Table 8
Concentrations of organochlorine pesticides in relation to pre-pregnant body weight (pg/g wet mass).

Compounds	T1: ≤49 kg (n=66)		T2: 50–55 kg (n=64)		T3: ≥56 kg (n=56)		P value[a]
	Med (range)	GM (95% CI)	Med (range)	GM (95% CI)	Med (range)	GM (95% CI)	
DDTs							
o,p'-DDT	3.2 (<2.0–11)	3.2 (2.8–3.6)	3.7 (<2.0–13)	3.2 (2.8–3.8)	4.1 (<2–11)	3.8 (3.3–4.5)	0.03
p,p'-DDT	22 (6.8–120)	23 (20–26)	22 (5.6–70)	23 (20–26)	27 (8.1–105)	28 (24–32)	0.04
p,p'-DDD	1.4 (<1.0–7.2)	1.2 (1.0–1.5)	1.2 (<1.0–9.0)	1.1 (0.91–1.4)	1.5 (<1.0–5.5)	1.4 (1.2–1.7)	0.35
o,p'-DDE	1.4 (<1.0–5.7)	1.1 (0.95–1.3)	1.3 (<1.0–6.2)	1.1 (0.97–1.3)	1.6 (<1.0–4.6)	1.5 (1.3–1.7)	0.03
p,p'-DDE	550 (120–4600)	560 (480–650)	610 (120–2400)	620 (520–730)	690 (160–2200)	670 (570–800)	0.09
p,p'-DDE + *p,p'*-DDT	580 (130–4700)	580 (500–670)	630 (120–2500)	640 (540–760)	710 (170–2200)	700 (600–830)	0.08
Chlordanes							
cis-Chlordane	2.1 (<0.8–17)	2.2 (1.9–2.5)	2.1 (0.88–6.4)	2.1 (1.9–2.4)	2.0 (0.83–18)	2.1 (1.8–2.4)	0.57
trans-Chlordane	0.66 (<0.6–2.9)	0.64 (0.54–0.76)	0.65 (<0.60–3.8)	0.63 (0.53–0.74)	<0.60 (<0.60–2.1)	0.51 (0.43–0.61)	0.07
Oxychlordane	42 (9.4–250)	41 (36–48)	43 (14–110)	42 (37–47)	38 (7.9–200)	38 (33–43)	0.44
cis-Nonachlor	10 (1.6–34)	10 (8.7–12)	11 (2.4–28)	10 (8.8–12)	11 (2.0–38)	11 (9.3–12)	0.47
trans-Nonachlor	68 (14–490)	73 (63–85)	73 (21–200)	73 (64–83)	68 (17–510)	69 (59–79)	0.64
cis-Heptachlorepoxide	26 (7.1–150)	27 (24–30)	28 (9.8–73)	27 (24–30)	32 (8.4–200)	32 (27–37)	0.09
Dieldrin	16 (7.5–52)	16 (14–18)	18 (5.8–59)	17 (15–19)	21 (9.7–54)	21 (19–23)	<0.01
HCB	96 (37–250)	99 (90–110)	100 (47–190)	100 (95–110)	110 (35–190)	100 (95–110)	0.25
HCHs							
β-HCH	140 (32–770)	130 (110–160)	150 (34–530)	150 (130–180)	170 (20–1200)	170 (140–210)	0.046
γ-HCH	1.4 (<1.0–7.2)	1.3 (1.1–1.5)	1.2 (<1.0–6.7)	1.2 (0.96–1.4)	1.1 (<1.0–100)	1.1 (0.88–1.4)	0.15
Mirex	6.8 (2.3–24)	6.8 (6.0–7.8)	5.9 (<2.0–30)	6.0 (5.2–7.0)	5.7 (1.9–21)	5.7 (5.0–6.5)	0.06
Parlar 26	4.3 (<3.0–19)	4.1 (3.5–4.9)	5.3 (<3.0–14)	4.4 (3.7–5.1)	5.7 (<3.0–19)	6.2 (5.3–7.2)	<0.01
Parlar 50	6.4 (<4.0–27)	6.1 (5.2–7.2)	8.3 (<4.0–21)	6.6 (5.6–7.9)	8.4 (<4.0–24)	8.7 (7.5–10)	<0.01

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

Table 9
Concentrations of organochlorine pesticides in relation to pre-pregnant BMI (pg/g wet mass).

Compounds	T1: <19.9 (n=62)		T2: 19.9–21.5 (n=62)		T3: >21.5 (n=62)		P value[a]
	Med (range)	GM (95% CI)	Med (range)	GM (95% CI)	Med (range)	GM (95% CI)	
DDTs							
o,p'-DDT	3.2 (<2.0–13)	3.3 (2.8–3.8)	3.7 (<2.0–9.1)	3.2 (2.8–3.8)	4.0 (<2.0–11)	3.6 (3.1–4.2)	0.16
p,p'-DDT	23 (6.8–120)	24 (20–27)	22 (5.6–63)	22 (20–25)	26 (8.1–100)	26 (23–30)	0.26
p,p'-DDD	1.4 (<1.0–7.2)	1.2 (1.0–1.5)	1.3 (<1.0–9.0)	1.2 (0.96–1.4)	1.4 (<1.0–6.1)	1.3 (1.1–1.6)	0.59
o,p'-DDE	1.3 (<1.0–6.2)	1.1 (0.9–1.3)	1.4 (<1.0–4.4)	1.2 (1.0–1.4)	1.5 (<1.0–4.6)	1.4 (1.2–1.6)	0.09
p,p'-DDE	550 (120–4600)	600 (500–710)	620 (120–2400)	590 (510–700)	680 (160–2400)	640 (540–760)	0.44
p,p'-DDE + *p,p'*-DDT	580 (130–4700)	620 (530–740)	640 (120–2500)	620 (530–720)	710 (170–2400)	670 (570–790)	0.41
Chlordanes							
cis-Chlordane	2.1 (0.8–17)	2.2 (1.9–2.6)	2.3 (<0.80–5.9)	2.1 (1.8–2.4)	2.0 (0.83–18)	2.1 (1.8–2.4)	0.48
trans-Chlordane	0.65(<0.60–2.5)	0.59 (0.50–0.70)	0.66 (<0.60–3.8)	0.65 (0.55–0.78)	0.41 (<0.60–2.1)	0.55 (0.46–0.65)	0.52
Oxychlordane	45 (12–250)	45 (39–52)	41 (9.4–94)	39 (34–45)	38 (7.9–200)	38 (33–43)	0.13
cis-Nonachlor	11 (3.2–34)	11 (9.4–13)	10 (1.6–28)	9.3 (8.1–11)	10 (2.0–38)	11 (9.3–12)	0.88
trans-Nonachlor	75 (27–490)	79 (68–91)	70 (14–170)	68 (60–77)	65 (17–510)	69 (60–79)	0.24
cis-Heptachlorepoxide	30 (7.1–150)	30 (26–34)	25 (9.8–71)	25 (22–28)	31 (8.4–200)	30 (26–35)	0.94
Dieldrin	17 (7.5–53)	17 (15–19)	16 (5.8–59)	16 (14–18)	21 (9.7–54)	21 (18–23)	0.02
HCB	110 (39–250)	100 (94–110)	100 (37–190)	98 (89–110)	110 (35–190)	100 (96–110)	0.84
HCHs							
β-HCH	140 (32–770)	140 (120–170)	140 (34–640)	150 (120–170)	160 (20–1200)	160 (140–200)	0.13
γ-HCH	1.3 (<1.0–7.2)	1.2 (1.0–1.5)	1.4 (<1.0–5.4)	1.2 (1.0–1.5)	1.1 (<1.0–100)	1.1 (0.87–1.4)	0.22
Mirex	7.0 (2.5–22)	7.0 (6.2–8.0)	5.9 (<2.0–30)	6.0 (5.2–7.0)	5.5 (<2.0–21)	5.6 (4.9–6.4)	<0.01
Parlar 26	5.2 (<3.0–19)	4.4 (3.7–5.2)	4.3 (<3.0–14)	4.1 (3.5–4.9)	5.7 (<3.0–19)	5.9 (5.1–6.9)	<0.02
Parlar 50	7.2 (<4.0–27)	6.4 (5.3–7.6)	7.2 (<4.0–22)	6.4 (5.4–7.6)	8.5 (<4.0–24)	8.5 (7.4–9.7)	0.04

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

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Concentrations of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls in blood and breast milk collected from pregnant women in Sapporo City, Japan

Takashi Todaka [a,*], Hironori Hirakawa [b], Jumboku Kajiwara [b], Daisuke Onozuka [b], Seiko Sasaki [c], Chihiro Miyashita [c], Eiji Yoshioka [c], Motoyuki Yuasa [c], Reiko Kishi [c], Takao Iida [d], Hiroshi Uchi [e], Masutaka Furue [a,e]

[a] *Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan*
[b] *Fukuoka Institute of Health and Environmental Sciences, Mukaizano 39, Dazaifu-shi, Fukuoka 818-0135, Japan*
[c] *Department of Public Health, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 5, Kita-ku, Sapporo, Hokkaido 060-8638, Japan*
[d] *Kitakyusyu Life Science Center, 1-4 Nakabaru-shin-cho, Tobata-ku, Kitakyusyu 804-0003, Japan*
[e] *Research and Clinical Center for Yusho and Dioxin, Kyushu University Hospital, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan*

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ABSTRACT

We measured the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (PCBs), and non-dioxin-like PCBs in paired samples of blood and breast milk collected from 67 secundiparas in Sapporo City, Japan, and combined this data with those of the 30 secundiparas previously measured. The arithmetic mean total toxic equivalents (TEQ-WHO) concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in blood and breast milk of the 97 secundiparous subjects were 3.0–23 (mean: 13, median: 14) and 2.7–20 (mean: 8.6, median: 8.5) pg TEQ g^{-1} lipid, respectively. The sums of the concentrations of 56 non-dioxin-like PCB congeners that were measured in the subjects' blood and breast milk were 16–326 (mean: 107, median: 100) and 12–252 (mean: 73, median: 67) ng g^{-1} lipid, respectively. The partitioning ratios of individual congeners of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs from blood to breast milk in secundiparas were almost the same as those of primiparas that have been recently reported, suggesting that the partitioning ratios of these compounds from maternal blood to breast milk in women is little affected by delivery. Furthermore, the partition of PCB congeners with chlorine at the 2-, 3-, 4′-, and 5-positions or the 2-, 4-, 4′-, and 5-positions of the biphenyl ring from the blood to the breast milk tended to occur at a higher level than that of other congeners. In particular, the levels of tetraCB-74 and hexaCB-146 in the breast milk for both primiparous and secundiparous mothers were slightly higher than those in the blood.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are highly toxic environmental pollutants. These pollutants are distributed worldwide, and their lipophilic compounds are highly resistant to biodegradation in the environment, becoming concentrated in the food chain and accumulating in the fatty tissues of animals and humans (Liem et al., 2000; Schecter and Gasiewicz, 2003). PCDDs, PCDFs, and PCBs accumulated in the maternal body have been reported to be transferred from the mother to her fetus via the placenta during pregnancy and from mothers to infant via breast milk (Wang et al., 2004; Nakano et al., 2005). Human exposure to PCDDs, PCDFs, and PCBs result in many adverse health effects, including growth retardation in fetuses and infants (Yonemoto, 2000), thyroid deficiency (Pavuk et al., 2003), immune deficiency (Weisglas-kuperus et al., 2000), reproductive effects (Guo et al., 2000), and carcinogenic effects (Steenland et al., 1999; Demers et al., 2002). Moreover, several epidemiological studies have demonstrated the adverse effects of environmental exposure to these dioxin-like compounds on the neurobehavioral development of children (Schantz et al., 2003). Because fetuses and infants are considered to be significantly more sensitive to a variety of PCDDs, PCDFs, and PCBs compared with adults, the adverse effects of these toxicants on these fetuses and infants are of grave concern (Needham and Sexton, 2000; Charnley and Putzrath, 2001; Branum et al., 2003). To elucidate the influence

* Corresponding author. Tel.: +81 92 921 9946; fax: +81 92 928 1203.
E-mail address: todaka@fihes.pref.fukuoka.jp (T. Todaka).

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of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs on the health of fetuses and infants, researchers have conducted exposure surveys of these compounds in maternal blood and breast milk in various countries. However, exposure studies of dioxin-like compounds and non-dioxin-like PCBs regarding maternal blood are limited in comparison with studies of breast milk, and comparisons of the concentrations of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs in blood and breast milk collected from the same mothers have been performed in only a few trials (Schecter et al., 1998; Wang et al., 2004; Nakano et al., 2005; Wittsiepe et al., 2007; Nakamura et al., 2008). Therefore, few exposure studies have compared the levels of dioxin-like compounds and non-dioxin-like PCBs in paired samples of blood and breast milk collected from primiparous mothers with those from secundiparous mothers. The data obtained by the study will help us understand the partitioning ratios of individual congeners of these compounds from blood to breast milk in primiparous and secundiparous mothers and the effect of delivery on the partitioning ratios. We previously measured the concentrations of dioxin-like compounds and non-dioxin-like PCBs in paired samples of blood and breast milk collected from 30 primiparous and 30 secundiparous mothers living in Sapporo City, Hokkaido Prefecture, Japan (Hori et al., 2007; Todaka et al., 2008a). Subsequently, we measured the concentrations of these compounds in paired samples of blood and breast milk collected from 89 primiparas living in the same area, and reported the concentrations of these compounds in blood and breast milk for the total 119 (89 + 30 previous) primparas (Todaka et al., 2010).

In the present study, we measured the concentrations of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs in blood and breast milk collected from 67 secundiparous mothers in Sapporo City, Japan, and combined this data with those of the 30 secundiparous mothers previously collected. The objectives of our primary study were: (1) to study the relationships of these compounds between blood and breast milk for the total 97 secundiparas and (2) to compare our findings with the concentrations of these compounds in those for 119 primiparas.

2. Materials and methods

2.1. Sampling

In 2002, the Hokkaido University Graduate School of Medicine established a hospital-based prospective cohort study entitled the "Hokkaido Study on Environment and Children's Health" to investigate the possible adverse effects of PCBs, PCDDs/PCDFs, perfluopinated chemicals, and many other environmental contaminants on fetal growth and newrodevelopment. 514 pregnant women were enrolled in this cohort study between July 2002 and October 2005. All the subjects participating in this study were native Japanese and residents of Sapporo City or the surrounding area. Blood, cord blood and breast milk specimens were collected from mothers, after obtaining informed consent from them. After collection, the specimens were frozen and stored at −80 °C until analysis. Between June 2004 and June 2008, we measured the levels of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs in 462 maternal blood samples and 250 breast milk specimens. In the present study, blood samples in 67 subjects were taken from the maternal peripheral vein after the 2nd trimester during their second pregnancy. In the previous 30 cases, blood samples were taken during the first 1 week after birth (Todaka et al., 2008a). The breast milk specimens were collected during 28–30 days after delivery. The ages of the secundiparas examined in the present study were within 22–41 years (mean: 31.9 years, median: 32.0 years).

2.2. Materials

Native congeners of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs were purchased from Wellington Laboratories (Guelph, Canada). [$^{13}C_{12}$]-congeners of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs as internal standards, were also purchased from Wellington Laboratories. An active carbon column was prepared as follows: active carbon was purchased from Nacalai Tesque (Kyoto, Japan), refluxed 3 times with toluene for 1 h, and dried in vacuum, after which 500 mg of the active carbon was mixed with 500 g of anhydrous sodium sulfate (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). A silver nitrate/silica gel was purchased from Wako Pure Chemical Industries, Ltd. An active carbon-dispersed silica gel was purchased from Kanto Chemical Industries, Ltd., Tokyo, Japan. All reagents and solvents used in this experiment were of the analytic grade of dioxin that is commercially available.

2.3. Analysis of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs

The extraction and purification of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs from blood and breast milk specimens were performed using a previously reported method (Iida and Todaka, 2003; Todaka et al., 2008a,b). Concentrations of the PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs and concentrations of 56 non-dioxin-like PCB congeners were also performed using a previously reported method (Iida and Todaka, 2003; Todaka et al., 2008a,b).

2.4. Quality control

To evaluate the accuracy and reliability of the analysis of PCDDs, PCDFs, and dioxin-like PCBs, our laboratory completed quality control studies for the analysis of these compounds. Our laboratory has participated in a quality control study for the analysis of these dioxin-like compounds in dried milk powder (BCRRM 534), assisted by a Grant-in-Aid for scientific research from the Ministry of Health, Labour, and Welfare, Japan, in 2003. In our results, the difference between values of our laboratory and certification values in the reference material was within 10% of certification values. In 2004 and 2006, we prepared human blood samples using blood collected from five volunteers and attempted to carry out a quality control study of the analysis of PCDDs, PCDFs, and dioxin-like PCBs in human blood. In both studies, the average variation among the TEQ values in samples obtained by all participating laboratories was within 10% (Iida et al., 2004). In 2007, our laboratory prepared human blood samples using blood collected from five volunteers and breast milk samples prepared using 10 randomly selected specimens from 250 breast milk samples collected in this cohort study. In both samples, measurements of 56 non-dioxin-like PCBs congeners that were measured in the present study among 197 non-dioxin-like PCBs congeners requested from three different analysis organizations and their results were compared with our results. The average variation among the total non-dioxin-like PCBs levels in human blood and breast milk samples obtained by the four participating laboratories was within 10% and was considered acceptable (Kajiwara et al., 2008, 2009, 2010). These results indicated that our laboratory's analytical methods for PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs in human blood and breast milk specimens provided correct results.

2.5. Data analysis

To estimate the TEQ concentrations, we introduced ND (less than the detection limit) values to half values of the detection limit

and calculated based on the toxic equivalency factor (TEF) values proposed by the World Health Organization (WHO) (Van den Berg et al., 2006). The statistical analysis was conducted using Mann–Whitney U-test, Pearson correlation test, and Spearman correlation test in the software program from SAS Institute (SAS Inc.). Significant probabilities (p values) were calculated for the respective number of samples analyzed. All statistical testing was 2-side with a significance level of 5%.

3. Results and discussion

3.1. Concentrations of dioxin-like compounds in blood and breast milk

The arithmetic mean TEQ concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in blood and breast milk of 97 secundiparas were 6.8, 2.4, 4.2, and 0.3 pg TEQ g^{-1} lipid, respectively, and 3.7, 1.6, 3.1, and 0.3 pg TEQ g^{-1} lipid, respectively, with the total TEQ concentrations of these dioxin-like compounds equaling 3.0–23 (mean: 13, median: 14) and 2.7–20 (mean: 8.6, median: 8.5) pg TEQ g^{-1} lipid, respectively (Table 1). Among PCDDs, PCDFs, and dioxin-like PCB congeners that were measured in the present study, 1,2,3,7,8-PentaCDD, 1,2,3,6,7,8-HexaCDD, 2,3,4,7,8-PentaCDF, and 3,3′,4,4′,5-PentaCB (#126) showed particularly high concentrations in blood and breast milk of 97 secundiparas mothers; the total concentrations of these four congeners in blood and breast milk samples contributed approximately 80% of the total TEQ concentrations. The concentrations of individual congeners of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in blood of secundiparous subjects were notably higher than those of breast milk.

The relative contribution ratios of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs to the total TEQ concentrations in blood and breast milk of secundiparas were 51.8%, 18.1%, 32.4%, and 2.7%, respectively, and 42.4%, 18.2%, 35.9%, and 3.5%, respectively. These results are similar to those obtained previously in 119 primiparas (Todaka et al., 2010).

3.2. Concentrations of non-dioxin-like PCBs in blood and breast milk

We previously indicated that the 56 PCB congeners measured in our studies appear to be the predominant PCB congeners in human subjects among the 197 non-dioxin-like PCB congeners, based on the results of our studies and other studies previously reported in Japan (Todaka et al., 2008b).

The concentrations of PCB congeners in blood and breast milk of 97 secundiparas are presented in Table 2. Among the 56 PCB congeners that were measured in the present study, hexaCB-138, hexaCB-153, heptaCB-180, and heptaCB-182/heptaCB-187 showed high ratios to the total concentrations of 56 PCB congeners in maternal blood and breast milk. Other PCB congeners contributed less than 5% of the total concentrations of these PCB congeners. The patterns of the major PCB congeners in maternal blood were almost the same as those obtained in breast milk. Similar results were observed in 119 primiparas previously studied (Todaka et al., 2010).

The sums of the concentrations of 56 PCB congeners in blood and breast milk of 97 secundiparas were 16–326 (mean: 107, median: 100) ng g^{-1} lipid and 12–252 (mean: 73, median: 67) ng g^{-1} lipid, respectively, indicating that the total concentrations of PCB congeners in maternal blood tended to be slightly higher than those in breast milk. Indicator PCBs (PCB 28, 52, 101, 138, 153, and 180) have been selected by the European Food Safety Authority as the major congeners that are almost always present in various sample matrices at high concentrations. The arithmetic mean total concentrations of indicator PCB congeners in blood and breast milk of 97 secundiparous mothers were 6.8–168 (mean: 54, median: 49) and 6.2–140 (mean: 39, median: 36) ng g^{-1} lipid, respectively, indicating that the total concentrations of indicator PCBs in maternal blood were slightly higher than those in breast milk.

The relative contribution ratios of the concentrations of triCBs, tetraCBs, pentaCBs, hexaCBs, heptaCBs, octaCBs, and nonaCBs to the total concentrations of 56 non-dioxin-like PCB congeners in the blood and the breast milk of secundiparous mothers were 1.4%, 5.8%, 6.5%, 46.2%, 31.7%, 6.9%, and 1.0%, respectively, and 1.0%, 6.8%, 8.1%, 53.8%, 25.9%, 3.9%, and 0.4%, respectively. The ratios of concentrations of these PCB compounds from secundiparous mothers were almost the same as those previously obtained from 119 primiparous mothers (Todaka et al., 2010).

During the past few decades, extensive research on the presence of PCDDs, PCDFs, and dioxin-like PCBs in blood and breast milk have been conducted in various countries. A recent German study (n = 169; mean age, 31.2 years) reported that the mean total TEQ levels of PCDDs, PCDFs, and dioxin-like PCBs in maternal blood and breast milk were 28.4 and 27.3 pg TEQ g^{-1} lipid, respectively (Wittsiepe et al., 2007). These levels for the Tohoku cohort study (n = 49; mean age, 32.4 years) that have been recently reported in Japan were 15.4 and 18.8 pg TEQ g^{-1} lipid, respectively (Nakamura et al., 2008). In the present study, the levels of PCDDs, PCDFs, and dioxin-like PCBs in maternal blood and breast milk were slightly lower than those in these cohort studies. Furthermore, several reports on the total TEQ levels of PCDDs, PCDFs, and dioxin-like PCBs and the total levels of non-dioxin-like PCBs in similar specimens have been performed in Asia, European countries, Russia, and the United States (Liem et al., 2000; Polder et al., 2003; Schecter et al., 2005). In those reports, the levels of these contaminants were higher than the levels found in the present study. The exposure levels of dioxin-like compounds and non-dioxin-like PCBs in our studies were some of the lowest levels reported in human studies.

3.3. Relationships of the measured compounds between blood and breast milk

Statistically significant correlations were observed between maternal age and the total TEQ concentration of PCDDs, PCDFs, and dioxin-like PCBs (correlation coefficient $\rho = 0.392$, $p < 0.001$) or the total concentration of 56 non-dioxin-like PCB congeners (correlation coefficient $\rho = 0.498$, $p < 0.001$) in maternal blood, and significant correlations were also observed between maternal age and the total TEQ concentration of these dioxin-like compounds (correlation coefficient $\rho = 0.375$, $p < 0.001$) or the total concentration of 56 PCB congeners (correlation coefficient $\rho = 0.493$, $p < 0.001$) in breast milk.

The total TEQ concentration of PCDDs, PCDFs, and dioxin-like PCBs in individual subjects' maternal blood and breast milk showed a close correlation (correlation coefficient $\rho = 0.761$, $p < 0.001$), and there was also good correlation between the total concentration of 56 non-dioxin-like PCB congeners in subjects' maternal blood and breast milk (correlation coefficient $\rho = 0.879$, $p < 0.001$). Concentrations of PCDDs, PCDFs, non-*ortho* PCBs, mono-*ortho* PCBs, and indicator PCBs in individuals' maternal blood also showed the highest correlation to those in breast milk.

Pearson and Spearman correlation analyses showed a relationship between the total TEQ concentration of PCDDs, PCDFs, and dioxin-like PCBs and the total concentration of 56 non-dioxin-like PCB congeners in maternal blood (correlation coefficient $\rho = 0.817$, $p < 0.001$), and also showed an association between the total TEQ concentration of these dioxin-like compounds and the total concentration of 56 PCB congeners in breast milk (correlation coefficient $\rho = 0.843$, $p < 0.001$).

Table 1
Concentrations of PCDDs, PCDFs, and dioxin-like PCBs in blood and breast milk of 97 secundiparous mothers collected in Sapporo City, Japan.

Congeners	Concentration (pg g^{-1} lipid)										Ratio[1] (milk/blood)	*p* Values	Ratio[2] (milk/blood)
	Blood					Breast milk							
	Mean	Median	SD	Minimum	Maximum	Mean	Median	SD	Minimum	Maximum			
2,3,7,8-TetraCDD	0.8	0.5	0.5	0.5	2.5	0.5	0.5	0.2	0.5	1.5	0.65	<0.001	0.59
1,2,3,7,8-PentaCDD	3.9	3.7	1.7	0.5	7.9	2.1	2.0	1.0	0.5	5.3	0.55	<0.001	0.63
1,2,3,4,7,8-HexaCDD	1.6	1.0	1.4	1.0	14	ND							
1,2,3,6,7,8-HexaCDD	13	12	6.4	2.4	42	7.1	6.4	3.5	1.0	21	0.55	<0.001	0.58
1,2,3,7,8,9-HexaCDD	2.0	1.0	1.3	1.0	8.3	ND							
1,2,3,4,6,7,8-HeptaCDD	26	24	12	9.4	85	5.2	4.8	2.4	2.0	18	0.20	<0.001	0.23
OctaCDD	458	432	179	80	1306	39	34	20	8.2	167	0.08	<0.001	0.10
Total PCDDs	506	479	195	99	1426	56	50	26	19.3	218	0.11	<0.001	0.13
2,3,7,8-TetraCDF	0.7	0.5	0.5	0.5	3.1	ND							
1,2,3,7,8-PentaCDF	0.6	0.5	0.4	0.5	3.5	ND							
2,3,4,7,8-PentaCDF	5.3	5.3	2.4	0.5	11	3.6	3.4	1.6	0.5	8.5	0.67	<0.001	0.74
1,2,3,4,7,8-HexaCDF	2.0	2.1	1.1	1.0	6.1	ND							
1,2,3,6,7,8-HexaCDF	2.3	2.3	1.3	1.0	6.8	ND							
2,3,4,6,7,8-HexaCDF	ND					ND							
1,2,3,7,8,9-HexaCDF	ND					ND							
1,2,3,4,6,7,8-HeptaCDF	2.4	2.2	2.3	1.0	17	ND							
1,2,3,4,7,8,9-HeptaCDF	ND					ND							
OctaCDF	2.1	2.0	0.6	2.0	8.2	ND							
Total PCDFs	19	17	6.7	9.5	43	13	13	2.0	10	21	0.71	<0.001	0.69
TriCB-77	12	12	5.7	5.0	27	ND							
TriCB-81	ND					ND							
PentaCB-126	35	33	18	5.0	84	26	24	14	5.0	69.7	0.77	0.001	0.82
HexaCB-169	26	25	14	5.0	76	15	14	7.7	5.0	45.6	0.58	<0.001	0.67
Total Non-*ortho* PCBs	77	74	32	20	172	51	50	21	20	120	0.67	<0.001	0.70
PentaCB-105	1493	1347	749	283	3412	1336	1237	717	224	4096	0.89	0.120	0.98
PentaCB-114	363	348	210	5.0	1130	311	272	177	55	1156	0.86	0.062	0.92
PentaCB-118	6150	5849	3155	981	14434	5552	5218	2962	1080	17027	0.90	0.205	1.00
PentaCB-123	114	113	61	5.0	293	84	75	49	5.0	253	0.74	<0.001	0.84
HexaCB-156	2043	1892	1068	282.1	6026	1612	1402	901	238	5808	0.79	0.001	0.88
HexaCB-157	472	433	256	5.0	1303	379	350	196	62	1241	0.80	0.004	0.86
HexaCB-167	753	708	392	5.0	1926	564	517	318	92	1943	0.75	<0.001	0.81
HeptaCB-189	265	242	144	5.0	807	150	139	79	5.0	516	0.57	<0.001	0.63
Total Mono-*ortho* PCBs	11653	11042	5733	1778	27197	9990	9479	5192	1909	32022	0.86	0.037	0.95
TEQ from PCDDs	6.8	6.8	2.7	1.7	14	3.7	3.5	1.5	1.3	9.7	0.54	<0.001	0.59
TEQ from PCDFs	2.4	2.3	1.0	0.6	4.4	1.6	1.5	0.5	0.6	3.1	0.66	<0.001	0.70
TEQ from PCDDs/PCDFs	9.1	9.0	3.6	2.5	17	5.2	4.9	1.9	2.0	13	0.57	<0.001	0.61
TEQ from non-*ortho* PCBs	4.2	4.0	2.1	0.7	9.8	3.1	2.9	1.6	0.7	7.8	0.73	<0.001	0.79
TEQ from mono-*ortho* PCBs	0.3	0.3	0.2	0.1	0.8	0.3	0.3	0.2	0.1	1.0	0.86	0.037	0.95
TEQ from dioxin-like PCBs	3.9	3.5	2.2	0.4	9.4	3.4	3.2	1.7	0.7	8.8	0.87	0.121	0.80
Total TEQ	13.1	13.6	5.1	3.0	23.0	8.6	8.5	3.4	2.7	20.0	0.66	<0.001	0.68

The partitioning ratio from maternal blood from breast milk: ratio[1], secundiparous mothers; ratio[2], primiparous mothers.
ND: less than the determination limit.
SD: standard deviation.
CDD: chlorinated dibenzo-*p*-dioxin.
CDF: chlorinated dibenzofuran.
CB: chlorinated biphenyl.

Table 2
Concentrations of non-dioxin-like PCBs in blood and breast milk of 97 secundiparous mothers collected in Sapporo City, Japan.

IUPAC #	Concentration (pg g^{-1} lipid)										Ratio[1] (milk/blood)	*p* Values	Ratio[2] (milk/blood)
	Blood					Breast milk							
	Mean	Median	SD	Minimum	Maximum	Mean	Median	SD	Minimum	Maximum			
TriCB-28[a]	1142	1099	564	42	2795	678	643	341	5.0	1602	0.59	<0.001	0.67
TriCB-29	21	5.0	32	5.0	174	6	5.0	4	5.0	28	0.31	<0.001	0.32
TriCB-37	322	5.0	898	5.0	6185	11	5.0	13	5.0	74	0.03	0.731	0.02
TetraCB-44	243	233	214	5.0	1195	63	50	52	5.0	210	0.26	<0.001	0.28
TetraCB-47/48	362	324	277	5.0	1204	176	159	106	5.0	582	0.49	<0.001	0.48
TetraCB-49	196	175	168	5.0	850	56	47	42	5.0	177	0.29	<0.001	0.35
TetraCB-52/69[a]	650	590	474	5.0	2098	253	198	236	5.0	1279	0.39	<0.001	0.46
TetraCBs-56/60	289	261	146	5.0	693	214	165	164	29	850	0.74	<0.001	0.82
TetraCB-63	52	48	32	5.0	137	47	44	26	5.0	146	0.89	0.311	0.96
TetraCB-66	781	658	387	154	2066	690	634	368	152	2001	0.88	0.108	0.99
TetraCB-70	167	149	114	5.0	543	45	40	37	5.0	192	0.27	<0.001	0.32
TetraCB-71	99	82	99	5.0	708	26	5	32	5.0	164	0.26	<0.001	0.31
TetraCB-74	3358	3324	1592	691	8669	3438	3301	1766	752	9324	1.02	0.977	1.12
PentaCB-85	111	95	71	5.0	357	76	63	52	5.0	262	0.68	<0.001	0.67
PentaCB-87	281	256	156	5.0	784	162	147	92	5.0	553	0.58	<0.001	0.60
PentaCB-92	314	257	219	5.0	1001	247	204	165	5.0	741	0.79	0.042	0.86
PentaCB-93/95/98	410	368	253	5.0	1140	198	176	134	5.0	587	0.48	<0.001	0.56
PentaCB-99	4278	4028	2028	733	11168	4062	3715	2050	726	12183	0.95	0.380	1.03
PentaCB-101[a]	741	650	446	5.0	2242	578	508	367	5.0	1908	0.78	0.004	0.87
PentaCB-107/108	332	302	202	5.0	1091	311	300	186	5.0	901	0.94	0.501	0.98
PentaCB-110	208	178	155	5.0	700	107	83	83	5.0	489	0.51	<0.001	0.49
PentaCB-117	259	238	143	5.0	830	179	161	114	5.0	602	0.69	<0.001	0.73
HexaCB-128	384	328	251	5.0	1629	295	261	185	32	1287	0.77	0.009	0.75
HexaCB-130	2167	592	6261	5.0	37790	628	557	370	5.0	2115	0.29	0.441	0.32
HexaCB-132	131	112	108	5.0	452	91	77	69	5.0	300	0.69	0.009	0.72
HexaCB-134	17	5	21	5.0	89	11	5	11	5.0	52	0.64	0.197	0.76
HexaCB-135	183	148	120	5.0	633	143	132	91	5.0	544	0.78	0.021	0.87
HexaCB-137	820	787	395	79	2161	703	637	365	129	2443	0.86	0.026	0.93
HexaCB-138[a]	12822	12044	6423	1769	35382	11119	10577	5678	1851	38118	0.87	0.051	0.94
HexaCB-139/149	272	241	208	5.0	772	258	210	161	5.0	786	0.95	0.887	0.92
HexaCB-141	124	94	107	5.0	441	100	92	62	5.0	289	0.81	0.367	0.84
HexaCB-146	2561	2459	2342	5.0	10645	2784	2500	1588	315	10306	1.09	0.146	1.33
HexaCB-147	135	120	91	5.0	388	111	107	72	5.0	343	0.82	0.086	0.94
HexaCB-151	432	361	306	5.0	1438	322	265	221	5.0	1259	0.75	0.014	0.81
HexaCB-153[a]	23994	21388	12484	2937	63114	19416	17712	10455	2919	71922	0.81	0.007	0.90
HexaCB-163/164	4520	3976	2695	473	13653	3399	3048	1851	478	11266	0.75	0.004	0.85
HexaCB-165	841	5.0	1655	5.0	8665	ND							
HeptaCB-170	5219	4721	3206	615	22524	2989	2575	1655	399	10741	0.57	<0.001	0.58
HeptaCB-172	806	728	525	5.0	3566	425	374	260	46	1680	0.53	<0.001	0.53
HeptaCB-177	1662	1447	1012	5.0	6248	1203	1082	661	177	3773	0.72	<0.001	0.76
HeptaCB-178	1583	1301	1014	5.0	5940	1007	895	619	135	4029	0.64	<0.001	0.68
HeptaCB-179	93	76	73	5.0	308	80	71	55	5.0	289	0.87	0.394	1.00
HeptaCB-180[a]	15300	13399	9681	1476	67239	7890	6894	4474	1168	28094	0.52	<0.001	0.54
HeptaCB-181	32	25	27	5.0	122	13	10	11	5.0	54	0.43	<0.001	0.45
HeptaCB-182/187	7015	6072	4444	741	28509	4082	3583	2445	558	15638	0.58	<0.001	0.63
HeptaCB-183	1924	1651	1120	5.0	6387	1170	1064	613	216	3734	0.61	<0.001	0.65
HeptaCB-191	184	177	119	5.0	671	85	74	53	5.0	300	0.46	<0.001	0.53
OctaCB-194	1976	1721	1120	316	7090	796	656	464	116	3091	0.40	<0.001	0.44
OctaCB-195	500	443	264	85	1444	252	221	128	54	773	0.50	<0.001	0.56
OctaCB-196203	1895	1734	1109	217	7503	686	567	464	43	2913	0.36	<0.001	0.39

OctaCB-198/201	2213	1941	1433	269	10610	730	553	632	60	4300	0.33	<0.001	0.37
OctaCB-200	114	106	81	5.0	394	58	48	41	5.0	231	0.51	<0.001	0.57
OctaCB-202	576	503	374	5.0	2087	320	269	198	37	1376	0.56	<0.001	0.60
OctaCB-205	82	77	49	5.0	261	31	28	21	5.0	126	0.37	<0.001	0.42
NonaCB-206	666	595	380	86	2278	168	143	119	26	747	0.25	<0.001	0.29
NonaCB-207	135	114	86	5.0	549	34	33	23	5.0	143	0.25	<0.001	0.28
NonaCB-208	274	225	190	5.0	1045	73	58	61	5.0	420	0.26	<0.001	0.31
DecaCB-209	565	512	388	63	3300	67	58	50	5.0	377	0.12	<0.001	0.42
Total TriCBs	1485	1218	1009	339	7871	695	656	341	32	1681	0.47	<0.001	0.48
Total TetraCBs	6197	6089	2352	1693	11941	5008	4634	2400	1147	12764	0.81	<0.001	0.90
Total PentaCBs	6934	6184	3114	1150	17620	5920	5501	2943	1016	17809	0.85	0.017	0.93
Total HexaCBs	49405	44608	25921	6120	129372	39383	36215	20574	6057	140598	0.80	0.005	0.87
Total HeptaCBs	33818	29897	20713	3659	141409	18945	16947	10600	2742	68130	0.56	<0.001	0.59
Total OctaCBs	7356	6517	4256	1108	29270	2873	2311	1865	327	12773	0.39	<0.001	0.43
Total NonaCBs	1075	927	625	204	3506	275	238	192	67	1114	0.26	<0.001	0.29
Total DecaCBs	565	512	388	63	3300	67	58	50	5.0	377	0.12	<0.001	0.42
Total indicator PCBs	53508	49105	27780	6846	168276	39256	35707	20532	6231	140209	0.73	<0.001	0.80
Total PCBs	106835	99542	53758	16017	325539	73167	66829	37360	12231	252257	0.68	<0.001	0.75

The partitioning ratio from maternal blood from breast milk: ratio[1], secundiparous mothers; ratio[2], primiparous mothers.
ND: less than the determination limit.
[a] Indicator PCB; CB: chlorinated biphenyl; SD: standard deviation.

3.4. The partitioning ratios of the measured compounds from blood to breast milk

To understand the partitioning ratio (milk/blood) of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs from maternal blood to breast milk in secundiparous mothers, we estimated the partitioning ratio of individual congeners of these compounds. These results, including the data of primiparous mothers, are presented in Tables 1 and 2. The partitioning ratios of individual congeners of these compounds in secundiparous mothers were less than 1.0. In particular, the ratios of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (heptaCDD) and 1,2,3,4,5,6,7,8-octachlorodibenzo-*p*-dioxin (octaCDD) tended to be lower compared to those of other congeners. These results obtained in the present study are similar to those that have been recently reported (Schecter et al., 1998; Wittsiepe et al., 2007; Nakamura et al., 2008). The HepCDD and OctaCDD partition from maternal blood to breast milk tended to decrease with the increasing number of chlorines for the PCDD congeners. Additionally, 2,3,7,8-tetraCDD is mostly bound to lipoprotein (80%), as well as other plasma proteins (15%) and red blood cells (5%) in the blood (Henderson and Patterson, 1988). OctaCDD exists bound to lipoprotein (45%) and other plasma proteins (50%) in the blood (Patterson et al., 1989). Therefore, it can be assumed from these that the binding capacity of PCDD congeners to plasma proteins and the different lipophilicity for the PCDD congeners may influence the partition of PCDD congeners from blood to breast milk. The arithmetic mean partitioning ratios of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in primiparous and secundiparous mothers were 0.59, 0.70, 0.79, and 0.95, respectively, and 0.54, 0.66, 0.73, and 0.86, respectively, indicating that the partitioning ratios in primiparous mothers were almost the same as those in secundiparous mothers, and that the ratios of non-*ortho* PCBs and mono-*ortho* PCBs for both primiparous and secundiparous mothers tended to be higher than those of PCDDs and PCDFs. In the case of non-dioxin-like PCBs, these ratios of triCBs, tetraCBs, pentaCBs, hexaCBs, heptaCBs, octaCBs, and nonaCBs in primiparous and secundiparous mothers were 0.48, 0.90, 0.93, 0.87, 059, 0.43, and 0.29, respectively and 0.47, 0.81, 0.85, 0.80, 0.56, 0.39, and 0.26, respectively, indicating that each partitioning ratio in primiparas was also nearly the same as that in secundiparas, and that the ratios of tetraCBs, pentaCBs, and hexaCBs for both primiparous and secundiparous mothers tended to be higher compared to those of triCBs, heptaCBs, octaCBs, and nonaCBs. Among PCB congeners of tetraCBs, pentaCBs, and hexaCBs for both primiparous and secundiparous mothers, tetraCB-63, pentaCB-107/108, pentaCB-114, pentaCB-117, hexaCB-137, hexaCB146, hexaCB-147, and hexaCB-156 with chlorine atoms at the 2-, 3-, 4′-, and 5-positions of the biphenyl ring and tetraCB-74, pentaCB-99, pentaCB-114, pentaCB-118, hexaCB-137, hexaCB-138, hexaCB-153, hexaCB-156, and hexaCB-167 having chlorine atoms at the 2-, 4-, 4′-, and 5-positions of the biphenyl ring tended to partition from maternal blood to breast milk at higher levels than those of other congeners. In particular, the levels of tetraCB-74 and hexaCB-146 in the breast milk for both primiparous and secundiparous mothers were slightly higher than those in the blood. These findings suggested that the partitioning ratio of individual congeners of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs from maternal blood to breast milk in women is little affected by delivery, and that each congener of dioxin-like PCB and non-dioxin-like PCBs with chlorine at the 2-, 3-, 4′-, and 5-positions or the 2-, 4-, 4′-, and 5-positions of the biphenyl ring should be targeted in future assessments of these PCB congeners in the infant body.

4. Conclusion

This study extends our previous studies by reporting the levels of PCDDs, PCDFs, dioxin-like-PCBs, and non-dioxin-like PCBs in paired samples of blood and breast milk collected from secundiparous mothers. The present study was one of the few studies where the partitioning ratios of individual congeners of dioxin-like compounds and non-dioxin-like PCBs from maternal blood to breast milk in child-bearing women were determined. Therefore, these data may provide important information regarding the health risk of these compounds in infants. In the future, collection of these data from many more mothers is warranted. Further research must be undertaken in the context of epidemiological investigations to more accurately assess the effects of these compounds on children.

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Self-reported tobacco smoke exposure and plasma cotinine levels during pregnancy – A validation study in Northern Japan

Seiko Sasaki [a], Titilola S. Braimoh [a], Thamar A. Yila [b], Eiji Yoshioka [a], Reiko Kishi [b,*]

[a] Department of Public Health, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan
[b] Center for Environmental and Health Sciences, Hokkaido University, North 12 West 7, Kita-ku, Sapporo 060-0812, Japan

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ABSTRACT

Maternal smoking is a critical public health concern requiring the establishment of its prevalence rate and clinical impact. Maternal self-reported information of tobacco smoke exposure requires validation using accurate biochemical analysis. This study examined the association between self-reported exposure to tobacco smoke and plasma cotinine level in Japanese pregnant women. We collected information about smoking and secondhand smoke (SHS) exposure during pregnancy from 5128 pregnant women in a prospective cohort design, and analyzed biochemically maternal blood samples using the enzyme-linked immunosorbent assay (ELISA) technique. Based on self-reports, the subjects were classified into three groups: 650 smokers, 728 ex-smokers and 3750 non-smokers. Using the receiver operating characteristic (ROC) curve, plasma cotinine cut-off value of 11.48 ng/mL was established for separating smokers from non-smokers, resulting in a smoking prevalence of 14%. A cotinine cut-off value of 0.21 ng/mL for discriminating exposed and unexposed nonsmokers resulted in a 63% prevalence of exposure to tobacco smoke among nonsmokers. Cotinine biomarker analysis proved accurate in validating self-reported smoking information in the subjects. Lower validity of SHS exposure suggests a need to confirm questionnaire information with biochemical analysis.

1. Introduction

Exposure to tobacco smoke during pregnancy is hazardous to the health of both the mother and her fetus (Kelly et al., 2005; Wu et al., 2007; Sasaki et al., 2008). In spite of the knowledge of this fact, it is surprising that some pregnant women still smoke actively or passively. Accurate measurement of the true exposure level is somehow difficult. However, different methods employed range from administration of questionnaires to biochemical analysis of cotinine in body fluids (urine, blood, saliva) and hair. One of the limitations of studies that evaluated smoking status of pregnant women by self-administered questionnaires only is misclassification or recall bias (Windham et al., 2000; Fantuzzi et al., 2007; Jaddoe et al., 2008). Smokers have difficulty in reporting smoking behavior correctly and nonsmokers could not recall secondhand smoke (SHS) exposure precisely. Hence, the use of biomarkers to validate the evaluation of smoking habit and SHS exposure reported through questionnaire information was introduced lately (Lindqvist et al., 2002; McDonald et al., 2005; Chiu et al., 2008).

Cotinine, the major proximate metabolite of nicotine, is the biomarker for both active and secondhand exposure to tobacco smoke (Benowitz et al., 2009). Its analysis can be performed using blood, urine, hair or saliva (Al-Delaimy et al., 2000; Etter et al., 2000; George et al., 2006; Man et al., 2009). It has a relatively longer half-life of approximately 17 h, higher sensitivity and greater specificity than nicotine (Benowitz, 1996). During pregnancy, level of cotinine in the blood is reduced due to its increased clearance and shortened half-life (Dempsey et al., 2002). Although the mechanism is not yet known, it has been reported that pregnancy has varying and irregular effect on the metabolic clearance of drugs (Loebstein et al., 1997).

Prenatal tobacco smoke exposure is a critical public health concern. As such, it is important to assess the validity of pregnant women's smoking and exposure status for research purposes (McDonald et al., 2005; Man et al., 2009). Using blood plasma cotinine as a biomarker for assessment of smoking status, it was observed that though the validity of self-reported smoking is high, that of SHS is low (George et al., 2006). Among 406 self-reported nonsmokers, a study identified 6% smokers and 3% passive smokers (Lindqvist et al., 2002). An assay of second trimester maternal serum cotinine showed a low correlation ($r = 0.39$) between cotinine concentration and self-reports (DeLorenze et al., 2002), whereas a direct relationship between self-reported information on tobacco smoke exposure in pregnant women and the levels of cotinine in the umbilical cord–blood of the fetus was found in another study (Chiu et al., 2008).

Studies on the correlation of questionnaire information on maternal smoking status with cotinine concentrations have inconsistent results. Furthermore, standardized cut-off values for distinguishing smokers from nonsmokers, and exposed from unexposed nonsmokers are yet

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

2. Methods

2.1. Study, subjects and data collection

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

2.2. Cotinine analysis

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

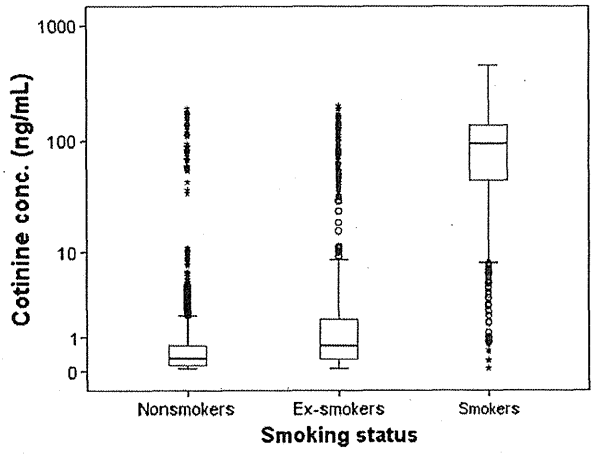

Fig. 1. Box and whisker plot of $\log_{10}$-transformed plasma cotinine concentrations in maternal blood samples based on self-reported exposure to tobacco smoke during pregnancy. Horizontal lines inside boxes signify the median, boxes indicate the interquartile range, whiskers represent the most extreme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

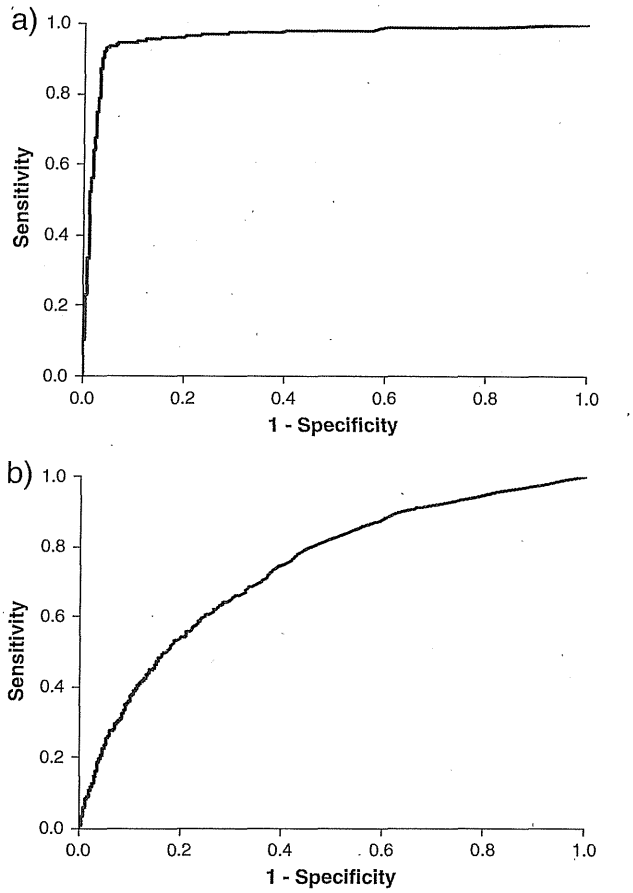

Fig. 2. Receiver operating characteristics curves to separate (a) smokers from nonsmokers and (b) exposed from unexposed nonsmokers.

2.3. Statistical analyses

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

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

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

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

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

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

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

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

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

3. Results

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

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

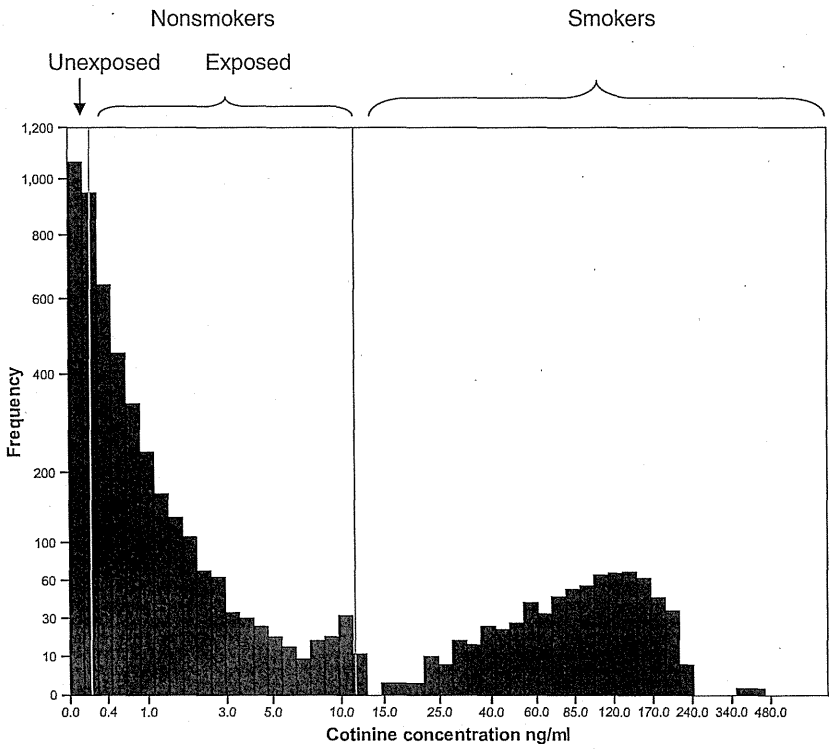

Fig. 3. Frequency distribution of plasma cotinine concentrations showing the cut-off points for unexposed and exposed non smokers and nonsmokers from smokers (N = 5128).

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

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

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

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

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

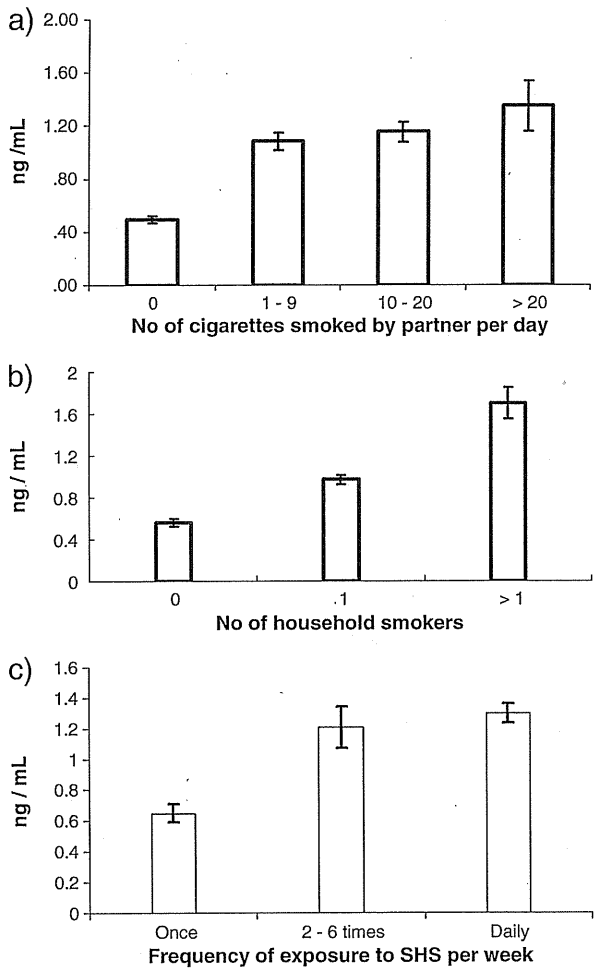

Fig. 4. Plasma cotinine concentrations and SHS exposure: relationship of plasma cotinine levels with (a) number of cigarettes smoked by partner daily; (b) number of household smokers; and (c) frequency of SHS exposure per week. All are significant at $P<0.01$. The standard errors are shown as vertical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

4. Discussion

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

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

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

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

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

5. Conclusion

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

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

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

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

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ABSTRACT

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

1. Introduction

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

Animal studies have demonstrated that fetal TCDD exposure inhibits cellular differentiation and maturation, particularly of T lymphocytes, causes thymic atrophy, and leads to immunosuppression in offspring (Yoshizawa et al., 2007). Offspring of

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

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

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

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

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

A few human studies have addressed prenatal exposure to environmental levels of PCBs/dioxins, although several of these studies were conducted in populations exposed to high levels. In the Rotterdam study, PCBs in maternal blood and dioxins in breast milk were significantly associated with a higher prevalence of otitis media and chicken pox, as well as a lower prevalence of shortness of breath with asthma. In addition, these indicators were related to a reduction in measles, mumps, and rubella reactivity after primary vaccination and an increased number of T lymphocytes at 42 months (Weisglas-Kuperus et al., 2000, 2004). On the other hand, in the Amsterdam study, dioxin levels in breast milk were associated with decreased allergies but not with any infections at 8 years of age (ten Tusscher et al., 2003). In Spain, PCB levels in cord blood were not related to the prevalence of asthma at 4 years of age (Sunyer et al., 2005). In Japan, DLC levels in breast milk were significantly associated with an increased lymphocyte subset ratio in the peripheral blood of breast-fed infants at 10 months (Nagayama et al., 2007), but no association was observed in another cohort of Japanese infants at 12 months of age (Kaneko et al., 2006).

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

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

2. Materials and methods

2.1. Study population

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

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

From recruitment to 18 months after delivery, 23 mothers were excluded for reasons of miscarriage (4), stillbirth (2), relocation (8), infant mortality (1), or voluntary withdrawal (8). At 18 months after delivery, follow-up questionnaires were mailed to 491 subjects, of whom 390 (79.4%) responded. From the questionnaires, we obtained information about potential confounding factors such as early feeding type (breast-feeding, bottle-feeding, or both), breast-feeding duration (weeks), age of starting solid foods, parental smoking status, living with a smoker excluding mother, living environment, day care attendance, vaccination history during the first 18 months of life, and infant height and weight at 18 months of age. We defined infants exposed to environmental tobacco smoke as those living with a smoker including their mother.

2.2. Assessment of infant allergies and infections

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

2.3. Exposure assessment

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

unavailable or insufficient sample volumes for measurement. One sample was excluded from the study because it contained extremely high levels of PCDFs.

2.4. Statistical analysis

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

3. Results

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

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

The number (%) of infants who developed allergies or infections during the first 18 months of life were as follows: food allergies: 62 (17.0) of whom 32 were male and 30 were female; eczema: 41 (11.3) of whom 22 were male and 19 were female; asthma: 32 (8.8) of whom 15 were male and 17 were female; otitis media: 68 (18.7) of whom 40 were male and 28 were female; rhinitis: 7 (1.9); pharyngitis: 3 (0.8); bronchitis: 4 (1.1); pneumonia: 8 (2.2); respiratory syncytial virus infection: 8 (2.2); chicken pox: 17 (4.7); other virus infections (rotavirus, cytomegalovirus, adenovirus, and herpes virus): 20 (5.5); and skin infection: 3 (0.8). There was no significant difference in the rate of developing illnesses by gender. Binominal analysis showed that positive allergic history in parents, day care attendance, long duration of breast-feeding (≥ 4 months), increasing maternal BMI, and multiparity were risk factors for developing allergies or infections (Table 3).

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

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

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

4. Discussion

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

For all infants, both PCDFs and 2,3,4,7,8-penta-CDF showed significant positive trends for a risk of otitis media. In Yucheng patients who were exposed to high levels of DLCs, 2,3,4,7,8-penta-CDF was described as the primary contributor to the toxic effects

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

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

Unknown smoking data for seven fathers (1.9%), beef intake during pregnancy for three mothers (1%), annual household income for one participant (0.3%), and distance from highway to home for one participant (0.3%).$p<0.05$, $p<0.01$; statistically significant differences following the Spearman's correlation test, Mann–Whitney U-test, Kruskal–Wallis test, and Univariate regression analysis for total dioxin TEQs.

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

because this compound accounted for 70% of the total dioxin TEQ levels in maternal blood (Masuda, 2001). Mastueda et al. (2007) indicated that both toxicity kinetics and the half-life for elimination of DLCs vary depending on the exposure source. Therefore, 2,3,4,7,8-penta-CDF may affect infant health more strongly than other DLC congeners, regardless of the exposure source.

In analyses stratified by gender, significant positive trends were observed for PCDDs, PCDFs, and total dioxins in male infants. Among female infants, however, a significant association was observed between the second quartile of PCDFs and the risk of otitis media. In rats treated with TCDD on gestational day 14, the maternal lowest-observed-averse-effect-level for immunosuppression in male offspring (median; 0.1 μg/kg) was lower

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

	DL	ND	%	Minimum	25th	Median	75th	Maximum
2,3,7,8-TCDD	1	198	54.4	0.50	0.50	0.50	1.28	3.44
1,2,3,7,8-PeCDD	1	8	2.2	0.50	2.88	3.93	5.15	12.90
1,2,3,4,7,8-HxCDD	2	227	62.4	1.00	1.00	1.00	2.28	13.60
1,2,3,6,7,8-HxCDD	2	0	0.0	2.37	9.60	13.27	17.51	113.84
1,2,3,7,8,9-HxCDD	2	160	44.0	1.00	1.00	2.18	3.00	25.10
1,2,3,4,6,7,8-HpCDD	2	0	0.0	8.35	18.31	23.31	30.99	85.38
OCDD	4	0	0.0	75.50	325.78	412.16	556.47	1491.50
Total PCDDs				92.69	365.51	460.90	608.97	1602.40
2,3,7,8-TCDF	1	290	79.7	0.50	0.50	0.50	0.50	8.41
1,2,3,7,8-PeCDF	1	335	92.0	0.50	0.50	0.50	0.50	4.60
2,3,4,7,8-PeCDF	1	2	0.5	0.50	4.08	5.54	7.16	19.93
1,2,3,4,7,8-HxCDF	2	146	40.1	1.00	1.00	2.22	2.88	12.47
1,2,3,6,7,8-HxCDF	2	109	29.9	1.00	1.00	2.49	3.26	10.09
2,3,4,6,7,8-HxCDF	2	345	94.8	1.00	1.00	1.00	1.00	3.86
1,2,3,7,8,9-HxCDF	2	ND						
1,2,3,4,6,7,8-HpCDF	2	149	40.9	1.00	1.00	2.22	3.07	19.53
1,2,3,4,7,8,9-HpCDF	2	ND						
[OCDD OR OCDF]	4	360	98.9	2.00	2.00	2.00	2.00	11.35
Total PCDFs				9.50	14.39	18.04	22.60	52.88
344′5-TCB (#81)	10	363	99.7	5.00	5.00	5.00	5.00	10.08
33′44′-TCB (#77)	10	132	36.3	5.00	5.00	11.09	14.20	41.54
33′44′5-PenCB (#126)	10	13	3.6	5.00	21.62	34.25	48.35	218.79
33′44′55′-HxCB (#169)	10	19	5.2	5.00	16.82	24.52	32.56	85.92
Total non-*ortho* PCBs				20.00	53.42	76.02	99.52	281.74
2′344′5-PeCB (#123)	10	6	1.6	5.00	68.64	112.71	156.26	941.94
23′44′5-PeCB (#118)	10	0	0.0	635.80	3875.39	5860.12	8454.33	25,243.31
2344′5-PeCB (#114)	10	6	1.6	5.00	230.99	343.92	475.61	1695.19
233′44′-PeCB (#105)	10	0	0.0	256.11	992.24	1479.96	2069.13	5991.69
23′44′55′-HxCB (#167)	10	1	0.3	5.00	482.22	713.17	1002.73	3430.73
233′44′5-HxCB (#156)	10	0	0.0	282.13	1345.64	1978.72	2726.27	9421.77
233′44′5′-HxCB (#157)	10	1	0.3	5.00	333.09	489.52	670.48	2712.74
233′44′55′-HpCB (#189)	10	3	0.8	5.00	168.63	238.74	337.06	950.16
Total mono-*ortho* PCBs				1724.33	7747.11	11,471.65	15,641.75	49,632.02
Total dioxins				1847.56	8149.66	11,968.14	16,432.17	50,477.45
PCDDs-TEQ				1.65	5.09	6.92	9.20	29.32
PCDFs-TEQ				0.64	1.79	2.38	3.06	7.77
Non-*ortho* PCBs-TEQ				0.65	2.75	4.22	5.86	23.17
Mono-*ortho* PCBs-TEQ				0.05	0.23	0.34	0.47	1.49
Total dioxins-TEQ				3.17	10.14	13.89	18.35	43.35

ND, not detectable; DL, detection limit. TEQs were calculated with toxic equivalency factor values (Van den Berg et al., 2006).

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

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

[a] Number (%) of infants who developed allergies or infections.
[b] Per increasing unit of BMI.
* $p<0.05$.
** $p<0.01$.

than that in female offspring (median; 0.3 μg/kg) (Luebke et al., 2006). A few human studies have shown gender-specific differences in the effects of prenatal exposure to DLCs with respect to gender ratio (Hertz-Picciotto et al., 2008; Mocarelli et al., 1996), lymphocyte subset rate (Nagayama et al., 2007), and birth weight (Sonneborn et al., 2008; Konishi et al., 2009). Similar to previous findings, our results show that male offspring may be more susceptible to DLCs than female offspring.

Significant positive associations were observed for PCDDs for the risk of otitis media among all infants and male infants. Borderline significant trends were observed for non-*ortho* PCBs among male infants. Uncertain associations were observed for mono-*ortho* PCBs. Each congener of OCDD, TCB-77, and HxCB-157 may be a partial cause of each association. However, these associations were independent of the magnitude for toxic potency such as half-lives, which are 3.7, 0.5, and 18 years for OCDD, TCB-77, and HxCB-157, respectively (Nakai et al., 2001). In addition, each contribution rate to the total dioxin TEQ is low. The rates (%) of OCDD, TCB-77, and HxCB-157 are 1.0, 0.01, and 0.11, respectively. These effects of each congener on otitis media may represent effects of other congeners because complex mixtures of DLCs in mammals may have multiple effects (Smialowicz et al., 2008). Our results indicate that not only PCDD/Fs but also DL PCBs may contribute to infections in infancy.

We found no relationship between DLC levels and infections except for otitis media. This finding may be due to unknown confounding factors that potentially influence the development of infections. For a few months after birth, the sustained effects of

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

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

[a] Adjusted for maternal age, pre-pregnancy BMI, parental allergic history, maternal educational level, parity, infant gender, duration of breast-feeding, environmental tobacco exposure, day care attendance, and blood sampling period.
[b] Adjusted for maternal educational level, parity, infant gender, duration of breast-feeding, environmental tobacco exposure, day care attendance, and blood sampling period.
[c] Quartiles applied to ordinal variables in the model.
* $p < 0.05$.

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

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

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

[a] Quartiles applied to ordinal variables in the model.
* $p < 0.05$.

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

Although no significant trend was observed for DLCs with respect to any particular allergy, the risk of food allergies was