikawa Medical University, 1-1-1, Higashi 2-Jo, Midorigaoka, Asahikawa 078-8510, Hokkaido, Japan

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ABSTRACT

The aim of this study was to document the exposure levels of pregnant women in Hokkaido to persistent organochlorine (POC) pesticides and the relationship between the body burdens of these pesticides and the study population's characteristics, such as age, pre-pregnancy body weight and calendar year in which blood was collected. From 2002 to 2005, whole blood samples were obtained from 186 pregnant women (aged 17 to 47 years) from the population of 514 women registered with the Sapporo Toho hospital cohort of the Hokkaido Study. Blood samples were analyzed by GC/NCIMS and GC/HRMS to quantify 29 POC pesticides. The subjects' demographic details were obtained from medical records and self-administered questionnaires. The Jonckheere–Terpstra test was used to determine relevant trends in the chemical concentrations of these pesticides and their relationship to the subjects' demographic details. Twenty-one of the 29 targeted compounds (including pesticides that have never been used in Japan, such as Mirex, Parlar-26 and Parlar-50) were detected in whole blood samples, and their log-transformed concentrations were found to significantly correlate with each other. The concentrations of *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, Parlar-26 and Parlar-50 declined from 2002 to 2005 ($p<0.05$). The pesticide concentrations appeared to have stronger associations with past conception than with parity, with most pesticide concentrations declining in a manner that appeared inversely related to past conceptions ($p<0.05$). Maternal age was positively associated with the following pesticide concentrations: *p,p'*-DDE, chlordanes group, *cis*-heptachlorepoxide, β-HCH and mirex. Maternal pre-pregnancy body weight was positively associated with the concentrations of dieldrin, HCB, β-HCH, Parlar-26 and Parlar-50, and appeared to be more strongly related to the body burdens of POC pesticides when compared with BMI associations. Further studies are required to evaluate the effects of POC pesticides on human health with regard to reproductive outcomes and child development.



## 1. Introduction

The use of most persistent organochlorine (POC) pesticides has been eliminated or restricted due to their persistence in the environment and their resulting bioaccumulation in humans and other animals. The lipophilic POCs are highly resistant to both abiotic and biotic degradation and appear to be transported long distances by weather systems (Bennett et al., 2001). The lipophilicity and long half-life of these chemicals, which are suspected endocrine disruptors (Routti et al., 2010; Elobeid et al., 2010), result in their distribution and accumulation on the body. In 2004, the Stockholm Convention on persistent organic pollutants (POPs) the production and usage of these compounds and encouraging environmental monitoring and awareness campaigns that focus on health concerns and ecosystems. At that time, the restricted compounds included nine POC pesticides, aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzen, mirex, toxaphene and dichlorodiphenyltrichloroethane (DDT). In 2009, α-hexachlorocyclohexane (HCH), β-HCH and γ-HCH were added to the restricted list (http://chm.pops.int). Most POC pesticides were prohibited from being applied to Japanese agricultural fields in the 1970s, although heptachlor, dieldrin and chlordane were used

*Abbreviations:* BMI, body mass index; CI, confidence interval; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; GC, gas chromatography; GM, geometric mean; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; LOQ, limit of quantitation; NCI-MS, negative-ion chemical-ionization mass spectrometry; PCB, polychlorinated biphenyl; POC, persistent organochlorine; POPs, persistent organic pollutants; SCLV, solvent cut large volume; SD, standard deviation; SIM, selected ion monitoring analysis.

* Corresponding author. Tel.: +81 11 706 4747; fax: +81 11 706 4725.
*E-mail address:* rkishi@med.hokudai.ac.jp (R. Kishi).

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in termite control until the 1980s, despite being banned from agricultural applications. Although it was never registered for agricultural use, hexachlorobenzene (HCB) was used in industry until the 1980s. Mirex and toxaphene have never been produced or used in Japanese industry or agriculture (http://www.env.go.jp/chemi/prtr/risk0.html).

The body burdens of POC pesticides are known to be associated with reproductive outcomes (Cocco et al., 2005; Longnecker et al., 2001; Ribas-Fito et al., 2002; Siddiqui et al., 2003; Tan et al., 2009; Toft et al., 2010; Pathak et al., 2010, 2011), cognitive anomalies (Fernandez et al., 2007; Torres-Sanchez et al., 2008) and neurodevelopment (Eskenazi et al., 2006; Ribas-Fito et al., 2006; Torres-Sánchez, et al., 2009), although the adverse effects of POC pesticides on human health are only suspected. For the last few decades, the body burdens of these chemicals have been gradually decreasing (Konishi et al., 2001; Solomon and Weiss, 2002; Craan and Haines, 1998; Schade and Heinzow, 1998).

To better understand the physical effects of persistent POC pesticides, studies that monitor the exposure levels of humans, especially pregnant women, to POC pesticides should be conducted. In addition, because POCs are suspected to cross the placental barrier (Ando et al., 1985; Saxena et al., 1981), the relationship between POC exposure and the health outcomes of infants and children should be evaluated. We have previously reported associations between maternal exposure to chemicals, such as polychlorinated biphenyl (PCB), perfluorooctane sulfonate and perfluorooctanoate, and birth weight (Konishi, et al., 2009; Washino et al., 2009) as well as associations between maternal PCB exposure and infants' mental and motor development (Nakajima et al., 2006). The present study analyzes whole blood samples to determine the exposure levels of pregnant women to 29 POC pesticides and evaluates the associations between these chemical concentrations, the subjects' physical characteristics and environmental variables.

## 2. Materials and methods

### 2.1. Study population

From 2002 to 2005, 514 pregnant women registered with a hospital-based prospective cohort study entitled the "Hokkaido Study on Environment and Children's Health" (Kishi et al., 2011). The concentrations of POC pesticides were determined in 186 of the 374 whole blood samples obtained from this population. Data regarding the subjects' age, height, pre-pregnancy body weight, past conception(s), and parity were obtained from the subjects' medical records. Data regarding their levels of education, annual household incomes and smoking statuses during pregnancy were obtained through self-administered questionnaires.

All the subjects supplied their written informed consent, and this study was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

### 2.2. Exposure measurement

The 29 POC pesticides evaluated in this study were *o,p'*-DDT, *p,p'*-DDT; *o,p'*-dichlorodiphenyldichloroethylene (DDE), *p, p'*-DDE, *o,p'*-dichlorodiphenyldichloroethane (DDD), *p, p'*-DDD, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, aldrin, dieldrin, endrin, heptachlor, *cis*-heptachlorepoxide, *trans*-heptachlorepoxide, HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, mirex, Parlar-26, Parlar-41, Parlar-40, Parlar-44, Parlar-50, and Parlar-62. The internal standards (for the clean-up and syringe spikes) were $^{13}C$-labeled isomers or d-isomers obtained from Cambridge Isotope Laboratory, Inc. (Andover, MA, USA). The organic solvents were of a grade appropriate for measurements of dioxins (Kanto Chemical Co. Inc., Tokyo; Wako Pure Chemicals, Osaka).

The concentrations of POC pesticides were measured according to the methods recommended by the Ministry of the Environment, as described below. All procedures were performed by IDEA Consultants, Inc. (Tokyo). Six milliliters of aqueous solution saturated with ammonium sulfate and 24 ml of 25% ethyl alcohol/hexane were added to 10 ml of whole blood with internal standards (for the clean-up spike) (0.1 ng each), and this mixture was shaken for 30 min. After the mixture was separated into two layers, the upper hexane layer was obtained as part of the extract solution. The procedure was then repeated with the lower layer and 20 ml hexane, and an additional upper hexane layer was obtained. The extract solution containing the combined hexane layers was washed with 20 ml of distilled water, dehydrated with sodium sulfate dehydrate, filtrated and concentrated to 10 ml under reduced pressure. This solution was then washed three times by shaking with 30 ml hexane-saturated acetonitrile to remove lipids. The resulting extract was washed with 3 ml distilled water, this mixture of organic and aqueous solvent was separated into two layers, and the upper hexane layer was removed. Next, 60 ml of distilled water was added to the acetonitrile extract, and the extraction procedure using 30 ml hexane was repeated twice. After washed with distilled water and dehydrated, the hexane extract was divided into two fractions (3:1, by volume): fraction 1 and fraction 2. Fraction 1 was purified using silica gel column chromatography (Wakogel C-200, Wako Pure Chemicals Industries; eluting solvent, 150 ml of hexane). Then internal standards (for syringe spikes) were added to fraction 1, and fraction 1 was injected into a gas chromatography/negative-ion chemical-ionization mass spectrometry (GC/NCI-MS) system for toxaphene measurement. Fraction 2 was purified using Florisil cartridge column chromatography (LC-Florisil, SUPELCO, Bellefonte, PA, USA; eluting solvent, 5% diethylether/hexane, 10 ml). Then, internal standards (for syringe spikes) were added to fraction 2, and fraction 2 was injected into a gas chromatography/high-resolution mass spectrometry (GC/HRMS) system for measurements of pesticides other than toxaphene.

For toxaphene measurements, a 6890 series GC system (Agilent Technologies, Ltd., Santa Clara, CA, USA) equipped with a DB-35ms column (i.d. 0.25 mm × 30 m, Agilent Technologies, Inc.) was connected to a mass spectrometer (BU20, JEOL Ltd., Tokyo). The injection was conducted in the splitless mode at 220 °C. The oven temperature was programmed as follows: 130 °C for 2 min, then a 15 °C/min increase up to 220 °C, a 2.5 °C/min increase up to 255 °C, a 10 °C/min increase up to 280 °C, and finally hold at 280 °C. The detector was operated in the NCI mode (interface temperature, 250 °C; ion source temperature, 180 °C; ionizing current, 300 μA; electron volt, 200 eV; acceleration volt, 2.5 kV). The target compounds' quantification and confirmation ions were as follows: Parlar-26, -40, -41 and -44, 378.8543, 376.8572; Parlar-50, 412.8153, 410.8182; Parlar-62, 376.8387, 374.8417.

Except for toxaphene, all pesticides were analyzed using an Agilent 6890 series GC connected to a mass spectrometer (AutoSpec-Ultima, Micromass Ltd., Manchester, UK). A BPX-Dioxin-I column (i.d. 0.15 mm × 30 m, Kanto Chemical Co. Inc.) was used for detection of chlordanes, drins and Heptachlor epoxide, while a RH-12ms column (i.d. 0.25 mm × 30 m, Inventx, Torrance, CA, USA) was used for detection of other pesticides. The injection was performed in Solvent Cut Large Volume (SCLV) mode, and the oven temperature was operated as follows: 160 °C for 1 min, then a 20 °C/min increase up to 300 °C, 300 °C for 8 min, a 70 °C/min decrease down to 160 °C, 160 °C for 1 min, a 4 °C/min increase up to 300 °C and finally hold at 300 °C. For the other pesticides, the injection was performed in splitless mode, and the oven temperature was programmed as follows: 130 °C for 1 min, then a 15°C/min increase up to 180 °C, a 4 °C/min increase up to 250 °C, a 15 °C/min increase up to 330 °C, and finally hold at 330 °C. The detector was operated in the selected ion monitoring analysis (SIM) mode using an interface temperature of 290 °C of interface temperature and an ion source temperature of 320 °C. The quantification and confirmation ions of the target compounds were as follows: HCH, 218.9116, 216.9145;

DDD, 235.0081, 237.0052; DDE, 246.0003, 247.9974; DDT, 235.0081, 237.0052; HCB, 283.8102, 285.8072; chlordane, 372.8260, 374.8230; nonachlor, 408.7840, 406.7870; oxychlordane, 386.8052, 388.8023; mirex, 271.8102, 273.8072; heptachlor, 271.8102, 273.8072; heptachlorepoxide, 352.8442, 354.8413; drins (aldrin, dieldrin and endrin), 262.8570, 264.8540.

The instrumental quantitation limit (IQL) was defined as 10 times the standard deviation (SD) of 7 measurements of the lowest standard concentration in the calibration curve(s). The limit of quantitation (LOQ) was calculated from the blood sample volume, the constant volume prior to injection and the injected volume.

### 2.3. Statistics

Age, pre-pregnancy body weight, height and pre-pregnancy body mass index (BMI) were divided into three categories using tertiles. Geometric means (GM) and 95% confidence intervals (CI) were calculated using GraphPad Prism 5 (San Diego, CA, USA). Regarding the 18 chemicals for which detection rates were above 50%, trends in each category's detected chemical concentrations were evaluated using the Jonckheere–Terpstra trend test in SPSS ver. 15 (SPSS, Inc., Chicago, Illinois). A value was considered significant when the $p$ value was less than 0.05.

## 3. Results

The demographic characteristics of the subjects are given in Table 1. At the time of enrollment, the subjects' ages ranged from 17 to 47 years with a median of 31 years. The numbers of registered women from year-to-year were as follows: 2002, 35 (18.8%); 2003, 64 (34.4%); 2004, 78 (41.9%); 2005, 9 (4.8%). The subjects' heights ranged from 140 to 172 cm with a median of 158 cm, and their pre-pregnancy body weights ranged from 38 to 95 kg with a median of 52 kg. The subjects' pre-pregnancy BMI values ranged from 16.2 to 35.8 with a median of 20.6. The subjects' parity values ranged from 0 to 5 times with a mean (±SD) of 0.7±0.8 times. The subjects' past conceptions ranged from 0 to 6 times with a mean (±SD) of 1.3±1.3 times. Subjects with smoking histories accounted for 49.5% of the group, and those who smoked during pregnancy accounted for 11.8%. Blood was collected during pregnancy (24.1 to 40.7 gestational weeks, 67.2%) or after delivery (32.8%).

Table 2 presents the LOQ values, the detection rates (% of>LOQ) and the concentrations of 29 chemicals detected in whole blood samples. The detection rates of the DDTs were as follows: *o,p'*-DDT, 87.6%; *p,p'*-DDT, 100%; *o,p'*-DDD, 0.5%; *p,p'*-DDD, 66.7%; *o,p'*-DDE, 72.0%; and *p,p'*-DDE, 100%. In terms of median values (and range), the highest concentration detected among the DDTs was 610 (120–4600) pg/g wet mass of *p,p'*-DDE, and the second highest concentration was 23 (5.6–120) pg/g wet mass of *p,p'*-DDT. The detection rates of chlordanes were as follows: *cis*-chlordane, 98.9%; *trans*-chlordane, 54.8%; oxychlordane, 100%; *cis*-nonachlor, 100%; *trans*-nonachlor, 100%. Among the chlordanes, the highest concentration detected was 69 (14–510) pg/g wet mass of *trans*-nonachlor followed by 40 (7.9–250) pg/g wet mass of oxychlordane. With regard to the heptachlors, *cis*-heptachlorepoxide was detected in every sample, but heptachlor and *trans*-heptachlorepoxide were not detected. The detection rates of the drins group were as follows: aldrin, 0.5%; dieldrin, 100%; endrin, 0%. The dieldrin concentration was 18 (5.8–59) pg/g wet mass. The HCB detection rate was 100%, and the concentration of HCB was 100 (35–250) pg/g wet mass. The detection rates obtained for the HCH isomers were as follows: α-HCH, 8.1%; β-HCH, 100%; γ-HCH, 64.5%; δ-HCH, 0%. The detection rate of mirex was 98.9%, and the concentration of mirex was 6.0 (<2–30) pg/g wet mass. Regarding the toxaphene measurements, two chemicals were detected as follows: Parlar-26, 82.8% and Parlat-50, 85.5%. Parlar-40, Parlar-41, Parlar-44 and Parlar-62 were not detected. The concentrations of Parlar 26 and Parlar 50 were 5.2 (<3–19) and 7.6 (<4–27) pg/g wet mass, respectively.

**Table 1**
Characteristics of subjects.

| Characteristics | N (%) | Median (range) | Mean±SD |
|---|---|---|---|
| Age (year) | 186 (100%) | 31 (17–47) | 30.8±4.8 |
| Height (cm) | 186 (100%) | 158 (140–172) | 158.3±5.3 |
| Pre-pregnancy body weight (kg) | 186 (100%) | 52 (38–95) | 53.8±9.4 |
| Pre-pregnancy BMI (kg/m$^2$) | 186 (100%) | 20.6 (16.2–35.8) | 21.4±3.5 |
| Educational level (year) | | | |
| 9–12 | 79 (42.4%) | – | – |
| ≥13 | 107 (57.6%) | – | – |
| Annual household income (million yen) | | | |
| <3 | 32 (17.2%) | – | – |
| 3–5 | 86 (46.2%) | – | – |
| 5–7 | 42 (22.6%) | – | – |
| ≥7 | 26 (14.0%) | – | – |
| Parity (times) | 186 (100%) | 1 (0–5) | 0.7±0.8 |
| 0 | 83 (44.6%) | – | – |
| 1 | 81 (43.5%) | – | – |
| 2 | 16 (8.6%) | – | – |
| 3 | 5 (2.7%) | – | – |
| 4 | 0 (0%) | – | – |
| 5 | 1 (0.5%) | – | – |
| Past conception (times) | 186 (100%) | 1 (0–6) | 1.3±1.3 |
| 0 | 52 (28.0%) | – | – |
| 1 | 65 (34.9%) | – | – |
| 2 | 41 (22.0%) | – | – |
| 3–6 | 28 (15.1%) | – | – |
| History of smoking | | | |
| Yes | 94 (49.5%) | – | – |
| No | 92 (50.5%) | – | – |
| Smoking during pregnancy | | | |
| Yes | 22 (11.8%) | – | – |
| No | 164 (88.2%) | – | – |
| Blood sampling period: | | | |
| 24.1–27.6 weeks of pregnancy | 14 (7.5%) | – | – |
| 30.0–40.7 weeks of pregnancy | 111 (59.7%) | – | – |
| After delivery | 61 (32.8%) | – | – |
| Year in which blood was collected | | | |
| 2002 | 35 (18.8%) | – | – |
| 2003 | 64 (34.4%) | – | – |
| 2004 | 78 (41.9%) | – | – |
| 2005 | 9 (4.8%) | – | – |

Table 3 displays Pearson's correlation coefficient among the log-transformed concentrations of chemicals including '*p,p'*-DDT+*p,p'*-DDE', chlordanes (the sum of *cis*-chlordane, *trans*-chlordane, oxychlordane, *cis*-nonachlor and *trans*-nonachlor), *cis*-hepatachlorepoxide, dieldrin, HCB, β-HCH, mirex and 'Parlar 26+Parlar 50'. All correlations found among the chemicals were statistically significant, and most of the chemicals were well correlated with the others (r=0.49–0.77). HCB was strongly correlated with chlordanes (r=0.77). There were also strong correlations between β-HCH and '*p,p'*-DDT+*p,p'*-DDE' and between dieldrin and *cis*-heptachlorepoxide. On the other hand, the correlations between mirex and *cis*-heptachlorepoxide and between mirex and dieldrin were not strong (r=0.39 and 0.32). The correlations of mirex or 'Parlar 26+Parlar 50' with the other five pesticides are visualized as scatter plots in Fig. 1.

Table 4 presents the concentrations of 18 chemicals from blood collections separated into calendar years. The detection rates of tabulated pesticides were above 50%. The chemical concentrations were compared within three calendar year groups: 2002, 2003 and '2004+2005'. Statistical significances were observed for *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDE, '*p,p'*-DDE+*p,p'*-DDT', *trans*-chlordane, Parlar 26 and Parlar 50, and except for *p,p'*-DDD, each of these chemical's concentrations decreased as the calendar year increased.

Table 5 presents the concentrations of POC pesticides in relation to past conceptions. It appears that most of the chemicals' concentrations declined as the history of past conception(s) increased. In this regard,

**Table 2**
Limits of quantification (LOQ), detection rates (percents of > LOQ) and concentrations of persistent organochlorine pesticides in the whole blood of pregnant women.

| Compounds | LOQ (pg/g of wet) | ≥LOQ | | Concentration (pg/g wet) | |
|---|---|---|---|---|---|
| | | (n) | (%) | Median (range) | GM (95% CI) |
| DDTs | | | | | |
| *o,p'*-DDT | 2 | 163 | 87.6 | 3.7 (<2–13) | 3.4 (3.1–3.7) |
| *p,p'*-DDT | 1 | 186 | 100 | 23 (5.6–120) | 24 (22–26) |
| *o,p'*-DDD | 1 | 1 | 0.5 | <1 (<1–1.1) | 0.50 (0.50–0.51) |
| *p,p'*-DDD | 1 | 124 | 66.7 | 1.3 (<1–9.0) | 1.2 (1.1–1.4) |
| *o,p'*-DDE | 1 | 134 | 72.0 | 1.4 (<1–6.2) | 1.2 (1.1–1.3) |
| *p,p'*-DDE | 2 | 186 | 100 | 610 (120–4600) | 610 (560–670) |
| Chlordanes | | | | | |
| *cis*-Chlordane | 0.8 | 184 | 98.9 | 2.1 (<0.8–18) | 2.1 (2.0–2.3) |
| *trans*-Chlordane | 0.6 | 102 | 54.8 | 0.60 (<0.6–3.8) | 0.60 (0.54–0.66) |
| Oxychlordane | 2 | 186 | 100 | 40 (7.9–250) | 41 (38–44) |
| *cis*-Nonachlor | 0.8 | 186 | 100 | 10 (1.6–38) | 10 (9.5–11) |
| *trans*-Nanachlor | 0.6 | 186 | 100 | 69 (14–510) | 72 (66–77) |
| Heptachlors | | | | | |
| Heptachlor | 2 | 0 | 0 | – | – |
| *cis*-Heptachlorepoxide | 2 | 186 | 100 | 28 (7.1–200) | 28 (26–30) |
| *trans*-Heptachlorepoxide | 0.9 | 0 | 0 | – | – |
| Drins | | | | | |
| Aldrin | 0.7 | 1 | 0.5 | <0.7 (<0.7–13) | 0.36 (0.34–0.37) |
| Dieldrin | 0.9 | 186 | 100 | 18 (5.8–59) | 18 (17–19) |
| Endrin | 2 | 0 | 0 | – | – |
| HCB | 2 | 186 | 100 | 100 (35–250) | 100 (97–110) |
| HCH isomers | | | | | |
| α-HCH | 2 | 15 | 8.1 | <1 (<1–3.9) | 1.1 (1.0–1.1) |
| β-HCH | 2 | 186 | 100 | 150 (20–1200) | 150 (140–170) |
| γ-HCH | 1 | 120 | 64.5 | 1.3 (<1–100) | 1.2 (1.1–1.3) |
| δ-HCH | 2 | 0 | 0 | – | – |
| Mirex | 2 | 184 | 98.9 | 6.0 (<2–30) | 6.2 (5.7–6.7) |
| Toxaphene | | | | | |
| Parlar 26 | 3 | 154 | 82.8 | 5.2 (<3–19) | 4.7 (4.3–5.2) |
| Parlar 40 | 2 | 0 | 0 | – | – |
| Parlar 41 | 3 | 0 | 0 | – | – |
| Parlar 44 | 3 | 0 | 0 | – | – |
| Parlar 50 | 4 | 159 | 85.5 | 7.6 (<4–27) | 7.0 (6.4–7.7) |
| Parlar 62 | 20 | 0 | 0 | – | – |

statistically significant results were obtained for *p,p'*-DDT, *o,p'*-DDE, *cis*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, *cis*-heptachlorepoxide, HCB, β-HCH, mirex, Parlar 26 and Parlar 50.

Table 6 presents the concentrations of POC pesticides in relation to parity. Similar to the correlation observed with regard to past conceptions, the concentrations of most chemicals appeared to decline with increased parity. In this regard, statistically significant results were obtained for measurements of *cis*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, HCB, β-HCH, Parlar 26 and Parlar 50.

Table 7 presents the concentrations of POC pesticides in relation to age. The chemical concentrations detected were compared with regard to three categories (divided into tertiles), and statistically significant age relationships were observed for *p,p'*-DDE, '*p,p'*-DDE + *p,p'*-DDT', *cis*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, *cis*-heptachlorepoxide, HCB, β-HCH, mirex and Parlar 50. It appears that these concentrations increased with maternal age.

Table 8 presents the concentrations of POC pesticides in relation to pre-pregnancy body weight. The chemical concentrations were compared with regard to three categories (divided into tertiles), and statistically significant relationships were observed for *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, dieldrin, β-HCH, Parlar-26 and Parlar-50.

Table 9 presents the concentrations of POC pesticides in relation to pre-pregnancy BMI. The concentrations of dieldrin, Parlar 26 and Parlar 50 significantly increased when BMI increased; however, the concentration of mirex significantly declined with an increase in BMI.

## 4. Discussion

Studies employing whole blood samples may have an advantage in that the influence of dietary lipids is less in whole blood than in serum or plasma. Although it has been found that POC pesticide concentrations are higher in sea-turtle plasma than in whole blood (Keller

**Table 3**
Correlation among log-transformed concentrations of major compounds[a].

| | *p,p'*-DDT + *p,p'*-DDE | Chlordanes | *cis*-Heptachlor epoxide | Dieldrin | HCB | β-HCH | Mirex | Parlar 26 + Parlar 50 |
|---|---|---|---|---|---|---|---|---|
| *p,p'*-DDT + *p,p'*-DDE | 1 | – | – | – | – | – | – | – |
| Chlordanes[b] | 0.61* | 1 | – | – | – | – | – | – |
| *cis*-Heptachlorepoxide | 0.54* | 0.62* | 1 | – | – | – | – | – |
| Dieldrin | 0.47* | 0.53* | 0.73* | 1 | – | – | – | – |
| HCB | 0.67* | 0.77* | 0.64* | 0.56* | 1 | – | – | – |
| β-HCH | 0.72* | 0.69* | 0.56* | 0.49* | 0.77* | 1 | – | – |
| Mirex | 0.54* | 0.72* | 0.39* | 0.32* | 0.61* | 0.50* | 1 | – |
| Parlar 26 + Parlar 50 | 0.54* | 0.63* | 0.56* | 0.63* | 0.68* | 0.53* | 0.57* | 1 |

[a] Log-transformed concentrations of chemicals were tested with Pearson's correlation test after<LOQ was substituted with the value of LOQ/2.
[b] Chlordanes = *cis*-chlordane + *trans*-chlordane + oxychlordane + *cis*-nonachlor + *trans*-nonachlor.
* $P<0.01$.

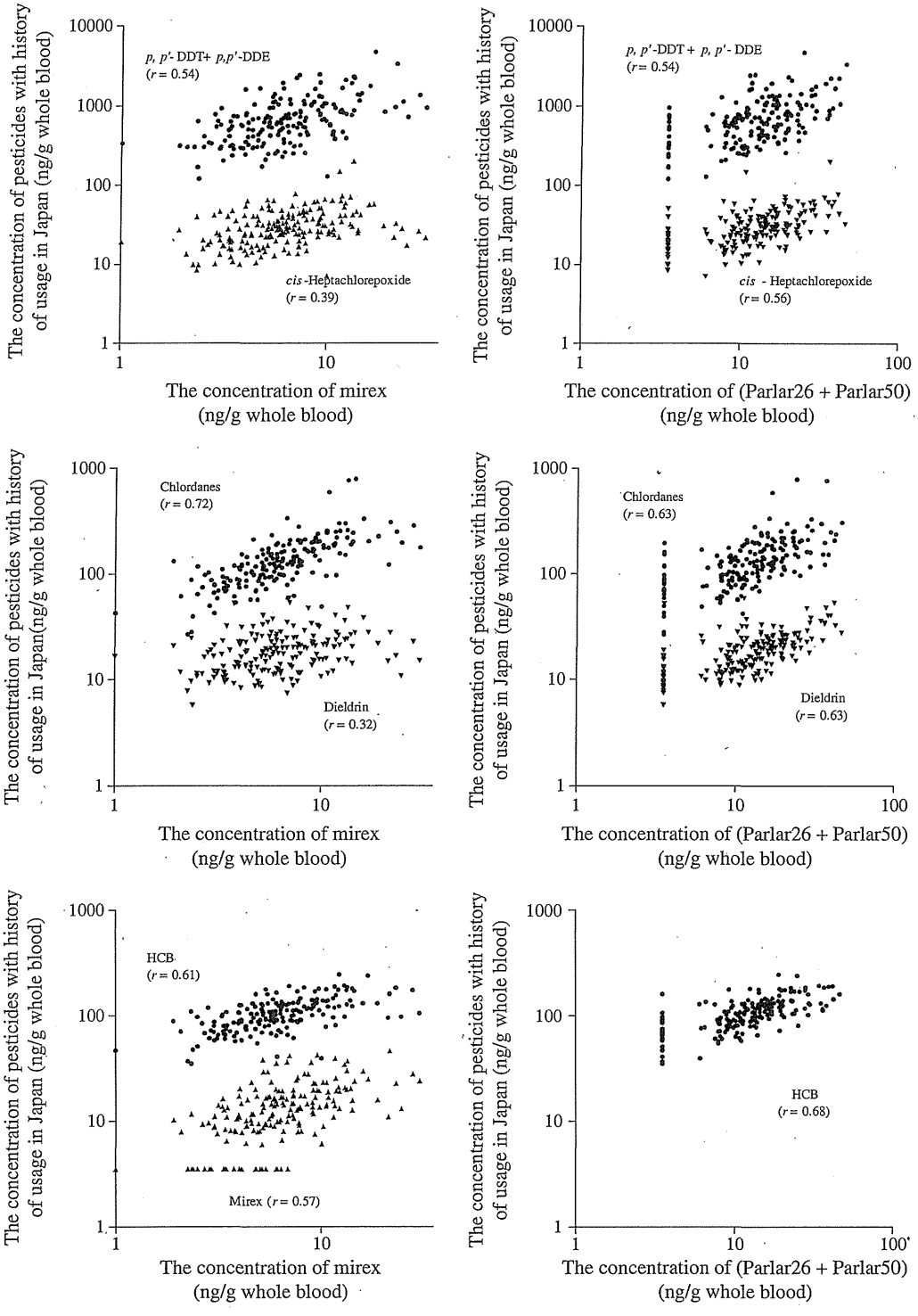


**Fig. 1.** Scatter plots of POC pesticide concentrations. Left row, mirex vs. others; right row, (Parlar 26 + Parlar 50) vs. others.

et al., 2004), published research regarding the presence of POC pesticides in whole blood samples has been limited (Iwasaki et al., 2008; Fukata et al., 2005; Sasaki et al., 1991; Ostrea et al., 2009; Sugiura-Ogasawara et al., 2003). Thus, comparisons of the present study with previous studies are necessarily limited, because most previously published studies involve POC pesticide concentrations detected in serum or plasma. Two previously published Japanese studies (Fukata et al., 2005; Sugiura-Ogasawara et al., 2003), which involved approximately the same time period as that employed in the present study, have reported POC pesticide concentrations that compare favorably to those reported herein. The concentrations of POC pesticides in the present study were similar to those in the previous studies. We have suggested that exposure levels are low in Japan, as indicated by reports of lower POC pesticide contamination levels in milk in Japan than in

**Table 4**
Concentrations of organochlorine pesticides in relation to year in which blood was collected (pg/g wet mass).

| Compounds | 2002 (n=35) | | 2003 (n=64) | | 2004–2005 (n=87) | | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | |
| DDTs | | | | | | | |
| *o,p'*-DDT | 4.0 (<2.0–13) | 4.1 (3.3–5.0) | 3.7 (<2.0–9.7) | 3.4 (2.9–3.9) | 3.4 (<2.0–11) | 3.2 (2.8–3.6) | 0.06 |
| *p,p'*-DDT | 25 (11–120) | 27 (22–33) | 22 (10–100) | 25 (21–28) | 22 (5.6–73) | 23 (20–25) | 0.32 |
| *p,p'*-DDD | <1.0 (<1.0–4.3) | 0.85 (0.67–1.1) | 1.3 (<1.0–6.1) | 1.1 (0.93–1.3) | 1.7 (<1.0–9.0) | 1.6 (1.3–1.8) | <0.01 |
| *o,p'*-DDE | 1.7 (<1.0–6.2) | 1.6 (1.3–2.1) | 1.3 (<1.0–4.6) | 1.2 (1.0–1.4) | 1.3 (<1.0–4.4) | 1.1 (1.0–1.3) | <0.02 |
| *p,p'*-DDE | 680 (250–4600) | 730 (570–940) | 660 (160–2400) | 640 (550–750) | 540 (120–2400) | 550 (480–620) | 0.04 |
| *p,p'*-DDE + *p,p'*-DDT | 690 (270–4700) | 760 (590–970) | 690 (170–2400) | 670 (580–780) | 560 (120–2500) | 570 (500–650) | 0.045 |
| Chlordanes | | | | | | | |
| *cis*-Chlordane | 1.8 (1.2–18) | 2.2 (1.8–2.8) | 2.1 (0.83–5.8) | 2.1 (1.8–2.3) | 2.1 (<0.80–17) | 2.2 (1.9–2.4) | 0.57 |
| *trans*-Chlordane | <0.60 (<0.60–1.7) | 0.3 (0.3–0.4) | 0.63 (<0.60–3.8) | 0.59 (0.51–0.69) | 0.8 (<0.60–2.9) | 0.74 (0.64–0.86) | <0.01 |
| Oxychlordane | 37 (18–200) | 41 (34–50) | 40 (11–110) | 40 (36–46) | 43 (7.9–250) | 40 (36–45) | 0.46 |
| *cis*-Nonachlor | 8.9 (4.7–38) | 11 (8.8–13) | 10 (3.2–26) | 9.9 (8.7–11) | 11 (1.6–34) | 10 (9.2–12) | 0.43 |
| *trans*-Nonachlor | 60 (37–510) | 73 (59–91) | 67 (25–170) | 69 (61–77) | 80 (14–490) | 73 (65–83) | 0.21 |
| *cis*-Heptachlorepoxide | 29 (11–200) | 31 (26–37) | 28 (9.9–78) | 28 (24–31) | 28 (7.1–150) | 28 (25–31) | 0.46 |
| Dieldrin | 22 (9.5–53) | 21 (18–24) | 17 (8.9–54) | 17 (15–19) | 17 (5.8–59) | 17 (16–19) | 0.08 |
| HCB | 100 (61–240) | 100 (94–120) | 110 (58–190) | 110 (98–120) | 100 (35–250) | 98 (90–110) | 0.47 |
| HCHs | | | | | | | |
| β-HCH | 154 (58–770) | 170 (140–200) | 160 (32–640) | 170 (140–190) | 140 (20–1200) | 140 (120–160) | 0.13 |
| γ-HCH | 1.0 (<1.0–6.7) | 0.97 (0.74–1.3) | 1.3 (<1.0–100) | 1.3 (0.99–1.6) | 1.3 (<1.0–6.6) | 1.2 (1.1–1.4) | 0.12 |
| Mirex | 6.0 (2.3–30) | 6.4 (5.2–7.9) | 5.6 (2.5–24) | 6.1 (5.4–6.9) | 6.2 (<2.0–28) | 6.2 (5.5–7.0) | 0.70 |
| Parlar 26 | 5.6 (<3.0–19) | 5.9 (4.7–7.3) | 5.1 (<3.0–18) | 4.8 (4.0–5.7) | 5.0 (<3.0–13) | 4.3 (3.8–5.0) | 0.046 |
| Parlar 50 | 8.8. (<4.0–27) | 8.9 (7.2–11) | 7.8 (<4.0–24) | 7.4 (6.2–8.8) | 6.4 (<4.0–23) | 6.1 (5.3–7.0) | <0.01 |

[a] *P* values resulted from two-tailed Jonckheere–Terpstra trend test.

China, Hong Kong, and Vietnam (Haraguchi et al., 2009; Solomon and Weiss, 2002).

Compared to the results of this study, high body-burden levels of POC pesticides were reported in the 1960s and 1970s. In a 1960s survey of 339 pregnant women in the USA, *p,p'*-DDT and *p,p'*-DDE were detected at levels of 15.0±8.8 and 53.9±35.3 ppb (pg/g serum), respectively (James et al., 2002). In a 1979 survey, the mean serum DDT concentration of 499 samples (men and women) detected (159.4 ng/ml) was 10 times the US geometric mean (15 ng/ml serum) (Kress et al., 1981). In the 1970s in Japan, 7.5±7.0 ppb of *p,p'*-DDT and 15.7±9.3 ppb of *p,p'*-DDE were detected in plasma samples from 86 women (Yamaguchi et al., 1975). After POC pesticides were banned, the body burdens of these chemicals remained at peak concentrations throughout the 1960s and 1970s and then decreased over several decades (Craan and Haines, 1998; Dallaire et al., 2002; Lackmann, 2005; Konishi et al., 2001; Solomon and Weiss, 2002). In contrast, the concentrations of related metabolites, such as DDE and oxychlordane, increased over this time period, resulting in higher bodily concentrations of metabolites versus their precursors. As presented in Table 2, the concentrations of metabolites, such as *p,p'*-DDE, oxychlordane and *cis*-heptachlorepoxide, determined in this study were higher than their precursors, such as *p,p'*-DDT, *cis*-chlordane, *trans*-chlordane, *cis*-heptachlor and *trans*-heptachlor. The ratios of DDE to DDT obtained in this and other recent studies (Fukata et al., 2005; Wittsiepe et al., 2008) are higher than those reported in the 1960s and 1970s (James et al., 2002; Yamaguchi et al., 1975).

Lackmann (2005) reported a decline in neonatal serum *p,p'*-DDE concentrations in Germany, obtaining a highest mean value of 1.49 ng/ml in 1984–1985 and a lowest mean value of 0.18 ng/ml in 2002. According to Dallaire et al. (2002), the mean cord blood concentrations of chlordanes, DDTs and HCB considerably declined in Canada from 1993 to 2000. In their analysis of 392 blood samples collected from 1993 to 2007 in Sweden, Hardell et al. (2010) observed approximately 10% reductions by year in the concentrations of PCBs, HCB, DDE and chlordanes. As presented in Table 4, most of the chemicals analyzed in the present study were also found to decrease from year to year, consistent with reports of their decreasing concentrations in milk in recent decades. Konishi et al. (2001) reported that the concentrations of contaminants in milk decreased from 1972 to 1998 in Osaka city, Japan. For instance, the concentrations of β-HCH and DDT declined to less than 3% of their peak levels by the mid-1970s. In Sweden, after peaking in the mid 1970s, the concentrations of dieldrin, β-HCH and DDT in human milk dramatically decreased throughout the 1980s and 1990s (Solomon and Weiss, 2002). In Canada, after peaking in the 1960s and 1970s, the concentrations of most POC contaminants in milk decreased, and the concentration of β-HCH decreased after peaking in the 1980s (Craan and Haines, 1998). In Northern Germany, the concentrations of DDT, HCB and β-HCH in milk declined by 10–20% over the decade from 1986 to 1997 (Schade and Heinzow, 1998). Thus, more recent reductions in the body burdens of POC pesticides appear to be a global trend.

Based on the samples collected from 1979 elderly Canadian in 2003, Medehouenou et al. (2011) reported that the lipid-adjusted POC concentrations of *p,p'*-DDTs, Chlordanes, β-HCH and HCB strongly correlated with one another. The present study, which used samples collected from 2002 to 2005, also revealed strong correlations among the concentrations of eight POC pesticides (Table 3). The correlations between *p,p'*-DDTs and chlordanes and between β-HCH and HCB found within our study appear to be stronger than those reported in the Canadian study, of which sampling area involved the 36 urban and surrounding areas. The narrow sampling area used in our study, which was conducted using a single hospital-based population may have contributed to the higher correlation coefficients obtained.

Notably, mirex, Parlar-26 and Parlar-50, which have never been used in Japan, were detected in most samples (>LOQ%, 82.8% and 85.5%), and the log-transformed concentrations of these compounds were positively correlated with other pesticides used in the past ($p<0.01$). According to a public announcement of the Ministry of the Environment, mirex, Parlar-26 and Parlar-50 were not found in environmental samples in the 1983 survey; however, these chemicals were detected in the atmosphere, solid surface sediments, shellfish, fish and seabirds in 2003 (Ministry of the Environment, 2005, 2006). It is possible that, in the past, mirex pollution may have resulted from imported products containing mirex as a flame retardant. The Japanese environment may also be influenced by the usage of pesticides in neighboring countries. For example, China produced toxaphene from 1964 to 1980, though this chemical was banned for all purposes in 1987 (Wong et al., 2005). Given that persistent organic pollutants are known

**Table 5**
Concentrations of organochlorine pesticides in relation to past conceptions (pg/g wet mass).

| Compounds | None (n = 52) | | 1 time (n = 65) | | 2 times (n = 41) | | 3–6 times (n = 28) | | P |
|---|---|---|---|---|---|---|---|---|---|
| | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | Med (range) | GM (95%CI) | value[a] |
| DDTs | | | | | | | | | |
| *o,p'*-DDT | 3.6 (<2.0–13) | 3.5 (2.9–4.1) | 3.9 (<2.0–9.1) | 3.7 (3.3–4.3) | 3.5 (<2.0–11) | 3.2 (2.6–3.9) | 3.1 (<2.0–6.8) | 2.8 (2.2–3.5) | 0.15 |
| *p,p'*-DDT | 28 (<9.9–120) | 27 (23–31) | 25 (8.1–73) | 26 (23–29) | 20 (6.8–66) | 21 (18–25) | 22 (5.6–100) | 21 (16–26) | 0.03 |
| *p,p'*-DDD | 1.3 (<1.0–9.0) | 1.4 (1.1–1.7) | 1.3 (<1.0–6.1) | 1.2 (1.0–1.5) | 1.4 (<1.0–4.9) | 1.2 (0.94–1.5) | 1.3 (<1.0–5.5) | 1.1 (0.84–1.5) | 0.31 |
| *o,p'*-DDE | 1.4 (<1.0–6.2) | 1.4 (1.1–1.6) | 1.4 (<1.0–4.6) | 1.3 (1.1–1.5) | 1.3 (<1.0–3.4) | 1.1 (0.91–1.3) | 1.1 (<1.0–2.2) | 1.0 (0.82–1.2) | 0.02 |
| *p,p'*-DDE | 690 (160–4600) | 680 (560–810) | 670 (120–2400) | 650 (550–760) | 540 (220–1900) | 550 (450–660) | 550 (120–2100) | 520 (400–670) | 0.07 |
| *p,p'*-DDE + *p,p'*-DDT | 710 (170–4700) | 710 (590–850) | 690 (130–2400) | 670 (580–790) | 560 (230–1900) | 570 (470–690) | 570 (120–2200) | 540 (420–700) | 0.07 |
| Chlordanes | | | | | | | | | |
| *cis*-Chlordane | 2.4 (<0.80–18) | 2.4 (2.1–2.9) | 2.1 (0.84–17) | 2.2 (1.9–2.5) | 2.0 (0.83–5.8) | 2.0 (1.7–2.3) | 1.8 (0.8–3.9) | 1.7 (1.5–2.1) | <0.01 |
| *trans*-Chlordane | 0.61 (<0.60–2.5) | 0.56 (0.46–0.68) | 0.65 (<0.60–2.9) | 0.61 (0.51–0.72) | 0.66 (<0.60–3.8) | 0.63 (0.50–0.78) | 0.63 (<0.60–2.0) | 0.60 (0.45–0.74) | 0.66 |
| Oxychlordane | 45 (19–200) | 48 (42–55) | 43 (7.9–250) | 42 (37–49) | 38 (9.4–94) | 35 (30–41) | 34 (14–88) | 34 (28–40) | <0.01 |
| *cis*-Nonachlor | 12 (5.3–38) | 12 (11–14) | 10 (2.0–34) | 11 (9.3–12) | 9.3 (1.6–26) | 8.8 (7.4–10) | 9.4 (2.4–28) | 8.5 (6.7–11) | <0.01 |
| *trans*-Nonachlor | 79 (33–510) | 84 (72–98) | 71 (17–490) | 73 (64–84) | 60 (14–170) | 63 (53–74) | 58 (21–220) | 61 (50–75) | <0.01 |
| *cis*-Heptachlorepoxide | 30 (11–200) | 31 (27–36) | 31 (7.1–64) | 29 (25–32) | 24 (9.9–150) | 26 (22–31) | 24 (9.8–78) | 25 (20–31) | 0.02 |
| Dieldrin | 20 (8.8–53) | 19 (17–21) | 17 (8.4–47) | 17 (16–19) | 16 (7.5–59) | 17 (15–19) | 19 (5.8–54) | 17 (14–21) | 0.21 |
| HCB | 120 (65–240) | 110 (110–120) | 100 (35–250) | 110 (96–110) | 90 (37–190) | 90 (79–100) | 91 (47–190) | 91 (80–110) | <0.01 |
| HCHs | | | | | | | | | |
| β-HCH | 170 (38–770) | 170 (150–200) | 150 (20–640) | 150 (130–180) | 120 (32–1200) | 130 (100–160) | 140 (34–600) | 140 (110–190) | 0.049 |
| γ-HCH | 1.3 (<1.0–7.2) | 1.2 (0.96–1.5) | 1.3 (<1.0–100) | 1.3 (1.0–1.6) | 1.3 (<1.0–4.8) | 1.3 (1.0–1.6) | 0.78 (<1.0–3.0) | 0.89 (0.70–1.1) | 0.40 |
| Mirex | 6.5 (2.3–30) | 6.9 (5.9–8.1) | 6.5 (2.1–28) | 6.6 (5.8–7.5) | 5.6 (<2.0–23) | 5.3 (4.5–6.3) | 5.1 (1.9–21) | 5.5 (4.4–6.9) | 0.02 |
| Parlar 26 | 6.0 (<3.0–19) | 5.9 (5.0–7.0) | 5.2 (<3.0–17) | 5.0 (4.3–5.8) | 4.1 (<3.0–19) | 3.9 (3.1–4.8) | 4.0 (<3.0–18) | 3.8 (2.9–5.0) | <0.01 |
| Parlar 50 | 9.3 (<4.0–27) | 8.6 (7.2–10) | 7.9 (<4.0–22) | 7.5 (6.5–8.7) | 6.2 (<4.0–23) | 5.8 (4.7–7.2) | 6.0 (<4.0–24) | 5.3 (4.0–7.1) | <0.01 |

[a] *P* values resulted from two-tailed Jonckheere–Terpstra trend test.

to diffuse a great-distance from their source(s), *trans*-border environmental pollution is certainly possible.

Associations between the POC pesticide concentrations found in blood samples and the corresponding subjects' characteristics are described below.

Hardell et al. (2010) reported that there was the association between parity and the PCB blood concentrations, although there were not significant associations between parity and the concentrations of HCB, DDE and chlordanes. Cao et al. (2011) reported an association between parity and the serum concentrations of HCB, β-HCH and *p,p'*-DDE in 1483 samples collected from 2008 to 2009 in Shanghai. In the present study, the concentrations of most chemicals decreased with past conceptions and/or with parity and exhibited a stronger association with past conceptions than with parity.

The concentrations of most chemicals increased with the age of the subject (Table 7), consistent with a longer exposure period for older subject groups. Environmental pollutant levels and exposure levels should also be higher for subjects born during time periods that enabled greater exposure(s). In addition, associations between chemical concentrations appear more strongly associated with pre-pregnancy body weight(s) than with BMI (Tables 6 and 7). Pre-pregnancy body weight may be better than BMI, as a surrogate for a body pool size of lipophilic pollutants. There were no significant trends in the concentrations of most chemicals in relation to subjects' heights; however, there was a statistically significant effect of height on the concentration of *trans*-chlordane alone (determined by the Jonckheere–Terpstra trend test, data not shown). No significant associations were found between most of the measured chemical concentrations and the subjects' educational levels (9–12 years, ≥13 years), smoking during pregnancy or annual household incomes (<5 million yen and ≥5 million yen; determined by Mann–Whitney *U* test, data not shown). However, a significant association was found between detected mirex concentrations and smoking histories (determined by Mann–Whitney *U* test, data not shown; $p<0.05$ determined by Jonckheere–Terpstra trend test, data not shown). Samples grouped according to their collections in the 2nd trimester, 3 rd trimester and after delivery revealed a significant association among the concentrations of *p,p'*-DDD, *trans*-chlordane and mirex and the natal stage during which blood was collected (determined by Jonckheere–Terpstra trend test, data not shown).

Cao et al. (2011) have reported a positive association between age, pre-pregnancy weight and BMI with serum concentrations of *p,p'*-DDE and β-HCH. Their findings regarding the association of age and the concentrations of *p,p'*-DDE and β-HCH are consistent with our results. In addition, based on a study that age-matched 1055 healthy controls to breast cancer subjects in France, Bachelet et al. (2011) have reported a positive association between age and detected *p,p'*-DDE concentrations. Carrizo et al. (2006) have reported a positive correlation between POC contamination levels in cord blood and maternal age, indicating that fetal exposure levels are higher in older mothers than in younger mothers. Similarly, Wittsiepe et al. (2008) have shown that HCB levels increase significantly with age. In their survey of 53 men, Hue et al. (2007) have demonstrated a positive correlation between age and total POC concentrations in plasma (including PCB and several pesticides) and determined that there is no association between BMI an total POC concentrations in plasma. Schildkraut et al. (1999) have demonstrated a positive correlation between BMI and serum DDE concentration, and Modehouenou et al. (2011) have reported that age (65 to 85 over) and BMI are positively associated with β-HCH, HCB, *trans*-nonachlor and *p,p'*-DDE concentrations.

The present study involves persistent POC pesticide measurements in whole-blood samples from pregnant women in Hokkaido. We have detected 21 chemicals, including three chemicals with no history of use in Japan that may have been distributed through long-distance transport mechanisms. This study suggests that contamination levels of POC pesticides are low compared to previous studies and that exposure levels are decreasing with each passing year. We have evaluated

**Table 6**
Concentrations of organochlorine pesticides in relation to parity (pg/g wet mass).

| Compounds | None (n=83) | | 1 time (n=81) | | 2–5 times (n=22) | | P value[a] |
|---|---|---|---|---|---|---|---|
| | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | |
| DDTs | | | | | | | |
| *o,p'*-DDT | 3.6 (<2.0–13) | 3.5 (3.1–4.0) | 3.8 (<2.0–11) | 3.3 (2.9–3.9) | 3.5 (<2.0–7.3) | 3.1 (2.4–3.9) | 0.66 |
| *p,p'*-DDT | 25 (6.8–120) | 25 (23–28) | 22 (5.6–70) | 23 (21–26) | 20 (7.1–100) | 22 (17–29) | 0.27 |
| *p,p'*-DDD | 1.3 (<1.0–9.0) | 1.3 (1.1–1.5) | 1.5 (<1.0–6.1) | 1.3 (1.1–1.5) | <1.0 (<1.0–5.5) | 1.1 (0.71–1.6) | 0.65 |
| *o,p'*-DDE | 1.4 (<1.0–6.2) | 1.4 (1.2–1.6) | 1.3 (<1.0–4.6) | 1.1 (1.0–1.3) | 1.2 (<1.0–2.2) | 1.0 (0.78–1.3) | 0.07 |
| *p,p'*-DDE | 670 (160–4600) | 670 (580–760) | 630 (120–2400) | 590 (510–680) | 530 (190–2100) | 490 (370–660) | 0.07 |
| *p,p'*-DDE + *p,p'*-DDT | 700 (170–4700) | 690 (610–790) | 640 (120–2400) | 620 (530–710) | 560 (200–2200) | 520 (390–690) | 0.07 |
| Chlordanes | | | | | | | |
| *cis*-Chlordane | 2.4 (<0.80–18) | 2.4 (2.1–2.7) | 2.0 (0.80–5.9) | 2.0 (1.8–2.3) | 1.7 (0.83–4.7) | 1.8 (1.5–2.2) | 0.01 |
| *trans*-Chlordane | 0.61 (<0.60–2.5) | 0.56 (0.48–0.64) | 0.67 (<0.60–3.8) | 0.66 (0.57–0.78) | <0.60 (<0.60–2.0) | 0.51 (0.38–0.68) | 0.60 |
| Oxychlordane | 46 (19–250) | 48 (43–54) | 39 (7.9–110) | 37 (33–41) | 28 (9.4–88) | 29 (22–38) | <0.01 |
| *cis*-Nonachlor | 12 (3.7–38) | 12 (11–13) | 9.4 (2.0–28) | 9.5 (8.4–11) | 8.0 (1.6–28) | 8.0 (6.0–11) | <0.01 |
| *trans*-Nonachlor | 83 (33–510) | 83 (74–94) | 66 (17–170) | 66 (59–73) | 52 (14–220) | 57 (42–76) | <0.01 |
| *cis*-Heptachlorepoxide | 30 (9.9–200) | 30 (27–33) | 28 (7.1–150) | 28 (25–32) | 22 (9.9–78) | 24 (18–31) | 0.07 |
| Dieldrin | 20 (8.6–59) | 19 (17–20) | 17 (5.8–47) | 17 (16–19) | 15 (7.8–54) | 16 (13–20) | 0.19 |
| HCB | 120 (63–250) | 110 (110–120) | 97 (35–190) | 98 (91–110) | 74 (37–190) | 78 (65–94) | <0.01 |
| HCHs | | | | | | | |
| β-HCH | 170 (38–770) | 180 (160–200) | 150 (20–600) | 140 (120–160) | 110 (32–1200) | 120 (81–180) | <0.01 |
| γ-HCH | 1.3 (<1.0–7.2) | 1.2 (1.0–1.4) | 1.2 (<1.0–100) | 1.3 (1.0–1.5) | 1.1 (<1.0–3.2) | 0.98 (0.75–1.3) | 0.60 |
| Mirex | 6.5 (1.9–30) | 6.7 (5.9–7.5) | 6.0 (<2.0–28) | 6.0 (5.3–6.7) | 5.2 (2.3–21) | 5.4 (4.2–6.9) | 0.08 |
| Parlar 26 | 5.7 (<3.0–19) | 5.5 (4.8–6.3) | 4.4 (<3.0–19) | 4.4 (3.8–5.1) | 4.0 (<3.0–18) | 3.6 (2.7–4.9) | <0.01 |
| Parlar 50 | 8.6 (<4.0–27) | 8.1 (7.1–9.2) | 6.6 (<4.0–23) | 6.6 (5.6–7.6) | 6.0 (<4.0–24) | 5.2 (3.9–7.1) | <0.01 |

[a] *P* values resulted from two-tailed Jonckheere–Terpstra trend test.

**Table 7**
Concentrations of organochlorine pesticides in relation to age (pg/g wet mass).

| Compounds | T1: ≤29 years (n=77) | | T2: 30–33 years (n=55) | | T3: ≥34 years (n=54) | | P value[a] |
|---|---|---|---|---|---|---|---|
| | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | Med (range) | GM (95% CI) | |
| DDTs | | | | | | | |
| *o,p'*-DDT | 3.4 (<2.0–11) | 3.2 (2.8–3.7) | 3.8 (<2.0–13) | 3.6 (3.1–4.2) | 3.9 (<2.0–9.1) | 3.4 (2.8–4.0) | 0.32 |
| *p,p'*-DDT | 21 (5.6–120) | 22 (19–25) | 26 (9.9–73) | 26 (23–30) | 27 (6.8–100) | 25 (21–30) | 0.06 |
| *p,p'*-DDD | 1.2 (<1.0–7.2) | 1.1 (0.90–1.3) | 1.7 (<1.0–6.1) | 1.4 (1.2–1.7) | 1.3 (<1.0–9.0) | 1.3 (1.1–1.6) | 0.09 |
| *o,p'*-DDE | 1.3 (<1.0–5.7) | 1.1 (0.98–1.3) | 1.4 (<1.0–6.2) | 1.4 (1.2–1.6) | 1.4 (<1.0–4.6) | 1.2 (1.0–1.5) | 0.22 |
| *p,p'*-DDE | 510 (120–4600) | 500 (430–570) | 720 (190–2400) | 710 (610–820) | 760 (120–2400) | 700 (580–860) | <0.01 |
| *p,p'*-DDE + *p,p'*-DDT | 540 (120–4700) | 520 (450–600) | 740 (200–2400) | 740 (640–860) | 800 (130–2500) | 730 (600–890) | <0.01 |
| Chlordanes | | | | | | | |
| *cis*-Chlordane | 1.9 (0.84–18) | 2.0 (1.7–2.2) | 2.1 (<0.80–17) | 2.2 (1.9–2.6) | 2.5 (0.83–8.0) | 2.4 (2.0–2.8) | 0.02 |
| *trans*-Chlordane | 0.66 (<0.60–3.8) | 0.62 (0.53–0.72) | 0.65 (<0.60–2.5) | 0.64 (0.53–0.76) | <0.60 (<0.60–2.9) | 0.52 (0.43–0.63) | 0.18 |
| Oxychlordane | 37 (7.9–200) | 35 (31–40) | 44 (16–250) | 44 (38–50) | 49 (9.4–170) | 46 (40–53) | <0.01 |
| *cis*-Nonachlor | 9.1 (2.0–38) | 9.0 (7.9–10) | 10 (4.5–34) | 11 (9.7–13) | 11 (1.6–33) | 11 (9.8–13) | <0.01 |
| *trans*-Nonachlor | 60 (17–510) | 63 (55–71) | 69 (32–490) | 76 (66–87) | 87 (14–380) | 82 (71–95) | <0.01 |
| *cis*-Heptachlorepoxide | 26 (8.4–200) | 25 (22–28) | 30 (12–150) | 31 (27–35) | 28 (7.1–78) | 30 (26–35) | 0.02 |
| Dieldrin | 17 (5.8–59) | 17 (15–19) | 20 (8.8–39) | 19 (17–20) | 17 (7.8–54) | 18 (16–20) | 0.38 |
| HCB | 97 (35–250) | 96 (88–100) | 110 (55–180) | 110 (100–120) | 110 (37–190) | 100 (93–120) | 0.045 |
| HCHs | | | | | | | |
| β-HCH | 120 (20–770) | 110 (97–130) | 170 (47–400) | 170 (150–190) | 210 (30–1200) | 200 (170–250) | <0.01 |
| γ-HCH | 1.2 (<1.0–7.2) | 1.1 (0.92–1.3) | 1.3 (<1.0–100) | 1.4 (1.1–1.8) | 1.2 (<1.0–6.6) | 1.2 (0.94–1.4) | 0.59 |
| Mirex | 4.8 (<2.0–22) | 4.8 (4.2–5.4) | 6.5 (3.1–16) | 6.8 (6.1–7.5) | 8.6 (2.3–30) | 8.2 (7.0–9.6) | <0.01 |
| Parlar 26 | 4.4 (<3.0–19) | 4.2 (3.6–4.9) | 6.0 (<3.0–13) | 5.4 (4.7–6.3) | 5.3 (<3.0–19) | 5.0 (4.1–6.0) | 0.06 |
| Parlar 50 | 6.4 (<4.0–27) | 6.0 (5.1–7.1) | 8.5 (<4.0–23) | 8.2 (7.2–9.4) | 7.9 (<4–24) | 7.4 (6.1–8.9) | 0.048 |

[a] *P* values resulted from two-tailed Jonckheere–Terpstra trend test.

the associations between the subjects' characteristics and 18 compounds, a larger number than determined in similar, previously published studies. We have determined that the body burdens of these chemicals are associated with subjects' characteristics, such as age, pre-pregnancy body weight(s) and history/ies of past conception(s). Further studies are needed to evaluate the effects of these chemicals on the development of children, and further monitoring of human blood as well as foods and environments should be conducted.

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## Ethics

This study was conducted with all the subjects' written informed consent and was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

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