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

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

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

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

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

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

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

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

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

4. Discussion

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

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

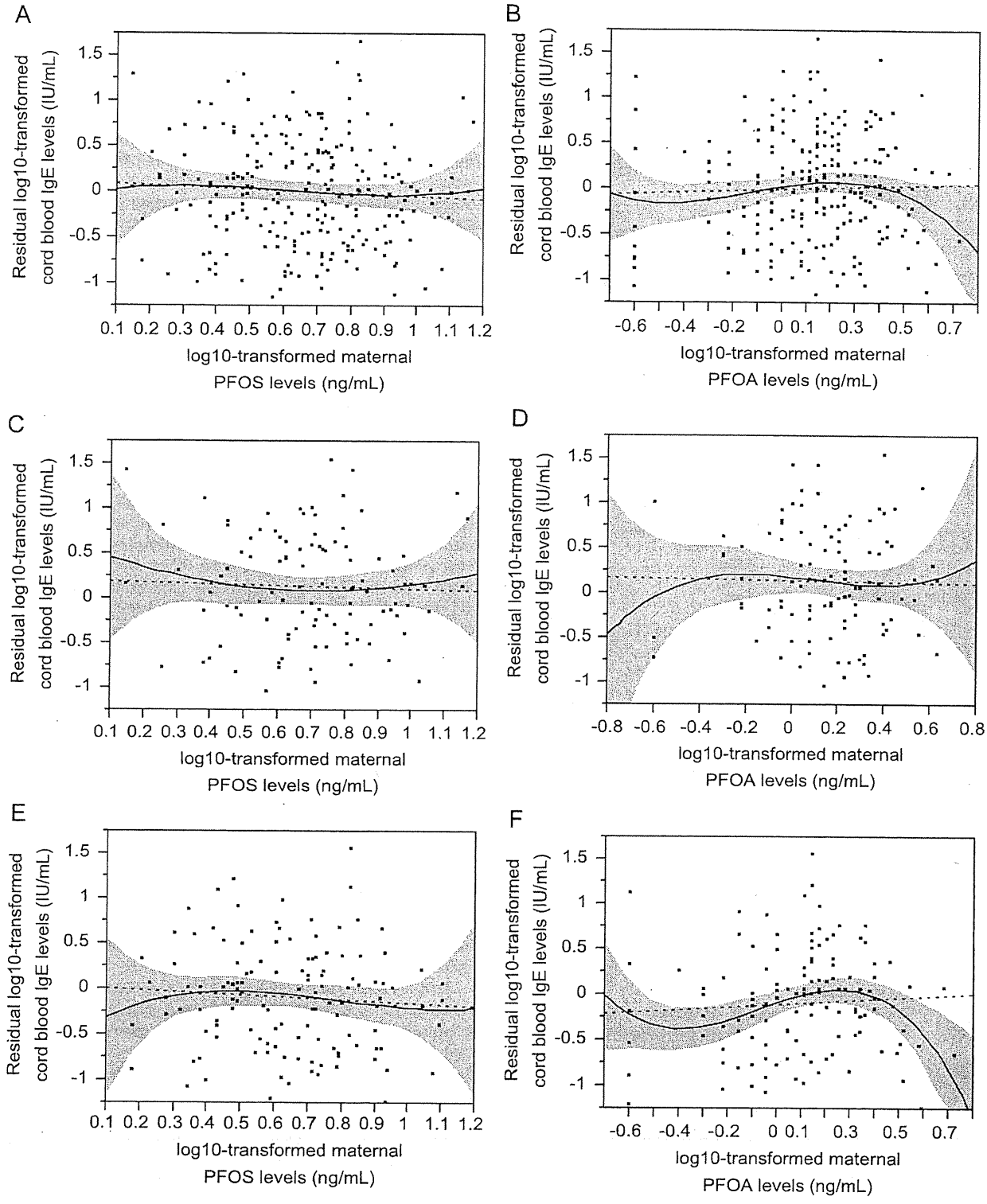

Fig. 1. Association between maternal $\log_{10}$-transformed PFOS and PFOA levels (ng/mL) and residual of potential confounding variables to $\log_{10}$-transformed cord blood IgE levels (IU/mL). The dashed lines denote the predicted fit from a linear regression model. The solid lines denote the predicted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

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

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

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

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

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

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

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

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

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

5. Conclusions

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

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特集　地域における母子保健縦断調査の活用

環境と子どもの健康に関する北海道コホートの成果と今後の課題

岸　玲子

1. 研究の背景

環境要因が子どもの健康に与える影響，とりわけ環境化学物質の胎児期曝露による影響に世界的な関心が高まっている．背景として，1996年発刊のColbornら[1]による「奪われし未来（Our Stolen Future）」において，環境化学物質の内分泌かく乱作用の影響は胎児期がもっとも感受性が高いとされたこと，翌年1997年の8カ国環境大臣会合において「マイアミ宣告」が採択され，子どもの環境保健は環境問題の最優先事項であり，政策の実施が緊急の課題となったことがあげられる．一方，“疾病の胎児期起源説（Fetal origins hypothesis）”として循環器疾患や2型糖尿病などへの罹患のしやすさが胎児期の低栄養等の影響を受けるというBarker仮説[2]によれば，胎児期に過酷な環境に適応し「倹約型」体質にプログラミングされ小児期の肥満や成人期疾患につながる懸念があることが指摘されている．さらに「Developmental Origins of Health and Diseases（DOHaD）」概念に発展し，胎児期から老年期まで生涯を通じたライフコースアプローチによる疫学研究が大きな関心をもたれるようになったこともあげられ[3]，子どもの健康に与える環境要因を解明するために，世界中の国と地域で出生コホート研究が実施されている．

1. 北海道研究と環境省エコチル調査の関係

わが国では1990年代には出生後の乳幼児を追跡する調査がいくつかの地域で実施されていたが，胎児期曝露に焦点をあて出生前から追跡した研究はほとんど存在しなかった．そこで，筆者らは厚生労働省および文部科学省の研究助成を受け，2001年から「環境と子どもの健康に関する北海道研究（北海道スタディ）」[4]を立ち上げた．胎児期の母親の血液，分娩時の臍帯血などを長期保存し，先天異常，出生時体格，神経発達，アレルギー疾患など種々のアウトカムについて，環境化学物質の実測値にもとづく曝露リスク評価をこれまで約10年間にわたって調査してきた．この北海道スタディは，514人と20,000人の大規模コホートで，北海道地域の母親と子どものサンプルから多くの科学的な成果が生まれ，すでに海外でも評価されるコホートとなっている[5,6]．

2011年1月から始動する環境省の「子どもの健康と環境に関する全国調査（エコチル調査）の基本設計段階では，その先駆け的な研究として北海道コホートから多くの関係資料を提供し，北海道コホートはその原型（計画のモデル）ともなった．したがって，今後の環境省エコチル研究では（厚生労働省や文部科学省という出所の違いはあるものの），北海道スタディや同じ時期にスター

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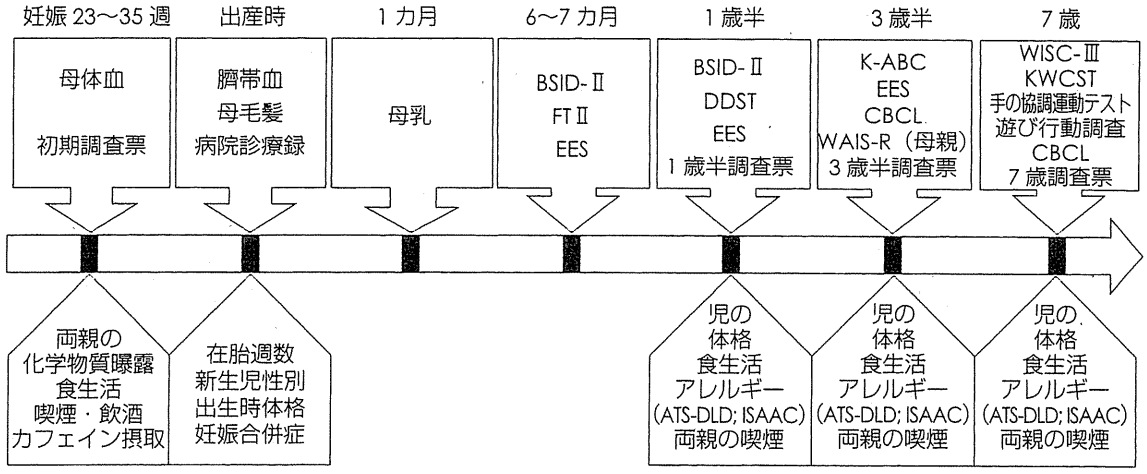

図1　環境と子どもの健康に関する北海道研究（札幌市内1産院コーホート）のアウトカム測定
各名称については，最後のページの注釈を参照いただきたい．

トしている東北スタディの結果をふまえて計画実施する方が，すべてこれらはわが国の公的資金により実施された研究であることを考えると，コスト的・労力的にみても妥当な進め方と思われる．そこで本稿では，北海道スタディの概要と最近の科学的知見を紹介し，最後に地域における子どもの健康と環境に関する今後の調査研究のあり方や課題についても述べることとする．

2.「環境と子どもの健康に関する北海道研究」の概要

1）研究デザインの特徴

「環境と子どもの健康に関する北海道研究：先天異常，発達，免疫アレルギー（北海道スタディ）：The Hokkaido Study of Environment and Children's Health, malformation, development & allergy」は2つのコホートで構成される．

特徴は，①低濃度の環境要因の影響解明に焦点を当てたこと，②前向き研究とし母体血および臍帯血の採取保存により，器官形成期など胎児期の環境要因について曝露測定を行なったこと，③先天異常，体格，神経行動発達，甲状腺機能，免疫機能など種々のアウトカムを対象に，④リスク評価を行なったこと，さらに⑤個人の感受性素因に着目し，環境と遺伝の交互作用を解明する目的で，化学物質代謝酵素・Ahレセプター・神経伝達物質受容体等の遺伝子多型も考慮したハイリスク群の発見と予防対策の検討を行なっていることである．

「札幌市内1産院コホート」では，妊娠週数23〜35週の妊婦514人とその出生児を前向きに追跡し，児の神経発達への影響を測るために詳細な対面調査を実施している（図1）．BSID-Ⅱを6カ月時と18カ月時に実施，Fagan testを7カ月時に，日本版DDSTを18カ月時に行ない，42カ月時には日本版K-ABCと母親のWAIS-Rを，43カ月時にはCBCLを実施し，就学時以降は児の行動発達調査を実施している．臍帯血IgEや出生後の感染症，アレルギーなど免疫系への影響を調べている．環境要因としては，PCB・ダイオキシン類，PFOS（perfluorooctanesulfonate：ピーフォス）・PFOA（perfluorooctanoate：ピーフォア），水銀の測定を行ない，農薬，ビスフェノールA，OH-PCBなどの測定も進み，アウトカムとの関係を報告している（表1）．

「北海道大規模コホート」は，全道の30の産科施設に協力をいただき（図2），器官形成期にあたる時期に母体血の採取と質問票の回収を行ない，臍帯血を採取し，マーカー奇形55種を調べ，生後は発育とアレルギー，行動発達に関し環境要因との関係を追跡している．20,000人を目標に

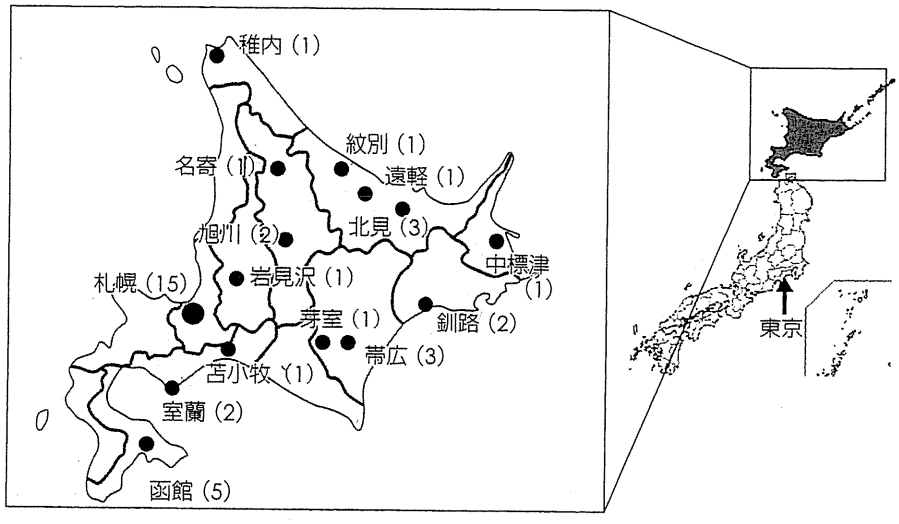

図2　環境と子どもの健康に関する北海道研究（先天異常・発達・アレルギー）
人口は560万人，疫学研究を実施しやすい規模，3医科大学の協力が可能であった．

表1　環境と子どもの健康に関する北海道スタディの測定項目

測　定	備　考
曝露評価	
PCDDs，PCDFs	母体血，臍帯血，母乳
PCBs	母体血，臍帯血，母乳
OH-PCBs	母体血，臍帯血，母乳
PFOS，PFOA	母体血，臍帯血，母乳
BPA，NP	母体血，臍帯血，母乳
DEHP	母体血，臍帯血，母乳
Pesticide	母体血，臍帯血，母乳
Heavy metals	母体血，臍帯血，母乳
MeHg	母体血，臍帯血，母毛髪
Cotinine	母体血，臍帯血，母毛髪
その他の生化学検査	
TSH，FT4	母体血，乳児血中
Folic acid	母体血，臍帯血
IgE，IgA	臍帯血

BPA：ビスフェノールA，NP：ノニルフェノール，
DEHP：フタル酸ビス（2-エチルヘキシル），
Pesticide：殺虫剤，Heavy metals：ヘビーメタル，
MeHg：メチル水銀，Cotinine：コチニン，
TSH：甲状腺刺激ホルモン，FT4：遊離サイロキシン，
Folic acid：葉酸，IgA：免疫グロブリンA

2010年6月現在，妊婦約19,100人が参加し，現在も登録を継続している．これまで尿道下裂，停留精巣の症例対照研究で環境要因とともに，エストロゲン代謝関連など遺伝的素因関与の可能性を報告してきたが，今後は先天異常やアレルギー，小児発達障害などについて大規模コホートの利点を活用した新知見の集積が期待される（図3）[7]．

2）これまで得られた成果

（1）環境化学物質の次世代影響

①PCBダイオキシン類と出生時体格

PCDDs，PCDFsおよびPCBsは，親油性かつ難分解性の有機塩素化合物で，環境中に広範囲に分布し，主に食物連鎖を介してヒトの体内に蓄積される．生体内の半減期が長く体内に長期に蓄積され，また母乳および臍帯を介して母親から児へ移行する．高精度のGC/MS分析を用いて，世界で初めて母体血中のPCDDs，PCDFsの同族異性体分析およびdioxin-like PCBs濃度を測定し，またWHOが設定したTEFを用いてダイオキシン類（29種類）のTEQ（毒性等価量）の算出を行ない，交絡要因を調整した結果，総PCDFs濃度，総PCDFs/TEQ濃度と出生体重との間に有意な負の関連を認めた．男児では総PCDDs濃度，総TEQ値が高いほどリスクを上げ出生時体重が有意に低かった．女児ではそのような傾向は認められなかった（表2）[8]．

②有機フッ素系難燃剤PFOS，PFOAと出生時体格

1950年頃から難燃剤として世界で使用されてきたPFOS，PFOAの濃度と出生体重との関連を

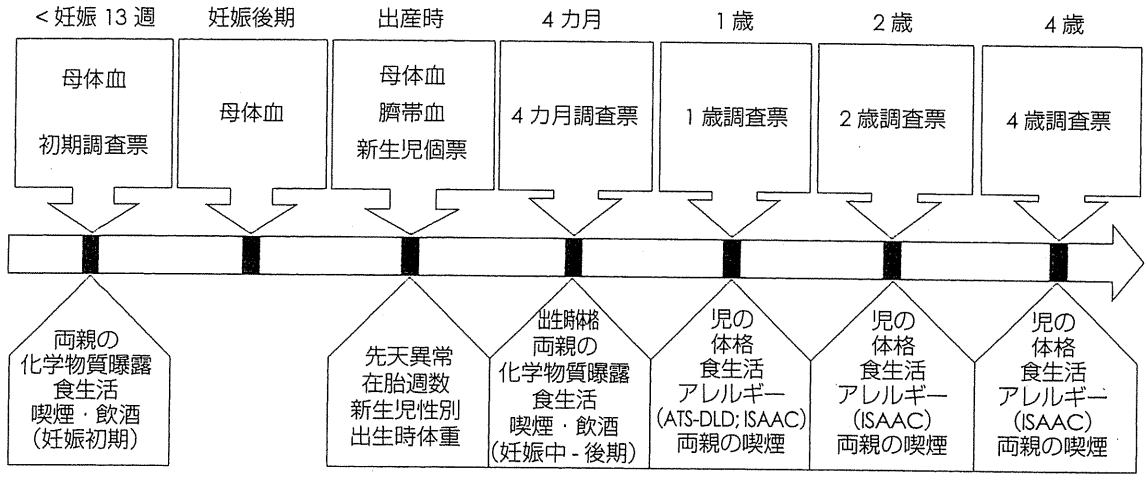

図3　環境と子どもの健康に関する北海道研究（北海道大規模コーホート）のアウトカム測定

表2　PCDDs/PCDFs および DL-PCBs と出生時体重の関係（多重回帰分析）

	全体※1)			男児※2)			女児※2)		
log 10 scale	ベータ※3)	(95%CI)	p 値	ベータ※3)	(95%CI)	p 値	ベータ※3)	(95%CI)	p 値
Total level (pg/g lipid)									
総 PCDDs 濃度	−92.5	(−282.2〜97.1)	0.338	−125.7	(−402.3〜150.8)	0.371	−19.3	(−294.0〜255.5)	0.890
総 PCDFs 濃度	−272.7	(−505.8〜−39.5)	0.022*	−237.6	(−595.2〜119.9)	0.191	−304.9	(−620.6〜10.7)	0.058
総 PCDDs/PCDFs 濃度	−101.7	(−294.6〜91.2)	0.301	−136.6	(−418.3〜145.1)	0.34	−28.7	(−307.5〜250.1)	0.839
総 non-*ortho* PCBs 濃度	−113.5	(−281.9〜54.9)	0.186	−90.7	(−350.4〜169.0)	0.491	−122.4	(−347.9〜103.2)	0.286
総 mono-*ortho* PCBs 濃度	−125.3	(−277.4〜26.8)	0.106	−138.6	(−372.7〜95.4)	0.244	−104.3	(−308.7〜100.1)	0.315
総 DL-PCBs 濃度	−125.9	(−278.2〜26.4)	0.105	−138.7	(−373.1〜95.7)	0.245	−105.3	(−309.9〜99.3)	0.311
総 PCDDs/PCDFs and DL-PCBs 濃度	−131.5	(−288.7〜25.6)	0.101	−148.5	(−391.1〜94.1)	0.229	−106.8	(−317.6〜103.9)	0.319
WHO-2006※4) (TEQ pg/g lipid)									
総 PCDDs/TEQ 濃度	−231.5	(−417.4〜−45.6)	0.015*	−331.4	(−607.4〜−55.5)	0.019*	−126.3	(−384.5〜131.9)	0.336
総 PCDFs/TEQ 濃度	−258.8	(−445.7〜−71.8)	0.007**	−269.8	(−561.5〜21.9)	0.07	−241.7	(−491.7〜8.4)	0.058
総 PCDDs/PCDFs/TEQ 濃度	−256.4	(−448.6〜−64.2)	0.009**	−338.7	(−628.2〜−49.1)	0.022*	−173.9	(−437.6〜89.8)	0.195
総 non-ortho PCBs/TEQ 濃度	−116.1	(−245.9〜13.7)	0.079	−107.3	(−306.1〜91.5)	0.288	−114.8	(−289.4〜59.8)	0.196
総 mono-ortho PCBs/TEQ 濃度	−125.3	(−277.4〜26.8)	0.106	−138.6	(−372.7〜95.4)	0.244	−104.3	(−308.7〜100.1)	0.315
総 DL-PCBs/TEQ 濃度	−119.9	(−252.3〜12.6)	0.076	−112.1	(−315.1〜91.0)	0.278	−117.5	(−295.6〜60.5)	0.195
総 TEQ 値	−220.5	(−399.2〜−41.9)	0.016*	−289.5	(−561.7〜−17.3)	0.037*	−144.2	(−386.7〜98.4)	0.243

※1）多重線形回帰モデルにて算出．妊娠期間，母親の年齢，妊娠前の母親の身長，母親の体重，妊娠中の喫煙状態，近海の魚の摂取量，採血期間，子どもの性別で調整．
※2）多重線形回帰モデルにて算出．妊娠期間，母親の年齢，妊娠前の母親の身長，母親の体重，妊娠中の喫煙状態，近海の魚の摂取量，採血期間で調整．
※3）β係数は出生時体重の減少を示す．PCDDs/PCDFs と DL-PCBs のレベルが 10 倍増加したときの値．
※4）TEQ（毒性等量）の算定は WHO2006（Van den Berg 2006）による．
*：$p < 0.05$，**：$p < 0.01$

調べたところ，母体血清PFOS濃度は出生体重との間に負の関連を認めた（log10-unit：β=-148.8g, 95% CI：-297.0 to -0.5）．しかし，PFOAの影響はみられなかった[9]．

③ダイオキシン・PCB類の児の神経発達に与える負の影響

児の神経発達に与える負の影響が示された．PCDFとPCDDの異性体濃度が高くなると生後6カ月時のBSID-IIの得点が低くなる負の関連が，特に運動発達に顕著にみられた．総TEQ値もBSID-IIの得点と有意な負の関連が運動発達でみられ低い得点と有意に関連した[10]．

④免疫・アレルギーへの影響

母体血中ダイオキシン類濃度が高いほど臍帯血IgEレベルが低下した[11]．18カ月までのアレルギー症状および感染症との関連を検討したところ，アレルギーとの有意な関係は認められなかったが，ダイオキシンレベルは中耳炎と関連が認められた．TEQ値はPCDFsが1増加すると中耳炎オッズ比が1.36倍と有意に増加した．男児のみ母体血中ダイオキシンレベル増加に伴い中耳炎オッズ比の有意な増加が認められ，女児では有意な関連が認められなかった[12]．

(2) 遺伝的感受性素因によるハイリスク群の存在

母親のCYP1A1遺伝子，AhR遺伝子，およびGSTM1についてみると，AhR遺伝子とCYP1A1遺伝子の特定の組み合わせで体重への影響がマイナス315gともっとも低かった．CYP1A1遺伝子TC/CC型ではTT型よりも酵素活性が上昇しているため，中間代謝物であるジオールエポキシドなどの発がん性物質の生成が促進され影響に個体差がみられたと考えられる[13]．

発がん物質ニトロソアミン類代謝活性化に関与するNQO1遺伝子の多型の検討も行なった．喫煙母親のNQO1遺伝子多型がCT/TT型では体重が77g低いのに対して，CC型では体重低下が-199gであった．NQO1遺伝子の多型は身長と頭囲にも影響がみられ，より大きな低下を示した[14]．妊娠初期に禁煙した場合は非喫煙の母親と変わらなかった．

3. 北海道コホートの今後の予定

以上，まとめると，北海道コホートでは主として環境化学物質の次世代影響を検討し世界ではじめて同属異性体レベルでPCB・ダイオキシン類の胎児期曝露影響が詳細に検討され，生下時体重に負の影響が示された．ダイオキシン類では生後発達への影響や感染症罹患リスクを上げる可能性も示唆された．総PCDDs濃度，総PCDFs/TEQ濃度と出生体重との関連には性差が認められ，男児の方が感受性は高かった．性差の原因については，Ah受容体とエストロゲン受容体の両者の作用で引き起こされる可能性が示唆されている．しかし，詳細なメカニズムは不明であるので，今後，種々の角度から検討を進める予定である．

PFOS・PFOAについては，すでにPOPs条約（ストックホルム条約）に入れられたが，わが国ではまだ規制が弱い．北海道（小規模）コホートでは出生体重への影響が認められたが，18カ月時までの感染症やアレルギー疾患に対しては影響がなかった．今後，大規模コホートでさらに検討を進める予定で準備をしている．今後，先天異常や生後発達への影響についても解析する必要がある．農薬やビスフェノールAについては現在測定を進めている．

北海道コホートでは喫煙に関して能動喫煙と受動喫煙をコチニン量で評価しているので，今後，それらと児の発達などの関係について解析を進める予定である．また，葉酸値を全員について測定しているので，葉酸サプリメントの摂取や葉酸値そのものと児の胎内成長や先天異常なども解析を進めている．

今後の課題として，①アレルギーやADHDなどがより明確になる学童期以降までの追跡，②複合曝露による影響の評価，③生後曝露の測定と評価，理由は出生コホートでは母親の胎内での胎児期曝露の出生時点，および生後の影響について主に観察解明が行なわれるが，同時に生後の発育過程では，どの子どもも栄養摂取状態が異なり，また家庭や学校の場などでの種々の曝露がある．胎

児期曝露のみならず，成長に合わせて各時期に確実に環境要因の評価とアウトカムの追跡していくことが重要である，④エピジェネティック作用の検討があげられる．

さらに特に，⑤「社会的要因」の重要性を指摘し，地域における子どもたちの健康と安全の問題に，これまで以上に積極的な関与と情報発信が不可欠であると考えている．特に，胎児期に参加した子どもが学童期を迎えた今，化学物質などの生活環境要因と遺伝要因に加えて今後は社会的要因の役割が高くなってきていることが推察される．子どもの成育環境として家庭や地域の社会経済的な環境は，環境化学物質と並ぶかそれ以上に子どもの健康を左右する重要な要因であることは明らかで，北海道コホートから，今年度の日本衛生学会に演題を出しているが，中島[15]は6カ月と18カ月と比較して，母体血PCB・ダイオキシン類濃度と6カ月のBSID-IIのMDI，PDIには有意な負の関連（濃度が高くなるとBSID-IIの得点が低くなる）がみられ，PDIでより多くその関連がみられた．しかし，18カ月ではMDIと1つの異性体，PDIでは2つの異性体と1つのTEQ値にしか有意な負の関連はみられず，6カ月に比べ減少した．海外でも乳幼児期における内分泌かく乱物質の神経発達への悪影響は，学齢期には母乳保育や適切な家庭環境による知的な刺激により改善する傾向にあると示唆している．北海道コホートの最新の結果でも，日本の子どもで同様の結果である可能性を示している．

すでに北海道コホートでは種々の家庭環境要因も測定しているので，発達過程に及ぼす社会環境要因の影響を含めて検討を行なうことになる．北海道コホートはすでにベイリーのスケールなど発達スコアを観察してきたが，今後はさらに社会環境要因と遺伝的な交互作用も発表していく予定である．2006年にはOECD統計によれば，わが国が先進国の中ではアメリカについで第2位の貧困層が多く，13人に1人が貧困といわれるからである．北海道スタディでは今後，それら社会的環境要因を含めた調査や解析によって健康障害や疾病のリスク要因を明らかにしていくことが重要と考えているからである．

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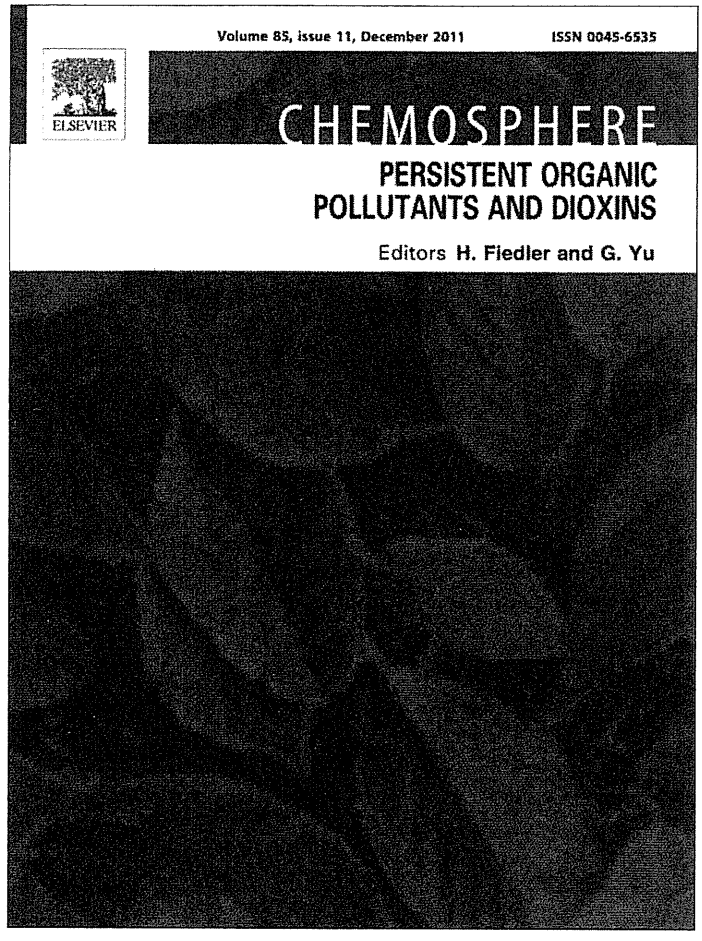
注

BSID-Ⅱ：The Bayley Scales of Infant Development (second edition)
FT Ⅱ：The Fagan Test of Infant Intelligence
EES：The Evaluation of Environmental Stimulation
DDST：The Denver Development Screening Test
K-ABC：The Kaufman Assessment Battery for Children
CBCL：The Child Behaviour Checklist
WAIS-R：The Wechsler Adult Environmental Stimulation
WISC-Ⅲ：The Wechsler Intelligence Scale for Children (third edition)
KWCST：Wisconsin Card Sorting Test (Keio Version)
ATS-DLD：American Thoracic Society-Division of Lung Disease
ISAAC：International Study of Asthma and Allergies in Childhood



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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

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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

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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		0.37
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

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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

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