Association of Maternal Serum Concentration of Hydroxylated Polychlorinated Biphenyls with Maternal and Neonatal Thyroid Hormones: The Hokkaido Birth Cohort Study

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Running title: Hydroxylated-PCB exposure and thyroid hormones

Abstract

Background: Evidence on the toxicity of hydroxylated metabolites of polychlorinated biphenyls (OH-PCBs) for thyroid hormones (TH) is limited, and the underlying mechanism remains unknown.

Objectives: We aimed to investigate the effects of environmental prenatal exposure to OH-PCBs and maternal and neonatal TH levels, taking the maternal-fetal TH transfer into account.

Methods: In this prospective birth cohort (the "Hokkaido study") we included 222 mother-neonate pairs. We measured five OH-PCB isomers in maternal serum samples either during pregnancy or within 5 days of delivery. Thyroid stimulating hormone (TSH) and free thyroxine (FT4) levels were obtained from maternal blood samples at an early gestational stage (median; 11.1 weeks) and from heel prick samples of neonates between 4 and 7 days after birth. Multiple linear regression analysis and structural equation modeling (SEM) were performed to investigate the associations between maternal OH-PCB and maternal and neonatal TH levels.

Results: Median concentration of Σ OH-PCBs was 25.37 pg/g wet weight. The predominant isomer was 4-OH-CB187, followed by 4-OH-CB146+3-OH-CB153. In the fully adjusted linear regression analysis, maternal Σ OH-PCBs was positively associated with maternal FT4, and 4-OH-CB187 was positively associated with both maternal and neonatal FT4 levels. Maternal OH-PCBs showed no significant association with TSH among mothers and neonates. Path analysis indicated the indirect pathway from 4-OH-CB187 exposure to increased neonatal FT4, via maternal THs and neonatal TSH.

Conclusions: These findings suggest that maternal exposure to OH-PCBs during

pregnancy may increase both maternal and neonatal FT4 levels. Neonatal FT4 is presumed to be increased by prenatal 4-OH-CB187 indirectly, and this process may be mediated by maternal THs and neonatal TSH.

Key words: Prenatal exposure; Hydroxylated polychlorinated biphenyls; Prospective cohort study; Thyroid hormone; Structural equation modeling

Abbreviations:

AGFI, adjusted goodness of fit index

AMC, antimicrosome antibody

ATG, antithyroglobulin antibody

CFI, comparative fit index

CI, confidence interval

FT4, free thyroxine

GFI, goodness of fit index

IQR, interquartile range

LC/MS/MS, liquid chromatography-tandem mass spectrometry

LOD, limit of detection

OH-PCBs, hydroxylated polychlorinated biphenyl

PCBs, polychlorinated biphenyl

T4, thyroxine

THs, thyroid hormones

TSH, thyroid stimulating hormone

TTR, transthyretin

Introduction

During the last decade, the advances in technology have made it possible to detect the metabolites of polychlorinated biphenyl (PCBs). Hydroxylated PCBs (OH-PCBs) are the predominant metabolites of PCBs, and are mainly formed by cytochrome P450 oxidation of PCBs (Letcher et al. 2000). There is increasing evidence that OH-PCBs accumulate in the environment, in animals, and in humans, at detectable levels (Bergman et al. 1994; Hovander et al. 2002; Park et al. 2007; Soechitram et al. 2004). They are water-soluble and therefore rapidly eliminated via the urine or excreta (Soechitram et al. 2004). However, a recent study reported that some OH-PCB congeners showed longer half-lives than the parent PCBs, with evidence of retention in the human blood for several years (Quinete et al. 2017). Furthermore, previous studies have reported that the ratio of ∑OH-PCB/∑PCBs in cord blood was higher than that in maternal blood (Kawashiro et al. 2008; Park et al. 2008; Soechitram et al. 2004). These reports indicate that OH-PCBs have a higher ability than PCBs in passing through the placenta and reaching the fetus. Although the adverse effects of prenatal exposure to PCBs on the health of children has been extensively reported (Bell 2014), there is a possibility that those effects may have been caused by OH-PCBs, and not PCBs. However, there are limited studies examining OH-PCB toxicity.

Thyroid hormones (THs) are crucial for fetal and neonatal neurodevelopment (Haddow et al. 1999; Zoeller et al. 2002). OH-PCBs are reported to possess a higher binding affinity for transthyretin (TTR) than thyroxine (T4) (Brouwer et al. 1998; Lans et al. 1993), which may disrupt TH levels. Animal studies previously reported that maternal exposure to 4-OH-CB107 was associated with decreased total and free T4 (FT4) levels and elevated thyroid stimulating hormone (TSH) in fetal plasma among

rats (Meerts et al. 2002). Additionally, one study among hooded seal pups showed that 4-OH-CB107 and 3-OH-CB138 were inversely associated with FT4: free triiodothyronine (FT3) and Total T3:FT3 ratios, respectively (Gabrielsen et al. 2011). As for human, three epidemiological studies involving Dutch and Japanese participants reported a significant association between prenatal exposure to OH-PCBs and neonatal or infant THs (Hisada et al. 2013; Otake et al. 2007; Soechitram et al. 2017). However, a Canadian study examining Inuit women and their infants found no significant association (Dallaire et al. 2009b). These findings suggest the adverse influence of OH-PCBs on newborn and infant THs; however, the findings, based on small sample sizes (under 100 participants) are controversial. Moreover, maternal THs are transferred to the fetus throughout pregnancy to support fetal development. The fetus is completely dependent on maternal TH supply during the first trimester of pregnancy, before fetal TH synthesis and secretion develops (Vulsma et al. 1989). Maternal hypotyroxinemia during early pregnancy, may be an important risk factor for impaired infant development (de Escobar et al. 2004; Pop et al. 1999). Therefore, disruption of maternal TH homeostasis in the early stages of pregnancy may impair fetal development. Early gestational screening tests for maternal THs have been implemented in many parts of the world to prevent abnormal fetal development. Nevertheless, few studies have investigated the association of exposure to OH-PCBs during pregnancy and neonatal TH concentration, taking the possibility of disruption of maternal THs by OH-PCBs into account.

The aim of this study is to determine whether maternal exposure to OH-PCBs at environmental levels is associated with TH levels in mothers and neonates. We also aim to investigate the indirect effect of maternal THs, affected by OH-PCB exposure, on

neonatal TH levels, using path analysis.

Material and Methods

Study design and population

This prospective birth cohort study was based on data from mothers and their neonates delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan (Sapporo cohort in Hokkaido Study on Environment and Children's Health). Details regarding the study population, data collection, and the questionnaires have been previously described (Kishi et al. 2011; Kishi et al. 2013; Kishi et al. 2017). In brief, pregnant women at 23-35 weeks of gestation and planning to deliver at one obstetric hospital, Toho Hospital, in Sapporo city were recruited between July 2002 and October 2005. All participants were native Japanese women residing in Sapporo and surrounding areas. Their children have been followed up as subjects in the prospective cohort. Out of a total of 514 women, we excluded those who experienced miscarriage, stillbirth, relocation, or voluntary withdrawal (n=10), and those who delivered twins (n=7). The following exclusion criteria were applied to the remaining 497 mother-neonate pairs: current maternal treatment for thyroid disease (n=14), lack of data on maternal OH-PCB levels (n=238), lack of data on both maternal and neonatal serum TH levels (n=23). Finally, data on 222 mother-neonate pairs were included in this analysis. The protocol for this study was approved by the ethics review board for epidemiological studies at the Hokkaido University Graduate School of Medicine and the Hokkaido University Center for Environmental and Health Sciences (14-10-1), and the study conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Data collection

At enrollment, participants completed a self-administered questionnaire to obtain relevant sociodemographic, medical, and behavioral information, as described previously (Kishi et al. 2011; Kishi et al. 2013; Kishi et al. 2017). In brief, the questionnaire extracted information related to medical history of thyroid diseases, dietary habits fish consumption, intake of seaweed and intake of iodine including supplements/eggs, smoking status, alcohol intake, household income, and educational levels, during pregnancy. We also obtained information from medical records on maternal age, maternal height, maternal pre-pregnancy weight, parity, pregnancy complications, gestational age, sex of the child, birth weight, and birth length.

Measurement of OH-PCB levels in maternal serum

Forty milliliters of maternal peripheral venous blood under non-fasting condition were collected during the third trimester at the time of the hospital examination following recruitment (Mean of gestational weeks: 35.95 ± 3.99) or within 5 days of delivery, and were stored at −80°C until analysis. We used the simultaneous measurement method for the concentration of OH-PCBs and their parent PCBs, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in the same maternal blood in Fukuoka Institute of Health and Environmental Sciences. The procedure of the measurement has been described in previous reports

(Tobiishi et al. 2013). In brief, each 5g of maternal blood sample was loaded into an extraction cell filled with Isolute. After freeze-drying, OH- [$^{13}C_{12}$]-PCBs was added as internal standards. Acetone: n-hexane was used as the extraction solvent for an accelerated solvent extractor. After the extract was evaporated to near dryness, it was dissolved in n-hexane and treated with sulfuric acid overnight. The separated hexane layer was applied to a silver nitrate/ silica gel column. Following the first fraction containing PCDDs, PCDFs and Co-PCBs, OH-PCBs were eluted with 15mL of 50% dichloromethane / n-hexane as the second fraction. The eluate was concentrated to near dryness with a multiple sample concentrator, and dissolved in 2mL of methanol. After the methanol solution was loaded onto an Envi-18 cartridge with 4mL of methanol, the eluate was concentrated under nitrogen flow and transferred to an LC injection vial with 0.2mL of methanol.

OH-PCBs concentrations in maternal serum were measured via liquid chromatography-tandem mass spectrometry (LC/MS/MS) at the Fukuoka Institute of Health and Environmental Sciences. The following isomers of OH-PCBs were measured in this study: 4-OH-CB107, 4-OH-CB146+3-OH-CB153, 4-OH-CB172, and 4-OH-CB187. We calculated the concentration of ∑OH-PCB as the sum of five congeners. Peaks of 4-OH-CB107, 4-OH-CB172, and 4-OH-CB187 were detected

clearly, but 4-OH-CB146 and 3-OH-CB153 could not be separated in this analytical condition. Therefore, we dealt with the concentrations of those two isomers as 4-OH-CB146+3-OH-CB153. Limits of detection determined as the concentrations corresponding to three times the standard deviation of the responses in chemical blanks were 2.2 pg/g wet weight of whole blood (wt) for each OH-PCB isomer. Samples exhibiting values below the limit of detection (LOD) of the test [2.2 pg/g wt] were assigned a value that was 50% of the LOD of the test for each OH-PCB isomer (1.1 pg/g wet wt).

TH measurement

As reported in our previous study (Kato et al. 2016), thyroid function and anti-thyroid antibody data were obtained by a mass screening test at the Sapporo City Institute of Public Health. The blood samples were collected from mothers between the 6^{th} and 18^{th} week of gestation and from heel prick samples of neonates between 4 and 7 days after birth under non-fasting condition. TSH and FT4 in maternal and neonatal blood was measured by Enzyme-Linked ImmunoSorbent Assay (ELISA) using Enzaplate Neo-TSH and Enzaplate N-FT4 (Bayer Medical, Tokyo, Japan), respectively. The normal reference values for the concentrations of THs were shown in the previous reports. Briefly, as for maternal THs, the normal range of FT4 (ng/dl) in pregnancy was defined as 0.70-2.00 at less than 6 gestational weeks (GW), 0.70-2.30 at 6-9 GW, 0.60-2.10 at 10-3 GW, 0.50-1.90 at 14-15 GW, 0.50-1.80 at 16-17 GW and 0.50-1.60 at

more than 17 GW, and the normal range of TSH was ≤ 6.0 mU/l throughout pregnancy for mothers (Honma et al. 1997). For neonates, TSH concentrations of < 10 μU/ml and 1.0 ≤ FT4 <4.0 ng/dl were defined as normal (Mass Screening in Sapporo City, 2016; Fujiwara et al. 2008). Both TSH and FT4 of mothers and neonates were measured from blood on filtered paper (TOYO ROSHI, Ltd., Tokyo, Japan). The FT4 values were determined for all samples. The TSH values of 31 (14.0 %) maternal and 16 (6.3 %) neonatal blood samples were below the lower limit. For samples with TSH levels below the LOD (0.50 μU/mL), we used a value of half the LOD (0.25 μU/mL). We also used the maternal screening data of antimicrosome antibody (AMC) and antithyroglobulin antibody (ATG) levels because they were known to affect the TH levels. AMC and ATG were measured using indirect agglutination reactions (AMC: Serodia AMC, Fuji-Rebio, Tokyo, Japan; ATG: Serodia ATG, Fuji-Rebio). Values of AMC and ATG above 100-fold of baseline levels were classified as positive (Sakaihara et al. 2000).

Data Analysis.

The analysis regarding the associations between maternal OH-PCB levels and maternal THs during pregnancy were designed as the cross-sectional study, and the analysis regarding to the associations between maternal OH-PCB levels and neonatal THs at birth were designed as the prospective study. The Mann-Whitney test and Spearman correlation test were conducted to examine associations between levels of maternal OH-PCBs, TSH, and FT4 in maternal and neonatal serum and the recorded characteristics of the subjects (Supplemental Tables 1 and 2). Correlations between maternal serum OH-PCB levels and TSH and FT4 levels of mothers and neonates were analyzed using Spearman's correlation test. We also performed multiple linear

regression analysis to evaluate the association between maternal serum OH-PCBs and maternal and neonatal THs. Levels of each OH-PCB and THs were transformed using a $\log_{10}$ scale to account for their skewed distribution. We conducted the directed acyclic graphs (DAGs) to determine potential confounders in the fully adjusted model. The set of variables were selected from the results in Supplemental Table 1, 2, and previous literatures. For maternal analyses, values were adjusted for maternal age at delivery (years), smoking during pregnancy (yes/no), annual household income (< 5 / ≥ 5 million Japanese yen), gestational weeks of maternal blood sampling for the measurement of maternal OH-PCB levels and THs. For neonate analysis, we selected maternal confounding factors (maternal age at delivery [years], annual household income [< 5 / ≥ 5 million Japanese yen], smoking during pregnancy [yes/no], Log_{10}TSH, and Log_{10}FT4), and neonatal sex. All statistical analyses were performed using JMP Pro software (version 12; SAS Institute Inc., NC, USA). P-values of < 0.05 were considered statistically significant. We then conducted path analysis using Analysis of Moment Structures (AMOS) 24.0, and extension software of SPSS (SPSS Inc., Chicago, USA). We tested several hypotheses to determine the relationships between maternal OH-PCBs and maternal and neonatal THs; 1) Maternal exposure to OH-PCBs influences maternal FT4 levels, subsequently, maternal FT4 levels transferred to fetus influences neonatal FT4. 2) Maternal exposure to OH-PCBs influences maternal FT4 and TSH levels, subsequently, maternal FT4 and TSH levels influences neonatal FT4 and TSH. 3) Maternal exposure to OH-PCBs influences maternal FT4 levels, which influence maternal TH parameters, subsequently, neonatal THs was influenced via maternal TH parameters transferred to fetus. In the hypothesis 3), we set a single latent variable to represent the maternal TH parameters which were unmeasured in this study.

Model fit was assessed using the following goodness-of-fit indices: Chi-square, Goodness of Fit Index (GFI), Adjusted Goodness of Fit Index (AGFI), Comparative Fit Index (CFI), and Root Mean Square Error of Approximation (RMSEA). Acceptable model fit was defined in accordance with a non-significant chi-square, GFI, AGFI, and CFI > 0.90, RMSEA < 0.07 (Browne and Cudeck 1993; Hu and Bentler 1999; Tabachnick and Fidell 2007).

Results

Table 1 shows the characteristics of the mothers and their neonates included in this study (n=222). The mean maternal age of delivery was 31.1 ± 4.9. 53.6 % of mothers were multiparous. 14.9 % of mothers smoked during pregnancy and 8.1 % of mothers had alcohol consumption during pregnancy. The mean gestational week of maternal blood sampling for THs was 11.1, and 18 mothers (8.1 %) had positive AMC and/or ATG results. The mean gestational age at birth was 38.9 weeks. Blood samples for neonatal TH tests were obtained at a mean 4.2 days after birth. Forty-nine (22.1 %) neonates were born through caesarian section. Participants included in the current study did no significantly differ from the excluded subjects without OH-PCB data in the original cohort (n=292) on any characteristics except for neonatal birth weight (Supplemental Table 4).

Table 1. Characteristics of included mothers and their children (n=222)

Characteristics		n (%)	Mean ± SD
Maternal characteristics			
Age at delivery (years)		222	31.1 ± 4.9
Pre-pregnancy BMI (kg/m^2)		222	21.1 ± 3.2
Parity	Primiparous	103 (46.4)	
	Multiparous	119 (53.6)	
Annual household income (million yen per year)	<5	160 (72.1)	
	≥5	62 (27.9)	
Educational level (years)	<13	101 (45.5)	
	≥13	121 (54.5)	
Fish consumption per week (g/day)		211	37.8 ± 33.2
Smoking during pregnancy	Nonsmoker	189 (85.1)	
	Smoker	33 (14.9)	
Alcohol consumption during pregnancy	Nondrinker	204 (91.9)	
	Drinker	18 (8.1)	
Intake of seaweed more than once per week	Yes	194 (87.4)	
	No	28 (12.6)	
Intake of iodine including supplements/eggs more than once per week	Yes	10 (4.5)	
	No	212 (95.5)	

Gargling with iodine more than once per month	Yes	25 (11.3)	
	No	197 (88.7)	
AMC and/or ATG positive	Yes	18 (8.1)	
	No	204 (91.9)	
Blood sampling period for OH-PCBs	Before delivery	138 (62.2)	
	After delivery	84 (37.8)	
Gestational week of blood sampling for THs		222	11.1 ± 2.5
Neonatal characteristics			
Sex	Male	105 (47.3)	
	Female	117 (52.7)	
Birth weight (g)	Male	105 (47.3)	3130.7 ± 348.5
	Female	117 (52.7)	3068.4 ± 376.2
Caesarean section	Yes	49 (22.1)	
	No	170 (76.6)	
Gestational weeks for birth (weeks)		222	38.9 ± 1.4
Blood sampling for TH tests (day)		222	4.2± 0.7

SD: standard deviation; BMI: body mass index; AMC: antimicrosomal antibody; ATG: antithyroglobulin antibody; TH: thyroid hormone.

The maternal serum concentrations and the detection frequencies of individual OH-PCB congeners and of the sum of congeners (∑OH-PCB) are shown in Table 2. The median concentration of ∑OH-PCB is 25.37 pg/g wet weight. The most prevalent congener is 4-OH-CB187, followed by 4-OH-CB146+3-OH-CB153. The median concentrations of 4-OH-CB187 and 4-OH-CB146+3-OH-CB153 were 11.07 (interquartile range [IQR]: 6.22–18.23) and 10.53 (IQR: not detected (ND)–15.94) pg/g

wet weight, respectively. 4-OH-CB107 and 4-OH-CB172 were detected in less than 50 % of samples (47.3 % and 24.8 % respectively). Therefore, we excluded these two isomers from the regression analysis. There was no significant difference of OH-PCB and TH concentrations between mothers whose blood were obtained before delivery and those after delivery (Supplemental Table 1 and 2).

Table 2. Distribution of OH-PCB concentrations in maternal serum

	Mean	Geometric Mean	Minimum	25th	Median	75th	Max	>LOD (%)
ΣOH-PCB	30.85	26.83	ND	13.23	25.37	42.23	140.50	
4-OH-CB107	5.27	9.15	ND	ND	ND	8.55	38.42	47.3
4-OH-CB146+3-OH-CB153	11.38	13.11	ND	ND	10.53	15.94	49.59	74.8
4-OH-CB172	1.17	4.13	ND	ND	ND	0.21	11.52	24.8
4-OH-CB187	12.92	12.84	ND	6.22	11.07	18.23	51.66	85.1
Maternal TSH (μU/mL)	1.4	1.0	ND	0.6	1.1	1.8	6.5	86.0
Maternal FT4 (ng/mL)	1.03	0.98	0.51	0.83	0.96	1.15	3.78	100
Neonatal TSH (μU/mL)	2.6	1.9	ND	1.2	2.0	3.4	25.2	93.7
Neonatal FT4 (ng/mL)	2.04	2.00	0.85	1.77	2.00	2.28	3.26	100

OH-PCB, OH-CB: hydroxylated polychlorinated biphenyl; Max: maximum; LOD: limit of detection. ND: Not detected

OH-PCBs in picogram per gram (pg/g) wet weight. ND samples exhibiting values below the limit of detection (LOD) of the test were assigned a value that was 50% of the LOD of the test for each OH-PCB isomer [1.1 pg/g wet weight of whole blood (wt)] and TSH (0.25 μU/mL) in the subsequent analysis.

As for TH status, the median levels of maternal TSH and FT4 levels were 1.1 (IQR: 0.6–1.8) μU/mL and 0.96 (IQR: 0.83–1.15) ng/mL, respectively. Neonatal TSH and FT4 levels were 2.0 (IQR: 1.2–3.4) μU/mL and 2.00 (IQR: 1.77–2.28) ng/mL,

respectively.

The results of the correlations between maternal OH-PCB concentrations and maternal and neonatal THs are shown in Supplemental Table 3. There were no significant correlations in either maternal or neonatal analysis.

Table 3 shows the association between maternal OH-PCB concentrations and maternal THs in the multivariate linear regression analysis. After adjusting for the potential confounders, for maternal age, maternal smoking during pregnancy and annual household income, ΣOH-PCB and 4-OH-CB187 concentrations showed a significant positive association with FT4 (β = 0.048; 95 % confidence interval [CI]: 0.003, 0.094; p = 0.038, β = 0.044; 95 % CI: 0.008, 0.079; p = 0.017). There was no significant association between maternal OH-PCB concentration and maternal TSH.

Table 3. Linear regression models for the associations between maternal OH-PCB concentrations and maternal thyroid hormone levels (n=222).

	Maternal TSH						**Maternal FT4**					
	Crude			**Adjusted**			**Crude**			**Adjusted**		
	β	**95% CI**	**p-value**	β	**95% CI**	**p-value**	β	**95% CI**	**p-value**	β	**95% CI**	**p-value**
Mothers												
Σ OH-PCB	0.020	–0.113, 0.153	0.765	0.003	–0.132, 0.137	0.970	0.031	–0.014, 0.076	0.175	**0.045**	**0.002, 0.089**	**0.042***
4-OH-CB146 +3-OH -CB15	0.015	–0.075, 0.105	0.747	0.017	–0.074, 0.107	0.717	0.017	–0.013, 0.048	0.267	0.022	–0.008, 0.051	0.149

3												
4-OH-CB187	0.016	–0.087, 0.120	0.754	0.003	–0.103, 0.108	0.958	0.032	–0.003, 0.067	0.076[#]	**0.042**	**0.008, 0.076**	**0.015***

OH-PCB: hydroxylated polychlorinated biphenyl; TSH: thyroid stimulating hormone; FT4: free thyroxine; β: partial regression coefficient; CI: confidence interval,

* Significant at the 0.05 probability level

[#] Significant at the 0.10 probability level

Values of OH-PCBs and TH are log transformed.

Adjusted for maternal age, maternal smoking during pregnancy, annual household income and gestational weeks of blood sampling for the measurement of maternal OH-PCB levels and THs.

In the neonatal analysis in Table 4, there were significant positive associations between maternal 4-OH-CB187 and all neonate FT4 (β = 0.029; 95 % CI: 0.002, 0.056; p = 0.034). The stratified analysis by neonatal sex showed a significant positive association between maternal ∑OH-PCB and 4-OH-CB146+3-OH-CB153 and male's FT4 levels, whereas no significant association was observed in female neonates. Neonatal TSH was not associated with maternal OH-PCBs.

Table 4. Linear regression models for the associations between maternal OH-PCB concentrations and neonatal thyroid hormone levels (n=222).

	Neonates TSH						**Neonates FT4**					
	Crude			**Adjusted**			**Crude**			**Adjusted**		
	β	**95% CI**	**p-value**	β	**95% CI**	**p-value**	β	**95% CI**	**p-value**	β	**95% CI**	**p-value**
All neonates [a]												

Σ OH-PCB	0.065	–0.072, 0.201	0.351	0.072	–0.068, 0.212	0.312	0.022	–0.010, 0.055	0.182	0.029	–0.005, 0.063	0.096#
4-OH-CB146+3-OH-CB153	0.040	–0.052, 0.132	0.392	0.039	–0.054, 0.132	0.411	0.006	–0.016, 0.028	0.600	0.008	–0.015, 0.031	0.520
4-OH-CB187	0.069	–0.037, 0.175	0.200	0.085	–0.024, 0.195	0.127	0.022	–0.003, 0.048	0.083#	**0.029**	**0.002, 0.056**	**0.034***
Male neonates [b]												
Σ OH-PCB	–0.014	–0.221, 0.193	0.896	0.021	–0.197, 0.238	0.850	0.033	–0.014, 0.079	0.168	0.049	–0.001, 0.098	0.055#
4-OH-CB146+3-OH-CB153	–0.002	–0.149, 0.144	0.975	0.012	–0.142, 0.166	0.879	0.022	–0.011, 0.055	0.190	0.032	–0.003, 0.067	0.076#
4-OH-CB187	0.059	–0.110, 0.228	0.491	0.082	–0.090, 0.255	0.346	0.019	–0.019, 0.057	0.329	0.029	–0.011, 0.069	0.154
Female neonates [b]												
Σ OH-PCB	0.150	–0.028, 0.329	0.098#	0.151	–0.033, 0.334	0.106	0.013	–0.034, 0.060	0.577	0.010	–0.039, 0.059	0.682
4-OH-CB146+3-OH-CB153	0.083	–0.034, 0.200	0.164	0.094	–0.025, 0.213	0.122	–0.007	–0.037, 0.024	0.670	–0.011	–0.043, 0.020	0.480
4-OH-CB187	0.088	–0.047, 0.222	0.198	0.092	–0.051, 0.235	0.204	0.027	–0.008, 0.061	0.133	0.027	–0.011, 0.064	0.158

OH-PCB: hydroxylated polychlorinated biphenyl; TSH: thyroid stimulating hormone; FT4: free thyroxine; β: partial regression coefficient; CI: confidence interval,

* Significant at the 0.05 probability level (association between maternal OH-PCB and thyroid hormone levels in the adjusted models)

[#] Significant at the 0.10 probability level (association between maternal OH-PCB and thyroid hormone levels in the adjusted models)

Values of OH-PCBs and THs are log transformed.

a; Adjusted for maternal age, maternal smoking during pregnancy, annual household income, Log_{10} maternal TSH, Log_{10} maternal FT4 and neonatal sex.

b; Adjusted for maternal age and maternal smoking during pregnancy and annual household income, Log_{10} maternal TSH and Log_{10} maternal FT4.

We conducted SEM analysis testing hypotheses described above to determine the relationships between maternal OH-PCBs and maternal and neonatal THs. 1)Maternal exposure to OH-PCBs influences maternal FT4 levels, subsequently, maternal FT4 levels transferred to fetus influences neonatal FT4. Figure 1 shows the SEM diagram by path analysis with the standardized estimates of the association between maternal 4-OH-CB187, maternal and neonatal TSH and FT4 in the total mother-neonate pairs following our a priori hypothesis 2) Maternal exposure to OH-PCBs influences maternal FT4 and TSH levels, subsequently, maternal FT4 and TSH levels influences neonatal FT4 and TSH. This diagram satisfied the definition of the goodness-of-fit indices and is the most likely to exist theoretically among our hypotheses (chi-square = 1.580, GFI = 0.997, AGFI = 0.979, and CFI = 1.000, RMSEA =0.000). As indicated in Figure 1, the direct direction of 4-OH-CB187 toward neonatal FT4 levels was not significant. On the other hand, 4-OH-CB187 was positively associated with maternal FT4. As for the association between maternal THs and neonatal THs, maternal TSH showed the highest loading onto neonatal TSH. Maternal FT4 did not show significant associations with neonatal THs. An indirect pathway from maternal FT4 directly increased by 4-OH-CB187 via maternal TSH and neonatal TSH (direction to increase) was presumed. In Supplemental Figure 1, the diagram was shown following our hypothesis 3) Maternal exposure to OH-PCBs

influences maternal FT4 levels, which influence “maternal TH parameters”, subsequently, neonatal THs was influenced via “maternal TH parameters” transferred to fetus, setting a single latent variable to represent the “maternal TH parameters” which were unmeasured in this study. 4-OH-CB187 did not influence neonatal FT4 significantly as well as Figure 1. Maternal TSH had the higher loading onto the latent variable “maternal TH parameters” compared to maternal FT4 influenced by 4-OH-CB187 exposure. “Maternal TH parameters” was positively associated with neonatal TSH, whereas, “maternal TH parameters” was not associated with neonatal FT4. Fit indices were all acceptable (chi-square = 7.194, GFI = 0.968, AGFI = 0.942, and CFI = 0.942, RMSEA = 0.033).

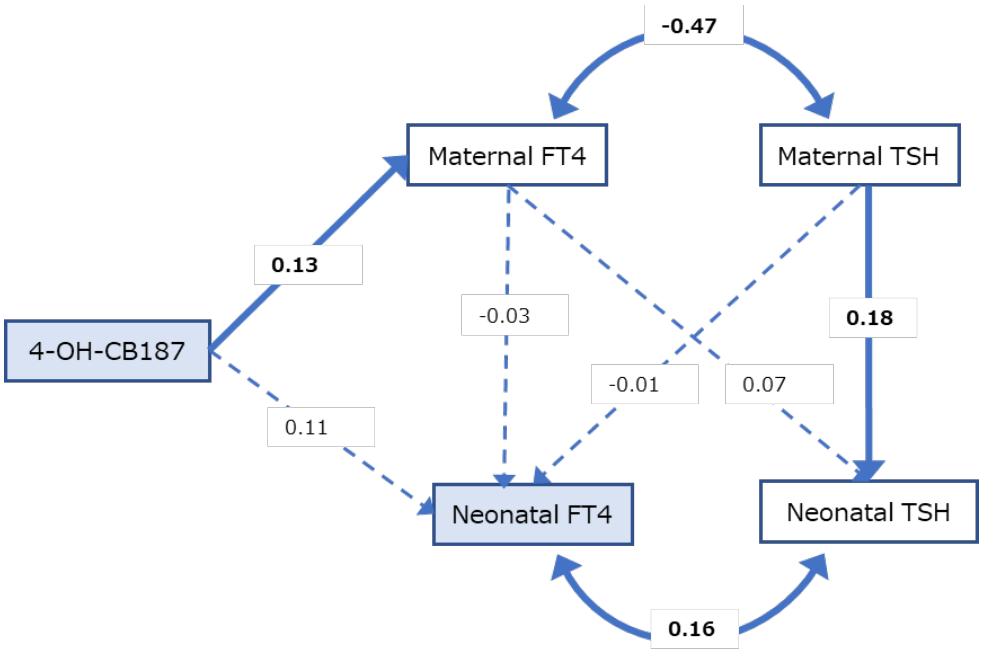

Figure 1.
Estimated pathway for the association between maternal 4-OH-CB187 exposure, maternal TSH and FT4 and neonatal TSH and FT4 through a structural equation model among the total mother-neonate pairs. Values on the arrows indicate standardized coefficients for the association between the two constructs. The two-sided arrows

represent the correlation coefficients. Arrows with the bold lines represent the significant associations and those with the dotted lines indicate the non-significant associations. Measurement errors were omitted for simplicity.

Discussion

This is the first study which suggested the possibility of maternal FT4 exposed to OH-PCBs indirectly affect neonatal FT4 levels via maternal TSH and neonatal TSH by conducting path analysis. We also found significant positive associations between OH-PCBs and FT4 in maternal serum during pregnancy and neonatal serum after adjustment for covariates.

Our previous study reported the absence of an association between maternal PCB levels and maternal and neonatal THs (Baba et al. 2018). However, current study suggested that OH-PCBs may have effects on fetal thyroid functions than PCBs. This may be explained by the fact that OH-PCBs have a stronger binding affinity for TTR than that of PCBs (Meerts et al. 2000). As the chemical structures of the OH-PCBs resemble T4, OH-PCBs are reported to competitively bind with TTR rather than T4, and TH homeostasis may be disrupted (Brouwer et al. 1998; Darnerud et al. 1996; Lans et al. 1993). Moreover, OH-PCBs bound to TTR can be delivered to the fetus (Landers et al. 2009; Lans et al. 1993). However, thyroxine-binding globulin (TBG) is a main transport protein in humans and the relative distribution of T4 in serum is reported to be 75 %, while only 20 % of T4 is bound to TTR in humans (Pappa et al. 2015). Further studies are required to elucidate the mechanisms of affected TH levels by PCBs or OH-PCBs.

The predominant OH-PCBs isomer identified in the maternal serum was 4-OH-CB187 (Median; 11.07 pg/g wet), followed by 4-OH-CB146+3-OH-CB153

(Median; 10.53 pg/g wet) in our study. As for 4-OH-CB187, the serum concentration in our participants was relatively lower than that previously reported (Berghuis et al. 2013; Fangstrom et al. 2002; Hisada et al. 2012). The profile of OH-PCBs may be mainly related to the precursor PCB profiles via dietary habit involving PCB source (Linderholm et al. 2007; Weiss et al. 2006). Since Japanese people intake more than 80 % of the PCBs via seafood, it is assumed that OH-PCB profiles in our participants are mainly derived from seafood (Mori et al. 2014). The concentrations of precursors of 4-OH-CB187, PCB-183 and PCB-187 (measured as PCB-182 and 187), were 1.6 and 5.8 ng/g lipid weight, respectively among participants in this study, which were predominant PCBs with rather high levels compared to other PCBs in our earlier study (Baba et al. 2018). Conversely, 4-OH-CB107, which has been reported as one of the main OH-PCB isomers, was detected only in 47.3 % of serum samples. While most of the OH-PCBs present in human blood are generated from PCBs, our earlier study examined maternal exposure to PCB-105 and 118 (precursors of 4-OH-CB-107) at rather high levels (Baba et al. 2018). In addition to the different concentrations of PCB precursors, the differences in OH-PCB profiles may be explained by the metabolic capacities by different CYP enzymes (Grimm et al. 2015).

We observed a significant positive association between maternal ΣOH-PCB and 4-OH-CB 187 and maternal FT4 in the linear regression analyses. Elevated T4 level during pregnancy might lead to hyperthyroidism, and fetuses of mothers with hyperthyroidism suffer from miscarriage, neonatal prematurity and low birth weight, though FT4 levels in our study were within normal range. Two epidemiological studies regarding OH-PCB exposure and THs during pregnancy have been reported (Dallaire et al. 2009b; Hisada et al. 2013), and both of these found no association between

OH-PCBs and FT4 levels. Increased FT4 in this study may be explained by the fact that some hydroxylated compounds inhibit the type 1 deiodinase (D1) activities which converts T4 to T3 or the inactive metabolites (reverse T3), leading to the increase of T4 level in the circulation (Schuur et al. 1998). The Inuit study reported a significant inverse association between OH-PCB concentrations and T3 levels in the blood among Inuit adults (Dallaire et al. 2009a). Although the disturbed conversion of T4 to T3 due to the inhibition of D1 or diiodothyronine sulfotransferases activities may be related to the increased FT4 in our study, it is impossible to determine this, because, we had no data on maternal T3 levels. Additionally, it is not clear whether OH-PCBs at low level could inhibit D1 activities as well as at high level exposure

The effects of OH-PCBs on neonatal THs have been investigated in previous studies, although, the results were not consistent (Table 5). Hisada et al. (2013) examined Japanese subjects (n=77) and reported a positive association between some OH-PCB isomers and neonatal TSH and FT4. Otake et al. (2007) reported a positive correlation between ∑OH-PCB and OH-PCB 187 in cord blood and neonatal FT4 levels among 23 Japanese subjects. Dallaire et al. (2009b) investigated Inuit women (n=41) and Soechitram et al. (2017) investigated Dutch subjects (n=100) and both research groups found no significant association between OH-PCBs and TH at birth. Our findings of a positive association between prenatal OH-PCBs and FT4 are partially consistent with two Japanese reports in which THs were measured using the heel-prick (Hisada et al. 2013; Otake et al. 2007). TH levels dramatically change over the first 24 hours after birth, and hormone levels from the cord blood may be affected by the stress of delivery, including labor pains, the duration of labor, uterotonic agents, and the lower temperature. Therefore, the data derived from the heel-prick blood on the fourth or fifth day after

birth in this study is stable and more suitable for TH measurements than data derived from cord blood samples. As shown in Table 5, the inconsistent results were also explained by differences in the OH-PCB levels. For example, Soechitram et al. (2017) reported a median 4-OH-CB187 concentration of 0.136 ng/g (136 pg/g) fresh weight, while according to Hisada et al. (2013) a median 4-OH-CB187 concentration was 29 pg/g wet weight, and in our study, median 4-OH-CB187 concentration was 11.07 pg/g wet weight. These discrepancies of blood sampling for THs and OH-PCB levels could account for the inconsistencies between the results of previous studies and the present findings. The inconsistencies among cited studies could also be due to the different hormonal fractions measured (free or total T4).

Table 5. Overview of previous reports on OH-PCB exposure and neonatal or infant thyroid hormone parameters

Reference	Country	Number of participants	Exposure to OH-PCBs			Thyroid hormone parameters						
			Sample	Number of isomers	Level of Σ OH-PCBs	Sample	TSH	T3	FT3	T4	FT4	Additional information
Current study	Japan	222	Maternal serum in the third trimester	5 OH-PCBs	25.37 pg/g wet wt.	Newborn's whole blood	→				↑	
Soechitram et al. (2017)	The Netherlands	100	Maternal serum in the third trimester	6 OH-PCBs	3860 pg/g fresh weight	Cord blood	→	→		→		OH-PCBs also associated with rT3 and T4S.
						3 month's blood	↓	↑		↑		
						18 month's	↓	↑		↑		

						blood				
Hisada et al. (2013)	Japan	77	Maternal serum (11.1 ± 1.9 weeks of gestation)	16 OH-PCBs	120 pg/g wet wt.	Newborn's whole blood	↑		↑	
Dallaire et al. (2008)	Canada	41	Maternal plasma at delivery or within the subsequent weeks and Cord blood	11 OH-PCBs	Maternal; 316 pg/g wet wt. Cord; 246 pg/g wet wt.	Cord blood	→	→	→	No association with cord TBG, too. OH-PCBs associated with maternal T3 positively.
Otake et al. (2007)	Japan	23	Cord tissue	6 OH-PCBs	10 pg/g wet wt.	Newborn's whole blood	→		↑	Analyzed by Spearman's rho correlations.

Arrows are indicated as follows: ↓ inversely associated ($p < 0.05$); ↑ positively associated ($p < 0.05$), and → no significant association. Blanks indicate not measured in the study.

4-OH-CB187 appears to disrupt the TH balance. Conversely, 4-OH-CB146+3-OH-CB153 showed no significant association with THs in mothers and total neonates, even though its concentration in maternal serum was comparable to 4-OH-CB187. The cord/maternal ratio of 4-OH-CB187 concentration was reported as 0.68, while 4-OH-CB146 and 3-OH-CB153 were 0.78 and 0.68, respectively, which was much higher than the concentration of PCB isomers (Park et al. 2008). It is estimated that 7.52 pg/g wet weight of 4-OH-CB187 and 7.16-8.21 pg/g wet weight of

4-OH-CB146+3-OH-CB153 were transferred from the mothers to the fetuses in our study, leading to fetal exposure to approximately the same levels of isomers.

This is the first study to conduct the SEM analysis to explore the association between OH-PCB exposure and maternal and neonatal TH. The path diagram suggests that there is no direct pathway from OH-PCBs to the neonatal FT4. According to Figure 1, the estimated possible indirect pathway is as follows: 1) maternal FT4 was increased as a result of OH-PCB exposure; 2) maternal TSH was decreased as a result of increased FT4 in the negative feedback system; 3) maternal TSH increased neonatal TSH. 4) neonatal FT4 was increased by neonatal TSH. Although maternal TSH is reported to have been poorly transferred to the fetus, high level of maternal TSH during early pregnancy was significantly associated with high neonatal TSH in previous study (Orito et al. 2009). Therefore, we linked the arrow directly from the maternal TSH to the neonatal TSH. On the other hand, we also showed Supplemental Figure 1 to test another hypothesis setting a latent variable, “maternal TH parameters”, since we did not have data of THs except for TSH and FT4. For example, T3 and thyrotropin-releasing hormone were reported to be transported from mother to fetus (Sahay et al. 2012). Considering of poor transfer of maternal TSH to fetus, the diagram in Supplemental Figure 1 is likely to be existing mechanism to influence neonatal FT4, however, it is difficult to make conclusion on the absence of the measurement of “maternal TH parameters”. In this analysis, the direction of correlation coefficient of TSH and FT4 between mothers and neonates was in opposite direction. It is presumed that negative correlation value in mothers are due to the negative feedback system in THs. In neonates, however, the function of negative feedback system is not mature at birth. In addition, the normal term newborn has a physiologic surge of TSH within 30 minutes of

birth, that stimulates thyroidal T4 secretion, with peak FT4 levels at 24-36 hours of life. TSH levels decline to normal levels by the day 3 to 5, but FT4 level is still high for several weeks after birth (Loh et al. 2015). Therefore, it looks like the measurement of neonatal TH (included TSH and FT4) within 1 month would still influenced by the natural physiological response. And the neonatal THs within 1-2 weeks would be affected or dominated by the above phenomena. Development of SEM analysis is indispensable to understand the complete pathway and the mechanism of OH-PCB exposure in further study.

The key strength of this study is the SEM analysis. This allowed the estimation of indirect pathways of OH-PCB on neonatal THs, contributing to the expansion of the current knowledge of the hormone transport from mothers to fetus. Moreover, selection bias is relatively small, though we included a limited number of participants from the original cohort (n=514) due to the lack of OH-PCB data. Due to the limited amount of blood obtained from mothers, we could only measure OH-PCB concentrations from samples with an adequate amount of blood after the measurement of other compounds. We compared the characteristics of included participants with those of excluded subjects in Supplemental Table 4. Although infant birth weight among included participants was heavier than among excluded subjects, we did not find significant differences in other maternal and infant characteristics.

This study has some limitations. Firstly, 4-OH-CB146 and 3-OH-CB153 were measured collectively due to the difficulty in distinguishing their peaks. Both of them were derived from PCB153. Our study suggests that exposure to PCB153 have less toxicity on THs. Secondly, maternal THs and neonatal THs were not obtained simultaneously due to the differences in the time of administering the THs screening test

to reduce the burden for drawing more blood. It has been clearly proven that even maternal subclinical hypothyroidism during early pregnancy not only affects poor development in the infants, but also carries complications in pregnancy, such as spontaneous abortion, intrauterine death, and preeclampsia (Casey et al. 2006; De Groot et al. 2012; Lazarus and Premawardhana 2005). Early gestation screening tests of THs are strongly recommended. This study used data derived from maternal TSH and FT4 in early pregnancy (median; 11.1 weeks of gestation). High correlations were reported between the maternal TSH in the early gestation and that in the late gestation, as well as maternal FT4 (Lambert-Messerlian et al. 2008), therefore, our data could reflect the actual relationship nearly the same as designated in path analysis in Figure 1. Thirdly, we only had data of two thyroid parameters, TSH and FT4, though several THs were working to regulate many body functions. Due to the limitation of TH level in our subjects were obtained from the municipal screening program, we only assessed the potential influence of two thyroid hormones, TSH and FT4, in this study. To understand the overall effects of OH-PCBs on TH circulation, the data of other THs and transport proteins, such as T3 or TBG, will be needed in next study. Lastly, there was a sequential concern in the effect of OH-PCB exposure on maternal THs. Our data analyzed the association between OH-PCB concentrations in maternal blood obtained at late pregnancy stage (mean of gestational weeks of blood sampling was 35.95 ± 3.99) and TH levels at early pregnancy stage. Additionally, the potential influence of physiological change varies within certain range according to the pregnancy stage. Glynn et al. reported that OH-PCB levels in maternal serum would be highly correlated between early and late pregnancy stage, though the concentration values of OH-PCBs would change during pregnancy due to the change of blood flow (Glynn et al. 2011).

Although we believe that a woman whose concentrations of OH-PCBs were high at third trimester would have also high levels of OH-PCBs at first trimester, the effect of OH-PCBs on maternal THs in this study should be carefully interpreted.

Conclusion

In this study, we reported a positive association between ∑OH-PCB and 4-OH-CB187 with maternal and neonatal FT4 among populations with low environmental exposure levels in Japan. We also showed intermediation paths between OH-PCB exposure and neonatal FT4 via maternal THs and neonatal TSH using path analysis. It is important to investigate the long-term effect of the disrupted THs at birth on later stages of in a prospective cohort study.

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