Title

Association of prenatal exposure to PCDD/Fs and PCBs with maternal and infant thyroid hormones: The Hokkaido Study on Environment and Children's Health

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List of abbreviations

AMC, anti-microsomal antibodies
ATG, anti-thyroglobulin antibodies
DLCs, dioxin-like compounds
DL-PCBs, dioxin-like PCBs
FT4, free thyroxine
CI, confidence interval
ND, non-detectable
NDL-PCBs, non-dioxine-like PCBs
NIHN, National Institute of Health and Nutrition, Japan
PCBs, polychlorinated biphenyls
PCDDs, polychlorinated dibenzo-p-dioxins
PCDFs, polychlorinated dibenzofurans
PFOA, perfluorooctanoate
PFOS, perfluorooctane sulfonate
POPs, persistent organic pollutants
TEF, Toxic equivalency factor
TEQs, Toxic equivalents
THs, thyroid hormones
TSH, thyroid stimulating hormone
T4, thyroxine

Manuscript

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are persistent organic pollutants (POPs) that are ubiquitous in the global environment. The levels of these POPs tend to be relatively higher in the breast milk of mothers in industrialized countries, where a downward time-trend is reported (van den Berg et al., 2017).

Seven PCDDs, ten PCDFs and 12 PCBs are considered to show toxic effects that are mainly mediated through the aryl hydrocarbon receptor (AhR). A World Health Organization (WHO) panel has assigned a toxic equivalency factor (TEF) for each of these 29 dioxin-like compounds (DLCs) (Van den Berg et al., 2006).

PCDDs affect dermal, developmental, immunological, and reproductive systems, and PCDFs affect hepatic and immunological systems (ATSDR, 1994; ATSDR, 1998). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is also a known human carcinogen (IARC, 2012).

A single dose of TCDD administered to rats showed a negative, dose-dependent association with thyroxin (T4) and free thyroxine (FT4) (Viluksela et al., 2004) and a positive association with thyroid stimulating hormone (TSH) (Nishimura et al., 2002).

Human and/or animal studies have reported PCB effects in multiple organ systems (ATSDR, 2000). Toxicities have been reported for not only dioxin-like PCBs (DL-PCBs), but also non-dioxin-like PCBs (NDL-PCBs). Exposure to NDL-PCBs are reported to be associated with neurodevelopmental deficits (Schantz et al., 2003), reproductive effects (Toft et al., 2004), and thyroxin deficiency (Brouwer et al., 1999).

Thyroid hormone (TH) homeostasis is considered vital in fetal development of the brain and other organs, and is markedly observed in congenital hypothyroidism. A systematic review showed that low maternal T4 increases the risk for delayed cognitive development (pooled random relative risk = 3.3) (Wang et al., 2016). A large prospective study found seven percent increase in ADHD symptom score at eight years of age in children who were exposed to mild maternal hypothyroxinemia (FT4 < 0.85 ng/dL) (Modesto et al., 2015). Our previous report showed an inverse association of prenatal DLCs exposure including coplanar PCBs and mental and motor development at six months of age in the same cohort (Nakajima et al., 2006).

PCDD/Fs and PCBs can affect TH homeostasis in a number of mechanisms (Crofton, 2008). However, epidemiological reports of the relationships between DLC/PCBs and human thyroid homeostasis are inconsistent. None of the five studies on DLC effect on THs of pregnant women reported significant associations for TSH or FT4 (Chevrier et al., 2014; Koopman-Esseboom et

al., 1994; Sauer et al., 1994; Zhang et al., 2010). Such reports on neonates were all from cross-sectional studies, and mostly reported non-significant associations (Darnerud et al., 2010; Koopman-Esseboom et al., 1994; Masuda, 2001; Pluim et al., 1993; Sauer et al., 1994; Wang et al., 2005). A systematic review of PCBs and THs of pregnant women and neonates found inconsistent results (El Majidi et al., 2014). Another systematic review of PCBs and adult thyroid functions reached no clear conclusion about the association between PCBs and TSH or FT4 (Salay and Garabrant, 2009).

The main emission source of DLCs in Japan was waste incinerators, followed by iron foundries at the time of sample collection as well as in 2015 (MOE, 2017). In annual food market surveys in Japan, seafood and meat accounted for 56-88 % and 8-31 % of all dietary intake of DLCs between 1999 and 2015, respectively. In the same surveys dietary intake was estimated to account for 94 to 99 % of all DLC exposure (TMG, 2016). In the same surveys, seafood intake accounted for 72 to 100 % of dietary PCB intake between 2005 and 2014. However, in our cohort, not fish intake, but only beef intake had significant correlation with maternal NDL-PCBs (Miyashita et al., 2015).

The mean TEQ of PCDD/Fs and PCBs in breast milk of the same, but primiparous mothers in our cohort were 6.8 and 4.4 pg/g lipid, respectively (Todaka et al., 2010). Compared to results from global surveys by WHO/UNEP (van den Berg et al., 2017), our results for PCDD/F-TEQ would place at between the 14th and 15th out of 27 countries in 2000-2003 survey, and 7^{th} and 8^{th} out of 36 countries in 2005-2010 survey. For PCBs, our results would be placed between the 15 and 16th out of 26 countries in the 2000-2003 survey and 7^{th} and 8^{th} out of 36 countries in the 2005-2010 survey. Considering our survey period (2003-2005), it would roughly be between the 20 percentile and the median for PCDD/F-TEQ, and between the 20 and 60 percentile for PCB-TEQ globally, which was typical for an area in an industrialized country.

We have previously published analyses of relationships between THs and Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), Di (2-ethylhexyl) phthalate (DEHP), and Bisphenol A in the Sapporo cohort of Hokkaido Study.

The purpose of the current study is to evaluate the effect of dioxins and PCBs during pregnancy on maternal and neonatal TH levels in a prospective birth cohort.

2. Materials and Methods

2.1 Study Population

This prospective birth cohort study involved pregnant women who were recruited at a hospital in Hokkaido, Japan. The hospital provided perinatal care to mostly uncomplicated pregnant women. Details of the study have been described previously (Kishi et al., 2017; Kishi et al., 2013; Kishi et

al., 2011). A total of 514 pregnant Japanese women were enrolled at 23–35 weeks of gestation between July 2002 and October 2005.

2.2 Data collection

A self-administered questionnaire was completed upon enrolment to collect baseline information, including demographics, medical history, supplement intake, and dietary habits during pregnancy. Within five days of delivery, women filled out a separate food frequency questionnaire on seafood intake. Medical information was derived from medical records including parity, maternal body measures (such as the height and bodyweight at delivery), delivery mode, gestational age at birth, infant gender, and birth size.

The study design was approved by the ethics committee of Hokkaido University Graduate School of Medicine. The study protocol conformed to the ethical rules of the Japanese government. All participants had the right to withdraw from the study at any time.

2.3. Exposure measurement

Detailed methods for analyzing PCDD/F and PCBs have been described previously (Todaka et al., 2007; Todaka et al., 2008b). In brief, maternal blood was drawn from peripheral veins of the mothers after the 30^{th} week of gestation.

PCDD/F and PCB levels were measured using high-resolution gas chromatography and high-resolution mass spectrometry (AutoSpec-Ultima E (Micromass, UK)) between 2003 and 2007. For DLCs, 29 congeners that have TEF (Van den Berg et al., 2006) were measured in 5 ml of maternal whole-blood samples. Non-dioxin-like PCBs were measured separately in 5 ml of maternal whole-blood samples. No contaminants were measured in cord blood or neonatal blood. Among the 197 non-dioxin-like PCB congeners, 58 congeners were identified. Limit of detection (LOD) varied between 1 and 10 pg/g lipids for the individual PCDD/F and PCB congeners. Detailed distributions of individual congener concentrations are provided in previous reports (Todaka et al., 2007; Todaka et al., 2008b). Maternal serum cotinine levels were measured on two occasions by ELISA using Cozart EIA cotinine kits (Cozart). Mercury levels were measured in maternal hair (Yasutake et al., 2003), which were examined as a potential confounder.

2.4. Quality Control

To evaluate the accuracy and reliability of the analysis of PCDD/Fs, and dioxin-like PCBs, our laboratory completed quality control studies for the analysis of these compounds. 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 PCDD/Fs, 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%. In 2007, our laboratory prepared human blood

samples using blood collected from five volunteers. 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 serum total non-dioxin-like PCBs levels in human blood samples obtained by the four participating laboratories was within 10% and was considered acceptable (Todaka et al., 2011). In addition, our laboratory's analytical method for non-dioxin-like PCBs demonstrated high reproducibility based on experiments conducted using the same control blood sample for 10 weeks. These findings indicate that our laboratory's analytical method for PCDD/Fs, dioxin-like PCBs and non-dioxin-like PCBs in human blood provides correct results (Todaka et al., 2011; Todaka et al., 2008b).

2.5. Measurement of thyroid hormones

The TSH and FT4 levels of mothers at an early gestational stage and newborns were measured using enzyme-linked immunosorbent assay (ELISA) kits at Sapporo Institute of Public Health for a mass screening program organized by the City of Sapporo. TSH and FT4 levels were determined using single 0.3 cm disks punched from filter paper blood samples, which were obtained from the mother's fingers or infant heels. The detection limit for TSH was 0.50 μU/mL, whereas there was no detection limit for FT4. Anti-thyroglobulin antibody (ATG) and anti-microsome antibody (AMC) were measured simultaneously using a gelatin particle agglutination method.

2.6. Calculations

PCDD/F/PCBs concentrations were adjusted based on the lipid content (pg/g lipid). The LOD/√2 was substituted in the analysis of individual PCDD/F/PCB congeners and THs with a concentration below the LOD.

PCBs were grouped according to their dioxin-like toxicological activity. Non-dioxin-like PCBs (NDL-PCBs) were defined as all PCBs except coplanar PCBs that had a TEF value as determined by WHO in 2005. Toxic equivalent (TEQ) values for PCDD/Fs and coplanar PCBs were calculated using the same TEF values (Van den Berg et al., 2006).

The gestational age at the time of maternal blood sampling for THs was calculated using records from the public screening program and estimates of gestational age made by obstetricians.

2.7. Data analysis

Among 514 women, we excluded seventeen (seven voluntarily withdrew, two had a miscarriage or stillbirth, one relocated, seven who had twins). We then excluded those with no data for serum

PCDD/F and PCB levels (n = 67), those receiving treatment for thyroid disease (five with Hashimoto disease and three with Graves disease, three had unspecified thyroid condition), and those of whom maternal or infants' TH data were not available (n = 47 or 19, respectively). One sample was excluded because of extremely high PCDF levels (2877.3 pg/g lipids). Another sample was excluded because the blood sampling for PCDD/F/PCBs was extremely late (45 weeks after the delivery). Finally, 386 pregnant women and 410 neonates were analyzed in the study.

PCDD/F/PCB, TSH, TSH x FT4, and PFOS levels were converted to a $\log_{10}$ scale because the data followed a log-normal distribution.

Parity was coded as zero versus one or more previous live births. We determined active smoking status using a combination of recorded smoking during pregnancy and/or at least one of the two measurements of serum cotinine > 10.0 ng/mL (CDC, 2009). AMC and ATG were coded as elevated if AMC or ATG > 0. Educational level was categorized and assessed as less than twelve years, or 12 or more years. Household annual income of millions of yen in Japanese currency was ranked in four levels. Alcohol consumption was assessed as never drink or still drinking during pregnancy. The blood sampling period was categorized into four groups: 23-31 weeks of gestation, 32-34, 35-41, and within a week after delivery.

Spearman's rank correlation coefficient test or analysis of variance was used to test for correlations among PCB levels and characteristics of the mothers and infants.

Multiple linear regression analyses were performed to adjust for potential covariates. Separate models were considered for maternal TH levels and neonatal TH levels. The models were adjusted for variables that were significantly associated with TSH or FT4 levels at $p < 0.05$ level in the univariate analysis (data not shown). PFOS was included in the model for maternal THs because Kato et al. (2016) previously reported that, in multivariate analyses, PFOS were significantly associated with maternal TSH in the same cohort. Mercury was associated with total TEQ/PCBs, but not with THs, therefore it was not adjusted for in the multivariate model. Blood sampling period for PCDD/F/PCBs showed significant association with POP levels ($p < 0.05$), and was included in the model regardless of the insignificance of association with THs (Konishi et al., 2009). Maternal FT4 and Log 10 TSH were also adjusted in the model for neonatal THs because they were significantly associated with log 10 neonatal FT4 when using analysis of variance at p = 0.1, 0.05 level, respectively. The variables considered in the adjusted model for maternal THs were maternal age at delivery, education, gestational age at blood sampling for PCDD/Fs and PFOS, Body Mass Index at recruitment, gestational age at TH blood sampling, increased AMC or ATG, pork intake, chicken intake, ham or sausages intake, and bacon intake. The variables considered in the adjusted model for neonatal THs were Log10 maternal TSH, maternal FT4, log10 PFOS, parity, active smoking status, inshore fish intake, infant age at blood

sampling, dairy product intake, gestational age at birth, and birth weight.

For exposures, we examined total dioxin (PCDD/F/PCBs) TEQ, total PCDD/Fs, total PCDDs, total PCDFs, individual PCDD/F congeners, total PCBs, total coplanar PCBs, non-ortho PCBs, mono-ortho PCBs, total NDL-PCBs, and individual PCB congeners in each analysis for the possible effects on THs.

Further stratified analysis was performed based on the infant gender for neonatal THs.

All statistical analyses were performed using JMP Pro 12 for Windows (SAS Institute Inc., NC, USA). Results were considered statistically significant if the *p* value was below 0.05.

3. Results

Demographic information, food frequency, and pregnancy, delivery and neonatal characteristics are shown in Table 1.

The median concentration of total PCDDs, PCDFs, PCBs were 455, 18.0 and 104700 pg/g lipid, respectively. The median and mean total TEQ in maternal serum was 13.8 and 14.7 pg/g lipid, respectively. Among the PCDD/F/PCB congeners, 1,2,3,7,8-PeCDD (4.10 pg/g lipid), 33'44'5-PenCB (#126) (3.72 pg/g lipid), and 2,3,4,7,8-PeCDF (1.72 pg/g lipid) were the three congeners with the highest mean TEQs (Table 2). There was no significant difference in the total dioxin-TEQ between mothers who delivered boys and those who delivered girls (14.67 and 14.93 pg/g lipid, respectively) (data not shown).

Concentrations of THs are presented in Table 3. Five mothers (1.3 %) had an abnormal TSH or FT4 value. Two neonates (0.5 %) had an abnormal TSH value (> 15 μU/ml) (data not shown).

Multiple linear regression analysis showed that total dioxin-TEQ and total coplanar PCBs have significant positive association with neonatal FT4 (beta = 0.224, 0.206, respectively) (Table 4). The associations were stronger in boys (beta = 0.299, 0.282, respectively) (Table 5), whereas the associations were not significant in girls. Non-ortho and mono-ortho PCBs were also positively associated with neonatal FT4. Ten out of seventeen PCDD/Fs, nine out of thirteen DL-PCBs (#81, 105, 118, 123, 126, 146, 157, 167, 169) and three out of 58 NDL-PCBs (#37, 202, 206) were significantly associated with neonatal FT4, all positively. No groupings or congeners had a significant association with neonatal TSH or TSH x FT4. More PCDD/F or PCB isomers were significantly associated with neonatal FT4 in boys than in girls (data not shown).

As for the maternal THs, multiple regression analysis showed that total Non-ortho PCBs was positively associated with maternal FT4 (beta = 0.185, p = 0.023). No other PCDD/F/PCB groupings or PCDD/F congeners had a significant association. Three PCB congeners had significant positive association(s) with maternal THs (#47 and #52 with TSH, #77 with FT4, #52 with TSH x FT4) (data not shown).

4. Discussion

This study provided the first evidence that non-accidental exposure to DLCs during pregnancy increases neonatal FT4, using the congener-specific analyses of DLC exposure, adjusting for many covariates including other pollutants, and with prospective study design and one of the largest sample sizes to date (Tables 6 and 7).

Compared with other areas in Japan, the levels of DLCs and PCBs were lower in the study population, especially NDL-PCBs (Todaka et al., 2007; Todaka et al., 2008b). More detailed reports on concentrations of PCDDs, PCDFs or PCBs in maternal blood and breast milk were published by Todaka et al. (2007; 2008a; 2010; 2011; 2008b), and analysis between demographic and dietary characteristics, and POPs concentrations by Miyashita et al. (2015).

Our study is the first to report a positive association between dioxin-TEQ and neonatal FT4. Koopman-Esseboom et al. (1994) reported a negative association between dioxin-TEQ and FT4, whereas four other previous studies that examined the same association did not demonstrate significance (Darnerud et al., 2010; Matsuura et al., 2001; Pluim et al., 1993; Wang et al., 2005) (table 7.).

Total NDL-PCBs were not associated with any maternal or neonatal THs. This suggests that the thyroid toxicity of non-accidental PCBs exposure is mainly through its dioxin-like activity.

The current study was conducted in Japan, where seafood supply per capita per year was 48.9 kg whereas the world average was 19.7 kg in 2013 (FAO, 2016) and the average iodine intake is estimated to be 1.5 mg/day, 1.2 mg of which is estimated to be from kombu seaweed (MHWL, 2010). Ingestion of iodine above the recommended daily intake is generally well-tolerated. However, in certain susceptible individuals, including those with pre-existing thyroid disease, the elderly, fetuses and neonates, or patients with other risk factors, the risk of developing iodine-induced thyroid dysfunction (hypothyroidism or hyperthyroidism) might be increased (Leung and Braverman, 2014). The average intake of 1.5 mg/day is far above the recommended dietary allowance and below the tolerable upper intake level (UL) for iodine for pregnant women in Japan (240 μg and 2000 μg, respectively) (NIHN, 2015), and iodine deficiency disorders are considered to be rare, but excessive intake is a possibility, especially in pregnant women whose UL is 1000 μg lower than general adults. The large intake of iodine in the Japanese population could have led to non-accidental dioxin exposure showing the opposite effect (increased neonatal FT4) to what is expected from animal experiments such as the one by Viluksela et al. (2004). In contrast with compulsory iodization of salt in many countries, iodine is not listed as a food additive in Japan (MHWL, 2011) and it is not permissible to produce or import any iodized food, including salt. These factors might affect the applicability of our study results to other

cultures or settings, although seaweed eating habits are assumed to be becoming increasingly common as Japanese food and restaurants gain popularity globally.

A higher effect of PCDD/F/PCBs on FT4 was observed in boys compared with girls, although there was little difference in dioxin-TEQ between mothers with a male fetus and those with a female fetus. Su et al. (2010) also reported a stronger effect of PCDD/Fs on TSH x FT4 in boys who were between two and five years old. A stronger effect on male infants was also observed for birth weight in our previous report (Konishi et al., 2009). Among adults over 18 years old, Abdelouahab et al. (2008) reported that for men only, serum T4 was inversely related to DLCs. One study on mice reported that median lethal dose was less than one tenth for the males compared with females (Pohjanvirta et al., 2012). The mechanism for the gender difference in the thyroid toxicity of dioxins is yet to be discovered.

Our study indicated that total dioxin-TEQ was not associated with maternal FT4, with a positive association only from non-ortho PCBs. No previous study has reported a positive association between DLCs and FT4 in pregnant women or adults. However, Croes et al. (2014) showed that marker PCBs, dl-PCBs and PCDD/Fs were positively correlated with FT4 in adolescents. The reason why only non-ortho PCBs showed effect is unknown. Future studies should examine this possible effect.

The fact that not TSH, but only FT4 was affected in this study indicates that dioxin toxicity on thyroid homeostasis involves similar mechanisms to resistance to thyroid hormone (RTH), where mutant thyroid hormone receptor beta gene is identified in majority of cases. It is notable that 40 to 60 percent of RTH patients are reported to have attention deficit hyperactivity disorder and 30 % learning disability among other symptoms. (Refetoff and Dumitrescu, 2007).

In our previous report, the levels of total PCDD/Fs, total PCDDs, and one PCDD congener (1,2,3,4,6,7,8-HpCDD) showed significant negative association with the mental developmental index (MDI), and the levels of two PCDD congeners (1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD) and three PCDF congeners (2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 1,2,3,6,7,8-HxCDF) showed significant negative association with the psychomotor developmental index (PDI) (Nakajima et al., 2006). The groupings and congeners that had significant associations were not the same for THs and motor or mental development. The differences in the effect could be due to possible differences in the toxicity of groupings or congeners for different systems, which suggests that dioxin-like toxicity in infant development does not necessarily go through the thyroid system. There was no association between DL-PCB congeners and development, which could be due to the smaller sample size (n = 134) of the previous study.

Strengths and limitations

The main strengths of the present study are prospective assessment and congener-specific analysis of PCDD/Fs and PCBs in maternal serum, a relatively large sample size, and consideration for various covariates including several other contaminants.

All six previous studies that examined the relationship between DLCs including PCDD/Fs and neonatal thyroid hormones, used breast milk or placenta to measure DLC exposure, making our study the only study that conducted a prospective assessment of exposure (Darnerud et al., 2010; Koopman-Esseboom et al., 1994; Matsuura et al., 2001; Pluim et al., 1993; Sauer et al., 1994; Wang et al., 2005).

To the best of our knowledge, our study had the second largest number of subjects for children of any ages among studies that conducted congener-specific analysis of 13 or more PCDD/Fs after the one by Matsuura et al. (2001). It is one of the few full congener-specific analyses of PCBs to test the association between PCBs and neonatal THs. We measured the two most important THs for both mothers and neonates as recommended by DeVito et al. (1999). This study had the highest number of adult participants among those that measured > 24 PCB congeners to test relationships with TSH and FT4.

Several important potential covariates, including smoking, fish intake and environmental contaminants such as PFOS and mercury were considered. Our previous reports on the same cohort showed that PFOA, Mono-2-ethylhexyl phthalate, Bisphenol A are not associated with maternal or neonatal TH levels (Kato et al., 2016; Minatoya et al., 2016; Minatoya et al., 2017).

Our study has several limitations. Numerous chemicals are detected in human bodies; it is possible that other unmeasured, but important factors such as Polybrominated diphenyl ethers (PBDE) may have affected the relationship between PCDD/F/PCBs and thyroid function although a survey in Japan showed that PBDE concentration in human breast milk was constantly below 2.4 ng/g lipid between 1973 and 2000, and also there was a voluntary discontinuance of production and import of PBDEs other than octa-BDE and deca-BDE by Japanese companies in 1995 (Akutsu et al., 2003).

Our samples were recruited from one secondary obstetric hospital in Japan. Therefore, the results might not be applicable to other setting or areas.

In conclusion, our study showed that non-accidental exposure to DLCs was associated with elevated neonatal FT4, especially in male infants. Future studies may want to test the suggested relationship in locations with different dietary habits and ethnicities and over the long-term, and further investigate the effect of maternal and infant TH disturbances on infant development.

References

Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, et al. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). Environmental Research 2008; 107: 380-92.

Akutsu K, Kitagawa M, Nakazawa H, Makino T, Iwazaki K, Oda H, et al. Time-trend (1973–2000) of polybrominated diphenyl ethers in Japanese mother's milk. Chemosphere 2003; 53: 645-654.

ATSDR. Toxicological Profile for Chlorodibenzofurans. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 1994.

ATSDR. Toxicological Profile for Chlorinated Dibenzo-p-Dioxins. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 1998.

ATSDR. Toxicological Profile for Polychlorinated Biphenyls (PCBs), Atlanta, GA: Agency for Toxic Substances and Disease Registry
2000.

Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, et al. Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs. Environ Health Perspect 1999; 107 Suppl 4: 639-49.

CDC. Fourth Report on Human Exposure to Environmental Chemicals. Atlanta, GA: Centers for Disease Control and Prevention. available: http://www.cdc.gov/exposurereport/ [accessed 1 November 2011]. In: Prevention CfDCa, editor, 2009.

Chevrier J, Warner M, Gunier RB, Brambilla P, Eskenazi B, Mocarelli P. Serum dioxin concentrations and thyroid hormone levels in the Seveso Women's Health Study. American Journal of Epidemiology 2014; 180: 490-8.

Croes K, Den Hond E, Bruckers L, Loots I, Morrens B, Nelen V, et al. Monitoring chlorinated persistent organic pollutants in adolescents in Flanders (Belgium): concentrations, trends and dose-effect relationships (FLEHS II). Environment International 2014; 71: 20-8.

Crofton KM. Thyroid disrupting chemicals: mechanisms and mixtures. International Journal of Andrology 2008; 31: 209-23.

Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. Environment International 2010; 36: 180-7.

DeVito M, Biegel L, Brouwer A, Brown S, Brucker-Davis F, Cheek AO, et al. Screening methods for thyroid hormone disruptors. Environ Health Perspect 1999; 107: 407-15.

El Majidi N, Bouchard M, Carrier G. Systematic analysis of the relationship between standardized biological levels of polychlorinated biphenyls and thyroid function in pregnant women and newborns. Chemosphere 2014; 98: 1-17.

FAO. Food Balance Sheets 2013, FAO Yearbook of Fishery and Aquaculture Statistics, Food and Agriculture Organization. 2016.

IARC. Chemical Agents and Related Occupations. Lyon, France: International Agency for Research on Cancer. 2012.

Kato S, Itoh S, Yuasa M, Baba T, Miyashita C, Sasaki S, et al. Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environ Health Prev Med 2016; 21: 334-344.

Kishi R, Araki A, Minatoya M. The Hokkaido Birth Cohort Study on Environment and Children's Health: cohort profile—updated 2017. 2017.

Kishi R, Kobayashi S, Ikeno T, Araki A, Miyashita C, Itoh S, et al. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile--updated 2013. Environ Health Prev Med 2013; 18: 429-50.

Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. Cohort profile: the Hokkaido study on environment and children's health in Japan. Int J Epidemiol 2011; 40: 611-8.

Konishi K, Sasaki S, Kato S, Ban S, Washino N, Kajiwara J, et al. Prenatal exposure to PCDDs/PCDFs and dioxin-like PCBs in relation to birth weight. Environ Res 2009; 109: 906-13.

Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, et al. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatric Research 1994; 36: 468-73.

Leung AM, Braverman LE. Consequences of excess iodine. Nat Rev Endocrinol 2014; 10: 136-42.

Masuda Y. Fate of PCDF/PCB congeners and change of clinical symptoms in patients with Yusho PCB poisoning for 30 years. Chemosphere 2001; 43: 925-30.

Matsuura N, Uchiyama T, Tada H, Nakamura Y, Kondo N, Morita M, et al. Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan--the second report from research on environmental health. Chemosphere 2001; 45: 1167-71.

MHWL J. p237, Dietary Reference Intakes for Japanese -2010-. Tokyo, Japan: Ministry of Health, Welfare and Labor, Japan (in Japanese). available: http://www.mhlw.go.jp/shingi/2009/05/s0529-4.html [accessed 20 December 2011]. 2010.

MHWL J. Registered food additives. Ministry of Health, Welfare and Labor, Japan (in Japanese). available: http://www.mhlw.go.jp/seisakunitsuite/bunya/kenkou_iryou/shokuhin/syokuten/index.html [accessed 20 December 2011]. 2011.

Minatoya M, Naka Jima S, Sasaki S, Araki A, Miyashita C, Ikeno T, et al. Effects of prenatal phthalate exposure on thyroid hormone levels, mental and psychomotor development of infants: The Hokkaido Study on Environment and Children's Health. Sci Total Environ

2016; 565: 1037-43.

Minatoya M, Sasaki S, Araki A, Miyashita C, Itoh S, Yamamoto J, et al. Cord blood bisphenol A levels and reproductive and thyroid hormone levels of neonates: The Hokkaido Study on Environment and Children's Health. Epidemiology 2017; Special Issue. In press.

Miyashita C, Sasaki S, Saijo Y, Okada E, Kobayashi S, Baba T, et al. Demographic, behavioral, dietary, and socioeconomic characteristics related to persistent organic pollutants and mercury levels in pregnant women in Japan. Chemosphere 2015; 133: 13-21.

Modesto T, Tiemeier H, Peeters RP, Jaddoe VW, Hofman A, Verhulst FC, et al. Maternal Mild Thyroid Hormone Insufficiency in Early Pregnancy and Attention-Deficit/Hyperactivity Disorder Symptoms in Children. JAMA Pediatr 2015; 169: 838-45.

MOE. ダイオキシン類の排出量の目録（排出インベントリー） (Emission source inventry of dioxin-like compounds) (in Japanese), Ministry of Environment, Japan. 2017.

Nakajima S, Saijo Y, Kato S, Sasaki S, Uno A, Kanagami N, et al. Effects of prenatal exposure to polychlorinated biphenyls and dioxins on mental and motor development in Japanese children at 6 months of age. Environmental Health Perspectives 2006; 114: 773-8.

NIHN. p39, Dietary Reference Intakes for Japanese -2015-. Tokyo, Japan: National Institute of Health and Nutrition, Japan. available: www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/overview.pdf [accessed 20 January 2017]. 2015.

Nishimura N, Miyabara Y, Sato M, Yonemoto J, Tohyama C. Immunohistochemical localization of thyroid stimulating hormone induced by a low oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in female Sprague-Dawley rats. Toxicology 2002; 171: 73-82.

Pluim HJ, de Vijlder JJ, Olie K, Kok JH, Vulsma T, van Tijn DA, et al. Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environmental Health Perspectives 1993; 101: 504-8.

Pohjanvirta R, Miettinen H, Sankari S, Hegde N, Linden J. Unexpected gender difference in sensitivity to the acute toxicity of dioxin in mice. Toxicol Appl Pharmacol 2012; 262: 167-76.

Refetoff S, Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. Best Pract Res Clin Endocrinol Metab 2007; 21: 277-305.

Salay E, Garabrant D. Polychlorinated biphenyls and thyroid hormones in adults: a systematic review appraisal of epidemiological studies. Chemosphere 2009; 74: 1413-9.

Sauer PJ, Huisman M, Koopman-Esseboom C, Morse DC, Smits-van Prooije AE, van de Berg KJ, et al. Effects of polychlorinated biphenyls (PCBs) and dioxins on growth and development. Human & Experimental Toxicology 1994; 13: 900-6.

Schantz SL, Widholm JJ, Rice DC. Effects of PCB exposure on neuropsychological function in

children. Environ Health Perspect 2003; 111: 357-576.

Su PH, Chen JY, Chen JW, Wang SL. Growth and thyroid function in children with in utero exposure to dioxin: a 5-year follow-up study. Pediatric Research 2010; 67: 205-10.

TMG. 都民の化学物質等摂取状況調査結果報告書（平成 11 年度から平成 26 年度までの 16 年間のまとめ）(in Japanese), Tokyo Metropolitan Government. 2016.

Todaka T, Hirakawa H, Kajiwara J, Hori T, Tobiishi K, Onozuka D, et al. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere 2007; 69: 1228-37.

Todaka T, Hirakawa H, Kajiwara J, Hori T, Tobiishi K, Onozuka D, et al. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in blood and breast milk collected from 60 mothers in Sapporo City, Japan. Chemosphere 2008a; 72: 1152-8.

Todaka T, Hirakawa H, Kajiwara J, Hori T, Tobiishi K, Yasutake D, et al. Relationship between the concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls in maternal blood and those in breast milk. Chemosphere 2010; 78: 185-92.

Todaka T, Hirakawa H, Kajiwara J, Onozuka D, Sasaki S, Miyashita C, et al. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls in blood and breast milk collected from pregnant women in Sapporo City, Japan. Chemosphere 2011; 85: 1694-700.

Todaka T, Hori T, Hirakawa H, Kajiwara J, Yasutake D, Onozuka D, et al. Congener-specific analysis of non-dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere 2008b; 73: 923-31.

Toft G, Hagmar L, Giwercman A, Bonde JP. Epidemiological evidence on reproductive effects of persistent organochlorines in humans. Reprod Toxicol 2004; 19: 5-26.

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, et al. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences 2006; 93: 223-41.

van den Berg M, Kypke K, Kotz A, Tritscher A, Lee SY, Magulova K, et al. WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit-risk evaluation of breastfeeding. Arch Toxicol 2017; 91: 83-96.

Viluksela M, Raasmaja A, Lebofsky M, Stahl BU, Rozman KK. Tissue-specific effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the activity of 5'-deiodinases I and II in rats. Toxicology Letters 2004; 147: 133-42.

Wang P, Gao J, Zhao S, Guo Y, Wang Z, Qi F. Maternal Thyroxine Levels During Pregnancy and Outcomes of Cognitive Development in Children. Mol Neurobiol 2016; 53: 2241-8.

Wang SL, Su PH, Jong SB, Guo YL, Chou WL, Papke O. In utero exposure to dioxins and

polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. Environmental Health Perspectives 2005; 113: 1645-50.

Yasutake A, Matsumoto M, Yamaguchi M, Hachiya N. Current hair mercury levels in Japanese: survey in five districts. Tohoku J Exp Med 2003; 199: 161-9.

Zhang J, Jiang Y, Zhou J, Wu B, Liang Y, Peng Z, et al. Elevated body burdens of PBDEs, dioxins, and PCBs on thyroid hormone homeostasis at an electronic waste recycling site in China. Environmental Science & Technology 2010; 44: 3956-62.

Table 1. Characteristics of subjects participating in the Hokkaido Study on Environment and Children's Health, Sapporo, Japan, 2002–2005

	N	Mean	SD	Median	Range
Maternal age at delivery (years)	379	31.18	4.73	31	20-48
BMI at recruitment (kg/m2)	378	21.09	3.09	20.45	16-36
Maternal BMI at delivery (kg/m2)	348	25.29	2.89	24.97	19-36
Gestational age at maternal blood sampling for THs (weeks)	378	10.93	2.18	10	2-28
Gestational age at delivery (weeks)	379	39.34	1.36	39.57	31-42
Birth weight (g)	379	3078	383	3068	1594-4292
Infant age at blood sampling (days)	379	4.401	0.887	4	3-7
Gestational age at maternal blood sampling for dioxins/PCBs/mercury (weeks)	379	34.56	4.16	35	23.29-41.29
					(%)
Household income (millions of yens per year)					
< 3	65			17.2%	
3 - 5	185			48.9%	
5 - 7	83			22.0%	
7 ≦	45			11.9%	
Education (years) (≦12/12 <)	160/219			42.2%	/ 57.8%
Parity (primiparous/multiparous)	177/202			46.7%	/ 53.3%
Alcohol intake more than once a week (yes/no)	116/263			30.6%	/ 69.4%
Current smoker (Reported or cotinine >10 ng/ml) (yes/no)	108/250			30.2%	/ 69.8%
Elevated AMC/ATG (yes/no)	36/343			9.5%	/ 90.5%
Inshore fish intake more than once a week (yes/no)	168/211			44.3%	/ 55.7%
Pelagic fish intake more than once a week (yes/no)	202/177			53.3%	/ 46.7%
Beef intake more than once a week (yes/no)	89/290			23.5%	/ 76.5%
Pork intake more than once a week (yes/no)	344/35			90.8%	/ 9.2%
Chicken intake more than once a week (yes/no)	316/63			83.4%	/ 16.6%
Ham or sausage intake more than once a week (yes/no)	238/141			62.8%	/ 37.2%
Bacon intake more than once a week (yes/no)	136/243			35.9%	/ 64.1%
Dairy product intake more than once a week (yes/no)	358/21			94.5%	/ 5.5%
Spontaneous Unassisted Vaginal Delivery (yes/no)	196/183			51.7%	/ 48.3%
Infant Gender (male/female)	182/197			48.0%	/ 52.0%

SD, standard deviation

Table 2. Concentration of PCBs and dioxins in maternal whole blood (pg/g lipid) (n = 379)

	DL	DR (%)	Min.	Median	Max.	Geometric Mean	Mean TEQ (geometric)
Total TEQ		100.0	3.2	13.8	43.4	14.7	
Σ PCDD/Fs		100.0	103.2	476	1638	527	9.80
Σ PCDDs		100.0	92.7	455	1602	507	7.26
2,3,7,8-TCDD	1	44.4	ND	ND	3.4	0.938	0.938
1,2,3,7,8-PeCDD	1	97.9	ND	3.88	12.9	4.10	4.10
1,2,3,4,7,8-HxCDD	2	36.5	ND	ND	13.6	1.63	0.163
1,2,3,6,7,8-HxCDD	2	100.0	2.37	13.0	113	14.4	1.44
1,2,3,7,8,9-HxCDD	2	55.1	ND	2.10	25.1	2.25	0.226
1,2,3,4,6,7,8-HpCDD	2	100.0	8.35	23.2	85.4	25.9	0.259
OCDD	4	100.0	75.5	407	1491	457.4	0.137
Σ PCDFs		99.5	ND	18.0	192	20.1	2.53
2,3,7,8-TCDF	1	21.3	ND	ND	8.41	0.713	0.0713
1,2,3,7,8-PeCDF	1	7.3	ND	ND	4.60	0.579	0.0174
2,3,4,7,8-PeCDF	1	99.5	ND	5.38	19.9	5.73	1.72
1,2,3,4,7,8-HxCDF	2	59.3	ND	2.23	12.5	2.20	0.220
1,2,3,6,7,8-HxCDF	2	68.5	ND	2.43	10.1	2.56	0.256
2,3,4,6,7,8-HxCDF	2	4.7	ND	ND	3.86	1.07	0.106
1,2,3,7,8,9-HxCDF	2	0.0	ND	ND	ND	ND	0.100
1,2,3,4,6,7,8-HpCDF	2	60.6	ND	2.23	162.5	3.08	0.0308
1,2,3,4,7,8,9-HpCDF	2	0.0	ND	ND	ND	ND	0.0100
OCDF	4	1.6	ND	ND	31.0	2.17	0.000650
Σ PCBs		100.0	17820	104700	362500	117000	
Σ NDL-PCBs		100.0	16020	92700	329300	105000	
PCB 153	10	100.0	2820	20500	77690	23600	
Σ Dioxin-like PCBs		100.0	1744	11094	36570	12300	4.87
Σ Non-ortho PCBs		99.0	ND	75.5	553.6	80.3	4.50
PCB 77	10	65.6	ND	11.2	475	12.2	0.000150
PCB 81	10	0.3	ND	ND	10.1	5.0	0.000367
PCB 126	10	96.3	ND	33.8	157	37.2	3.72
PCB 169	10	94.5	ND	24.0	85.9	25.8	0.775
Σ Mono-ortho PCBs		100.0	1724	11030	36380	12200	0.369
PCB 105	10	100.0	256	1430	5421	1610	0.0482
PCB 115	10	99.0	ND	335	1695	379	0.0114
PCB 118	10	100.0	635	5776	20200	6440	0.193
PCB 123	10	98.2	ND	108	458	121	0.00361
PCB 156	10	100.0	282	1891	6430	2130	0.0639
PCB 157	10	100.0	50.6	467	1537	519	0.0156
PCB 167	10	100.0	65.0	686	2275	765	0.0230
PCB 189	10	99.5	ND	228	806	259	0.00777

DL, detection limit, DR, detection rate, ND, not detected, TEQ, toxic equivalents, NDL, non-dioxin-like
* PCB numbers are based on Mills et al. 2007.

Table 3. Concentrations of thyroid hormones in filter blood samples from mothers and neonates

	N	DR (%)	Minimum	25th	Median	75th	Maximum	Geometric Mean
Maternal TSH (μU/ml)	386	77.8	ND	0.5	1.0	1.7	6.5	1.26
Maternal FT4 (ng/dl)	386	100.0	0.51	0.86	0.98	1.15	3.78	1.04
Maternal TSH x FT4	386	77.8	ND	0.57	0.96	1.55	6.13	1.20
Neonatal TSH (μU/ml)	410	94.9	ND	1.2	2.20	3.7	25.2	2.73
Neonatal FT4 (ng/dl)	410	100.0	0.85	1.78	2.03	2.30	3.26	2.05
Neonatal TSH x FT4	410	94.9	ND	2.44	4.25	7.84	57.96	5.63

ND, not detected

Table 4. Multiple linear regression analyses of maternal whole-blood PCB levels and neonatal thyroid hormone levels (n = 385)

	Log_{10} TSH			FT4			Log_{10} TSH x FT4		
	β	(95 % CI)		β	(95 % CI)		β	(95 % CI)	
Σ Dioxin TEQ	-0.041	-0.215	0.133	0.224*	0.016	0.433	0.010	-0.171	0.191
Σ PCBs	-0.025	-0.173	0.123	0.139	-0.038	0.317	0.005	-0.149	0.159
Σ NDL-PCBs	-0.025	-0.172	0.123	0.130	-0.047	0.307	0.003	-0.150	0.157
Σ Dioxin-like PCB	-0.017	-0.161	0.127	0.206*	0.034	0.378	0.029	-0.121	0.178

Adjusted for log10 maternal TSH, maternal FT4, log 10 perfluorooctane sulfonate, cotinine >10 or reported smoker, inshore fish intake, dairy product intake, parity, gestational age at birth, birth weight, and infant age at blood sampling.
NDL, non-dioxin-like, CI, confidence interval
*: $p < 0.05$

Table 5. Multiple linear regression analysis of dioxins or PCBs and neonatal thyroid hormones in 167 boys

	Log_{10} TSH			FT4			Log_{10} TSH x FT4		
	β	(95 % CI)		β	(95 % CI)		β	(95 % CI)	
Σ Dioxin TEQ	-0.188	-0.467	0.090	0.299*	0.011	0.587	-0.120	-0.406	0.167
Σ PCBs	-0.097	-0.339	0.146	0.240	-0.011	0.490	-0.042	-0.291	0.207
Σ NDL-PCBs	-0.092	-0.334	0.150	0.231	-0.019	0.481	-0.040	-0.288	0.209
Σ Dioxin-like PCBs	-0.146	-0.376	0.084	0.282*	0.040	0.524	-0.082	-0.320	0.155

Adjusted for log10 maternal TSH, maternal FT4, log 10 perfluorooctane sulfonate, cotinine >10 or reported smoker, inshore fish intake, dairy product intake, parity, gestational age at birth, birth weight and infant age at blood sampling.
NDL, non-dioxin-like, CI, confidence interval
*: $p < 0.05$

Table 6. Summary of previous studies on the association of DLCs and thyroid hormones in pregnant women

Author (first) / Year	Country / region	Prospective /cross-sectional	No of subjects	PL	Congeners analyzed (PCDD/F, Dioxin-like PCBs)	Other chemicals	TSH	FT4	T3	T4
Lignell 2016	Uppsala, Sweden	C	97 for PCDD/Fs /281 for PCBs	N	17 PCDD/F congeners 4 mono-ortho PCBs	PBDE	NS	NS	Down (PCDD/Fs)	-
Darnerud, 2010	Uppsala, Sweden	C	211 (Same subjects as Lignell et al. 2016)	N	17 PCDD/F congeners 4 mono-ortho PCBs	P,p'-DDE	NS	NS	Down (PCDD/Fs)	-
Zhang 2010	China	(THs measured at 16 wks, exposure was measured after delivery)	50 (25 exposed, 25 control)	Y/N (e-waste site)	17 PCDD/F, 12 DL-PCBs	PBDE	NS	-	NS	Down (PCDD/Fs or PCBs)
Foster 2005	South Western Ontario, Canada	C	145	N	Dioxin responsive-chemically activated luciferase expression assay	None	NS	-	NS	-
Koopman-Esseboom 1994	Netherlands	P	78 mothers	N	5 PCDD and 3 PCDF congeners, 2 non-ortho PCBs	None	NS	NS	Low (TEQ)	Low (TEQ)

NS, not significant, -, not measured, TH, thyroid hormone, PL, Highly polluted area/accidental exposure, Y, yes, N, no

Table 7. Summary of previous studies on the association of DLCs and thyroid hormones in neonates

Author (first) / Year	Country / region	Prospective /cross-sectional	No of subjects, age at blood sampling for THs	Polluted	Chemical analysis of PCDD/F /Dioxin-like PCBs	Other chemical	TSH	FT4	T3	T4
Darnerud 2010	Uppsala, Sweden	C	150 at 3 weeks 115 at 3 months	N	17 PCDD/F congeners 4 mono-ortho PCBs	P,p'-DDE	NS	NS	NS	-
Wang 2005	Taiwan	C	119 (day of delivery, 2 weeks)	N	17 PCDD/Fs 12 dioxin-like PCB congeners	None	Low (Total TEQ in females)	Low (non-ortho PCBs)	NS	High (PCDD/Fs)
Koopman-Esseboom 1994	Netherlands	P	78 (2^{nd} week and 3rd month)	N	5 PCDDs and 3 PCDFs 2 non-ortho PCBs	None	High (TEQ)	NS	NS	NS
Pluim 1993	Netherlands	P	38 (Birth, 1 wk, 11 wks)	N	17 PCDD/Fs	None	NS (High at 11wks)	NS	NS	NS

NS, not significant, -, not measured, TH, thyroid hormone, PL, Highly polluted area/accidental exposure, Y, yes, N, no