Associations between prenatal exposure to organochlorine pesticides and thyroid hormone levels in mothers and infants: The Hokkaido Study on Environment and Children's Health

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Abstract

Organochlorine pesticides (OCPs) are environmental contaminants with potentially adverse effects on neurodevelopment. Previous findings on the association between prenatal exposure to OCPs and the maternal or infant thyroid hormone system are inconsistent. Moreover, the influence of exposure to multiple OCPs and other chemical compounds is not clearly understood. Our study therefore aimed to examine the association between OCP exposure and both maternal and infant thyroid hormone systems. We also explored multiple exposure effects of OCPs and the influence of each compound using weighted quantile sum (WQS) methods. The study population included 514 participants in the Hokkaido study, recruited from 2002 to 2005 at one hospital in Sapporo, Japan. To quantify 29 OCPs, maternal blood samples were analyzed using gas chromatography/mass spectrometry. Blood samples for measuring thyroid stimulating hormone (TSH) and free thyroxine (FT4) levels were obtained from mothers during the early gestational stage (mean 11.4 weeks), and from infants between 7 and 43 days of age. The data of 333 mother child pairs with OCP and thyroid hormone measurements were included in the final analyses. Multivariate regression models showed a negative association between maternal FT4 and levels of *o,p'*-dichlorodiphenyldichloroethylene (DDE), *o,p'*-dichlorodiphenyltrichloroethane (DDT), and dieldrin. The WQS analysis showed that *o,p'*-DDT (48.6%), *cis*-heptachlorepoxide (22.8%), dieldrin (15.4%) were the primary contributors to the significant multiple exposure effect of OCPs on maternal FT4. For infants, we found a positive association between FT4 and *cis*-nonachlor and mirex. The most contributory compounds in the multiple exposure effect were *trans*-nonachlor (27.1%) and *cis*-nonachlor (13.8%), while several compounds contributed to the WQS via small weights (0.4-9.1%). These results indicate that OCPs, even at very low levels, may influence maternal and child thyroid hormone levels, which could modulate child development.

Keywords:

Thyroid hormone

Organochlorine pesticides

Pregnancy exposure

Birth cohort

Introduction

Organochlorine pesticides (OCPs) are environmental contaminants categorized as persistent organic pollutants. OCPs persist in the environment and bioaccumulate through the food chain in human and animal fatty tissues (Shen et al., 2005; Sheng et al., 2013). These compounds have been banned or restricted under the Stockholm Convention since 2004 but can still be found in the environment. In Japan, most OCPs were prohibited from agricultural use in the 1970s (Nakai et al., 2004). However, heptachlor, dieldrin, and chlordane were used for termite control until the 1980s despite being banned from agricultural applications. Although never registered for agricultural use, hexachlorobenzene (HCB) was used for industrial purposes until the 1980s. In Japan, mirex and toxaphene have never been produced or used for industrial or agricultural purposes (Ministry of the Environment Government of Japan, 2017). However, these compounds have been detected in air, soil, and water, as well as biosamples from animals and humans (Ministry of the Environment Government of Japan, 2014). In our previous study, 21 OCPs were detected in the serum of Japanese pregnant women (Kanazawa et al., 2012).

Growing evidence has demonstrated the adverse effects of prenatal OCP exposure on neurodevelopment (Boucher et al., 2013; Eskenazi et al., 2006; Puertas et al., 2010; Ribas-Fito et al., 2003a; Ribas-Fito et al., 2006; Torres-Sanchez et al., 2007). These results might be due to a mediation effect on the thyroid hormone (TH) levels of mothers and infants, as TH in particular plays an essential role in brain development (Bernal, 2007). Fetuses depend on their mother’s supply of THs in early gestation; hence, disruption of maternal TH homeostasis at this stage might impair fetal development. In fact, mild maternal hypothyroxinemia during pregnancy increases the risk of adverse effects on fetal neurodevelopment (Haddow et al., 1999; Kooistra et al., 2006; Pop et al., 2003; Pop et al., 1999).

An association between exposure to OCPs and disruption of the maternal TH system has been reported in several human epidemiological studies (Chevrier et al., 2008; Kim et al., 2015; Lopez-Espinosa et al., 2009; Steuerwald et al., 2000; Takser et al., 2005). Chevrier et al. (2008) reported a

negative association between HCB with free thyroxine (FT4) and total thyroxine (TT4). Lopez-Espinosa et al. (2009) reported a negative association between *p,p'*-dichlorodiphenyldichloroethylene (DDE) level in maternal serum and FT4. Takser et al. (2005) reported an association between three OCPs (*p,p'*-DDE, *cis*-nonachlor, and HCB) and decreased total triiodothyronine. On the other hand, in a study by Steuerwald et al. (2000) which measured *p,p'*-DDE, as well as in a study by Kezios et al. (2013) that measured *p,p'*-dichlorodiphenyltrichloroethane (DDT) and *p,p'*-DDE, no associations were observed between pesticide exposure and maternal TH. There were no previous studies which consider about multiple exposure effect of OCPs, even though people are exposed to a mixture of various OCPs in our daily life.

In infants, several studies have measured TH levels after birth. Kim et al. (2015) measured infant thyroid stimulating hormone (TSH) level 2 days after birth, and found a positive association with exposure to ΣDDT and *p,p'*-DDE. Lopez-Espinosa et al. (2010), Ribas-Fito et al. (2003b), and Alvarez-Pedrerol et al. (2008) also found increased TSH in infants' blood 2-5 days after birth; these increases were associated with OCP levels in cord serum. All four of these studies measured only TSH (rather than additional thyroid hormones). Cordier et al. (2015) was the only study to measure multiple TH levels in infants after birth. Specifically, they measured TSH, FT3, and FT4 for male and female infants, and reported a positive association between cord chlordecone and male infant TSH. Previous studies measuring TSH levels in infant blood soon after birth consistently indicated positive associations with OCPs. It is necessary to examine association between other THs and OCP exposure.

The purpose of this study is to examine the association between OCP exposure and both maternal and infant thyroid hormone systems. However, people are exposed to a mixture of various OCPs in our daily life, and hence it is necessary to estimate as many of these compounds as possible in order to most accurately evaluate multiple exposure effects.

Materials and Methods

Study population

This study was conducted using data from a prospective study: the Sapporo Cohort of the Hokkaido Study on Environment and Children's Health (Kishi et al., 2017; Kishi et al., 2013; Kishi et al., 2011). Briefly, we recruited 514 pregnant women between July 2002 and July 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. All participants were native Japanese women residing in Sapporo and the surrounding areas. The participants completed a self-administered questionnaire after the second trimester of their pregnancy, which obtained baseline information such as education level, family income, tobacco/smoking history, and alcohol consumption. Clinical perinatal data for mothers and infants were collected from participants' medical records.

Among the 514 participants, 379 had OCP data. We included the data of 342 participants who had measurements for both maternal and infant thyroid hormone levels. Furthermore, we excluded the data of 6 mothers who were undergoing treatment for thyroid disease during pregnancy, 1 mother who withdrew from the cohort study, and 2 mothers for whom basic questionnaire data were lacking. We included data from participants who had toxemia of pregnancy (n=28) or gestational diabetes (n=1). Finally, we included 333 mothers and infant pairs for examination of multi-exposure effects. 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 was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Exposure measures

Maternal blood samples to measure OCP levels were obtained at the time of patients' hospital examinations following recruitment at 23–35 weeks of gestation (n = 379). If a blood sample for OCPs could not be obtained during pregnancy because of maternal anemia, a sample was collected during

post-partum hospitalization within a week after delivery (n = 116). All samples were stored at −80 °C until analysis. The 29 OCPs evaluated in this study were six DDTs (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD), five chlordanes (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane), three drins (aldrin, dieldrin, and endrin), three heptachlors (heptachlor, *cis*-heptachlor epoxide [HCE], and *trans*-HCE), HCB, four hexachlorocyclohexane (HCH) isomers (α-HCH, β-HCH, γ-HCH, and δ-HCH), mirex, and six toxaphenes (Parlar-26, Parlar-41, Parlar-40, Parlar-44, Parlar-50, and Parlar-62). The internal standards (for the clean-up and syringe spikes) were ^{13}C-labeled isomers or d-isomers obtained from Cambridge Isotope Laboratory, Inc. (Andover, MA, USA). The concentrations of OCPs were measured according to the methods recommended by the Ministry of the Environment, Japan using gas chromatography/negative-ion chemical-ionization mass spectrometry and gas chromatography/hi-resolution mass spectrometry. All procedures were performed by IDEA Consultants, Inc. (Shizuoka, Japan). The detailed methodology for the measurement of OCPs has been described in our previous report (Kanazawa et al., 2012).

Thyroid hormone measurement

As reported in our previous study (Kato et al., 2016), thyroid function and anti-thyroid antibody (AMC: antimicrosomal antibody; ATG: antithyroglobulin antibody) data were obtained by a mass screening test at the Sapporo City Institute of Public Health. For thyroid hormone measurement, the blood samples were collected from mothers between the 6th and 18th week of gestation and from heel prick samples of neonates between 4 and 7 days after birth under non-fasting conditions. TSH and FT4 levels in maternal and neonatal blood were 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, 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 10-13 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)(Fujiwara et al., 2008). Both TSH and FT4 of mothers and neonates were measured from blood on filtered paper (TOYO ROSHI, Ltd., Tokyo, Japan). FT4 values were determined for all samples. For samples with TSH levels below the LOD (0.50 μU/mL), we used a value of half the LOD (0.25 μU/mL). Additionally, we calculated TSH × FT4 value as a measure of FT4 feedback to stimulate the hypothalamus (Su et al., 2010).

Data analysis

We analyzed the correlations between TH values and maternal and infant characteristics using Pearson's correlation coefficient test and Student's t-test. The values of OCP and TH were log10 transformed, as the levels of TH and OCP showed non-normal distributions. We performed a multivariate regression analysis to examine associations between TH and the OCP concentrations in maternal serum samples. Potential confounders were selected according to findings from previous studies (Herbstman et al., 2008; Kim et al., 2015). Subsequently, we used directed acyclic graphs to determine adjustment factors (Textor et al., 2011); these graphs for maternal and infant models are illustrated in Supplemental materials – see Supplemental Figs. 1 and 2. For mothers, variables included in the adjusted model were maternal age at delivery, body mass index (BMI), smoking during pregnancy, and education level. For infants, sex and first-born status were included in the multivariate models, in addition to the covariates included for the maternal multivariate models. The blood sampling periods for OCP and TH measurements were included in models *a priori* for both mothers and infants. Furthermore, we added total dioxin toxic equivalency (TEQ) measured from same sample as OCPs, because our group previously reported that levels of maternal dioxin congeners are positively

associated with neonatal FT4 levels (Baba et al., 2018). Although OH-PCB also increased both maternal and neonatal FT4 levels in our another previous study (Itoh et al., 2018), we added OH-PCB levels as adjustment factor only in supplemental analysis because the number of participants who had both OCP and OH-PCB data were small (n=188). We performed these statistical analyses using SPSS (Version 22.0; SPSS, Chicago, IL, USA). To facilitate interpretation of study results, *post hoc* power analysis was conducted using G* Power3 software (Faul et al., 2007). Multivariate regression analysis was conducted with 10 predictors (for example, these comprised OCP exposure and adjustment factors in the model for infants), and the detectable effect size f^2 was 0.05 (n = 333) (α = 0.05, β = 0.2). According to the guideline developed by Cohen (1988), $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively. Therefore, our analysis was sufficiently powered detect small to medium effect sizes.

As a secondary analysis, we conducted weighted quantile sum (WQS) regressions (Carrico et al., 2015) using the R package gWQS, taking into account the statistically significant associations found in first multiple regression analysis. WQS can assess associations between multiple OCP and TH, and is stable for strong correlations between OCPs, as distinct from regression analysis (Czarnota et al., 2015). WQS index allows us to make generalized inferences about multiple exposure effects of OCPs, and estimation of chemical weights (represented as percentages contributing to the WQS index) can be used to identify individual chemicals most strongly associated with TH (Czarnota et al., 2015). WQS estimates the empirical weights for each exposure based on associations between individual exposures and the outcome of interest and constructs a weighted index of exposure. The WQS analysis was applied for 15 chemical compounds detected in more than 80% of the participants. Dioxin TEQ levels was highly correlated with several OCPs in our previous study (Yamazaki et al., 2017). Therefore, we included dioxin TEQ as within the WQS index. We did not split our data into training/validation samples, which is one of the standard procedures for constructing a WQS index, because of the small sample size. We performed 100 bootstrapping steps and focused on the direction

of the observed significance in multivariate regression models. All WQS models were adjusted for the confounders used in the multiple regression model.

Results

The basic characteristics of the study population and TH values of mothers and infants are shown in Table 1. The average maternal age at delivery was 31.3±4.7 years (mean ± standard deviation [SD]), and maternal BMI was 21.1±3.1 kg/m^2. More than half (58.3%) of the mothers had an education longer than 13 years. Most of mothers (85.9 %) did not smoke during pregnancy. Among the infants, males comprised 46.5%, and 46.8% of infants were first-borns. TSH levels from 74 (22.2 %) maternal blood samples and 16 (4.8%) infant blood samples were below the LOD. The median levels of TSH, FT4, and TSH×FT4 levels were 1.00 IU/mL (95% confidence interval [CI] 1.14, 1.37 IU/mL), 0.98 ng/mL (95% CI 1.00, 1.06 ng/mL), and 0.93 (95% CI 1.08, 1.28) for mothers, respectively, and 2.20 IU/mL (95% CI 2.51, 3.03 IU/mL), 2.01 ng/mL (95% CI 2.00, 2.08 ng/mL), and 4.20 (95% CI 5.14, 6.26) for infants, respectively. Furthermore, we compared participant characteristics between analysis groups (those included in comparison to those excluded from the analysis), and between the blood sampling period groups (blood samples conducted during pregnancy or after delivery) in Supplemental table 1. Participants who were included in the analysis had higher maternal age, higher smoking rate, a longer blood sampling period for infant TH, and higher total dioxin TEQ than the excluded group. For the OCP blood sampling period, group with sampling during pregnancy had a lower maternal age, longer gestational age (days), and a lower first-born rate in infant. Supplemental table 2 shows a comparison of TH levels between the same groups. We found no differences between both the analysis groups and the blood sampling groups.

Maternal serum OCP concentrations are presented in Table 2. Of 29 OCPs, 15 compounds were detected among >80% of the participants. Correlations between OCPs, and dioxin are shown in Supplemental table 3, and comparison of OCP levels between analysis groups and between the blood

sampling period groups are shown in Supplemental table 4. Several OCP levels were significantly lower in the included group than in the excluded group, and there was no difference between participants sampled during pregnancy or postpartum.

Table 3 shows associations between pregnancy exposure to OCPs and maternal THs. We found a significant negative association between FT4 and *o,p'*-DDE (β = -0.04; 95% CI: -0.07, -0.01), *o,p'*-DDT (β = -0.06; 95% CI: -0.10, -0.02), and dieldrin (β = -0.08; 95% CI: -0.14, -0.02) in the adjusted model. The association between o,p'-DDT and FT4 remained significant after Bonferroni correction for tests conducted regarding 15 OCPs, for each TSH, FT4, and TSH×FT4 ($p < 0.003$). Supplemental table 5 shows a model with OH-PCB adjustment. The results were very similar to that shown in Table 3.

Table 4 shows associations between prenatal exposure to OCPs and infant TH levels. We found a significant positive association between FT4 and *cis*-nonachlor (β = 0.05; 95% CI: 0.001, 0.10) and mirex (β = 0.05; 95% CI: 0.003, 0.10) in Adjusted model. We did not observe any statistically significant associations following Bonferroni correction. OH-PCB adjusted model in Supplemental table 6 shows same direction of results, but number of OCPs with significant associations with FT4 were increased.

We applied WQS to maternal and infant TH levels to reveal the multiple exposure effect and relative weights of each OCP. Table 5 shows WQS results, in which both positive and negative associations were observed for maternal and infant TH. WQS models were statistically significant for maternal FT4 in the negative direction (β = -0.017; 95% CI: -0.030, -0.004), and were significant for infant FT4 in the positive direction (β = 0.017; 95% CI: 0.006, 0.028). The left panel of Figure 1 shows each OCP weight (represented as % in the WQS index) for maternal FT4. The most dominant OCP was *o,p'*-DDT (weighted at 48.6%), followed by *cis*-HCE (22.8%), and dieldrin (15.4%). In total, these three compounds accounted for 86.8% of WQS for maternal FT4. For infant FT4, the right panel of Figure 1 indicates that WQS were divided into several OCPs. The most dominant chemical was *trans*-

nonachlor (27.1%), followed by *cis*-nonachlor (13.8%), Parlar-50 (9.1%), mirex (8.3%), dieldrin (7.3%), and *p,p'*-DDT (6.8%). These six OCPs accounted for 72.4% of WQS for infant FT4. In addition, total dioxin (9.1%) was also account for relatively high weight.

Discussion

In this study, we observed negative associations between maternal FT4 and levels of *o,p'*-DDE, *o,p'*-DDT, and dieldrin using multivariate regression analysis. Subsequently, the WQS analysis also showed that *o,p'*-DDT (48.6%), *cis*-HCE (22.8%), and dieldrin (15.4%) were the primary contributors to a significant multiple exposure effect of OCPs on maternal FT4. For infants, we found a positive association between FT4 and *cis*-nonachlor and mirex. The compound with the greatest contribution to this association was *trans*-nonachlor (27.1%); several other compounds contributed to WQS in small weights (2-16%). This is the first study to examine the association between multiple exposures to OCPs and both maternal and infant FT4 levels.

As we previously reported, maternal OCP concentrations observed in Japan are relatively low as compared to other countries (Kanazawa et al., 2012; Yamazaki et al., 2017). The OCP concentrations in this study were similar to those reported in two previous Japanese studies (Fukata et al., 2005; Sugiura-Ogasawara et al., 2003), which involved participants exposed to OCPs during approximately the same period as in the present study. According to Govarts et al. (2012), the level of *p,p'*-DDE exposure in the previous Japanese cohorts and in the present study were close to the mean value in European cohorts.

We summarized previous studies for maternal and infant THs in Table 6. For maternal TH, two previous studies reported inverse associations between OCPs and FT4, as found in this study. This includes a study published by Lopez-Espinosa et al. (2009) in a Spanish birth cohort, which reported *p,p'*-DDE levels that were similar to those in the present study (*p,p'*-DDE = 176 ng/g lipid). The other study, published by Chevrier et al. (2008), using data from the United States, showed that very high

exposure levels among U.S. participants (*p,p'*-DDE = 1302.1 ng/g). In four additional studies, however, no association was found between OCP exposure and FT4. Specifically, OCP levels were very low in a Canadian study (*p,p'*-DDE = 0.38 to 0.47 ng/ml) (Takser et al., 2005), a Mexican study (p,p'-DDE = 1.20 ng/ml) (Hernandez-Mariano et al., 2017), as in a study conducted in California (*p,p'*-DDE = 38.7 ng/ml) (Kezios et al., 2013). The fourth study, from the Faroe Islands, reported high levels of OCP exposure (*p,p'*-DDE = 720 ng/g), and found that PCB was significantly associated with FT4, but not OCPs (Steuerwald et al., 2000). Therefore, the inconsistency in these studies would not be solely explained by OCP exposure levels. In our study, *p,p'*-DDE did not significantly associated with any THs, but *o,p'*-DDE, *o,p'*-DDT, and dieldrin had significant inverse associations, which was same direction in Lopez-Espinosa et al. (2009), and Chevrier et al. (2008). Further studies will be needed to explore the cause of inconsistency, because these studies were different in OCP levels and profile, TH levels, sampling period of THs etc.

For infant TH, most previous studies measuring infant blood TH levels after birth reported an increase in TSH in association with ∑DDT, *p,p'*-DDE (Kim et al., 2015), or *β*-HCH (Alvarez-Pedrerol et al., 2008; Lopez-Espinosa et al., 2010; Ribas-Fito et al., 2003b), as shown in Table 6. In the present study, TSH was not associated with any OCPs. The *p,p*'-DDE level in the present study was three times higher than reported by Kim et al. (2015), and the *β*-HCH level was lower than in the three previous studies conducted in Spain. The lack of the TSH modulation in our study might be explained by mechanism of hypothalamic–pituitary axis, however, we did not find any modulation in TSH×FT4. Additionally, we found infants' FT4 level was positively associated with several OCPs. Although no previous study has examined infants' FT4 after birth, two studies evaluating cord serum samples reported an association between FT4 and exposure to OCPs. The findings of Dallaire et al. (2008) were consistent with our results, in which FT4 increased as HCB was increasing. An inverse result was reported by Maervoet et al. (2007), who found that HCB and *p,p'*-DDE were negatively associated with FT4. The levels of HCB in the present study were approximately half of those reported by Dallaire

et al. (58.4 ng/g lipid), and similar to the levels reported by Maervoet et al. (22.4 ng/g lipid), indicating that the inconsistency of results could not be explained by the differences in exposure levels. We note that the data derived from heel-prick blood samples after at least four days following birth in our study should not be directly compared to the results of these studies. TH levels dramatically change during the first 24 hours after birth, and hormone levels from cord blood may be affected by the stress of delivery, including labor pains, the duration of labor, uterotonic agents, and the lower temperature (Paul, 2006). Further, the association between exposure to OCPs and maternal TH might have affected these study results, as 30–60% of thyroid hormones in cord blood are of maternal origin (Fisher, 1997; Thorpe-Beeston et al., 1991). Further evidence using blood spot FT4 data is needed.

In the present study, we investigated the multiple exposure effect of 16 compounds, including OCPs and dioxin, using WQS methods. We found mixture of OCPs were associated with maternal FT4 inversely, and children's FT4 positively. OCP highly correlated each other, and WQS method allowed us to examine the relative weight of each compound in the multiple exposure effect within a single multivariate model for each THs. Thus, our findings suggest that OCPs exposure may alter FT4 levels not only as a sole chemical but also as a whole. We found that mixture of OCPs was specifically associated with FT4 in both maternal and infant blood. Although T4 modulation should feed back to the hypothalamus and thereby stimulate the anterior pituitary to secrete TSH in the normal hypothalamic–pituitary axis, we did not find any change in TSH or TSH×FT4 in this study. It is necessary to examine modulation of other THs (such as FT3) in future analyses. Regarding the mixture effect of 15 OCPs and dioxin exposures, the direction of association was negative in maternal FT4 and positive in infant FT4. A direct relationship between maternal and infant FT4 cannot be evaluated because the sampling periods were different for mothers and infants. Additionally, the fact that OCPs, which were associated with FT4, differed between mothers and infants might indicate that the observed results have completely different underlying mechanisms. For mothers, the sum of o,p’-DDT, dieldrin, and cis-HCE equaled 86.8% of WQS, while six OCPs above 5% explained 72.4% of WQS for infant.

This difference of number of influential compounds might reflect infants' vulnerability to chemical compounds, because their FT4 could be affected by mixture of OCPs, even if toxicity of each chemical might be low and difficult to find an association as an individual. These findings also suggest the importance of examining mixture effects in the future study.

A major strength of our study was that both maternal and infant TH levels were assessed. No previous study has measured the effect of OCP exposure on infant FT4 after birth. Investigating TH status after birth was key, as TH has been found to vary at different points in time throughout pregnancy and after birth. Furthermore, we measured the level of exposure for multiple OCPs, and thus could estimate the multiple exposure effect using WQS methods. Chemical compounds of different OCPs and dioxin are strongly correlated with each other (Supplemental table 2). The high correlations observed between these compounds justified the use of WQS analysis. The present study is the first study to estimate the multiple exposure effect of OCPs on TH as well as the relative weight of each compound.

One limitation of this study is concern regarding multiple testing. We performed 120 statistical tests, and hence may expect 6 false significant results just by chance. After Bonferroni correction for multiple comparison, we observed significant associations only between maternal FT4 and *o,p'*-DDT. However, WQS analysis, which accounts for 15 OCPs and dioxin simultaneously within individual regression models, showed similar results to the multivariate regression models for both mothers and infants. An additional limitation of our study relates to the sequencing of exposure and outcome measurements for the maternal analysis. Specifically, the data for maternal TH was collected between 6 and 18 weeks of gestation, whereas blood sampling for OCPs occurred between 23-35 weeks gestation or at a postpartum visit. However, OCPs have a long half-life and are lipophilic and hence stored in adipose tissue (Needham et al., 1990). Hence, OCP levels remain consistent over the course of months to years, even after delivery (Takser et al., 2005). The stability of OCP levels in maternal blood ensures the validity of the observed association between OCPs and maternal TH. Another potential limitation is about bias by blood sampling period of OCPs, however, we confirmed that there

was no difference in OCP exposure levels between during pregnancy and after delivery in our data (Supplemental table 4). We have to note about possibility of systematic differences between participants included in (n=333) and excluded from (n=46) the analysis. Age at delivery was higher, prevalence of smoking and total dioxin were lower in the included group than in the excluded group (Supplemental table 1). We might underestimate the contribution of total dioxin to multiple exposure effect. Importantly, levels of four OCPs (Supplemental table 4) were lower in the included group than in the excluded group. These results suggest the possibility that we underestimated the effect of OCPs. We measured a limited number of chemicals other than OCPs. There are many chemical compounds in the environment, and a single study could not measure them all. However, in the present study, we suggest the possibility to exploring overall exposure profiles or exposomes in Japan. Finally, because TH levels in our participants were obtained from a 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 OCPs on TH circulation, data regarding other THs, such as T3, will be needed in future studies.

In conclusion, pregnancy OCP exposure was associated with higher maternal FT4 and lower infant FT4, but was not associated with TSH or TSH×FT4. These results suggest that OCPs might disrupt TH homeostasis differently from normal negative feedback mechanisms. The weight of each compound contributing to the multiple exposure effect reflects differing mechanisms for associations observed with maternal and infant FT4 levels. In the present study, we did not investigate TH modulation from a clinical perspective, but our findings may suggest pathways leading to future developmental concerns.

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Highlights

- We examined association between pregnancy OCPs and thyroid hormone (THs).
- OCPs were negatively associated with maternal free thyroxine.
- OCPs were positively associated with infant free thyroxine.
- WQS (weighted quantile sum) analysis revealed profile of OCP contributions.
- Our results indicate that even low levels of pesticides could modulate THs.

Table 1. Characteristics and thyroid hormone levels of mothers and infants (n=333).

Characteristic		Mean±SD and range or N (%)		
Maternal characteristics				
Age at delivery (year)		31.3	±4.7	17-48
BMI before pregnancy		21.1	±3.1	16.0-36.1
Educational attainment (years)	<13	139	(41.7)	
	≥13	194	(58.3)	
Annual income (yen)	<300	56	(16.8)	
	≥300	276	(82.9)	
Smoked during pregnancy	No	286	(85.9)	
Alcohol intake during pregnancy	No	231	(69.4)	
Seaweed intake per week	No	97	(29.1)	
Iodine per month (e.g. supplements/eggs)	No	233	(70.0)	
AMC and/or ATG positive	No	301	(90.4)	
Blood sampling period of OCPs	During pregnancy	217	(65.2)	
	After delivery	116	(34.8)	
Blood sampling period of TH (day)		80.0	±15.3	56-200
Total dioxin TEQ (pg/g lipid)		14.5	±6.4	3.2-42.9
Infant characteristics				
Infant sex	Male	155	(46.5)	
	Female	178	(53.5)	217-292
Gestational age (day)		275.5	±9.8	
First-born	Yes	156	(46.8)	
	No	179	(53.3)	
Birth weight (g)		3071.8	±383.2	1594-4292
Blood sampling (day after birth) of TH		4.4	±0.9	3.0-7.0
Thyroid hormone levels	**Median**	**95%CI**		
Maternal				
TSH(mU/L)	1.00	1.14-1.37		
FT4 (ng/dL)	0.98	1.00-1.06		
TSH×FT4	0.93	1.08-1.28		
Infant				
TSH(mU/L)	2.20	2.51-3.03		
FT4 (ng/dL)	2.01	2.00-2.08		
TSH×FT4	4.20	5.14-6.26		

SD: standard deviation; CI: confidence interval; BMI: body mass index; AMC: antimicrosomal antibody; ATG:

Table2. Concentrations of OCPs (pg/g wet weight) in maternal serum (n=333).

OCP		Detection limit	Detection rate	Minimum	Percentile			Max
					25th	50th	75th	
Chlordanes	oxychlordane	0.9	100.0	7.9	26.9	39.2	55.9	250.9
	cis-Nonachlor	0.4	100.0	1.6	6.5	9.7	14.0	38.1
	trans-Nonachlor	0.5	100.0	13.1	49.3	69.8	102.3	513.5
DDTs	p,p'-DDD	0.4	89.7	0.2	0.9	1.5	2.3	9.0
	o,p'-DDE	0.4	85.0	0.2	0.7	1.3	1.8	6.2
	p,p'-DDE	0.6	100.0	99.5	394.2	635.6	1013.3	4575.7
	o,p'-DDT	0.6	97.6	0.3	2.3	3.6	4.9	17.1
	p,p'-DDT	0.4	100.0	2.4	16.2	22.8	34.0	121.5
Drins	Dieldrin	0.8	100.0	4.1	12.1	16.3	22.4	71.5
Heptachlors	cisHeptachlorepoxide	0.4	100.0	6.2	18.8	26.0	37.4	200.5
HCB	HCB	0.9	100.0	34.9	79.1	101.6	128.8	239.8
HCHs	ßHCH	0.6	100.0	20.0	104.7	153.7	235.6	1667.1
Mirex	Mirex	0.5	100.0	0.9	4.1	5.9	8.3	35.0
Toxaphenes	Parlar26	1.0	97.1	0.5	2.8	4.3	6.4	20.8
	Parlar50	2.0	96.0	1.0	4.3	6.4	9.5	29.3

Among 26 compounds, the 15 compounds include here were those detected in over 80% of the participants.

OCP: organochlorine pesticide; DDD: dichlorodiphenyldichloroethane; DDE: dichlorodiphenyldichloroethylene; DDT: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; HCH: hexachlorocyclohexane

Table3. Associations between prenatal exposure to OCPs and maternal TH (n = 333)

	TSH								**FT4**									**TSH×FT4**							
	Crude				**Model**				**Crude**				**Model**					**Crude**				**Model**			
	β	95%CI		*p*	β	95%CI		*p*	β	95%CI		*p*	β	95%CI		*p*		β	95%CI		*p*	β	95%CI		*p*
oxychlordane	-0.03	-0.20	0.14	0.73	0.03	-0.20	0.25	0.81	0.01	-0.04	0.06	0.76	0.00	-0.07	0.06	0.89	#	-0.02	-0.18	0.13	0.77	0.02	-0.18	0.23	0.83
cis-Nonachlor	-0.01	-0.17	0.16	0.95	0.04	-0.18	0.26	0.70	-0.02	-0.07	0.02	0.30	-0.05	-0.11	0.01	0.10	#	-0.03	-0.18	0.12	0.69	-0.01	-0.21	0.19	0.94
trans-Nonachlor	0.00	-0.16	0.16	0.96	0.04	-0.16	0.25	0.70	-0.01	-0.06	0.03	0.56	-0.03	-0.08	0.03	0.36	#	-0.02	-0.16	0.13	0.80	0.01	-0.17	0.20	0.88
p,p'-DDD	0.07	-0.04	0.17	0.22	0.06	-0.05	0.17	0.28	-0.02	-0.05	0.01	0.28	-0.01	-0.04	0.02	0.45	#	0.05	-0.05	0.15	0.31	0.05	-0.05	0.15	0.34
o,p'-DDE	0.01	-0.10	0.12	0.82	0.03	-0.08	0.15	0.57	-0.03	-0.06	0.00	0.06	**-0.04**	**-0.07**	**-0.01**	**0.02**	#	-0.02	-0.11	0.08	0.74	0.00	-0.11	0.10	0.94
p,p'-DDE	0.00	-0.14	0.13	0.98	0.01	-0.15	0.17	0.93	-0.02	-0.06	0.02	0.38	-0.02	-0.07	0.02	0.32	#	-0.02	-0.14	0.10	0.76	-0.014	-0.16	0.13	0.84
o,p'-DDT	0.03	-0.11	0.16	0.69	0.05	-0.10	0.20	0.52	**-0.06**	**-0.09**	**-0.02**	**0.005**	**-0.06**	**-0.10**	**-0.02**	**0.002**	#	-0.03	-0.15	0.10	0.66	-0.02	-0.15	0.12	0.81
p,p'-DDT	-0.01	-0.18	0.15	0.88	0.04	-0.15	0.23	0.67	-0.03	-0.07	0.019	0.26	-0.05	-0.10	0.00	0.07	#	-0.04	-0.19	0.11	0.60	-0.01	-0.18	0.17	0.94
Dieldrin	-0.01	-0.21	0.20	0.94	0.00	-0.23	0.23	0.99	**-0.06**	**-0.12**	**0.00**	**0.046**	**-0.08**	**-0.14**	**-0.02**	**0.01**	#	-0.07	-0.25	0.12	0.48	-0.08	-0.29	0.13	0.45
cis-HCE	0.00	-0.17	0.18	0.97	-0.02	-0.22	0.18	0.86	-0.04	-0.09	0.006	0.09	-0.04	-0.10	0.01	0.10	#	-0.04	-0.20	0.12	0.62	-0.06	-0.24	0.12	0.50
HCB	-0.07	-0.32	0.19	0.60	0.00	-0.35	0.35	1.00	-0.01	-0.09	0.06	0.72	-0.04	-0.14	0.05	0.36	#	-0.08	-0.31	0.15	0.49	-0.04	-0.36	0.27	0.78
βHCH	0.02	-0.11	0.16	0.74	0.02	-0.16	0.20	0.82	-0.01	-0.05	0.03	0.67	-0.01	-0.06	0.04	0.72	#	0.01	-0.11	0.14	0.82	0.01	-0.15	0.17	0.89
Mirex	0.00	-0.16	0.17	0.96	0.01	-0.20	0.23	0.90	0.00	-0.04	0.05	0.89	0.01	-0.05	0.07	0.79	#	0.01	-0.14	0.15	0.93	0.02	-0.18	0.22	0.83
Parlar26	0.01	-0.13	0.14	0.90	0.00	-0.16	0.16	0.98	-0.03	-0.06	0.012	0.18	-0.03	-0.07	0.01	0.20	#	-0.017	-0.14	0.10	0.78	-0.026	-0.17	0.12	0.72
Parlar50	-0.05	-0.19	0.09	0.47	-0.07	-0.24	0.10	0.41	-0.03	-0.07	0.013	0.18	-0.03	-0.08	0.01	0.14	#	-0.08	-0.21	0.05	0.22	-0.10	-0.26	0.05	0.18

Models were adjusted for maternal age, maternal BMI, smoking during pregnancy, maternal education, blood sampling period of TH and OCP, Total dioxin.

The values of OCP, TH, and Total dioxin were log10 transformed.

OCPs: organochlorine pesticides; TH: thyroid hormone; CI: confidence interval; DDD: dichlorodiphenyldichloroethane; DDE: dichlorodiphenyldichloroethylene; DDT: dichlorodiphenyldichloroethylene; HCE: Heptachlorepoxide; HCB: hexachlorobenzene; HCH: hexachlorocyclohexane

Table4. Associations between prenatal exposure to OCPs and infant TH (n = 333)

	TSH								FT4									TSH × FT4							
	Crude				Model				Crude				Model					Crude				Model			
	β	95%CI		*p*	β	95%CI		*p*	β	95%CI		*p*	β	95%CI		*p*		β	95%CI		*p*	β	95%CI		*p*
oxychlordane	-0.04	-0.20	0.12	0.62	0.06	-0.16	0.28	0.58	0.03	-0.005	0.07	0.09	0.04	-0.02	0.09	0.17	#	-0.01	-0.18	0.17	0.94	0.10	-0.13	0.33	0.40
cis-Nonachlor	-0.02	-0.17	0.14	0.84	0.05	-0.16	0.26	0.62	**0.04**	**0.0004**	**0.08**	**0.05**	**0.05**	**0.001**	**0.10**	**0.04**	#	0.02	-0.14	0.19	0.79	0.11	-0.12	0.33	0.35
trans-Nonachlor	-0.04	-0.19	0.11	0.63	0.05	-0.15	0.24	0.64	0.03	-0.002	0.07	0.06	0.04	-0.005	0.09	0.08	#	0.00	-0.16	0.16	0.97	0.09	-0.12	0.30	0.40
p,p'-DDD	-0.04	-0.14	0.06	0.45	-0.03	-0.14	0.07	0.52	0.01	-0.01	0.04	0.36	0.01	-0.02	0.04	0.44	#	-0.03	-0.14	0.08	0.62	-0.02	-0.13	0.09	0.67
o,p'-DDE	0.01	-0.09	0.12	0.81	0.03	-0.08	0.14	0.60	0.02	-0.01	0.04	0.12	0.02	-0.01	0.05	0.14	#	0.03	-0.08	0.14	0.56	0.05	-0.07	0.17	0.40
p,p'-DDE	0.00	-0.13	0.13	0.98	0.03	-0.12	0.18	0.70	0.02	-0.02	0.05	0.32	0.01	-0.03	0.05	0.55	#	0.02	-0.12	0.16	0.80	0.04	-0.12	0.20	0.62
o,p'-DDT	0.08	-0.05	0.21	0.23	0.09	-0.05	0.23	0.22	0.00715	-0.02	0.04	0.66	0.00	-0.03	0.04	0.84	#	0.09	-0.05	0.22	0.21	0.09	-0.06	0.24	0.23
p,p'-DDT	-0.02	-0.17	0.14	0.82	0.00	-0.18	0.19	0.97	0.03	-0.003	0.07	0.07	0.04	-0.004	0.08	0.08	#	0.02	-0.15	0.18	0.85	0.04	-0.15	0.24	0.66
Dieldrin	0.10	-0.10	0.29	0.34	0.14	-0.08	0.36	0.21	0.03	-0.01	0.08	0.17	0.04	-0.01	0.10	0.12	#	0.13	-0.08	0.34	0.22	0.18	-0.05	0.41	0.12
cis-HCE	-0.02	-0.19	0.15	0.84	0.01	-0.18	0.19	0.96	-0.01	-0.05	0.03	0.51	-0.02	-0.06	0.03	0.46	#	-0.03	-0.21	0.15	0.74	-0.01	-0.21	0.19	0.91
HCB	-0.02	-0.26	0.22	0.88	0.12	-0.22	0.46	0.49	0.06	-0.003	0.11	0.06	0.07	-0.01	0.15	0.10	#	0.04	-0.22	0.29	0.78	0.19	-0.17	0.54	0.31
βHCH	0.00	-0.13	0.13	0.96	0.09	-0.09	0.26	0.33	0.02	-0.01	0.05	0.29	0.02	-0.02	0.07	0.26	#	0.01	-0.12	0.15	0.85	0.11	-0.07	0.30	0.24
Mirex	0.01	-0.14	0.17	0.87	0.08	-0.12	0.29	0.42	0.03	-0.01	0.07	0.10	**0.05**	**0.003**	**0.10**	**0.04**	#	0.04	-0.12	0.21	0.60	0.14	-0.08	0.36	0.22
Parlar26	0.05	-0.08	0.18	0.46	0.09	-0.07	0.24	0.26	0.01	-0.02	0.05	0.36	0.03	-0.01	0.06	0.16	#	0.06	-0.07	0.20	0.36	0.11	-0.05	0.28	0.17
Parlar50	0.01	-0.12	0.14	0.88	0.04	-0.13	0.20	0.66	0.03	-0.01	0.06	0.12	0.04	-0.003	0.08	0.07	#	0.04	-0.11	0.18	0.62	0.07	-0.10	0.24	0.41

Models were adjusted for maternal age, maternal BMI, smoking during pregnancy, maternal education, infant sex, first-born status, blood sampling period of TH and OCP, and Total dioxin.

The values of OCP, TH, and Total dioxin were log10 transformed.

OCPs: organochlorine pesticides; TH: thyroid hormone; CI: confidence interval; DDD: dichlorodiphenyldichloroethane; DDE: dichlorodiphenyldichloroethylene;

DDT: dichlorodiphenyldichloroethylene; HCE: Heptachlorepoxide; HCB: hexachlorobenzene; HCH: hexachlorocyclohexane

Table 5. Associations between prenatal exposure to OCPs and maternal and infant TH calculated by WQS

		WQS β	CI		Direction of WQS
Mother	TSH	0.011	-0.039	0.061	positive
		-0.030	-0.079	0.019	negative
	FT4	0.005	-0.009	0.002	positive
		-0.017	**-0.030**	**-0.004**	negative
	TSH×FT4	0.007	-0.041	0.050	positive
			not estimated		negative
Infant	TSH	0.030	-0.017	0.077	positive
		-0.006	-0.052	0.040	negative
	FT4	**0.017**	**0.006**	**0.028**	positive
			not estimated		negative
	TSH×FT4	0.039	-0.012	0.090	positive
			not estimated		negative

Mother: Adjusted for maternal age, maternal BMI, smoking during pregnancy, maternal education, and

Infant: Adjusted for maternal age, maternal BMI, maternal education, smoking during pregnancy, infant sex,

Table6. Summary of previous studies about OCP exposure and TH.

No.	Author	Year	Place & Cohort	N	Matrix		OCP	Summary of results					Exposure Level	Thyroid hormone Level	Results
					OCP	TH		fT3	TT3	fT4	TT4	TSH			
Maternal															
1	Hernández-Mariano	2017	floriculture area of Estado, Mexico	430	Maternal serum	Maternal serum	p,p'-DDE	→	↑	→	→	→	p,p'-DDE: 1.2 ng/mL (Median)	TSH: 0.85 mIU/L, FT3: 2.12 pg/mL, TT3: 1.32 mg/mL, FT4: 0.75 ng/mL, TT4: 5.77 μg/dL (Median)	A significant positive association between p,p'-DDE and total T3 serum levels was observed.
2	Kezios	2013	Oakland, California	600	Maternal serum	Maternal serum	p,p'-DDT p,p'-DDE o,p'-DDT	- - -	- - -	→ → →	- - -	→ → →	p,p'-DDT: 10.8, p,p'-DDE: 38.7, o,p'-DDT: 0.43 ng/mL (Median)	FT4: 1.23 ng/dL, TSH: 1.29μIU/mL (Median)	Neither FT4 nor TSH was related to exposures.
3	Lopez-Espinosa	2009	Valencia, Spain INMA	157	Maternal serum	Maternal serum	p,p'-DDE	-	→	↓	-	↑	p,p'-DDE: 176 ng/mL lipid (Median)	TT3: 2.5 nmol/L, FT4: 11pmol/L, TSH: 1.2 mIU/L (Median)	p,p'-DDE were associated with increased TSH and reduced FT4 but not TT3 levels.
4	Chevrier	2008	Salinas Valley, California. CHAMACOS	334	Maternal serum	Maternal serum	DDT/DDE isomer HCB	- -	- -	→ ↓	→ ↓	→ →	p,p'-DDT: 18.8, o,p'-DDT: 1.7, p,p'-DDE: 1302.1, Hexachlorobenzene: 65.8 ng/g (GM)	TT4: 10.6μg/dl, FT4: 0.82ng/dl (Mean) TSH: 1.16mIU/l (GM)	None of the DDT/DDE isomers were associated with any of the thyroid hormone measurements. Hexachlorobenzene was negatively associated with both TT4 and FT4, but not with TSH.

5	Takser	2005	Southwest Québec, Canada	149	Maternal blood	Maternal blood	p,p'-DDE cic-nanochlor HCB 3 other OCPs	− − − →	↓ ↓ ↓ →	→ → → →	− − − →	→ → → →	1st trimester (n =39) trans-Nanochlor: 0.03, Oxychlordane: 0.02, Hexachlorobenzene: 0.04 , DDT: 0.01, p,p'-DDE: 0.38, beta-BHC: 0.03μg/L 2nd trimester (n = 145) trans-Nanochlor: 0.04, Oxychlordane: 0.03, Hexachlorobenzene: 0.06 , DDT: 0.03, p,p'-DDE: 0.43, beta-BHC: 0.04μg/L at delivery (n = 101) trans-Nanochlor: 0.05, Oxychlordane: 0.03, Hexachlorobenzene: 0.06 , DDT: 0.04, p,p'-DDE: 0.47, beta-BHC: 0.16μg/L (Median)	1st trimester (n =40) TSH: 2.1 mIU/L, FT4: 14.3 pmol/L, TT3: 2.7 nmol/L 2nd trimester (n = 147) TSH: 2.2 mIU/L, FT4: 12.8 pmol/L, TT3: 3.2 nmol/L at delivery (n = 100) TSH: 2.6 mIU/L, FT4: 11.6 pmol/L, TT3: 3.3 nmol/L (Median)	Negative correlation between maternal total TT3 and p,p'-DDE, cis-nanochlor, and hexachlorobenzene were found.
6	Steuerwald	2000	Faroe Islands	182	Maternal serum	Maternal serum	18 pesticides, p,p'-DDE	→	-	→	→	→	maternal serum p,p'-DDE: 0.72 μg/g (GM)		Thyroid function did not correlate with any OCPs.

Infant

1	Kim	2015	Korea	104	Maternal serum, cord serum, blood spot	Bloodspot at 2 days infant, cord serum	Maternal serum βHCH ΣCHD(Chlordane) ΣDDT p,p'-DDE Cord blood ΣCHD(Chlordane) p,p'-DDE HCB	↓ → → → → → →	↓ → → → → → →	→ ↓ → → → → →	→ → → → → → ↓	→→ → → → ↑ → ↑ ↑ → →↑ → → Left: cord serum Right: Blood spot	Maternal serum β-HCH: 7.5, ΣCHD: 3.9, ΣDDT: 62.3, p,p'-DDE: 55.2, HCB: 5.5 ng/g lw Cord blood β-HCH: 7.5, ΣCHD: 2.6, ΣDDT: 65.2, p,p'-DDE: 63.0, HCB: 12.7 (Median)	Cord serum FT3:1.39 pg/mL, TT3:0.63ng/mL, FT4:1.24 ng/dL, TT4 8.61μg/mL, TSH: 8.24μIU/mL Bloodspot TSH: 5.05uiU/mL (Median)	Maternal exposure to β-HCH, ΣCHD, ΣDDT, and p,p'-DDE were associated with neonatal thyroid hormones. Cord serum exposure to ΣCHD, p,p'-DDE, and HCB showed significant associations with TT4 or TSH in cord serum.
2	Lopez-Espinosa	2010	Valencia, Spain INMA	453	Cord serum	Infant blood after 2-5days delivery	βHCH 11 other OCPs	- -	- -	- -	- -	↑ →	Maternal serum β-HCH: 7.5, ΣCHD: 3.9, ΣDDT: 62.3, p,p'-DDE: 55.2, HCB: 5.5 ng/g lw Cord blood β-HCH: 7.5, ΣCHD: 2.6, ΣDDT: 65.2, p,p'-DDE: 63.0, HCB: 12.7 (Median)	blood sample spotted TSH Median 1.4, CI=1.2, 1.4 mIU/l	Neonatal TSH levels tended to be higher in newborns with bβ-HCH levels in umbilical cord above 90th percentile (104 ng/g lipid) than in those with levels below the median (34 ng/g lipid), with an adjusted increment in neonatal TSH levels of 21% (95% confidence interval= -3, 51; P =0.09).
3	Alvarez-Pedrerol	2008	Spanish Balearic Island of Menorca	387	Cord serum	Infant plasma at 3days	βHCH 3 other OCPs	- -	- -	- -	- -	↑ →	For low TSH group (n=382) HCB = 0.70, p,p'-DDE = 1.08, p,p'-DDT = 0.07, beta-HCH = 0.24 ng/ml (GM)		Levels of β-HCH was positively related to TSH concentrations

4	Ribas-Fito	2003	Flix, Catalonia, Spain	70	Cord serum	Plasma of 3 days old newborns	β-HCH HCB p,p'DDE	- - -	- - -	- - -	- - -	↑ → →	HCB: 1.14, p,p'-DDE: 0.85, β-HCH: 0.54 ng/ml (Median)	TSH in plasma of 3 days old newborns	p,p'-DDE,β-HCH) were related to higher concentrations of TSH, although only significant for β-HCH
5	Cordier	2015	Guadeloupe (French West Indies) Timoun mother-child cohort	111	Cord blood & Milk	Child blood at 3 moths	Cord Chlordecone Milk Chlordecon Cord and Milk p,p'-DDE	→ ↓ →	- - -	→ ↓ (girl) →	- - -	↑ (boy) → →	chlordecone：0.13, p,p'-DDE : 0.30 μg/L	TSH:Total 1.98, Boys 2.23, girls 1.44 mIU/L FT3: Total 4.50, Boys 4.40, Girls 4.70 pg/mL FT4: Total 14.30, Boys 14.30, Girls 13.90 pg/mL (Median)	Cord chlordecone was associated with an increase in TSH in boys, whereas postnatal exposure was associated with a decrease in FT3 overall, and in FT4 among girls.
6	Dewailly	1993	Inuit woman at Kativik region, Quebec, Canada	92	Milk	Infant blood	8 OCPs	-	-	-	-	→	-	-	TSH blood level and OCPs (heptachlor epoxide, alpha and ganma-chlordane, endrin, HCB, dieldrin, DDE, mirex) exposure did not show any statistically significant associations.
7	Nagayama	2007	Fukuoka, Japan	Cretinism group 65 Nomal group 108	Breast milk collected within 4 weeks after childbirth	Infant serum at 5-20 days after birth	DDT HCH Chlordane HCB	OR of cretinism 10 2.8 (n.s.) 6.6 22					PCBs TEQ: case 0.62, normal 0.28 pg TEQ/g DDT: case 7.72, normal 3.67 ng/g HCH: case 2.99, normal: 1.25 ng/g Chlordane: case 1.85, normal 0.76 ng/g HCB: case 0.34, normal 0.18 ng/g	Mean TSH: case 47.6, normal 8.31 μIU/ml Mean FT3: case 2.80, normal 3.58 pg/ml Mean FT4: case 0.90, normal 2.46 ng/dl	Odds ratios of cretinism increased as DDT, Chlordane, and HCB level in maternal breast milk.

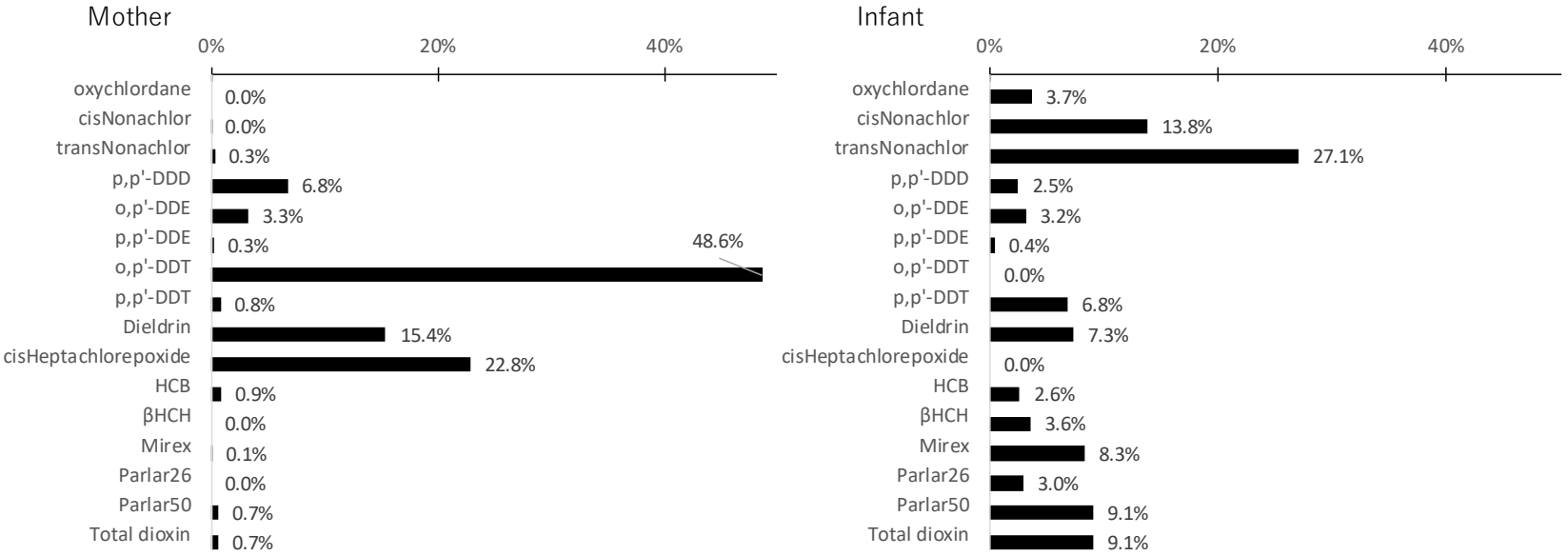

Figure 1. Contribution of OCPs and total dioxin TEQ to WQS percentage for maternal and infant FT4.
The percentage for each compound indicates the compound's contribution to the WQS index value.

OCPs: organochlorine pesticides; TEQ: toxic equivalency; WQS: Weighted quantile sum, FT4: free thyroxine
HCH: hexachlorocyclohexane; HCB: hexachlorobenzene; DDT: dichlorodiphenyldichloroethylene; DDE: dichlorodiphenyldichloroethylene; DDD: dichlorodiphenyldichloroethane