보건학 박사학위논문

Exposure to several persistent organic pollutants (POPs) in pregnant women and newborn infants and associated endocrine disruption effects

산모-태아의 잔류성 유기오염물질 노출과 내분비계 교란 영향

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김 선 미

Abstract

Exposure to persistent organic pollutants (POPs) in pregnant women and newborn infants and associated endocrine disruption effects

Sunmi Kim
Graduate School of Public Health
Seoul National University

Many of the persistent organic pollutants (POPs) had been banned several decades ago. However, most of these compounds, including organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) have been frequently detected in various environmental media, biota, and human biological samples worldwide. In addition, new POPs - such as polybrominated diphenyl ethers (PBDEs) - have also been frequently detected because of their extensive use and bioaccumulative characteristics. Among various toxic effects of POPs, endocrine disruption that may link to changes in growth and development, metabolic disorders and obesity is one of the important adverse health effects.

Thyroid hormones and adipokines are key hormones related to normal development and energy metabolism. Early life stages are particularly susceptible to these hormones and the endocrine disruption. Endocrine disrupting effect during these sensitive periods may lead to permanent adverse effects in later stages of life. Therefore, association between POPs exposure and endocrine

disruption among the susceptible human populations, including fetuses, newborn infants, and pregnant women deserves scrupulous investigation. However, current understanding on endocrine disruption effects of POPs is still limited among these vulnerable populations and previously reported results are frequently controversial. In order to address these issues, the present study investigates the association between POPs exposure and thyroid hormones or adipokine levels among pregnant women or matching newborns.

For this purpose, pregnant women and their matching newborn infants without any known occupational exposure pathways to major POPs were recruited from five university hospitals located in four cites of South Korea in 2011-2012 (Children's Health and Environmental Chemicals of Korea Panel: CHECK Panel). Maternal and cord blood serum samples were collected at delivery, and breast milk samples were collected between 15^{th} and 30^{th} day of lactation. Target chemicals including 19 OCPs, 19 PCBs, and 19 PBDEs, and hormones were measured in serum samples, and chemicals which were detected > 60% and sum of the isomers (ΣPCB, ΣPBDE, Σdichlorodiphenyltrichloroethane (DDT), Σchlordane (CHD), Σhexachlorhexane (HCH)) were used in statistical analysis. This study was conducted in three parts.

In the first part, the associations between major groups of POPs and thyroid hormone balances among pregnant women were assessed. Blood samples were collected within a day before delivery from 105 pregnant women of CHECK Panel in 2011. Serum was then analyzed for target POPs along with five thyroid hormones (free and total T3 and T4, and TSH). Several PCBs, such as CB 28, -52, and -118, showed negative associations with T3 or T4. BDE 47 and ΣPBDEs showed significant associations with T3 or T4. For OCPs, ΣDDT and hexachlorobenzene (HCB) were generally associated with the reduction of T3 or T4. While the thyroid hormone levels of all subjects were within the reference

range, the levels of exposure to several target POPs were clearly associated with alteration of thyroid hormone balance among pregnant women without any known occupational sources of exposure.

In the second part, the associations between prenatal exposure to major POPs and thyroid hormone levels among newborn infants were investigated (n=104). As thyroid hormone levels in cord blood serum could be influenced by the input of thyroid hormones of maternal origin, thyroid hormone concentrations of the matching mothers at delivery were adjusted. In addition, TSH measured in bloodspot samples of newborn infants on 2 day after birth was used. In cord serum, BDE 47, BDE 99, ΣCHD, and *p,p′*-dichloro-diphenyldichloro-ethylene (DDE) showed significant positive associations with cord blood serum or bloodspot TSH. At the same time, *p,p′*-DDE and HCB showed negative association with total T3 and T4 in cord serum, respectively. Maternal exposure to *β*-hexachlorhexane (*β*-HCH), ΣCHD, ΣDDT, or *p,p′*-DDE were also associated with neonatal thyroid hormones. Although the sample size was small and the thyroid hormone levels of the subjects were within the reference range, our observation clearly supported endocrine disrupting effects of several POPs among newborn infants at the levels occurring in the general population.

In the third part, the associations between several kinds of maternal POPs exposure and the levels of adipokines in breast milk were investigated (n=50). As the effect marker hormones related to obesity and diabetes, leptin and adiponectin in breast milk were selected. Significant negative association between breast milk leptin concentration and *oxy*-chlordane (oxyCHD), ∑CHD, BDE 47, or CB 138 levels in maternal serum was observed. *P,p'*-DDT, oxyCHD, *trans*-Nonachlordane (tNCHD), ∑CHD, BDE 47, CB 153, or ∑PCB body burden were significantly associated with increased adiponectin concentrations in breast milk samples. Thus,

the results of this study provide a line of evidence that POPs at the current level of exposure may link to the alteration of lipid metabolism, which might possibly lead to obesity in later stages of life.

Through a series of cross-sectional studies, it was established that the current levels of exposure to POPs could be associated with the disruption in thyroid hormones and lipid metabolism among pregnant/lactating women or newborn infants. These findings are supported by previous experimental studies, but should be confirmed in prospective birth cohorts with a greater number of subjects for the biological significance. Considering the importance of thyroid hormones and adipokines during gestation and early life stages, health implication of endocrine disruption effects by low level POPs exposure deserves further investigation.

Keywords: POPs, Polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs), Organochlorine pesticides (OCPs), pregnant women, fetus, thyroid hormone, adiponectin, leptin, breast milk, CHECK Panel

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Chapter 1. Introduction

1.1. Background

Persistent organic pollutants (POPs) exposure in general population

Among numerous man-made chemicals, persistent organic pollutants (POPs) represent a global public health concern because of their persistence in environment, bioaccumulation potential, and adverse health effects. To address this emerging public health threat, a total of 152 countries have committed to discontinue or restrict the use of major POPs by ratifying the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention). Such POPs include organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and, more recently, polybrominated diphenyl ethers (PBDEs). Many OCPs, including dichlorodiphenyltrichloroethane (DDT) had been widely used to control disease vectors or harmful insects since World War II. However, due to environmental persistence, serious ecosystem damages, and potential human health implications of OCPs, the use of most of them has been banned since 1970s. Previously, PCBs were extensively used as transformer, hydraulic fluids and additives of paints and oils; several decades ago, however, their use was also restricted in most countries due to similar reasons. PBDEs had been used as flame retardants worldwide and applied in numerous products, such as polyurethane foam, furniture, mattresses, synthetic textiles, and in electrical instruments. PBDEs are a new group of POPs that were added in Stockholm Convention in 2009 and their ubiquitous occurrences and potential health consequences are a growing worldwide concern.

Penta-, octa-, and deca BDE mixtures are predominant commercial flame retardant products. PBDEs are added to the surface of products, and bromines are

released to the air and then replace oxygen when the product meets fire. They are released into the environment during manufacturing operations or when the products containing PBDEs are disposed. Due to toxicity PBDEs, their use in consumer products or production has been banned since 2004 in many countries, including those of the European Union. Testicular cancer, decreased birth weight, cryptorchidism, decreased sperm quality were linked with PBDEs body burden in human cross-sectional studies (Hardell et al., 2005; Chao et al., 2007; Main et al., 2007; Akutsu et al., 2008), and thyroid disruption effects were also reported in infant and adult populations (Herbstmann et al., 2008; Kim et al., 2009; Hagmar et al., 2001; Turyk et al., 2008; Meeker et al., 2009).

Because of their persistent and accumulative characteristics, numerous POPs that were banned several decades ago are still detected in human biological samples like serum, adipose tissue, and breast milk (Govarts et al., 2012). PCBs concentrations in adipose tissue in Korean adults were lower than in Japanese, but higher than in US American and Singaporean populations (Moon et al. 2012). Considering that DDTs and chlordane (CHD) concentrations are relatively greater in Korean cetaceans (Moon et al., 2010), accumulation of PCBs and OCPs should still be monitored in Korea. Even the new POPs were frequently detected in Korea. In Korean general population, PBDEs, one of the new POPs, were detected on average 7.0 to 8.6 ng/g lipid weight (lw) with 96~97% of detection frequency in two separate studies (n=400~450) (MFDS, 2008; MFDS, 2009). The levels reported in Korea are similar to those of other Asian populations, and lower than those of North America (MFDS, 2008; MFDS, 2009). Many studies have been conducted to find exposure sources of PBDEs in general populations. As shown by previous studies, food and dust ingestion are known as major intake routes of PBDEs. Among the three possible pathways of PBDEs intake (namely, dust, air, and breast milk), dust and human milk account for almost 100%, while air inhalation accounts for a very insignificant part (Toms et al., 2009). Especially in

case of children, dust ingestion is the major exposure route, while intake amounts of PBDEs from two exposure routes, seafood and dust are similar in adult population of Korea (Lee et al., 2013a).

Pregnant women and newborn infants as susceptible populations

Fetuses and infants are considered to be among most susceptible populations for the exposure to POPs, because of their endocrine disrupting toxcities. Previous experimental studies show that POPs may lead to decrease of reproduction success, alteration of thyroid hormone regulation, developmental neurotoxicity, as well as physical developmental delay, weight velocity, and metabolic disorders. In addition, POPs can cause oxidative stress, genotoxicity, and disturbance of steroidogenic system (Gao et al., 2009; He et al., 2008; Reistad and Mariussen, 2005).

Among several kinds of toxicity following POPs exposure, the number of studies describing thyroid dysfunction in animal and human related to environmental chemical exposures has increased in the past decade. Fetal exposure to PCBs caused neuro-developmental toxicity (Huisman et al., 1995a; Weisglas-Kuperus, 1998), and leading to significant negative effects in infancy (Huisman et al., 1995b). Postnatal exposure to PBDEs was also continuously reported to be associated with neurodevelopmental toxicity in infants (Chao et al., 2011; Gascon et al., 2011). More recent studies show that prenatal POPs exposure directly causes growth inhibition, and overweight of babies (Murphy et al., 2010; Valvi et al., 2012). Likewise, slight decreases in thyroid function (subclinical or mild hypothyroidism) may lead to negative health outcomes, especially over a long term and during pregnancy. Even though thyroid hormone levels are within the reference range, small changes (< 25%) of maternal T4 or TSH during the early fetal period are associated with adverse health outcomes. Thus, the exposure to POPs at early stages of life could have considerable and long-lasting advers health consequences. Therefore, exposure to POPs among pregnant and lactating women has received growing attention.

Importance of thyroid hormones and adipokines and their association with POPs exposure

In early stages of life, growth related hormones play an important role in metabolism and development. Thyroid hormones are among the most important hormones that regulate normal development. Disturbance of the thyroid hormone regulation system by POPs exposure was observed in experimental studies and human epidemiological studies (Boas et al., 2012). Many reports showed that the associations between POPs body burden and developmental delay or metabolic disorders were linked to the thyroid hormone regulation system. Substantial evidence has been accumulated on thyroid hormone toxicity of most POPs, including PBDEs in animal or cell line toxicity test.

Compared to experimental results, relatively few studies exploring the relationship between POPs exposure and thyroid hormone homeostasis in human population were published. In PubMed search, titles including both name of POPs chemical and thyroid hormone were found in over 550 reports; however, there is still insufficient direct evidence in the human literature supporting the hypothesis that effects on thyroid hormone signaling mediate the association between chemical exposures and human disease (WHO/UNEP, 2012).

Adipokines are another important group of hormones that play a role in regulation and development of the lipid metabolic system in our body. Adiponectin and leptin are among the most studied adipokines, which are secreted in the adipocytes. Both are active players in the development and regulation of metabolism during early stages of life, e.g. fetal and infant period. As adiponectin levels in breast milk were highly correlated with the levels in serum of mothers, breast milk can serve as an alternative biological specimen for measurement of adipokines.

Since thyroid hormones affect adipose tissue metabolism, which regulate

adipokine secretion, there is an obvious relationship between thyroid hormones and adipokines function. A continuous interaction between the thyroid hormone and regulatory mechanisms localized in adipose tissue and brain is important for the human body weight control and maintenance of optimal energy balance (Santini et al., 2014). It has been established that leptin is an important factor in the development of hypothalamic pathways, which involve in the regulation of energy balance (Savino et al., 2011). For hypothalamic-pituitary-thyroid axis, leptin acts on the expression of thyrotropin releasing hormone (TRH), and decreased T4 and T3 levels can be reversed by leptin (Ahima et al., 1996; Flier et al., 2000). The *in vivo* administration of human TSH can induce the proportional release of leptin compared to the adipose mass, thus confirms the function of TSH receptors on the surface of white adipocytes (Santini et al., 2014). Adiponectin secretion increases insulin action by increasing fat oxidation, which is controlled by thyroid hormone activity (Lihn et al., 2005). Although the role of thyroid hormones in adipokine modulation remains unclear (Luvizotto et al., 2012), adverse health consequences, such as elevated cholesterol level or diabetes, could be caused by slight decreases of thyroid hormone levels within the reference range.

Considering the importance of thyroid hormones and adipokines and their interaction during fast developmental stage, finding the association between there hormone levels and POPs exposure is of a great public health interest and needs to be addressed.

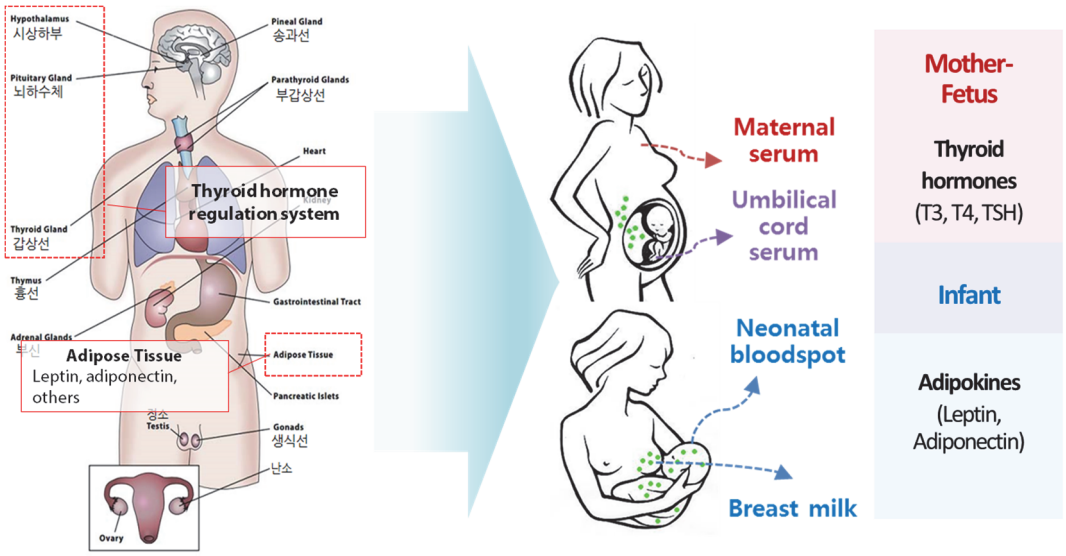

Figure 1-1. Active players in development and regulation of metabolism during fetal-newborn-infants period

(Source: WHO/UNEP, 2012 (The left panel)).

1.2. Thyroid hormone disruption by POPs in pregnant women and neonates

Thyroid hormone system plays a crucial role in maintenance of homeostasis, activation of metabolic function, neurodevelopment, and cognitive functioning. Thyroid hormones are particularly relevant for normal growth and development of the fetus throughout the gestation period (Forhead and Fowden, 2014). Several POPs have been documented for their potentials to alter thyroid hormone balance. For example, PCBs and their metabolites which have structural similarity to T4 may compete with endogenous thyroid hormones for thyroid binding globulins, which would eventually lead to clearance of thyroid hormones. PBDEs also share similar structure with T4, and cause thyroid hormone disruption through the same mechanism (Zhou et al., 2001). Thyroid disruption toxicity of many OCPs has been reported in experimental studies (Darras, 2008; Hallgren and Darnerud, 2002). Several kinds of environmental chemicals have been identified that can directly interfere with thyroid hormone receptor or other processes controlling the thyroid hormone regulation system (Figure 1-2).

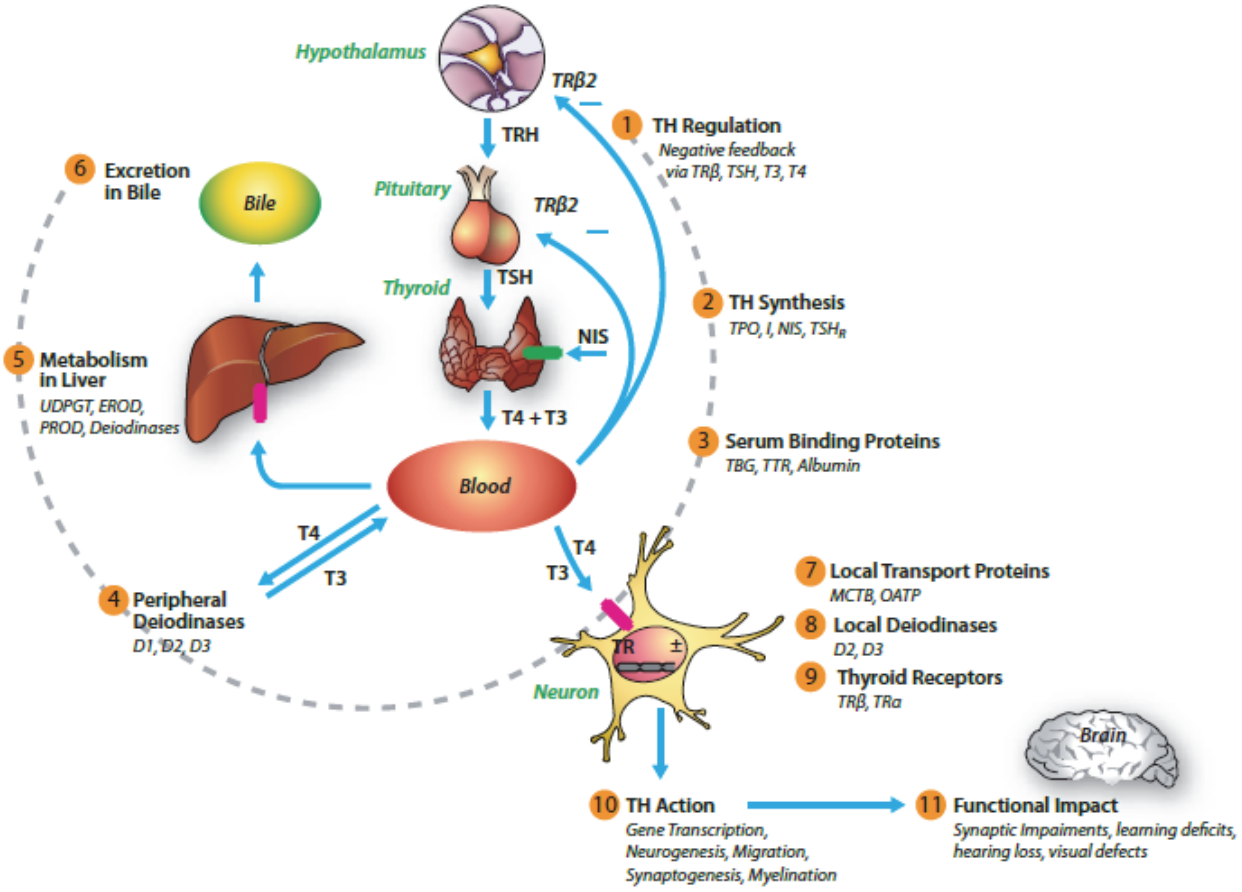

Figure 1-2. Possible sites of action of environmental contaminants on the hypothalamic-pituitary-thyroid (H-P-T) axis (Source: WHO/UNEP, 2012)**.**

As accurate regulation of thyroid hormone balance is essential for developing fetus and newborn infants, even smaller-scale changes in thyroid hormones should be considered with caution. Moderate changes of thyroid hormone levels may be associated with adverse outcomes for the mother or her offspring (Berbel et al., 2009; Idris et al., 2005; Sahu et al., 2010). In previous studies, within the reference range, higher maternal thyroid stimulating hormone (TSH) levels were associated with an increased risk of miscarriages, fetal and neonatal distress (Benhadi et al., 2009) and preterm delivery (Stagnaro-Green et al., 2005). In addition, high free thyroxin (T4) levels within the normal reference range were associated with the reduced preterm delivery rate (Torremante et al., 2011).

Relatively fewer studies have been conducted on the association between POPs exposure and thyroid hormones among newborn infants compared to the body of research that focused on pregnant women. Moreover, epidemiological observations generally yielded inconsistent results. For example, while several studies reported the increase of TSH and decreases of T3 or T4 with PBDEs, PCBs, and OCPs body burden (Abdelouahab et al., 2013; Chevrier et al., 2007; Maervoet et al., 2007), the associations in opposite directions or no association were also reported (Chevrier et al., 2011; Zhang et al., 2010), even within the same study (Ribas-Fito et al., 2003). The reason for these inconsistent associations may be explained by (1) confounding effect by the maternal origin to cord serum thyroid hormone levels (Thorpe-Beeston et al., 1991; Vulsma et al., 1989), and (2) influence of maternal, fetal, and delivery conditions to thyroid status of the fetus (Herbstman et al., 2008). Thyroid hormones measured in cord serum could be influenced by the physiological or environmental factors that could affect maternal thyroid hormone levels. Also, in studies involving human populations, controlling labor-related factors beforehand is difficult, those variables that may influence the fetal thyroid hormones should be identified and adjusted in the

statistical analytical model. Thus, it is quite challenging to identify the true association, if any, between POPs exposure and thyroid hormone levels in cord serum. In this context, bloodspot sample by hill prick method from newborns can be regarded as an alternative to cord serum. Since TSH level may vary significantly within 24 hrs from delivery, bloodspot TSH levels of the newborn infants on 2 day post-partum are considered relatively independent from the influence of the maternal thyroid hormones (Kim et al., 2005). Also, compared to the use of the cord serum TSH, the use of bloodspot TSH was regarded to have a greater sensitivity for screening congenital hypothyroidism (Hardy et al., 2008).

1.3. Adipokines disturbance and POPs exposure

There is a growing body of evidence on many POPs may act as environmental obesogens, by altering energy balance, promoting adiponenesis and lipid accumulation (Grun and Blumberg, 2006). OCPs, particularly DDE, have been linked to the increased BMI in children (Valvi et al., 2012). In addition, low-dose exposure to OCPs and PCBs is associated with a greater risk of type 2 diabetes and obesity (Lee et al., 2007; Lee et al., 2011; Lim et al., 2010; Taylor et al., 2012).

Imbalance in adipokines production and adipose tissue dysfunction are suggested as risk factors for obesity and associated metabolic disorders. Since the discovery of leptin and adiponectin in adipocytes in 1994 and 1995, several other adipokines, i.e., ghrelin, resistin, and obestatin, have also been documented. These adipokines play a role in the inhibition of fatty acids synthesis, improvement of insulin sensitivity, inhibition of adipocyte differentiation, and modulation of insulin secretion. Leptin is the most well-known adipokine that is associated with increased energy expenditure, satiety signals in hypothalamus, and caloric intake reduction (Metwally et al., 2008).

POPs can be stored within the adipose tissue and may affect the function of adipocytes. The association between POPs exposure and adipokine expressions has been studied in order to explain the underlying mechanisms of obesity-related disorders caused by POPs. In experimental studies, expressions of adipokine and leptin have been altered following the exposure to several POPs. *P,p'*-DDE, *oxy*-chlordane (oxyCHD), and CB 153 exposure alters adipogenesis, and both adiponectin and leptin are increased in adipocytes (Howell and Mangum, 2011; Taxvig et al., 2012). *In vivo* studies have also shown alteration of adipokines in mouse and rat (Provost et al., 2007; Wahlang et al., 2013), but the trend was inversed by exposure duration (Provost et al., 2007). Based on previous results, it

is obvious that alteration of adipokine by POPs exposure is one of the mechanisms of obesogenic effect. However, very few cross-sectional studies has been conducted regarding the association between POPs exposure and adipokine concentrations. Two cross-sectional studies have consistently shown negative associations between PCBs levels and adiponectin concentrations (Lim and Jee, 2014; Mullerova et al., 2008). Serum leptin was decreased by CB 138, CB 180, and BDE 153 levels in obese adult (Pereira-Fernandes et al., 2014), and similarly, *β*-HCH and *p,p'*-DDE exposure was negatively related with serum leptin levels in 8-9 years old boys (Burns et al., 2011). Among these five epidemiological studies, only one study targeted general population (Lim and Jee, 2014) and looked only at the levels of PCBs. Generally, while circulating adiponectin is inversely associated with overweight and obesity in children (Asayama et al., 2003; Stefan et al., 2002), the biological explanation for the positive association of adiponectin in milk with BMI of mothers is still challenging and difficult to interpret. Breast milk adipokines reflect the serum adipokine levels of lactating mothers (Savino et al., 2012).

In breast-fed infants, serum adiponectin is significantly related to the breast milk adiponectin concentrations, suggesting possible transport across the human intestinal mucosa. It was reported that adiponectin could be absorbed through the intestinal tract of mice (Newburg et al., 2010). Therefore, dietary intake of adipokines may directly affect the metabolic processes in infants. However, no study to date has reported the association between breast milk adipokines and POPs exposure.

1.4. CHECK Panel study

Since 2011, Children's Health and Environmental Chemicals in Korea Panel, or CHECK Panel, was developed with matching pregnant women and their newborn infant pairs. CHECK Panel is composed of pregnant women-fetus pairs without any known occupational exposure pathways to major POPs such as OCPs, PCBs, and PBDEs; the pairs were recruited from six university hospitals located in Seoul, Anyang, Ansan and Jeju of South Korea. These cities are representative for residential (Seoul and Anyang), industrial (Ansan), and rural (Jeju) regions of Korea. By 2013, a total of 352 pairs of mothers and their matching newborn infants have been recruited.

CHECK Panel was developed (1) to determine the levels of the exposure to various environmental pollutants including POPs in newborns and their mothers, and (2) to assess potential adverse health effects due to the exposure to POPs. For these purposes, several biological specimens were collected from the panel participants and were measured for several chemical contaminants. Questionnaire surveys were also carried out (Figure 1-3).

Biological samples from pregnant women included blood at 6 months of pregnancy, blood, spot urine, and placenta tissue at the delivery. Umbilical cord blood, fetal meconium, and neonatal urine were also collected. Breast milk samples were collected at 7 days, 14 days, 1 month, and 3 months after the delivery. In addition, duplicate food samples including homemade babyfood, repeated urine and hair samples of infants at 6-27 months age were collected. After birth, questionnaire about living patterns, use of furniture or home appliance, and house dust sampling were conducted to find out exposure sources of target environmental chemicals.

Alongside with 19 OCPs, 19 PCBs and 19-21 PBDEs, endocrine disruptors including phthalates, bisphenol A, and heavy metals including lead and mercury

were analyzed in appropriate biological samples. Possible effect biomarkers to assess negative health effects, i.e., stress-related markers, endocrine hormones, growth, as well as development-related markers were selected and measured.

On the CHECK Panel study, several findings related to exposure, risks, and association with health effects have been published up to now, i.e., the occurrence of PBDEs in maternal-fetal serum samples (Choi et al., 2014), POPs concentrations and risk assessment via breast milk consumption (Lee et al., 2013b; Lee et al., 2013c), baby food consumption (Jeong et al., 2014a; Jeong et al., 2014b), and phthalate metabolite concentrations in breast milk (Kim et al., 2015).

In a study by Choi et al. (2014), 198 maternal blood samples and 118 matching umbilical cord blood samples were determined for 19 PBDE congeners. Average concentration of total PBDEs in maternal blood serum was 3.34±8.42 ng/g lw at delivery and 3.14±7.46 ng/g lw at 6 months of pregnancy, respectively. In cord blood serum, an average of 9.37 ± 12.60 ng/g lw was detected. Among the measured PBDE congeners, BDE 47, BDE 99 and BDE 153 were most dominant in both maternal and cord blood sera. Relatively higher levels of BDE-99 were detected in cord blood serum. Strong positive correlations were found between maternal and cord blood serum samples, indicating the importance of maternal transfer.

After the birth, breast milk samples during lactation period were collected from 89 mothers at <7, 15, 30, and 90 days. ΣPBDE ranged from 0.23 to 68.4 (mean: 2.73) ng/g lw, and were within the ranges reported for European and Asian countries. Within a month of lactation after delivery, no significant changes were found in the PBDE concentrations. The predominance of BDE 153 rather than BDE 47 was found in the most samples that BDE 153 was detected, and was likely to be associated with stepwise debromination of BDE209. No associations were found between PBDE concentrations in breast milk and demographic parameters, except for ΣPBDE with maternal age and delivery mode. Certain

types of diet such as corn, seafood and nut correlated significantly with PBDEs levels in breast milk. The estimated daily intakes of ΣPBDE for breast-feeding infants were lower than the guidelines proposed by the USEPA, indicating limited health risk from PBDEs through breastfeeding (Lee et al., 2013b).

In Lee et al. (2013a), 19 PCBs and 19 OCPs were analyzed in 206 breast milk samples at <7, 15, 30, and 90 days. The concentrations of ΣPCBs and ΣOCPs ranged from <LOQ to 84.0 (median: 12.1) ng g/lw and from <LOQ to 559 (median: 144) ng g/lw, respectively. The residue levels of PCBs and OCPs in our study were relatively lower than those reported for European, African and Asian populations. Within a month postpartum typically after day seven the levels of ΣPCB and ΣOCP significantly increased. Some OCP compounds were correlated with maternal age, BMI, parity, and delivery mode. Certain types of dietary habits such as seafood and noodle consumption were significantly associated with ΣPCB and ΣOCP. The estimated daily intakes of ΣPCB and ΣOCP were 45.2-127 ng/kg bw-day and 625–1259 ng/kg bw-day during lactation, respectively, which are lower than the threshold values proposed by the US EPA and Health Canada. The exposure of Korean infants to CHDs via breast milk had a potential health risk which deserves further investigation.

PBDEs, PCBs, and OCPs were also analyzed in babyfood samples (Jeong et al., 2014a; Jeong et al., 2014b). 24 PBDE congeners were determined in 147 homemade babyfood samples collected from 97 households for 6-, 9-, 12-, 15-, and from 24 to 27-month-old infant groups. The concentrations of ΣPBDE ranged from 24.5 to 6000 (mean: 263) pg/g fresh weight, higher than those found in commercial formulae from the United States. The predominant congeners were BDE 209 and BDE 47, accounting for 92 % of the ΣPBDE concentrations, reflected by high deca-BDE consumption in Korea. The detected levels and detection rates of BDE 47 in the babyfood samples showed an increasing trend with an increase in infant ages, probably due to changes in the food ingredients

from hypoallergenic to greasy. The daily intakes of BDEs 47 and 209 via babyfood consumption ranged from 0.04 to 0.58, 0.80 to 20.3, and 1.06 to 22.3 ng/kg bw-day for 6-, 9- , 12-, 15-, and 24–27-month-old infant groups, respectively; these intakes were lower than theoral reference doses proposed by the USEPA. Together with three exposure sources, babyfood, breastmilk and dust ingestion for 6-month-old infants, the daily intake of ΣPBDE was 25.5 ng/kg bw-day, which was similar to the intake via babyfood consumption only for over 24-month-old infants in our study. This indicates that babyfood is an important exposure pathway of PBDEs for over 24-month-old infants (Jeong et al., 2014a).

The average concentrations of ΣPCBs, ΣDDTs, ΣHCHs and ΣCHD in baby food samples were 37.5, 96.6, 26.0, and 13.2 pg/g fresh weight, respectively. The major compounds were CBs 28, 153, 52, and 33 for PCBs and *p,p′*-DDE, *p,p′*-DDT and *β*-HCH for OCPs. The contribution of DDTs to the total OCPs concentrations increased from 30% (6-month-old infants) to 67% (15-month-old infants) with increasing infant age, while the concentrations of PCBs, HCHs and CHLs gradually decreased with increasing infant age, suggesting that highest priority for risk reduction of DDTs. The estimated daily intakes (EDIs) of OCs in Korean infants from baby food consumption were lower than the thresholds proposed by the United States Environmental Protection Agency and Health Canada, implying limited potential health risks. However, considering simultaneous exposure from baby food and breast milk consumption, CHDs and heptachlor epoxide posed potential health risks (Jeong et al., 2014b).

Two studies above are valuable as the first report on the occurrence and exposure assessment of PBDEs via homemade babyfood (Jeong et al., 2014a), and also add the information on the occurrence and exposure assessment of PCBs and OCPs by homemade babyfood (Jeong et al., 2014b).

Phthalates were analyzed in breast milk samples. There is limited information available on phthalate exposure and its associated risks among breast-

fed newborn infants. Thus, breast milk samples were collected from 62 lactating mothers at 1 month post-partum from four cities of Korea in 2012 and were evaluated for six phthalate metabolites (mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono(2-ethyl-hexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and monoethyl phthalate (MEP)). MEP was detected in all samples, with a median concentration of 0.37 μg/L, and MiBP, MnBP and MEHP were detected in 79-89 % of samples, with median concentrations of 1.10, 1.70, and 2.08 μg/L, respectively. However, MEHHP and MEOHP, the oxidized forms of di-ethyl-hexyl phthalate (DEHP), were detected in only one sample. For exposure assessment, the levels of phthalate diesters were estimated based on the parent : metabolite ratios in the breast milk that are reported elsewhere. For risk assessment, the endocrine-related toxicity of the monoester was assumed to be the same as that of its diester form. Median daily intake estimates of phthalates, including both monoester and diester forms, through breastmilk consumption ranged between 0.91 and 6.52 μg/kg body weight (bw) for DEHP and between 0.38 and 1.43 μg/kg bw for di-n-butyl phthalate (DnBP). Based on the estimated daily intake, up to 8 % of infants exceeded the reference dose of anti-androgenicity (RfD AA) for DEHP, and 6 % of infants exceeded the tolerable daily intake (TDI) for DnBP (Kim et al., 2015).

Follow-up studies are being designed with >160 pairs of the subjects to develop thorough exposure profiles for participating children, as well as to understand the association of these profiles with health effects during young childhood.

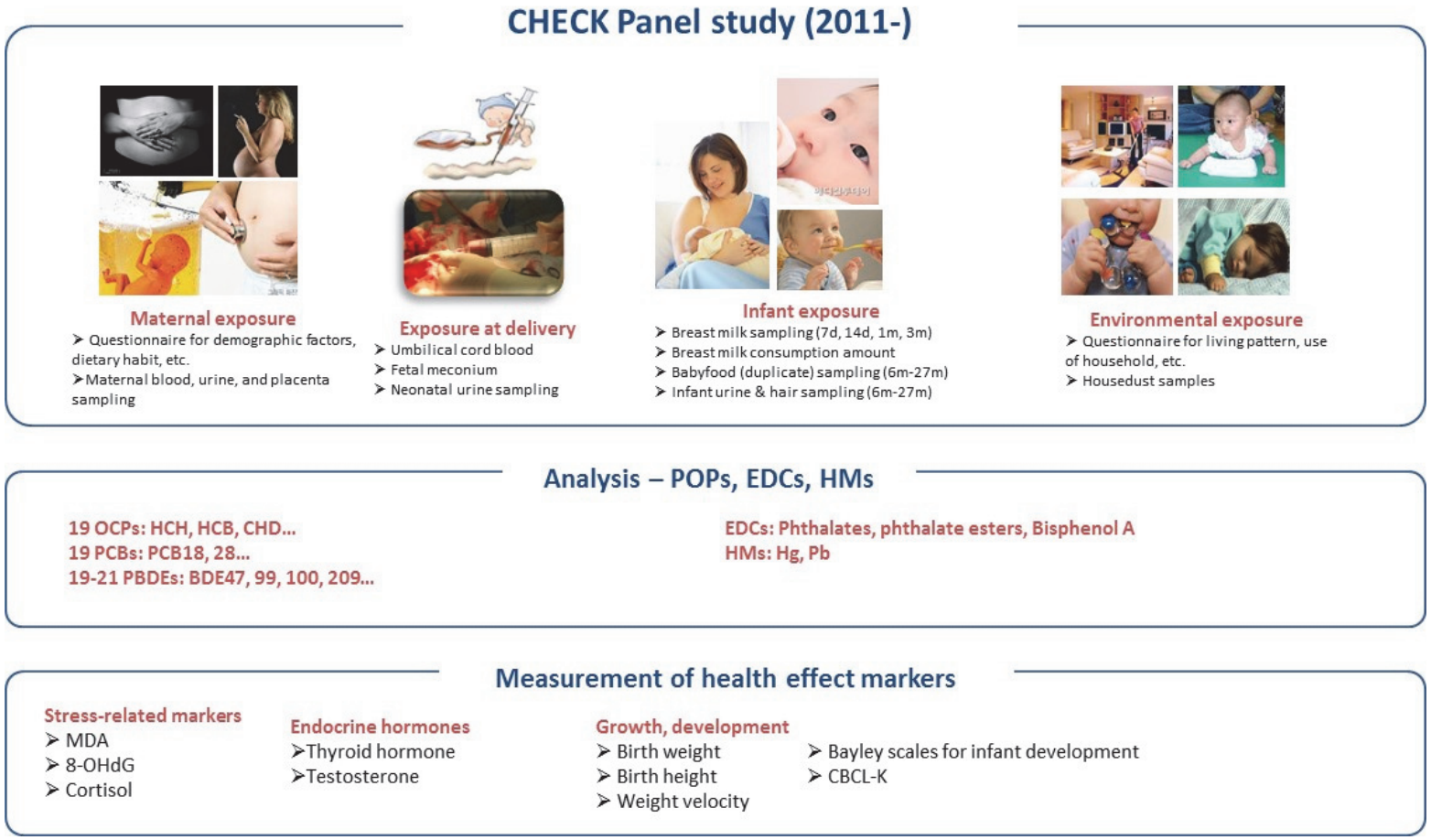

Figure 1-3. Summary of CHECK Panel study.

1.5. Study design and objectives

There are two knowledge gaps that should be filled to better understand the effects of POPs on endocrine systems of susceptible human population, namely: (1) current knowledge about toxicity of POPs on thyroid hormone and adipokine regulation system is still limited in vulnerable population; (2) existing human epidemiological studies often show inconsistent directions of association between POPs exposure and endocrine effects.

This study consists of three parts (Figure 1-4). In the first part (Chapter 2), the association between major groups of POPs and thyroid hormone balances was investigated in pregnant women. Furthermore, the association between thyroid hormone levels and prenatal POPs exposure was assessed using both cord serum thyroid hormone measurement and bloodspot sample measurement (Chapter 3). In the third part (Chapter 4), to establish the link between POPs exposure and adipokine regulations which can directly affect infants, maternal POPs levels and breast milk adipokine concentrations were compared. Taken together, our results allow for identification of adverse endocrine effects by POPs exposure in vulnerable populations.

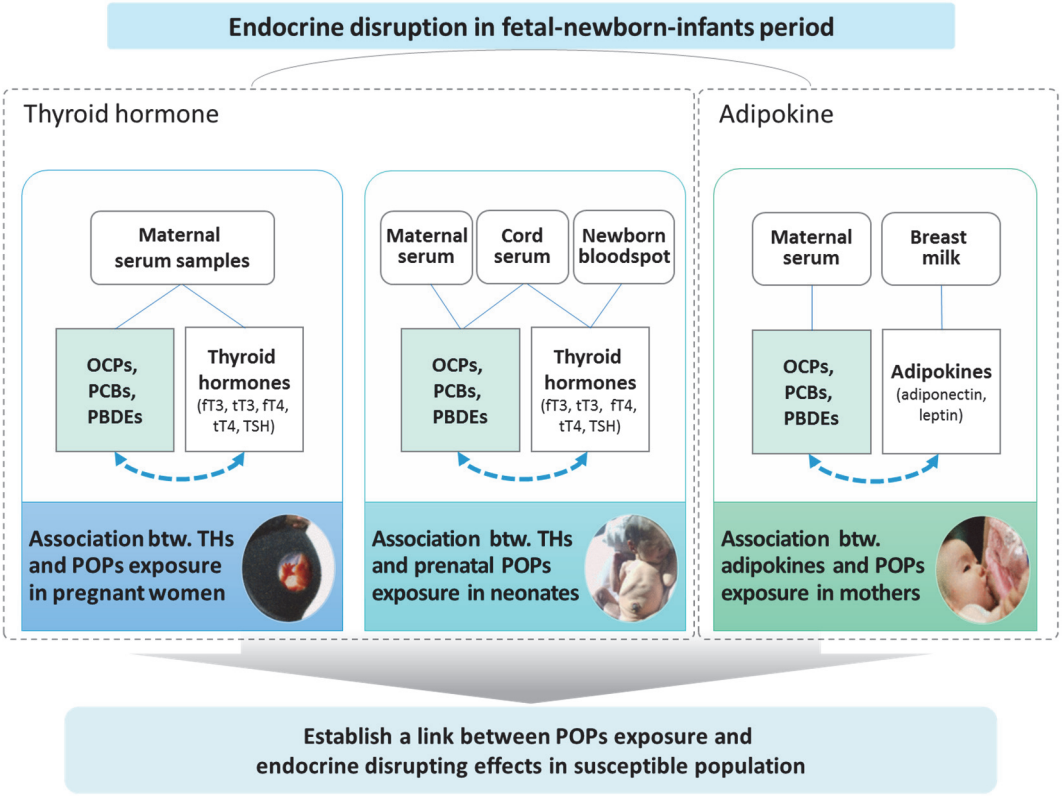

Figure 1-4. Study contents to investigate endocrine disrupting effects of POPs exposure in susceptible population.

Chapter 2. Association between several persistent organic pollutants and thyroid hormone levels in serum among the pregnant women of Korea

2.1. Introduction

Widespread exposure to POPs among general human populations raises concerns about their potential public health consequences. Potential toxicities of POPs include disruption of thyroid function. For PCBs and their metabolites, relatively firm evidences on thyroid hormone disruption are present in humans (Boas et al. 2012). PCBs and their hydroxylated metabolites are structurally close to thyroxine (T4), hence may disturb thyroid hormone balance by competing for thyroid binding proteins. Even at environmentally occurring concentrations, PCBs may decrease blood triiodothyronine (T3) or T4 concentrations or elevate TSH in humans (Chevrier et al., 2008; Osius et al., 1999; Persky et al., 2001; Schell et al., 2008; Takser et al., 2005). PBDEs are structurally more similar to T4 than PCBs, and have been hypothesized to interfere with thyroid hormone transport and metabolism (Birnbaum and Staskal, 2004; McDonald, 2002).

Results of animal experimental studies generally support the thyroid disrupting effects of PBDEs. For example, several PBDEs were shown to reduce the circulating thyroid hormones in rats and fish (Lema et al., 2008; Stoker et al., 2004; Zhou et al., 2001). PBDEs are also suspected to cause thyroid disruption in humans (Dallaire et al., 2009; Turyk et al., 2008), but whether PBDEs are negatively associated with T3 or T4 is not clear. Turyk et al. (2008) reported, for

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example, a negative association between serum PBDE and T3 or TSH, and a positive association with T4. OCPs such as DDT and HCB are also reported to alter thyroid hormones (Bloom et al., 2003; Gocmen et al., 1989; Sala et al., 2001). For example, accidental exposure to HCB led to an enlarged thyroid in humans (Gocmen et al., 1989). Several other studies have reported negative associations between HCB and total thyroid hormones (Bloom et al.,, 2003; Sala et al.,, 2001) but neither for TSH nor for free thyroid hormones (Sala et al., 2001).

Thyroid hormones are crucial for neurodevelopment and cognitive functioning of fetus. Even marginal changes of T4 level in a pregnant mother may affect the cognitive function of the fetus (Berbel et al., 2009; Haddow et al., 1999; Pop et al., 2003; Porterfield, 1994). Therefore even subtle effects on thyroid hormone balances due to prenatal exposure to POPs could be of potential public health concern (Zoeller et al., 2002).

In the present study, we examined the association between the exposure to several POPs and the thyroid hormone status in pregnant women of Korea. While relatively great number of studies has investigated the association between exposure to POPs and thyroid status, most studies have been focused on narrow range of POPs, or limited to occupational or general human populations. Due to the susceptibility of fetus to prenatal exposure to POPs, and to disturbance of maternal thyroid hormone balance, pregnant women are important group which deserves such investigation. In the present study, we measured extensive list of chemicals that incorporate three groups of major POPs that have been suggested for thyroid disruption in the literature. The results of this study will help identify important environmental chemicals that may influence the thyroid hormone balance in pregnant women.

2.2. Materials and methods

Study population and sample collection

Study population of this study was one of the subgroup of a matched pregnant woman and fetus panel of Korea, i.e., Children's Health and Environmental Chemicals in Korea Panel. CHECK Panel is composed of pregnant women-fetus pairs without any known occupational exposure pathways to major POPs such as OCPs, PCBs, and PBDEs and was recruited from five university hospitals located in Seoul, Anyang, Ansan and Jeju of South Korea since 2011. Upon the recruiting, occupational exposure, gestational diabetes, thyroid disease, surgical disease, and congenital deformity cases were excluded. Blood samples were collected from the women within a day before delivery. Blood serum was separated on site and stored in polypropylene cryovials at -70^{o}C until analysis. For this study, 138 pregnant women were recruited and collected appropriate amount maternal blood samples between February and December, 2011.

One-on-one interview with participating pregnant women were conducted at the time of enrollment for demographic parameters, physiological data, and pregnancy history. Medical records regarding current or previous health status and gestational period were abstracted. The present study was conducted with 105 participating pregnant women finally, because of missing information such as pre-pregnancy body mass index (BMI) in some subjects.

Institutional Review Boards of School of Public Health, Seoul National University, and all participating university hospitals approved the study, and the informed consents were obtained from the participating women. All samples and data were processed blind.

Chemical analysis

A total of 19 PCB congeners, 19 PBDE congeners and 19 OCPs were measured in serum samples. Measured PCB congeners were CB 18, 28, 33, 44, 52, 70, 101, 105, 118, 128, 138, 153, 170, 180, 187, 194, 195, 199 and 206. Measured PBDE congeners were BDE 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184 and 191. OCPs included DDTs, CHDs, HCHs, HCB, heptachlor, heptachlor epoxide and mirex. DDTs included *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDT and *o,p'*-DDT; CHDs included oxyCHD, *trans*-chlordane, *cis*-chlordane, *trans*-Nonachlordane (tNCHD) and *cis*-nonachlordane; and HCHs included α-, β-, γ- and δ-HCH. For quantification, ^{13}C-labeled 8 PCBs (EC9605-SS; Wellington Laboratories, Guelph, ON, Canada), ^{13}C-labelled 6 PBDEs (MBDE-MXE; Wellington) and ^{13}C-labeled 19 OCPs (ES-5349-L; Cambridge Isotope Laboratories, Andover, MA, USA) were used as surrogate internal standards.

Methanol (MeOH), hexane and dichloromethane (DCM) of ultra-trace residue-analysis grade were purchased from J. T. Baker (Phillipsburg, NJ, USA), and nonane (pesticide analysis grade) was purchased from Sigma-Aldrich (St Louis, MO, USA). Formic acid (90%) was purchased from Merck (Darmstadt, Germany). A Sep-Pak VacC_{18} (500 mg/6 cc), Sep-Pak Plus NH_2 (360 mg), Sep-Pak Vac silica gel (1 g/6 cc) and Sep-Pak Vacflorisil (500 mg/6 cc) cartridges (Waters, Milford, MA, USA) were used for solid phase extraction (SPE). Empty polypropylene columns (6 mL) for clean-up were purchased from Supelco (Bellefonte, PA, USA).

Sample preparation

The experimental procedures of analysis of OCPs, PCBs and PBDEs in serum were optimized with some modifications of previous studies (Dmitrovic et al., 2002; Kang et al., 2008). In brief, serum samples (2 mL) were fortified with formic acid and Milli-Q water for protein denaturation, after ^{13}C-labeled OCPs, PCBs and PBDEs were spiked. The samples were extracted by SPE using Sep-Pak C_{18} SPE cartridge, which was pre-washed with MeOH and conditioned with Milli-Q water. The extracted cartridge was rinsed with Milli-Q water and subsequently dried. A Sep-Pak Plus NH_2 cartridge, pre-washed with 6 mL of hexane, was connected to the lower end of the C_{18} cartridge. Eight milliliter of hexane was passed through the combined NH_2-C_{18} cartridges and was collected. After removing C_{18} cartridge, 6 mL of 5% DCM in hexane was passed through NH_2 cartridge and was combined to a previous fraction. The pooled eluents were cleaned up onto a silica gel/florisil SPE cartridge, using 12mL of 50% DCM in hexane. The purified eluents were concentrated and dissolved in 100μLnonane for instrumental analysis. POPs concentrations were normalized by lipid weight of serum. Total lipid (mg/dL) was calculated from concentrations of total cholesterol and triglyceride that were analyzed by enzymatic methods in a commercial clinical laboratory, by the equation of Total lipid = 2.27 * total cholesterol + triglyceride + 62.3 (Bernert et al., 2007). Measured lipid content of maternal serum was on average 882 mg/dL in the present population.

Instrumental analysis and quality control

A high-resolution gas chromatography interfaced with a high-resolution mass spectrometer (HRGC/HRMS; JMS 800D, JEOL, Tokyo, Japan) was used for the identification and quantification of OCPs, PCBs and PBDEs. Details of instrumental parameters have been reported elsewhere (Moon et al., 2007; Moon et al., 2009). In brief, OCPs, PCBs and PBDEs were quantified using the isotope dilution method based on relative response factors of individual compounds. The HRMS was operated under positive EI mode, and ions were monitored by selected ion monitoring using molecular ions of target compounds. A DB5-MS (30 m length, 0.25 mm inner diameter, 0.25μm film thickness; J&W Scientific, Palo Alto, CA, USA) was used for the separation of OCPs and PCBs. A DB5-MS (15 m length, 0.25 mm inner diameter, 0.1 μm film thickness; J&W Scientific) was used for the separation of from tri- to heptaBDE congeners.

The recoveries of spiked 13C-labeled compounds were 91 ± 10% (average± SD) for OCPs, 62 ± 5.5% for PCBs and 87 ± 13% for PBDEs. Solvents injected before and after the injection of standards showed negligible contamination or carryover. Procedural blanks (n = 10) were processed with every set of 15 serum samples to check laboratory contamination. Blanks did not contain quantifiable amounts of target contaminants. Limit of quantification (LOQ) was calculated as 10 times the signal to noise ratio. The respective LOQs for OCPs, PCBs and PBDEs were from 0.7 to 1.7 ng/g lw, from 0.8 to 8.3 ng/g lw and from 0.2 to 0.8 ng/g lw. All the POP concentrations were adjusted to the lipid contents of serum.

Hormone analysis

Concentrations of free and total T3, free and total T4, and TSH of serum samples were measured using the electrochemiluminescence immunoassay at Samkwang Medical Laboratories (Seoul, Korea). Commercial kits for T3, T4 and TSH were used and conducted with Elecsys automatics analytics Modular E170 (F. Hoffmann-La Roche Ltd., Basel, Switzerland). Determinations and quality control were made following the manufacturer's instruction. Calibration curve and master curve from 2-point and 5-point calibration were used for calculating concentrations of hormones by relative light unit measurements, and tolerance limit for quality control was three times of standard deviation of repeated measurement. Assay ranges were 0.26-32.55 pg/mL, 0.20-6.51 ng/mL, 0.02-7.77 ng/dL,0.42-24.86 μg/dL and 0.01-100.00 μlU/mL for free T3, total T3, free T4, total T4 and TSH, respectively.

Statistical analysis

Multiple regression analysis was conducted to evaluate associations between the levels of POPs and thyroid hormones among the study population. Thyroid hormone levels as dependent variables and POPs levels as independent variables of regression model were natural log-transformed and normality assumption of residuals was confirmed. Covariates that have been reported for associations with the thyroid hormones elsewhere (Franklin et al., 1985; Herbstman et al., 2008) were adjusted using a multivariate model (PROC GLM in SAS 9.1). These covariates include age, pre-pregnancy BMI, gestational duration, mode of delivery (e.g., vaginal or C-section delivery), or parity. In addition to general linear model analysis, a multiple logistic regression was carried out with hormone levels expressed as a dichotomous variable of high and low. Cutoff for high or low concentrations was arbitrarily selected at top or bottom 20th percentile depending on the direction of hypothesized association with POPs (Chevrier et al., 2010). Since we hypothesized that POPs exposure would lead to lower levels of T3 or T4, and higher levels of TSH, the bottom 20th percentile value was selected as a cutoff for T3 or T4, while top 20th percentile was selected for that of TSH. None of the participating pregnant women declared smoking during pregnancy, therefore smoking was not considered as covariate. Non-detects were treated with proxy value (LOQ divided by square root 2) following each detection frequency, to prevent biased estimators and incorrect statistical conclusions. For chemicals that were detected >=75% of the population, a proxy value was used to replace the non-detect and was used for statistical analysis. For chemicals that were detected in <75% but >=60%, however statistical analysis was conducted with the detected values only, in order to minimize the influence of non-detects.

2.3. Results

Characteristics of study population

The participating pregnant women comprised mostly in their early 30's (mean of 33 years of age, and SD of 4 years), and about a half of the women were primipara. Two thirds of the participating women gave birth to babies by spontaneous, vaginal delivery. Most women were within normal weight range before pregnancy, with mean pre-pregnancy BMI of 22.1 kg/m^2 (SD 10). General characteristics of the participating women along with the levels of measured hormones are summarized in Table 2-1. Maternal total T3 and total T4 concentrations were influenced by pre-pregnancy BMI, and TSH levels at delivery were also significantly associated with parity and mode of delivery.

Table 2-1. Demographic characteristics of the study population

	n (%)	Thyroid Hormone levels Median (IQR)				
		Free T3 (pg/mL)	Total T3 (ng/mL)	Free T4 (ng/dL)	Total T4 (ug/mL)	TSH (ulU/mL)
Pregnant women	**105**	**2.51 (2.24-2.70)**	**1.42 (1.24-1.61)**	**0.87 (0.80-0.95)**	**9.03 (7.84-9.81)**	**1.79 (1.25-2.91)**
Age (year)						
22-29	14 (13)	2.49 (2.19-2.67)	1.40 (1.12-1.63)	0.86 (0.77-0.91)	8.16 (7.35-9.33)	1.37 (1.19-2.33)
30-39	81 (77)	2.48 (2.24-2.71)	1.41 (1.24-1.61)	0.89 (0.80-0.97)	9.16 (8.01-9.85)	1.79 (1.27-2.89)
40-46	10 (10)	2.67 (2.42-2.70)	1.53 (1.39-1.62)	0.89 (0.84-1.03)	9.60 (8.30-10.3)	1.90 (1.25-3.14)
Parity						
0	51 (49)	2.45 (2.19-2.66)	1.37 (1.17-1.59)	0.89 (0.81-0.99)	8.95 (7.84-9.61)	**1.92*** (1.41-3.14)
≥1	54 (51)	2.54 (2.28-2.74)	1.47 (1.30-1.61)	0.87 (0.80-0.95)	9.25 (7.67-10.1)	**1.64†** (1.12-2.38)
Delivery						
Normal	70 (67)	2.52 (2.25-2.74)	1.42 (1.26-1.61)	0.87 (0.80-0.95)	8.95 (7.97-9.61)	**1.70*** (1.14-2.62)
C-section	35 (33)	2.47 (2.19-2.66)	1.41 (1.17-1.60)	0.91 (0.82-0.98)	9.46 (7.59-10.6)	**2.34†** (1.50-3.14)
BMI before pregnancy (kg/m^2)						
<18.5	14 (13)	2.43 (2.23-2.65)	**1.41*†** (1.15-1.62)	0.83 (0.73-0.91)	**8.58*** (7.59-9.20)	1.69 (1.10-2.91)
18.5-22.9	56 (53)	2.52 (2.28-2.73)	**1.43*†** (1.24-1.61)	0.87 (0.80-0.98)	**8.85*†** (7.53-9.69)	1.72 (1.22-2.81)
23.0-24.9	13 (13)	2.53 (2.47-2.67)	**1.55*** (1.50-1.62)	0.92 (0.86-0.99)	**9.89†** (9.61-10.7)	1.88 (1.35-3.35)
≥25	22 (21)	2.37 (2.23-2.71)	**1.33†** (1.17-1.54)	0.87 (0.80-0.94)	**8.94*†** (8.30-9.66)	1.90 (1.62-3.01)
Gestational age at delivery (days)						
≤259 (37wks)	4 (4)	2.30 (1.96-2.32)	1.21 (1.02-1.32)	0.91 (0.85-0.93)	8.90 (8.02-9.49)	2.02 (1.41-2.69)
260-294 (37-42wks)	101 (96)	2.52 (2.24-2.71)	1.43 (1.25-1.61)	0.87 (0.80-0.96)	9.03 (7.84-9.85)	1.79 (1.25-2.91)

Values in parentheses are interquartile range (IQR), showing the 25^{th} and 75^{th} percentile values. Different symbols (*, †) means significant differences between groups ($p < 0.05$) based on Wilcoxon rank-sum test or ANOVA post-hoc LSD test.

POPs concentrations in serum

Several target compounds were detected with high frequencies (Table 2-2). Among the target POPs, CB 138, CB 153, BDE 47, *β*-HCH, *p,p'*-DDE, and *p,p'*-DDT were detected in 100% of serum samples (n=105).

Table 2-2. Serum concentrations of OCPs, PCBs, and PBDEs in pregnant women (n=105)

Variable	N>LOQ	Detection frequency (%)	Median (IQR)
PCB			
CB 28	65	62	2.70 (1.80-4.16)
CB 52	60	57	1.88 (1.24-2.80)
CB 118	66	63	2.29 (1.67-3.20)
CB 138	105	100	4.60 (1.90-6.76)
CB 153	105	100	9.02 (5.86-12.17)
Total PCB	99	94	24.69 (15.63-34.35)
PBDE			
BDE 47	105	100	1.05 (0.59-2.10)
Total PBDE	94	90	2.13 (1.35-4.34)
OCP			
β-HCH	105	100	7.58 (4.02-11.42)
Total HCH	87	83	9.62 (5.81-12.36)
p,p'-DDE	105	100	57.37 (38.85-78.87)
p,p'-DDT	105	100	5.20 (2.94-8.99)
Total DDT	104	99	64.40 (42.15-92.35)
OxyCHD	73	70	1.83 (1.44-2.40)
tNCHD	87	83	2.27 (1.85-3.03)
Total CHD	96	91	3.75 (2.58-5.03)
HCB	76	72	9.48 (5.10-14.59)

Units of concentration are in ng/g lipid weight. Values in parentheses are interquartile range (IQR) showing the 25^{th} and 75^{th} percentile values. Values were presented only when the frequency of detection was > 60%.

Association between POPs and hormone concentrations in serum

Several POPs showed significant associations with levels of thyroid hormones and TSH, after adjustment of covariates (Table 2-3). In pregnant women, several PCBs showed consistently significant negative associations with free /total T3, and free/total T4 concentrations (Table 2-3, Figure 2-1). CB 52 and CB 118 showed significant negative association with free or total T3 concentrations. CB 28 showed significant negative associations with free T4. Total PCB showed significant negative association with total T3 and total T4. While not significant, all PCB congeners that were detected in frequencies of >60 % showed the same negative trends with thyroid hormones. For TSH, all PCBs showed positive associations, but statistical significance was not observed. By logistic regression analysis, results similar to those by liner regression were observed (Table 2-4): PCBs were generally related to increased odds of low (<20th percentile) T3 or T4 levels. CB 118 was related to significantly increased odds of low free T3 (adjusted odd ratio (OR_{adj}) of 1.92) and total T3 (OR_{adj} of 2.65). CB 153 was also related to significantly increased odds of low free T4 (OR_{adj} of 1.11) and total T4 (OR_{adj} of 1.08). In addition, CB 138 was associated with increased odds of low total T4 (OR_{adj} of 1.15).

BDE 47 showed significant negative associations with total T3 (Table 2-3). Total PBDE showed significant negative association with total T3 and free T3; however, positive relationship between total PBDE and free T4 concentration was also observed at the same time. BDE 47 levels were significantly related to increased odds of low total T4 concentrations.

Some OCPs showed negative trends of association with T3 or T4 levels, but other directions of association were also noted (Table 2-3). Most of the associations were not statistically significant. Among OCPs, *p,p'*-DDT and total DDT showed negative associations with free T4 and total T3, respectively. HCB

was also negatively associated with free T4 concentration, and was related to increased odds of low total T4 concentration (OR_{adj} of 1.08). While tNCHD, HCB, and *p,p'*-DDT showed generally negative trends with T3 or T4 but positive trend with TSH, statistical significance was absent or marginal ($p<0.1$) in multiple linear regression analysis (Table 2-3).

Table 2-3. Associations between serum POPs concentrations and thyroid hormone levels in pregnant women (n=105)

	Free T3		Total T3		Free T4		Total T4		TSH	
	β	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI
PCB										
ΣPCB	**-0.053***	-0.108, 0.002	**-0.117****	-0.198, -0.036	-0.029	-0.084, 0.027	**-0.056****	-0.109, -0.002	0.195	-0.167, 0.556
CB 28^{+}	-0.015	-0.057, 0.027	-0.025	-0.100, 0.050	**-0.068****	-0.117, -0.019	-0.030	-0.081, 0.020	0.172	-0.165, 0.510
CB 52^{+}	**-0.092****	-0.159, -0.026	**-0.113****	-0.223, -0.003	-0.011	-0.067, 0.044	-0.023	-0.081, 0.035	0.192	-0.146, 0.530
CB 118^{+}	-0.020	-0.091, 0.051	**-0.114****	-0.223, -0.005	-0.049	-0.136, 0.038	-0.047	-0.134, 0.040	0.389	-0.183, 0.960
CB 138	-0.008	-0.039, 0.023	-0.033	-0.079, 0.013	-0.005	-0.037, 0.027	-0.018	-0.048, 0.011	0.154	-0.040, 0.347
CB 153	-0.011	-0.046, 0.025	-0.038	-0.092, 0.016	-0.013	-0.050, 0.024	-0.018	-0.052, 0.017	0.020	-0.207, 0.246
PBDE										
ΣPBDE	**-0.049****	-0.088, -0.009	**-0.112****	-0.170, -0.054	**0.058****	0.016, 0.100	0.007	-0.032, 0.046	-0.055	-0.318, 0.209
BDE 47	-0.021	-0.049, 0.007	**-0.042****	-0.084, -0.000	0.028	-0.001, 0.057	0.006	-0.021, 0.033	0.024	-0.154, 0.203

Table 2-3. (Continued)

	Free T3		Total T3		Free T4		Total T4		TSH	
	β	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI	*β*	95% CI
OCP										
ΣHCH	-0.030	-0.084, 0.023	-0.002	-0.090, 0.086	-0.007	-0.069, 0.055	0.010	-0.052, 0.072	-0.307	-0.706, 0.091
β-HCH	0.030	-0.006, 0.066	0.027	-0.028, 0.082	0.005	-0.033, 0.043	0.014	-0.021, 0.049	-0.135	-0.363, 0.094
ΣCHD	-0.005	-0.058, 0.049	-0.034	-0.120, 0.052	-0.025	-0.082, 0.032	-0.028	-0.084, 0.029	0.201	-0.158, 0.559
oxyCHD^{+}	-0.033	-0.109, 0.042	-0.125	-0.251, 0.001	0.035	-0.055, 0.125	-0.003	-0.095, 0.090	-0.268	-0.754, 0.218
tNCHD	-0.033	-0.095, 0.029	-0.085	-0.188, 0.018	-0.049	-0.118, 0.021	**-0.059***	-0.128, 0.010	0.225	-0.160, 0.609
ΣDDT	**-0.052***	-0.104, 0.000	**-0.096****	-0.179, -0.013	-0.045	-0.106, 0.016	-0.035	-0.093, 0.022	0.299	-0.072, 0.670
p,p'-DDE	0.028	-0.021, 0.078	0.003	-0.073, 0.079	-0.049	-0.101, 0.002	-0.008	-0.057, 0.040	-0.013	-0.330, 0.303
p,p'-DDT	0.002	-0.035, 0.039	0.004	-0.053, 0.061	**-0.045****	-0.083, -0.008	-0.015	-0.051, 0.021	0.145	-0.090, 0.380
HCB^{+}	-0.012	-0.061, 0.037	-0.035	-0.108, 0.038	**-0.047****	-0.095, -0.000	-0.023	-0.071, 0.026	0.091	-0.183, 0.365

Signs ** and * indicate statistical significance at p=0.05, and 0.1, respectively. All POPs concentrations are in ng/g lipid and were natural log –transformed. Results of association were adjusted for age, gestation period, mode of delivery, parity, and pre-pregnancy BMI. Chemicals that were detected >=75% of the population at concentrations greater than the limit of quantification, a proxy value of 'LOQ divided by square root 2' was used. For chemicals that were detected in <75% but >=60%, statistical analysis was conducted with detected values only. Such chemicals are indicated by '+'.

Table 2-4. Adjusted ORs and the associated 95% CIs for thyroid hormone levels in response to POPs in pregnant women

	Free T3 Low vs. highest 80%		Total T3 Low vs. highest 80%		Free T4 Low vs. highest 80%		Total T4 Low vs. highest 80%		TSH High vs. lowest 80%	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
PCB										
ΣPCB	1.01	0.99, 1.03	1.01	0.99, 1.03	1.02	1.00, 1.04	1.02	1.00, 1.05	1	0.97, 1.02
CB 28^{+}	1.04	0.98, 1.11	1.05	0.98, 1.11	1.03	0.97, 1.10	1.04	0.97, 1.10	0.95	0.82, 1.11
CB 52^{+}	1.1	0.89, 1.36	1.1	0.89, 1.36	1.2	0.96, 1.50	1.27	1.00, 1.63	1.16	0.92, 1.47
CB 118^{+}	**1.92****	1.05, 3.51	**2.65****	1.26, 5.58	1.23	0.68, 2.25	1.32	0.73, 2.40	0.55	0.25, 1.19
CB 138	0.98	0.87, 1.11	1	0.89, 1.12	1.06	0.96, 1.17	**1.15****	1.02, 1.29	1.07	0.96, 1.18
CB 153	1.01	0.95, 1.08	1.02	0.96, 1.09	**1.11****	1.03, 1.19	**1.08****	1.01, 1.15	1.02	0.96, 1.08
PBDE										
ΣPBDE	1.01	0.84, 1.22	1.09	0.91, 1.30	1.12	0.93, 1.34	1.12	0.93, 1.36	1.01	0.84, 1.20
BDE 47	0.99	0.78, 1.27	1.09	0.87, 1.37	1.2	0.95, 1.51	**1.35****	1.07, 1.71	1.1	0.89, 1.37

Table 2-4. (Continued)

	Free T3 Low vs. highest 80%		Total T3 Low vs. highest 80%		Free T4 Low vs. highest 80%		Total T4 Low vs. highest 80%		TSH High vs. lowest 80%	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
OCP										
ΣHCH	0.99	0.90, 1.09	1.02	0.93, 1.13	0.96	0.85, 1.07	0.96	0.84, 1.08	0.95	0.85, 1.06
β-HCH	0.98	0.89, 1.07	0.99	0.90, 1.09	0.91	0..81, 1.02	0.93	0.84, 1.04	0.95	0.86, 1.04
ΣCHD	0.94	0.69, 1.27	1.11	0.81, 1.51	1.1	0.86, 1.42	0.96	0.75, 1.23	0.92	0.69, 1.21
oxyCHD^{+}	0.73	0.28, 1.85	2.42	0.85, 7.11	1.59	0.73, 3.48	1.12	0.49, 2.52	1.58	0.75, 3.33
tNCHD	0.71	0.46, 1.12	0.81	0.51, 1.28	1.06	0.78, 1.49	0.92	0.65, 1.29	0.81	0.56, 1.18
ΣDDT	1	0.98, 1.01	1	0.99, 1.02	1	0.99, 1.01	1	0.99, 1.01	1	0.99, 1.01
p,p'-DDE	1	0.98, 1.01	1	0.99, 1.02	1	0.98, 1.01	1	0.99, 1.01	1	0.98, 1.01
p,p'-DDT	0.99	0.93, 1.06	1	0.94, 1.06	1.09	0.98, 1.22	1	0.95, 1.06	0.94	0.83, 1.06
HCB^{+}	1.02	0.96, 1.09	1.06	1.00, 1.13	1.06	1.00, 1.13	**1.08****	1.01, 1.17	1	0.94, 1.06

Asterisk (**) indicates statistical significance at p=0.05. All POPs concentrations are in ng/g lipid and were natural log–transformed. Results of association were adjusted for age, gestation period, mode of delivery, parity, and pre-pregnancy BMI. Cutoff for high or low concentrations was arbitrarily selected at top or bottom 20th percentile depending on the direction of hypothesized association, i.e., the bottom 20th percentile value as a cutoff for T3 or T4, and the top 20th percentile for that of TSH.

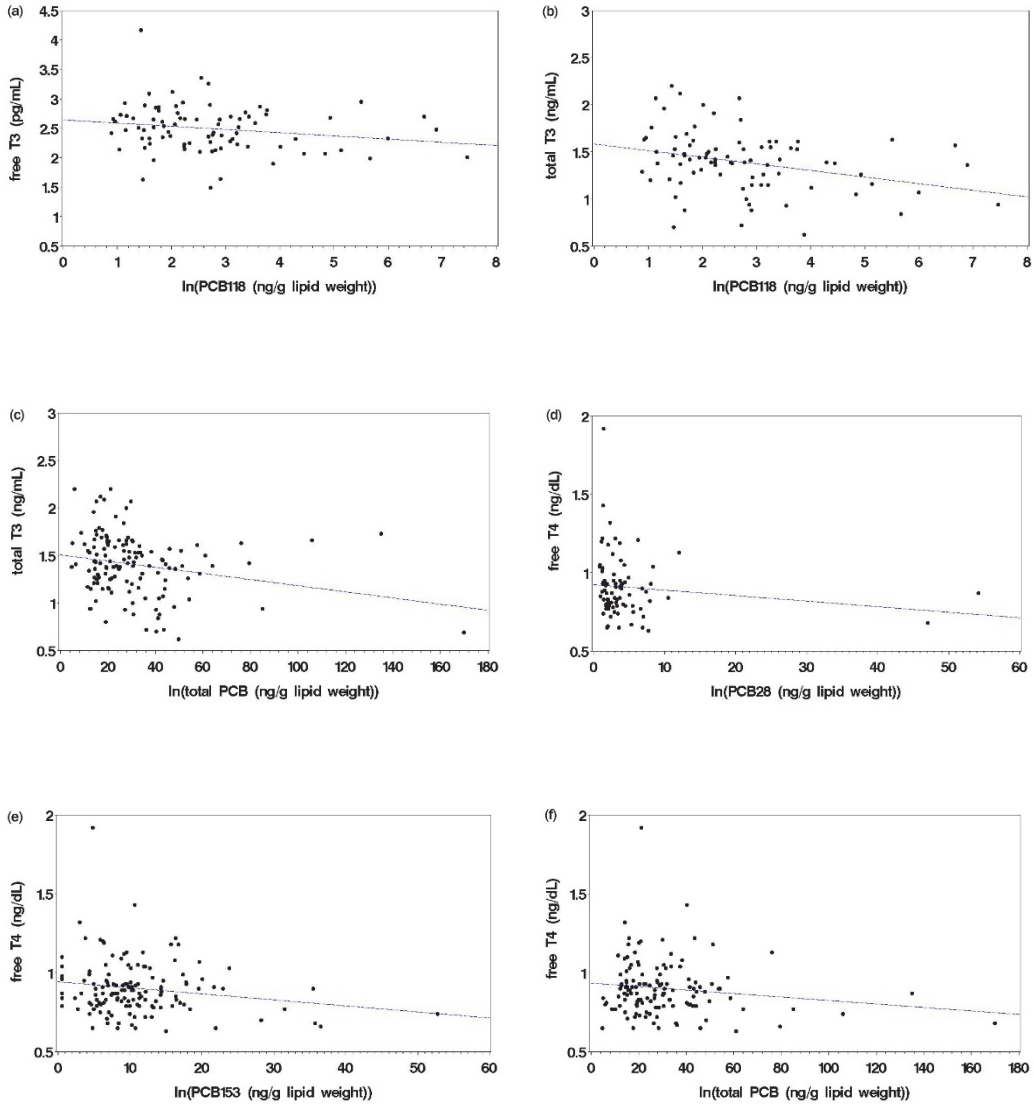

Figure 2-1. Simple association between thyroid hormone concentrations and PCB levels in blood of pregnant women (n=105). (a) Free T3 and CB 118, (b) total T3 and CB 118, (c) total T3 and total PCB, (d) free T4 and CB 28, (e) free T4 and CB 153, and (f) free T4 and total PCB. PCBs that showed significant associations with thyroid hormones based on multiple regression analysis are shown. All PCBs concentration was natural log transformed.

2.4. Discussion

Our observations that several PCBs are significantly associated with thyroid hormones (Table 2-3 and 2-4; Figure 2-1) were generally comparable to those observed in most of epidemiological and experimental studies that demonstrated decrease of thyroid hormones, e.g., T3 or T4. For PBDEs and OCPs, our observations suggest their potential thyroid disrupting effects, however, the associations were not consistent.

PCBs and thyroid hormones

Our observations of significant inverse associations between several PCB congeners such as CB 28, 52, and 118, or total PCB, and thyroid hormones in pregnant women clearly support the thyroid disrupting effects of PCBs at the levels occurring among general population. Because of structural resemblance, PCBs have been considered to compete for common receptors and hence decrease the levels of T3 or T4 (Boas et al., 2012). In addition, PCB may enhance hepatic metabolism that may lead to elimination of thyroid hormones (Lyn 2009). Although a few evidences have been suggested otherwise, e.g., among adults > 60 years of age participated in either 1999-2000 and 2001-2002 cycles NHANES, PCB levels showed a positive association with TSH in women but a negative association among men (Turyk et al., 2008); Bloom et al. (2009) reported a positive association between PCB170 and free T4 in the New York State Angler Cohort (n=38); and Alvarez-Pedrerol et al. (2009) reported PCBs 138, 153, or 180 measured at first trimester of pregnancy showed negative associations with T3 but positive associations with T4 in two pregnant women cohort (n=520~570) of Spain, most epidemiological studies on general population indeed showed negative associations between PCBs and thyroid hormones like T3 or T4 (Osius et

al., 1999; Persky et al., 2001; Schell et al., 2008; see Table 2-5), and positive associations with TSH (Schell et al., 2008). For pregnant women, however, only limited number of reports is available, but is generally comparable to our observations. For example, Takser et al. (2005) reported negative correlation between three non-coplanar PCBs such as CB 138, 153, and 180, and total T3 levels among pregnant women. Chevrier et al. (2008) also reported negative association between total PCB or PCB44, 52, or 183, and free T4 among 334 pregnant women in California, USA. Our observations among the pregnant women in Korea again confirm the same negative association between PCBs and thyroid hormones.

PBDEs and thyroid hormones

The significant negative associations between BDE 47 and total T3, and between total PBDE and free T3 or total T3 observed in the present study (Table 2-3) suggest the potential thyroid disruption effects of PBDEs in pregnant women. The effect of PBDEs on circulating thyroid hormone balance has been quite consistently demonstrated in experimental animal studies (Fernie et al., 2005; Kodavanti et al., 2010; Zhou et al., 2001). Occupationally exposed human populations, e.g., Chinese workers in an electronic waste dismantling site, who had high serum PBDE levels (n=49) did also exhibit higher TSH concentrations than control (Yuan et al., 2008). However epidemiological evidences of such association between PBDEs and thyroid hormones are generally limited (Meeker, 2012). Instead, the opposite direction of association has been frequently reported (Table 2-5), and our study also showed positive relationship between total PBDE and free T4 levels. Among 140 pregnant women, BDE 47, 99, 100, or 153 were positively associated with free or total T4 after adjustment of smoking, maternal age, race, gestational age, and parity (Stapleton et al., 2011). Positive associations between PBDEs and T_4 levels have been also reported in other human populations (Meeker et al., 2009; Turyk et al., 2008).

While inconsistent with free T4, the significant negative associations between T3 and BDE 47 or total PBDE observed in the present study, however support the observations of animal experimental studies, and add another line of evidence for potential effects of PBDEs on lowering thyroid hormones in humans at the levels occurring among general pregnant women. PBDEs are thought to influence thyroid hormone balance by induction of hepatic enzymes involved in conjugation or by down-regulation of trans-membranal thyroid hormone transport, which would eventually lead to enhanced biliary elimination of thyroid hormones (Boas et al., 2012; Lyn, 2009).

Interactions among the POPs including PBDEs are possible and may complicate the interpretation of the data. The Spearman correlation analysis showed that BDE 47 concentrations were significantly positively associated with those of HCB, CB 28, CB 52, and CB 118, but the associations between BDE 47 and total PCB, and total PBDE and individual PCB congeners were not significant (Table 2-6). Since some PBDEs especially with less number of bromine atoms may share the same source, e.g., food, with PCBs, the observed significant associations between PBDEs and thyroid hormones may in fact be related to PCBs, which were identified as significant modulators of serum thyroid hormones. While total PBDE concentration was not associated any other POPs in our study, BDE 47 showed weak correlations with HCB, CB 28, CB 52, or CB 118 (Table 2-6). Among these, CB 52 was identified as significant determinant of free T3, and CB 52/CB 118, as significant determinants of total T3 in multivariate model. The significant associations between BDE 47 and free T3 or total T3 were not remained when the PCBs were included as covariates in the model: With free T3, the significance of CB 52 remained but that of BDE 47 became marginal (Table 2-7). Therefore, the influence of BDE 47 on thyroid hormones appeared to relatively weak compared to that of PCBs among the study population. This observation should be confirmed in other populations to differentiate the effect of PBDE exposure on thyroid hormones in relation to that of PCBs.

Table 2-5. Significant associations between POPs in serum concentrations and thyroid hormone levels in previous studies

Chemical	Population	n	Direction of change by increasing POPs levels	Reference
BDE 47, 99, 100, 153, 154, 183	Pregnant women	9	No association	Mazdai et al., 2003
BDE 28, 47, 99, 100, 153	Pregnant women	270	TSH decrease, free T4 increase	Chevrier et al., 2010
BDE 47, 99, 100, 153	Pregnant women	140	free T4 or total T4 increase	Stapleton et al., 2011
PBDEs	Adults	38	free T4 increase	Bloom et al., 2008
BDE 153	Adults	120	total T3 increase	Dallaire et al., 2009
PBDEs	Adults	405	free T4 or total T4 increase, TSH decrease	Turyk et al., 2008
CB 170	Adults	38	free T4 increase	Bloom et al., 2009
CB 138, 153, 180	Pregnant women	520-570	T3 decrease, T4 increase	Alvarez-Pedrerol et al., 2009
***p,p'*-DDE**	Adults	341	total T3 and free T4 increase	Meeker et al., 2007
PCBs, *p,p'*-DDE, HCB	Adults	464	TSH decrease	Langer et al., 2006

Table 2-5. (Continued)

Chemical	Population	n	Direction of change by increasing POPs levels	Reference
PBDEs	Workers	49	TSH increase	Yuan et al., 2008
CB 118, 138, 153, 180, 183, 187	7-10 yrs children	320	free T3 decrease or TSH increase	Osius et al., 1999
PCBs	Adults	178	free T4 or total T4 decrease	Persky et al., 2001
PCBs, HCB	Pregnant women	334	free T4 or total T4 decrease	Chevrier et al., 2008
CB 138, 153, 180, *p,p'*-DDE	Pregnant women	120	total T3 decrease	Takser et al., 2005
PCBs, HCB	Adolescent	232	free T4 decrease, TSH increase	Schell et al., 2008
HCB	Adults	66	total T4 decrease	Bloom et al., 2003
CHDs	Spouses of workers	16529	odds of hypothyroidism increase	Goldner et al., 2010
***p,p'*-DDE**	Adults	196	TSH increase	Rylander et al., 2006
tNCHD, CB 138	Pregnant women	105	TSH increase	Present study
HCB, PBDEs, BDE 47, PCBs, CB 28, 138, 153			free T4 or total T4 decrease	
PBDEs, BDE 47, PCBs, CB 118			free T3 or total T3 decrease	

Table 2-6. Spearman correlation coefficients between measured POPs in pregnant women

		***β*-HCH**	**HCH**	***p,p'*-DDE**	***p,p'*-DDT**	**DDT**	**oxyCHD**	**tNCHD**	**∑CHD**	**HCB**	**BDE 47**	**∑PBDE**	**CB 28**	**CB 52**	**CB 118**	**CB 138**	**CB 153**	**∑PCB**
	ρ	-0.124	-0.023	0.101	0.081	0.106	0.109	0.172	0.163	**0.330**	1.000	**0.540**	**0.268**	**0.316**	**0.273**	-0.053	0.031	0.085
BDE 47	*p*>|r|	0.208	0.831	0.304	0.409	0.284	0.361	0.110	0.113	**0.004**		**<.0001**	**0.031**	**0.014**	**0.027**	0.592	0.752	0.405
	n	105	87	105	105	104	73	87	96	76	105	94	65	60	66	105	105	99
	ρ	-0.027	0.067	0.159	-0.013	0.143	0.189	-0.039	0.061	-0.003	**0.540**	1.000	-0.143	0.100	0.056	0.060	0.094	**0.205**
∑PBDE	*p*>|r|	0.798	0.556	0.125	0.900	0.173	0.129	0.733	0.576	0.982	**<.0001**		0.275	0.466	0.672	0.568	0.365	**0.054**
	n	94	80	94	94	93	66	79	86	69	94	105	60	55	60	94	94	89

Table 2-7. Adjusted associations with significant PCBs as covariates between serum concentrations of PBDEs and free or total T3 in pregnant women

Variables	free T3	
	β	**95% CI**
BDE 47	-0.039*	-0.081, 0.003
CB 52	-0.072**	-0.141. -0.004
Variables	**total T3**	
	β	**95% CI**
BDE 47	-0.006	-0.076, 0.063
CB 52	-0.114	-0.305, 0.076
CB 118	-0.078	-0.259, 0.104

Significant results from multiple regression analysis using each individual congener (Table 2-3) were reanalyzed by adding CB 52 for testing relationship between BDE 47 and free T3, and CB 52 and 118 in model for testing relationship between BDE 47 and total T3 as covariates. Here, CB 28, 52, and 118 showed significant relationships with BDE 47 in Spearman rank correlation test. **, * indicate statistical significance at p=0.05, and 0.1, respectively.

OCPs and thyroid hormones

While several OCPs appeared to be associated with T3, T4, or TSH in the present study, the directions of association were less consistent and generally insignificant. DDTs and HCB were shown to be generally associated with reduction of T3 or T4, or increase of TSH (Table 2-3). Serum HCB concentrations were also shown to be related with increased odds of low (≤20%) total T4 levels (Table 2-4). The effects of HCB exposure on thyroid disruption have been reported in several studies. For example, negative associations between HCB and free or total T4 were observed among 334 pregnant women in California, USA (Chevrier et al., 2008), but in their study, HCB and PCB were strongly correlated together, thus independent association of HCB with thyroid function could not be confirmed. Similar negative association between HCB and total T4 was reported in a preliminary study with a selected sample of New York State Angler Cohort (n=66) (Bloom et al., 2003). While DDTs are reported to inhibit TSH receptor and may result in decreased circulatory T3 and T4 concentrations (Lyn, 2009), most epidemiological studies that reported potential associations failed to control the influence of other chemicals with possibly more powerful thyroid disrupting potentials, e.g., PCBs. Since PCBs may often have similar sources of exposure with DDTs depending on the population, therefore true association between DDTs and thyroid hormones might be masked. In the present study, strong associations were observed between DDTs and several PCBs, and their significant associations on thyroid hormones disappeared when CB 52, CB 118, or total DDT were included as covariates in the regression model. Thus thyroid hormone disrupting potential of DDTs suggested in the present study need to be further investigated in other populations.

2.5. Summary and implications

In this study, several PCBs such as CB 28, 52, and 118 showed negative associations with T3 or T4 in pregnant women. BDE 47 and total PBDE showed significant associations with T3 or T4. For OCPs, DDTs and HCB were generally associated with reduction of T3 or T4.

Major limitations of the current study include (1) no measurement of iodine intake or urinary iodine concentrations, and (2) relatively small sample size. Iodine ions are necessarily for synthesis of thyroid hormones, and additional intake of iodine is recommended for pregnant women (WHO/UNICEF, 2007). However the influence of iodine intake on the association between POPs exposure and thyroid hormone levels is not clear yet in humans. Based on a large sample of pregnant women from two population-based cohort studies of Spain (n=1090), iodine intake was identified not to modify the association between organochlorine compounds and thyroid hormones (Alvarez-Pedrerol et al., 2009). However potential influence of iodine intake should not be ignored and should be subject to further assessment. The sample size of the present study (n=105) is relatively small compared to those of other studies (n=140-570; Stapleton et al., 2011; Chevrier et al., 2011; Chevrier et al., 2008; Alvarez-Pedrerol et al., 2009). However, the present study is unique in that we have measured as many as 57 different chemicals including PCBs, PBDEs and OCPs, and all five types of thyroid hormones at the same time. With the limited sample size, the results of the present study clearly show consistent effect of several POPs on thyroid function, similar to those reported from several animal and epidemiological studies. While the validation of our result in larger populations would be needed, the results of the present study provide a strong line of evidence that the current POPs exposure among humans in sensitive life stage could influence the balance of thyroid hormones.

Thyroid hormone levels in blood are tightly regulated within an individual, hence intra-individual variation would often be negligible compared to the inter-individual variations or the wide reference ranges. Therefore, small changes in thyroid hormone levels by the exposure to environmental chemicals may not be easily detected in a small human population (Boas et al., 2012). While all the subjects were within the reference range, PCBs and PBDEs at the current exposure levels among pregnant women are clearly related with potential for disrupting thyroid hormone homeostasis in the present study. Although subtle, the changes in thyroid hormones should be seen with caution because even minor changes within a given pregnant woman may have significant consequences especially on sensitive population like fetus. Therefore it is important to identify and control the potential sources of POPs exposure among the pregnant women.

Chapter 3. Association between several persistent organic pollutants and thyroid hormone levels in cord blood serum and bloodspot of the newborn infants of Korea

3.1. Introduction

POPs have been detected in various environmental media and biota worldwide, even though many of these compounds including OCPs, and PCBs had been banned for use several decades ago. DDT, one of best known OCPs, which had been widely used for vector control, has been frequently detected in human worldwide (Fujii et al., 2011; Smith, 1999). PBDEs are a group of emerging POPs that have been relatively recently recognized for widespread contamination and adverse health effects (Eriksson et al., 2001; Siddiqi et al., 2003). Both in wildlife animals and humans, adverse reproductive, developmental, neurologic, and endocrine health effects have been well-documented for many POPs (EPA, 2009).

POPs can cross the placenta during pregnancy (Vizcaino et al., 2014), occur in breast milk (Lee et al., 2013b; Lee et al., 2013c; Gomara et al., 2007), and therefore affect the endocrine system of fetuses and breastfed infants. The early life stages of life are particularly vulnerable to chemical exposures because of incomplete metabolic activities (Dencker and Eriksson, 1998), and rapid somatic growth and development (Birnbaum, 1994). The exposure to chemicals including POPs of developing fetus has been suggested to be possibly linked to the health of later stages of life (Eriksson and Talts, 2000; Gascon et al., 2011).

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Exposure to POPs has been associated with disruption of thyroid hormones in several human epidemiological studies (Alvarez-Pedrerol et al., 2009; Meeker et al., 2007; Stapleton et al., 2011). Thyroid hormones are essential for normal growth and development of the fetus during the most of gestation period (Forhead and Fowden, 2014). Among pregnant women, even moderate changes of thyroid hormone levels may be associated with adverse outcomes for the mother or her offspring (Berbel et al., 2009; Idris et al., 2005; Sahu et al., 2010). In previous studies, while within reference range, higher maternal TSH levels were associated with an increased risk of miscarriages, fetal and neonatal distress (Benhadi et al., 2009) and preterm delivery (Stagnaro-Green et al., 2005). In addition, high free T4 levels within the normal reference range were associated with reduced preterm delivery rate (Torremante et al., 2011). Thus, considering the importance of thyroid hormones in developing fetus and newborn infants, even small changes in thyroid hormones in these vulnerable populations are of potential concern. Associations between prenatal POPs exposure and thyroid hormone levels among newborn infants have been investigated by several groups, but the results are generally inconsistent and often controversial (Abdelouahab et al., 2013; Chevrier et al., 2007; Chevrier et al., 2011; Eggesbo et al., 2011; Herbstman et al., 2008b; Longnecker et al., 2000). Since thyroid hormone levels of cord blood serum are significantly influenced by maternal hormones (Fisher, 1997; Thorpe-Beeston et al., 1991b) and show significant fluctuations during and shortly after the delivery (Kim et al., 2005), it is quite challenging to identify the true association between POPs exposure and thyroid hormone levels in cord serum.

This study is conducted as a part of a matched pregnant woman and fetus panel of Korea, i.e., CHECK Panel study, which has been investigated the levels of POPs exposure and their associations with adverse health outcomes including thyroid hormone levels (Lee et al., 2013a; Lee et al., 2013b; Choi et al., 2014). The CHECK Panel is composed of pregnant women-fetus pairs without any

known occupational exposure pathways to major POPs such as OCPs, PCBs, and PBDEs and was recruited since 2011 from four cities of Korea. In Chapter 2, negative associations between PBDEs/PCBs exposure and thyroid hormone levels from the pregnant women (n=105) of this panel were reported.

In the present study, the associations between prenatal exposure to various POPs, and thyroid hormone status of the newborn infant population of the same CHECK Panel were investigated. In order to account for the influence of maternal thyroid inputs, cord serum models were adjusted for maternal thyroid hormone concentrations. The results of this study will help better understand the influences of prenatal POPs exposure on newborn infants, and identify the areas that warrant further investigations in the future.

3.2. Materials and methods

Study population and sample collection

A total of 148 healthy pregnant women with neither pre-pregnancy thyroid disease history nor pregnancy induced thyroid diseases and diabetes were recruited before delivery from five university hospitals located in four cities of Korea, i.e., Seoul, Anyang, Ansan, and Jeju. These four cities were representatives of a residential megacity, a mid-sized residence city, a mid-sized industrial city, and a mid-sized island city, respectively. Their matching fetuses were also recruited after full-term normal delivery. Details about the participating women can be found in Chapter 1 and 2. During delivery, maternal blood and umbilical cord blood were collected, separated for serum on site, and stored in polypropylene cryovials at −70 °C until analysis.

Among them, POPs and thyroid hormones were analyzed in blood serum of 104 matching pairs. In addition, on day 2 post-partum, bloodspot was obtained from each participating newborn infant by heel prick method, except for 5 infants who were sampled at day 5 and 7 post-partum.

Institutional Review Boards of School of Public Health, Seoul National University, and all participating university hospitals (Soon Chun Hyang University Seoul Hospital Institutional Review Board, Hallym University Sacred Heart Hospital Institutional Review Board, Institutional Review Board of Korea University Ansan Hospital, and Institutional Review Board of Jeju National University Hospital) approved the study, and the informed consents were obtained from the participating women. All samples and data were processed blind.

Data collection

Umbilical cord serum samples were processed and measured for 19 PCB congeners, 19 PBDE congeners, and 19 OCPs, consistently with previous study (Chapter 2). In addition, levels of five thyroid hormones, i.e., free/total T3, free/total T4, and TSH, were obtained (Chapter 2). All thyroid hormones were analyzed by electrochemiluminescence immunoassay, and details for thyroid hormone measurements were provided in Chapter 2. For POPs quantification, ^{13}C-labeled 8 PCBs (EC9605-SS; Wellington Laboratories, Guelph, ON, Canada), ^{13}C-labelled 6 PBDEs (MBDE-MXE; Wellington) and ^{13}C-labeled 19 OCPs (ES-5349-L; Cambridge Isotope Laboratories, Andover, MA, USA) were used as surrogate internal standards.

Concentrations of five thyroid hormones in cord serum samples were measured using the electrochemiluminescence immunoassay at Samkwang Medical Laboratories (Seoul, Korea) consistently with Chapter 2. TSH levels from bloodspots (n=96) were analyzed as a part of a national screening program of Korea, by radioimmunoassay.

One-on-one interview with participating pregnant women were conducted at the time of enrollment. Demographic characteristics, physiological data, and pregnancy related record were obtained.

Sample preparation

The experimental procedures of analysis of OCPs, PCBs and PBDEs in serum were optimized with some modifications of previous studies (Dmitrovic et al., 2002; Kang et al., 2008). In brief, serum samples (2 mL) were fortified with formic acid and Milli-Q water for protein denaturation, after ^{13}C-labeled OCPs, PCBs and PBDEs were spiked. The samples were extracted by SPE using Sep-Pak C_{18} SPE cartridge, which was pre-washed with MeOH and conditioned with Milli-Q water. The extracted cartridge was rinsed with Milli-Q water and subsequently dried. A Sep-Pak Plus NH_2 cartridge, pre-washed with 6 mL of hexane, was connected to the lower end of the C_{18} cartridge. Eight milliliter of hexane was passed through the combined NH_2-C_{18} cartridges and was collected. After removing C_{18} cartridge, 6 mL of 5% DCM in hexane was passed through NH_2 cartridge and was combined to a previous fraction. The pooled eluents were cleaned up onto a silica gel/florisil SPE cartridge, using 12mL of 50% DCM in hexane. The purified eluents were concentrated and dissolved in 100μL nonane for instrumental analysis. POPs concentrations were normalized by lipid weight of serum. Total lipid (mg/dL) was calculated from concentrations of total cholesterol and triglyceride that were analyzed by enzymatic methods in a commercial clinical laboratory (Samkwang Laboratory, Seoul, Korea), by the following equation (Bernert et al., 2007).

Total lipid = 2.27 * total cholesterol + triglyceride + 62.3

Measured lipid content of maternal blood and cord blood serum was on average 881 mg/dL and 231 mg/dL in the present population.

Instrumental analysis and quality control

A high-resolution gas chromatography interfaced with a high-resolution mass spectrometer (HRGC/HRMS; JMS 800D, JEOL, Tokyo, Japan) was used for the identification and quantification of OCPs, PCBs and PBDEs. Details of instrumental parameters have been reported elsewhere (Moon et al., 2007; Moon et al., 2009). In brief, OCPs, PCBs and PBDEs were quantified using the isotope dilution method based on relative response factors of individual compounds. The HRMS was operated under positive EI mode, and ions were monitored by selected ion monitoring using molecular ions of target compounds. A DB5-MS (30 m length, 0.25 mm inner diameter, 0.25μm film thickness; J&W Scientific, Palo Alto, CA, USA) was used for the separation of OCPs and PCBs. A DB5-MS (15 m length, 0.25 mm inner diameter, 0.1 μm film thickness; J&W Scientific) was used for the separation of from tri- to heptaBDE congeners.

The recoveries of spiked ^{13}C-labeled compounds were 91 ± 10% (average± SD) for OCPs, 62 ± 5.5% for PCBs and 87 ± 13% for PBDEs. Solvents injected before and after the injection of standards showed negligible contamination or carryover. Procedural blanks (n = 10) were processed with every set of 15 serum samples to check laboratory contamination. Blanks did not contain quantifiable amounts of target contaminants. LOQ was calculated as 10 times the signal to noise ratio. The respective LOQs for OCPs, PCBs and PBDEs were from 0.7 to 1.7 ng/g lipid weight (lw), from 0.8 to 8.3 ng/g lw and from 0.2 to 0.8 ng/g lw. All the POP concentrations were adjusted to the lipid contents of serum.

Statistical analysis

Among 57 kinds of target POPs, 8 chemicals which were detected > 60% in cord serum and sum of the isomers (ΣPCB, ΣPBDE, ΣDDT, ΣCHD, ΣHCH) were used in statistical analysis. For chemicals that were detected ≥ 75% of the population, a proxy value, i.e., LOQ divided by square root 2, was used to replace the non-detects (Hornung and Reed, 1990). For chemicals that were detected in < 75% but ≥ 60%, statistical analysis was conducted only with the detected values, in order to minimize the influence of non-detects (Chapter 2).

Multivariate analysis was conducted with natural log transformed dependent and independent variables to minimize the effect of highly right-skewed data. To determine associations between POPs and thyroid hormone measurements in cord serum, covariates that have been reported for associations with the thyroid hormones elsewhere (Franklin et al., 1985; Herbstman et al., 2008a) including age (continuous variable), pre-pregnancy BMI (continuous), gestational day (continuous), mode of delivery (categorical), parity (categorical), maternal weight-gain during pregnancy (continuous) were included. In addition, where related, respective thyroid hormone concentrations of the matching mother were also added into the model because cord serum thyroid levels can be affected by the maternal input (Fisher, 1997; Thorpe-Beeston et al., 1991b). For example, we included maternal free T4 level as a covariate (continuous) in the cord free T4 regression model. Inclusion of maternal thyroid hormone was decided following Spearman correlation test with whole CHECK population pair (n=258). Unlike cord serum thyroid hormones, bloodspot TSH was considered to be independent of maternal TSH. As infant sex was determined to be significantly associated with bloodspot TSH, infant sex was added as covariate for analysis of bloodspot TSH. In multivariate analysis, ANOVA test results ($p < F$) for significance of linear model and normality of the residuals were checked. P for trend was determined

p<t for parameter estimates in general linear model. *P* for trend < 0.05 was set as significant results, but considering small sample size, we also commented on $p <$ 0.10 as marginal significance. To interpretation of regression results, we provided back-calculated value of regression coefficient to show a change in thyroid hormone levels associated with increase of interquartile range (IQR) in each target chemical concentrations.

Sensitivity analysis was conducted with covariates to identify major predictors among them. When two or more independent variables were determined as significant predictors to the same dependent variable, and also simultaneously correlated each other in Spearman correlation test, those predictors were included in the same multivariate model. SAS 9.3 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

3.3. Results

Characteristics of study population

General characteristics of the participating women and their newborn infants, along with the levels of thyroid hormones are summarized in Table 3-1. The ages of the participating pregnant women are generally in their early 30s (mean of 33 years of age, SD of 4 years), and about a half of the women were primiparae. Approximately two thirds of the participating women gave birth to babies by spontaneous vaginal delivery. Before pregnancy, most women were within normal weight range, with a mean pre-pregnancy BMI of 21.8 kg/m^2 (SD 3.4). All TSH measurements in bloodspot papers were below 20 μlU/mL and determined no newborn infants with congenital hypothyroidism (Kim et al., 2005; Lee et al., 2001).

Respective thyroid hormones were generally correlated each other in case of total T3, free T4, and total T4 in whole CHECK population (data not shown). Therefore, to keep consistency, matching maternal free T3, total T3, free T4, total T4, or TSH was included as a covariate in all respective multivariate models for each cord thyroid hormone.

Table 3-1. Characteristics of the study population

Variable	n	Mean ± SD	Median	Range
Maternal characteristics				
Maternal age (years)	104	33.3 ± 3.9	33	25-46
Gestational age (days)	104	276 ± 7.6	276	261-293
BMI (kg/m^2)	99	21.8 ± 21.1	21.1	15.6-33.6
Maternal weight gain during pregnancy (kg)	104	14.2 ± 13.7	13.7	3-32
Mode of delivery	104	NSVD[a]: 72 (69%), C-section[b]: 32 (31%)		
Parity	104	Primipara: 55 (53%), multipara: 49 (47%)		
Maternal serum hormones				
Free T3 (pg/mL)	104	2.53 ± 0.34	2.52	1.49-3.68
Total T3 (ng/mL)	104	1.44 ± 0.28	1.46	0.62-2.20
Free T4 (ng/dL)	104	0.92 ± 0.16	0.90	0.65-1.58
Total T4 (μg/mL)	104	9.18 ± 1.55	9.24	5.45-14.09
TSH (μlU/mL)	104	2.11 ± 1.20	1.87	0.01-5.61
Infants characteristics				
Infants sex	104	Female: 51 (49%), Male: 53 (51%)		
Birth weight (kg)	104	3.3 ± 0.4	3.3	2.5-4.3
Birth length (cm)	103	50.1 ± 1.9	50.0	45-54
Cord serum hormones				
Free T3 (pg/mL)	104	1.43 ± 0.23	1.39	1.00-2.39
Total T3 (ng/mL)	104	0.65 ± 0.11	0.63	0.48-1.08
Free T4 (ng/dL)	104	1.24 ± 0.11	1.24	0.94-1.52
Total T4 (μg/mL)	104	8.65 ± 1.19	8.61	5.65-11.59
TSH (μlU/mL)	104	10.27 ± 5.74	8.24	1.59-31.94
Bloodspot TSH[c] (ulU/mL)	96	5.59 ± 3.08	5.05	0.10-15.90

[a] Normal spontaneous vaginal delivery.

[b] Caesarean section.

[c] Bloodspot TSH was measured from bloodspot samples collected at day 2-7 post-partum. Most newborn babies were collected for bloodspot on day 2 (within 48 hrs) post-partum, but 3 and 2 infants were collected on day 5 and 7 post-partum, respectively.

POPs concentrations in cord blood serum

Several target compounds were detected in > 60% of samples (Table 3-2). Among the target POPs, only *p,p'*-DDE was detected in > 90% of cord serum samples. Detection levels of POPs were generally similar with maternal population data. However, detection frequencies were generally lower. Thus cord serum CB 28, CB 118, CB 138, oxyCHD, and *p,p'*-DDT were excluded from the statistical consideration because of their low detection frequencies (< 60%), and BDE 99 was included (> 60%), compared to the analysis in their maternal population. The concentration of ΣPCB and ΣPBDE were higher in the cord serum than in the maternal serum. In cord serum, ΣPCB and ΣPBDE were detected at a median of 34.7 ng/g lw (interquartile range (IQR) of 18.4-55.5) and 8.8 ng/g lw (4.9-14.3), respectively, while those were detected at 23.5 (15.7-33.5) and 2.23 ng/g lw (1.5-4.6) in maternal serum. Most of the POPs were correlated with each other in both cord and maternal serum. CB 153 was correlated with many other chemicals in cord serum, and *p,p'*-DDE was correlated with other chemicals like CB 153, BDE 47, *β*-HCH, and tNCHD in maternal serum.

Table 3-2. Cord blood serum concentrations of OCPs, PCBs, and PBDEs among the newborn infant population (n=104)

Chemical	Cord blood serum			Maternal serum		
	Detection frequency		Median (IQR) [a]	Detection frequency		Median (IQR)
	n>LOQ	(%)	(ng/g lw)	n>LOQ	(%)	(ng/g lw)
PCB						
ΣPCB	97	**93.3**	34.7 (18.4-55.5)	96	**92.3**	23.5 (15.7-33.5)
CB 52	66	**63.5**	5.4 (3.5-9.9)	69	**66.3**	1.0 (0.6-2.0)
CB 153	78	**75.0**	10.5 (7.2-14.1)	95	**91.3**	8.4 (5.9-11.3)
PBDE						
ΣPBDE	88	**84.6**	8.8 (4.9-14.3)	97	**93.3**	2.2 (1.5-4.6)
BDE 47	77	**74.0**	3.0 (2.0-4.5)	92	**88.5**	1.2 (0.6-2.1)
BDE99	67	**64.4**	3.0 (1.8-4.5)	29	**27.9**	0.7 (0.6-1)
OCP						
***β*-HCH**	70	**67.3**	7.5 (5.3-10.0)	88	**84.6**	7.5 (4.0-11.8)
ΣHCH	71	**68.3**	10.4 (7.8-13.9)	90	**86.5**	9.4 (6.0-12.9)
***p, p'*-DDE**	101	**97.1**	63.0 (44.0-91.5)	101	**97.1**	55.2 (38.7-73.9)
ΣDDT	103	**99.0**	65.2 (46.3-97.2)	102	**98.1**	62.3 (42.6-81.3)
tNCHD	70	**67.3**	1.8 (1.4-2.7)	92	**88.5**	2.1 (1.4-2.7)
ΣCHD	82	**78.8**	2.6 (1.6-3.9)	96	**92.3**	3.9 (2.8-5.1)
HCB	69	**66.3**	12.7 (2.8-22.3)	80	**76.9**	5.5 (1.5-12.2)

[a] Interquartile range (IQR) showing the 25th and 75th percentile values.

Only the compounds of which frequency of detection was > 60% in cord serum were shown here.

ΣPCB is the sum of all target PCB congeners (CB 18, 28, 33, 44, 52, 70, 101, 105, 118, 128, 138, 153, 170, 180, 187, 194, 195, 199 and 206), and ΣPBDE is the sum of all target PBDE congeners (BDE 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184 and 191). ΣHCH included *α-, β-, γ-* and *δ*-HCH, ΣDDT included *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD, *o,p′*-DDD, *p,p′*-DDT and *o,p′*-DDT, and ΣCHD included oxyCHD, trans-chlordane, cis-chlordane, tNCHD and cis-nonachlordane.

Association between cord serum POPs and thyroid hormone concentrations of newborn infants

Out of eight target POPs, four chemicals showed significant associations with thyroid hormone levels of newborn infants after adjustment of the covariates at p <0.05 (Table 3-3). Cord serum HCB and total T4 showed negative associations with each other, showing a 5.8 % (95% confidence interval (CI), -10.9 to -0.4) decline in total T4 by IQR increase in HCB. BDE 47, and BDE 99 showed positive associations with cord TSH and blood spot TSH, respectively. IQR increase of BDE 47 and BDE 99 was associated with a 30.4 % (95% CI, 2.6 to 65.7) increase of bloodspot TSH, and a 21.2 % (95% CI, 0.1 to 46.8) increase of cord TSH, respectively. *P,p'*-DDE showed significant positive association with bloodspot TSH, and marginal negative association with total T3, simultaneously. A 16.5 % (95% CI, 1.9 to 33.2) increase of bloodspot TSH was associated with IQR increase of *p,p'*-DDE in cord serum. ΣCHD and TSH in cord serum was positively related with marginal significance as well. In contrast, ΣPCB and *β*-HCH concentrations showed negative association with bloodspot TSH, but only at the level of p <0.1. Cord blood CB 52, CB 153, and tNCHD were not associated with any thyroid hormones in neonates in our study.

In the sensitivity analysis, all three marginally significant results were disappeared. For total PCB and total T3, positive association was disappeared after adjustment of *p,p'*-DDE, and negative association with bloodspot TSH and total PCB was disappeared after adjustment of BDE 47. Similarly, marginal significance of ΣCHD with bloodspot TSH became insignificant after inclusion of BDE 99 in the multivariate model. However, positive association between *p,p'*-DDE and bloodspot TSH (ΣCHD: β=0.041, p>0.1; p,p'-DDE: β=0.539, p<0.01) was remained significant even after the sensitivity analysis (Table 3-4).

Table 3-3. Associations between POPs concentrations and hormone levels in cord blood serum or in bloodspot

POPs (ng/g lw)	Cord blood						Bloodspot	
	n	Free T3 (pg/mL)	Total T3 (ng/mL)	Free T4 (ng/dL)	Total T4 (µg/dL)	TSH (µlU/mL)	n	TSH (µlU/mL)
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)		β (95% CI)
PCB								
ΣPCB	89	0.012 (-0.03, 0.05)	**0.034*** (0.00, 0.07)	-0.002 (-0.02, 0.02)	0.004 (-0.03, 0.04)	0.012 (-0.10, 0.13)	84	**-0.160*** (-0.33, 0.01)
CB 52[+]	62	-0.034 (-0.09, 0.02)	0.002 (-0.05, 0.06)	-0.017 (-0.05, 0.02)	-0.028 (-0.08, 0.02)	0.072 (-0.10, 0.24)	58	0.121 (-0.09, 0.33)
CB 153[+]	72	0.018 (-0.06, 0.10)	0.022 (-0.05, 0.09)	0.021 (-0.02, 0.06)	-0.003 (-0.07, 0.06)	0.012 (-0.22, 0.24)	68	0.150 (-0.16, 0.46)
PBDE								
ΣPBDE	81	0.026 (-0.01, 0.06)	0.023 (-0.01, 0.06)	0.002 (-0.02, 0.03)	0.011 (-0.03, 0.05)	0.076 (-0.05, 0.20)	77	-0.087 (-0.29, 0.12)
BDE 47[+]	70	-0.016 (-0.09, 0.06)	-0.017 (-0.09, 0.06)	-0.015 (-0.06, 0.03)	-0.002 (-0.07, 0.06)	0.077 (-0.15, 0.31)	66	**0.327**** (0.03, 0.62)
BDE 99[+]	62	0.008 (-0.06, 0.07)	0.013 (-0.05, 0.08)	0.002 (-0.04, 0.04)	0.013 (-0.05, 0.07)	**0.210**** (0.00, 0.42)	61	0.037 (-0.24, 0.32)

Table 3-3. (Continued)

POPs (ng/g lw)	n	Cord blood					n	Bloodspot
		Free T3 (pg/mL)	Total T3 (ng/mL)	Free T4 (ng/dL)	Total T4 (µg/dL)	TSH (µlU/mL)		TSH (µlU/mL)
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)		β (95% CI)
OCP								
ΣHCH	65	-0.047 (-0.14, 0.05)	0.016 (-0.08, 0.11)	-0.041 (-0.10, 0.01)	-0.004 (-0.09, 0.09)	0.097 (-0.20, 0.40)	64	-0.057 (-0.38, 0.27)
β-HCH^{+}	64	-0.023 (-0.11, 0.06)	-0.002 (-0.09, 0.08)	-0.019 (-0.07, 0.03)	-0.016 (-0.10, 0.07)	0.106 (-0.16, 0.37)	63	**-0.270*** (-0.57, 0.03)
ΣCHD	76	0.018 (-0.04, 0.07)	0.029 (-0.02, 0.08)	0.005 (-0.02, 0.03)	0.021 (-0.03, 0.07)	**0.149*** (0.00, 0.30)	73	0.197 (-0.05, 0.45)
tNCHD^{+}	65	0.012 (-0.06, 0.08)	0.029 (-0.04, 0.10)	0.012 (-0.02, 0.05)	0.031 (-0.03, 0.09)	0.116 (-0.08, 0.31)	64	-0.079 (-0.31, 0.16)
ΣDDT	95	-0.049 (-0.12, 0.02)	-0.060 (-0.13, 0.01)	-0.007 (-0.05, 0.03)	0.017 (-0.05, 0.08)	0.022 (-0.21, 0.25)	90	0.110 (-0.24, 0.46)
p,p'-DDE	96	-0.033 (-0.07, 0.01)	**-0.038*** (-0.08, 0.00)	-0.002 (-0.02, 0.02)	-0.016 (-0.05, 0.02)	0.082 (-0.04, 0.21)	91	**0.208**** (0.03, 0.39)
HCB^{+}	64	-0.019 (-0.05, 0.01)	-0.016 (-0.05, 0.02)	-0.004 (-0.02, 0.01)	**-0.029**** (-0.06, 0.00)	0.059 (-0.04, 0.16)	63	-0.109 (-0.28, 0.06)

Signs ** and * indicate statistical significance of regression parameter at p=0.05, and 0.1, respectively. For chemicals that were detected in <75% but >=60%, statistical analysis was conducted with detected values only. Such chemicals are indicated by '+'.

Table 3-4. Associations between serum POPs concentrations and thyroid hormones after adding all the POPs with significant association in the model

Cord POPs	Cord or Bloodspot Thyroid hormones	n	β (95% CI)
ΣPCB	TT3	89	0.02 (-0.02, 0.06)
p,p'-DDE	TT3		-0.04* (-0.09, 0.00)
BDE 99	Cord TSH	57	0.22** (0.00, 0.42)
ΣCHD	Cord TSH		0.13 (-0.06, 0.33)
ΣPCB	Bloodspot TSH	65	0.07 (-0.13, 0.27)
BDE 47	Bloodspot TSH		0.38** (0.08, 0.67)
ΣCHD	Bloodspot TSH	73	0.04 (-0.20, 0.28)
p,p'-DDE	Bloodspot TSH		0.54** (0.25, 0.82)
Maternal POPs	**Cord or Bloodspot Thyroid hormones**	**n**	**β (95% CI)**
β-HCH	fT3	95	-0.04* (-0.08, 0.01)
p,p'-DDE	fT3		-0.02 (-0.07, 0.03)
ΣCHD	fT4	88	-0.03 (-0.07, 0.01)
p,p'-DDE	fT4		-0.01 (-0.05, 0.02)
BDE 47	Bloodspot TSH	89	0.02 (-0.13, 0.18)
ΣDDT	Bloodspot TSH		-0.13 (-0.58, 0.32)
p,p'-DDE	Bloodspot TSH		0.45** (0.14, 0.76)

Signs ** and * indicate statistical significance of regression parameter (β) at p=0.05, and 0.1, respectively. 'CI' confidence interval; 'fT3' free T3; 'TT3' total T3; 'fT4' free T4. For the calculation of association, two or more independent variables that were determined as significant effectors to a given thyroid hormone, and at the same time were correlated each other, were added in the multiple regression analysis, in order to identify major effectors.

Association between maternal serum POPs and thyroid hormone concentrations of newborn infants

Among seven target chemicals, three kinds of maternal POPs, i.e., *β*-HCH, ΣCHD, ΣDDT, and *p,p'*-DDE, were significantly associated with thyroid hormones of newborn infants, and BDE 47, tNCHD, and HCB were marginally associated (Table 3-5). Maternal CB 52 and CB 153 were not associated with fetal thyroid hormone status (Table 3-5). *β*-HCH showed negative associations with free and total T3 both, and IQR increase of *β*-HCH was related with 4.7 % (95% CI, -8.4 to -0.7) decrease of free T3 and 4.1 % (95% CI, -8.0 to -0.1) decrease of total T3. ΣCHD were negatively related with free and total T4 both (*p* for trend = 0.07 for total T4), and 2.3 % (95% CI, -4.4 to -0.1) decline of free T4 was associated with IQR increase of ΣCHD in maternal serum. Although at *p* for trend <0.1 level, maternal tNCHD concentration was also negatively related with cord total T3 concentrations. Both ΣDDT, and *p,p'*-DDE showed significant positive associations with bloodspot TSH, but the negative associations between *p,p'*-DDE and free T3, free/total T4 were observed with marginal significance. Increased maternal BDE 47 was marginally related with increased bloodspot TSH, but no relationships were observed with the other cord thyroid hormones.

In sensitivity analysis, *p,p'*-DDE appeared to be a predominant effector on bloodspot TSH. Multivariate model analysis with bloodspot TSH and its three significant effectors, i.e., BDE 47, total DDT and *p,p'*-DDE, showed that the β coefficient of regression equation of *p,p'*-DDE became greater (*p,p'*-DDE: $\beta=0.451$, $p<0.01$) compared to the model with *p,p'*-DDE only ($\beta=0.209$, $p<0.05$) (Table 3-4). However, both maternal *β*-HCH and ΣCHD became insignificant after adjustment of maternal *p,p'*-DDE in the free T3 and free/total T4 models (Table 3-4).

Table 3-5. Associations between POPs concentrations in maternal blood and thyroid hormone levels in cord blood serum or in bloodspot

POPs (ng/g lw)	Cord blood						Bloodspot	
	n	Free T3 (pg/mL)	Total T3 (ng/mL)	Free T4 (ng/dL)	Total T4 (µg/dL)	TSH (µlU/mL)	n	TSH (µlU/mL)
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)		β (95% CI)
PCB								
ΣPCB	88	-0.029 (-0.08, 0.03)	-0.020 (-0.08, 0.03)	-0.004 (-0.04, 0.03)	0.005 (-0.06, 0.05)	-0.063 (-0.26, 0.13)	84	-0.157 (-0.44, 0.14)
CB 52[+]	95	-0.003 (-0.05, 0.04)	0.011 (-0.04, 0.06)	0.010 (-0.02, 0.04)	0.029 (-0.01, 0.07)	0.046 (-0.10, 0.19)	91	-0.023 (-0.26, 0.21)
CB 153	94	-0.011 (-0.05, 0.03)	-0.009 (-0.05, 0.03)	-0.004 (-0.03, 0.01)	0.011 (-0.03, 0.04)	-0.030 (-0.16, 0.10)	90	0.031 (-0.14, 0.23)
PBDE								
ΣPBDE	90	-0.006 (-0.04, 0.03)	0.004 (-0.03, 0.04)	-0.002 (-0.02, 0.02)	0.013 (-0.02, 0.04)	-0.068 (-0.18, 0.04)	86	-0.005 (-0.13, 0.21)
BDE 47	94	-0.006 (-0.04, 0.03)	-0.010 (-0.04, 0.02)	0.004 (-0.01, 0.02)	-0.008 (-0.03, 0.02)	-0.028 (-0.13, 0.07)	90	**0.130**[*] (-0.04, 0.29)

Table 3-5. (Continued)

POPs (ng/g lw)	Cord blood						Bloodspot	
	n	Free T3 (pg/mL)	Total T3 (ng/mL)	Free T4 (ng/dL)	Total T4 (μg/dL)	TSH (μlU/mL)	n	TSH (μlU/mL)
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)		β (95% CI)
OCP								
ΣHCH	83	-0.023 (-0.06, 0.01)	-0.014 (-0.05, 0.02)	-0.005 (-0.03, 0.01)	0.002 (-0.03, 0.03)	0.052 (-0.06, 0.17)	79	-0.031 (-0.15, 0.14)
β-HCH	95	**-0.044**** (-0.08, -0.01)	**-0.039**** (-0.08, 0.00)	-0.017 (-0.04, 0.00)	-0.028 (-0.06, 0.01)	0.066 (-0.06, 0.19)	91	0.041 (-0.12, 0.26)
ΣCHD	88	-0.005 (-0.08, 0.07)	-0.013 (-0.08, 0.05)	**-0.038**** (-0.08, -0.01)	**-0.056*** (-0.12, 0.00)	0.009 (-0.21, 0.23)	87	-0.221 (-0.41, 0.16)
tNCHD	95	-0.027 (-0.08, 0.03)	**-0.049*** (-0.09, 0.01)	-0.015 (-0.04, 0.01)	-0.027 (-0.07, 0.02)	-0.015 (-0.18, 0.15)	91	0.048 (-0.17, 0.39)
ΣDDT	94	-0.027 (-0.10, 0.05)	-0.023 (-0.10, 0.05)	-0.017 (-0.06, 0.02)	0.004 (-0.06, 0.07)	-0.013 (-0.25, 0.22)	90	**0.345**** (0.06, 0.81)
p,p'-DDE	95	**-0.036*** (-0.08, 0.01)	-0.028 (-0.07, 0.01)	**-0.020*** (-0.04, 0.00)	**-0.034*** (-0.07, 0.01)	0.001 (-0.13, 0.13)	91	**0.264**** (0.11, 0.50)
HCB	95	-0.005 (-0.04, 0.03)	-0.015 (-0.05, 0.02)	0.001 (-0.02, 0.02)	-0.011 (-0.04, 0.02)	**0.089*** (-0.02, 0.19)	91	-0.095 (-0.26, 0.09)

Signs ** and * indicate statistical significance of regression parameter at p=0.05, and 0.1, respectively. For chemicals that were detected in <75% but >=60%, statistical analysis was conducted with detected values only. Such chemicals are indicated by '+'. Since and BDE 99 were detected below 60% in maternal serum samples, they were not statistically analyzed.

3.4. Discussion

Influence of maternal thyroid hormones on the effect of POPs exposure to neonatal thyroid hormones

Relatively few studies have been conducted on the association between POPs exposure and thyroid hormones among newborn infants (Table 3-6), and these observations generally showed inconsistent results. For example, while several studies reported the increase of TSH and decreases of T3 or T4, which are similar to the results of experimental studies on PBDEs (Abdelouahab et al., 2013; Herbstman et al., 2008b; Kim et al., 2011; Lin et al., 2011), PCBs (Chevrier et al., 2007; Herbstman et al., 2008b; Zhang et al., 2010), or OCPs (Asawasinsopon et al., 2006; Maervoet et al., 2007; Ribas-Fito et al., 2003), the associations of the opposite directions or no association were also reported (Chevrier et al., 2011; Dallaire et al., 2008; Kim et al., 2009b; Longnecker et al., 2000; Takser et al., 2005; Zhang et al., 2010), even within the same study (Ribas-Fito et al., 2003). Seemingly inconsistent associations between prenatal POPs exposure and the thyroid hormones in cord serum may be explained by several reasons. First, thyroid hormone levels in cord serum could be confounded by maternal transfer of thyroid hormones. Maternal thyroid hormones can be transferred to the fetus by perfusion system of placenta and blood exchange via cord. Fetuses depend entirely on the maternal supply of T4 during the first trimester and continue to depend on to varying degrees throughout the pregnancy (Contempre et al., 1993). At birth, around 30-60 % of thyroid hormones in cord blood are of maternal origin (Fisher, 1997; Thorpe-Beeston et al., 1991a). Therefore, thyroid hormones measured in cord serum could be confounded by the physiological or environmental factors that could influence maternal thyroid hormone levels. Second, several factors related to maternal, fetal, and delivery conditions may influence the thyroid status of the fetus. For example, delivery associated factors,

such as mode of delivery, time of labor, emergency cesarean section, and induced labor, are reported to be related with intrapartum stress of mother and fetus (Herbstman et al., 2008a), leading to rapid changes of thyroid hormone levels shortly after birth (Kim et al., 2005). Because, in studies involving human populations, controlling labor-related factors beforehand is difficult, those variables that may influence the fetal thyroid hormones should be identified and adjusted in the statistical analytical model.

In the present study, in order to minimize the influences of the factors that would confound the between cord serum thyroid hormone levels and prenatal POPs exposure, we included maternal thyroid hormone level as covariate in the multivariate analysis model. In addition, TSH levels measured in bloodspot collected at 2 day post-partum were also analyzed for the association with prenatal POPs exposure. Since TSH is maintained consistent after 24 hrs of delivery, bloodspot TSH levels of the newborn infants at 2 day post-partum are considered relatively independent from the influence of the maternal thyroid hormones (Kim et al., 2005). In this context, Hardy et al. (2008) reported that bloodspot TSH showed better sensitivity than cord serum TSH, and greater sensitivity than cord serum T4 for screening congenital hypothyroidism. The use of maternal thyroid hormones as covariates and inclusion of bloodspot TSH are among the strengths of our study. Adjusting with maternal thyroid hormones appeared to successfully control, at least to certain extent, the confounding influences of maternal input on cord thyroid hormone levels. One example is the influence of maternal tNCHD on cord total T3. The association between maternal tNCHD and cord total T3 was marginally significant (β=-0.049, p=0.074, 3.2 % (95% CI: -6.4 to 0.0) of total T3 decline by tNCHD IQR increase) with maternal total T3 adjustment (Table 3-4). While, following the removal of maternal total T3 level as a covariate in the model, the association between maternal tNCHD and cord total T3 became insignificant (β=-0.040, p>0.10), 2.6 % (95% CI: -5.7 to 0.7) of total T3 decrease

by tNCHD IQR increase (Table 3-7), and this observation implies that the maternal total T3 could in fact confound the association and could lead to a wrong conclusion. In our previous study, maternal T3 was not affected by tNCHD exposure (Chapter 2). However, these observations warrant further confirmation in other pregnant women-fetus pair populations.

Table 3-6. Associations between prenatal POPs concentrations in either maternal or cord blood and thyroid hormone levels of newborn infants

Matrix	n	POPs	Thyroid hormone measurement						Reference
			fT3	TT3	fT4	TT4	TSH	matrix	
Cord serum	104	**PCBs**	-	↑	-	-	- / ↓s	Cord serum & Bloodspot	This study
		PBDEs	-	-	-	-	↑ / ↑s		
		OCPs	-	↓	-	↓	- / ↑s		
Cord serum	108	**PBDEs**			-		-	Cord serum	Kim et al. (2009b)
Cord serum	297	**PBDEs**			-	↓ / -s	-	Cord serum & Bloodspot	Herbstman et al. (2008b)
		PCBs			↓ / -s	- / ↓s	-		
Cord serum	39	**DDTs**			-	↓	-	Cord serum	Asawasinsopon et al. (2006)
Cord serum	92	**PCBs**	-			-	-	Cord blood	Takser et al. (2005)
Cord serum	9	**PBDEs**	-	-	-	-	-	Cord serum	Mazdai et al. (2003)
Cord serum	70	**HCB, PCBs, *p,p'*-DDE**					-s	Bloodspot	Ribas-Fito et al. (2003)
		***β*-HCH**					↑		
Cord plasma	410, 260	**PCBs**		-	-		-	Cord serum	Dallaire et al. (2008)
		HCB		-	↑		-		
Cord plasma	198	**PCBs, HCB**	↓		↓		-	Cord plasma	Maervoet et al. (2007)
		***p,p'*-DDE**			↓		-		
Cord blood	90	**PBDEs**	-	-	↓	-	-	Cord blood	Kim et al. (2011)
Cord blood	50	**PCBs**		-		↓	-	Cord blood	Zhang et al. (2010)
		PBDEs		-		-	-		
Cord blood	54	**PBDEs**	↓	↓	-	-	-	Cord blood	Lin et al. (2010)

Table 3-6. (Continued)

Matrix	n	POPs	Thyroid hormone measurement						Reference
			fT3	TT3	fT4	TT4	TSH	matrix	
Maternal serum	104	**PCBs**	-	-	-	-	- / -s	Cord serum & Bloodspot	This study
		PBDEs	-	-	-	-	- / ↑s		
		OCPs	↓	↓	↓	-	- / ↑s		
Maternal serum	79	**PCBs, OH-PCBs**			-s		↑s	Bloodspot	Hisada et al. (2014)
Maternal serum	260	**PBDEs**	-	-	↓	↓	-	Cord blood	Abdelouahab et al. (2013)
		PCBs	-	-	-	-	-		
Maternal serum	289	**PBDEs**					-	Cord serum	Chevrier et al. (2011)
Maternal serum	285	**PCBs**					↑s	Bloodspot	Chevirer et al. (2007)
Maternal blood	160	**PCBs**			-	-	-	Cord serum	Longnecker et al. (2000)

'-' no association; '↑' positive association; '↓' negative association; blank cell means data not available. 'fT3' free T3; 'TT3' total T3; 'fT4' free T4; 'TT4' total T4. In the present study, cord thyroid hormone levels were adjusted with maternal thyroid hormone levels in the model. Unless otherwise noted, all thyroid hormone measurements were from cord blood or cord serum. 's' indicates the measurement in bloodspot of newborn infant.

Table 3-7. Results of multivariate analysis with or without maternal thyroid hormone as covariates in the model

Fetal POPs	Neonatal thyroid hormone	Without maternal thyroid hormone		With maternal thyroid hormone	
		n	β (95% CI)	n	β (95% CI)
ΣPCB	Cord TT3	89	**0.03 (-0.07, 0.07)**	89	**0.03 * (0.00, 0.07)**
p, p'-DDE		96	-0.04 * (-0.08, 0.00)	96	-0.04 * (-0.08, 0.00)
HCB	Cord TT4	64	-0.03 ** (-0.06, -0.01)	64	-0.03 ** (-0.06, 0.00)
BDE 99	Cord TSH	62	0.22 ** (0.01, 0.42)	62	0.21 ** (0.00, 0.42)
ΣCHD		76	0.15 * (0.00, 0.31)	76	0.16 * (0.00, 0.30)
ΣPCB	Bloodspot TSH	84	-0.16 * (-0.33, 0.01)	81	-0.18 * (-0.36, 0.01)
BDE 47		66	0.33 ** (0.03, 0.62)	63	0.35 ** (0.04, 0.66)
p, p'-DDE		91	0.21 ** (0.03, 0.39)	88	0.25 ** (0.06, 0.44)
Maternal POPs	**Neonatal thyroid hormone**	**Without maternal thyroid hormone**		**With maternal thyroid hormone**	
		n	**β (95% CI)**	**n**	**β (95% CI)**
β-HCH	Cord TT3	95	-0.04 ** (-0.08, 0.00)	95	-0.04 ** (-0.08, 0.00)
tNCHD		95	**-0.04 (-0.09, 0.01)**	95	**-0.05 * (-0.10, 0.00)**
ΣCHD	Cord fT4	88	-0.04 ** (-0.08, -0.01)	88	-0.04 ** (-0.08, -0.00)
p, p'-DDE		95	-0.02 * (-0.04, 0.00)	95	-0.02 * (-0.04, 0.00)
ΣCHD	Cord TT4	88	-0.06 * (-0.12, 0.00)	88	-0.06 * (-0.12, 0.01)
p, p'-DDE		95	-0.03 * (-0.07, 0.01)	95	-0.03 * (-0.07, 0.00)
HCB	Cord TSH	95	0.10 * (0.00, 0.21)	95	0.09 * (-0.02, 0.19)
BDE 47	Bloodspot TSH	90	**0.13 * (-0.01, 0.27)**	87	**0.12 (-0.04, 0.23)**
ΣDDT		90	0.35 ** (0.00, 0.69)	87	0.43 ** (0.06, 0.81)
p, p'-DDE		91	0.26 ** (0.07, 0.45)	88	0.31 ** (0.11, 0.50)

Signs ** and * indicate statistical significance of regression parameter at p=0.05, and 0.1, respectively. Bold values (shaded) represent difference in significance of association after adjustment of maternal thyroid hormones in the model. 'CI' confidence interval; 'fT3' free T3; 'TT3' total T3; 'fT4' free T4; 'TT4' total T4.

Association between POPs exposure and thyroid hormones

Several POPs appeared to be associated with the changes in thyroid hormone levels in newborn infants, which is comparable to our recent observations on pregnant women (Chapter 2) and several experimental studies (Darras, 2008; Hallgren and Darnerud, 2002; Scollon et al., 2004; Zhou et al., 2001). Negative associations of several POPs exposure with total T3 and positive associations with cord serum or bloodspot TSH, which remained significant after controlling relevant covariates in multivariate models, clearly showed the thyroid disrupting potentials of POPs among newborn infants even at the current low exposure levels.

Our findings that prenatal exposure to OCPs (*p,p'*-DDE, HCB, *β*-HCH, and ∑CHD) appeared to be associated with T3, T4 of cord serum, and TSH of bloodspot was consistent with experimental study results. There are several experimental studies that support thyroid disruption toxicity of OCPs (Foster et al., 1993; Scollon et al., 2004; Alvarez et al., 2005). In the 30-days exposure, increased T4 to T3 conversion and enlarged liver were observed following HCB treatment in rats (Alvarez et al., 2005). In experiment using sparrows, *p,p'*-DDT exposure led to decreased thyroid hormone levels, and inhibition of TSH receptor function was one of the suggested thyroid hormone disruption of DDTs (Scollon et al., 2004; Santini et al., 2003). CHDs can interfere in the cellular uptake of thyroid hormones, resulted in T3 or T4 reduction (Shimada et al., 2004).

The effect of OCPs appeared to be more evident among newborn infants compared to their matching mothers (Chapter 2). Early developmental stages are considered to be more sensitive to exposure to OCPs (Vesselinovitch et al., 1979). Potential epigenetic transgenerational action of several OCPs on endocrine system (Schug et al., 2011; Skinner et al., 2013) could also in part explain the greater sensitivity among newborn infants.

The significant positive associations between BDE 47 and bloodspot TSH, and between BDE 99 and cord serum TSH, even after adjustment of other related chemicals (Table 3-3 and 3-5) suggest the effects of PBDEs thyroid hormone homeostasis disruption in newborn infants. PBDEs, especially BDE 47, were suggested to act through increase of hepatic enzyme related with glucuronidation, or decrease of transport protein transthyretin (Hallgren et al., 2001; Richardson et al., 2008). Although human study is still sparse and the results are not consistent, our observation among newborn infants is comparable to those of experimental studies (Abdelouahab et al., 2009; Hallgren et al., 2001; Kim et al., 2009a; Kodavanti et al., 2010), warranting further confirmation in the epidemiological studies.

There was no significant association between prenatal PCBs exposure and thyroid hormone levels among newborn infant population in the present study, opposite to the result in maternal serum which significant relationships between PCBs exposure and total T3 or T4 were observed (Chapter 2). Total PCB in cord serum shows only weak effects ($p < 0.10$) on cord total T3 and bloodspot TSH levels (Table 3-3). However, these weak influences of total PCB disappeared by adding *p,p'*-DDE and BDE 47 of cord serum in the model (Table 3-5), suggesting that the effects of total PCB on thyroid hormones was probably confounded by PBDEs and OCPs. This null-association is not consistent with experimental results in rats (Gauger et al., 2004; Donahue et al., 2004; Zoeller et al., 2000), however, several epidemiological studies regarding thyroid hormone and PCB in newborns also failed to detect such observations (Table 3-6). For example, no effects on thyroid hormones were observed with prenatal PCB exposure (Longnecker et al., 2000; Takser et al., 2005), even though significant associations with other POPs were observed in the same population (Abdelouahab et al., 2013; Dallaire et al., 2008; Ribas-Fito et al., 2003).

Why do PCBs influence thyroid hormone levels differently among pregnant

women and newborn infants? While direct answer to this question is not ready, a couple of reasons can be considered in the future studies. First, potential differences in thyroid-related metabolic pathways between pregnant women and newborn infants should be considered (Glinoer, 1997). Even in the same condition of marginal iodine deficiency, newborns were protected from hypothyroxinemia unlike mothers, suggesting different physiological responses (Glinoer et al., 1992). Second, compared to other chemicals, PCBs are regarded to possess more complex mechanisms of action of thyroid-disruption, e.g., on the function of the TSH receptor, binding transport proteins, hormone receptor and gene expression, and excretion/clearance of thyroid hormones (Boas et al., 2006). Such complex dynamics may have obscures true associations.

3.5. Summary and implications

We tried to explore the associations between thyroid hormone levels and prenatal POPs exposure in matching pregnant-infant pair, and several POPs were appeared as significant determinant of decrease of T3/T4 and increase of TSH concentrations. This study has some limitations: (1) cross-sectional study design and small sample size, and (2) possible measurement error of free form thyroid hormone. Cross-sectional origin can lead to achievement of statistical significance due to chance with numerous regression model generations. We tried to make complementary result with sensitivity analysis, and many kinds of POPs measurements made it possible although our sample size is not enough to have statistical power. Also, it believes that serum albumin, abnormal binding proteins, high free fatty acid, or hormone binding inhibitors may alter the free hormone immunoassay measurements (Spencer, 2013). Although the relationships between binding protein and free thyroid hormones were controversial in direct liquid chromatography-tandem mass spectrometry (LC/MS/MS) method (van Deventer et al., 2011; Jonklass et al., 2009), this should be considered and double-checked with TSH results when we interpret results for free thyroid hormones. These limitations might be lead that the associations in our study were somehow inconsistent within chemicals, across hormones (e.g. positive associations with TSH were not found in parallel with negative associations with fT4), or measured media (maternal vs cord serum, or cord serum vs bloodspot).

However, our study is unique in that we have measured three groups of POPs and all five thyroid hormones in both maternal and fetal serum samples at the same time. While the validation of our result in larger populations would be needed, the results of the present study provide another line of evidence that the current OCPs and PBDEs exposure among humans in sensitive life stage could influence the balance of thyroid hormones. Controlled for maternal thyroid

hormones, the observed associations are believed to more accurately reflect the response of cord serum thyroid hormones against the prenatal POPs exposure. The observed associations between POPs exposure and bloodspot TSH of newborn infants also support the same influence of the POPs toward thyroid hormone disrupting potential among newborn infants. Slight decreases in thyroid function (subclinical or mild hypothyroidism) may lead negative health outcomes, especially over the long term and during pregnancy. Even though thyroid hormone levels are within the reference range, small changes (< 25%) of maternal T4 or TSH during the early fetal period are associated with adverse health outcome (WHO/UNEP, 2012). Thus, considering the importance of thyroid hormones in rapidly developing bodies, public health implications of thyroid hormone disturbance among newborn infants should receive further investigations. This observation also emphasizes importance of further studies on later life stage implications of the hormonal alteration among developing infants.

Chapter 4. Association between several persistent organic pollutants in serum and adipokine levels in breast milk among Korean lactating women

4.1. Introduction

Several hormones, synthesized and secreted by adipocytes, i.e., adipokines, have been identified in serum and breastmilk, and associated with regulation of food intake and energy balance (Savino et al., 2013). For this reason, adipokines, such as leptin and adiponectin, have been often employed to understand the etiology of obesity among human population (Briffa et al., 2013; Boeke et al., 2013; Gillman and Mantzoros, 2007; Mantzoros et al., 2009). Recently several POPs have been suggested to cause obesity by alteration of lipid metabolism of humans (Lind and Lind, 2012; Thayer et al., 2012). For example, low-dose exposure to OCPs and PCBs has been associated with a greater risk of obesity (Lee et al., 2007; Lee et al., 2011; Lim et al., 2010; Taylor et al. 2012) even among childhood (Valvi et al. 2012).

POPs exposure has been associated with altered adipokine levels in many *in vitro* and *in vivo* experiments (Howell and Magnum, 2011; Wahlang et al., 2013; Taxvig et al., 2012; Provost et al., 2007). Epidemiological observations are relatively limited but also increasing in number. Serum leptin showed negative

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associations with serum CB 138, CB 180, and BDE 153 levels in obese adults (Pereira-Fernandes et al., 2014). Leptin mainly acts to regulate energy balance, and decreased leptin may lead to increased adiposity. Adiponectin acts to increase uptake and catabolism of fatty acids, and energy expenditure to regulate the energy homeostasis. Adiponectin is correlated to adiposity (Savino et al., 2013), but the directions of the association vary by population (Mullerova et al., 2008; Lim and Jee, 2014).

Among infants and children, blood adipokines have been also associated with obesity. In a prospective cohort study, perinatal leptin (maternal plasma at 26-28 weeks of gestation, and cord blood) was negatively correlated with adiposity at 3 years of age, but age 3 leptin was positively correlated with adiposity at age 7 (Boeke et al., 2013). Similarly, serum leptin levels were inversely correlated with *β*-HCH or *p,p'*-DDE exposure in boys of 8 to 9 years of age (Burns et al., 2011). Adiponectin in cord blood was inversely associated with weight gain for the first 6 months after birth and adiposity at 3 years of age (Mantzoros et al., 2009).

Adipokines in breast milk can influence their circulating levels in breastfed infants (Savino et al., 2012; Ucar et al., 2000). Furthermore, adiponectin in breast milk is reported to possess a greater biological activity and greater gastrointestinal absorption rate than the forms existing in blood (Newburg et al., 2010). Therefore breast milk adipokines have been recognized as important hormones that are associated with obesity (Ahima and Osei, 2008; Leal Vde and Mafra, 2013; Nakamura et al., 2014). Doneray et al. (2009) reported that breast milk leptin concentration is inversely associated with BMI increase during the first one month of age. Adiponectin levels in breast milk are positively associated with weight gain among 2 year old infants (Brunner et al., 2014; Woo et al., 2012).

Despite the importance of breast milk adipokines among infants, to our knowledge its association with maternal POPs exposure has never been

investigated. In the present study, we investigated the association between the breast milk adipokine levels and the serum POPs levels among lactating women of Korea. The utility of breast milk as an alternative medium to maternal serum for measuring adipokines could be assessed. In addition, since breast milk adipokines are important for infant growth and possibly for obesity in the later stages of the life, this study will also shed light on the implication of maternal POPs exposure on the potential adverse health outcome of her offspring through lactational transfer of adipokines.

4.2. Materials and methods

Study population and sample collection

A total of 138 healthy pregnant women without chronic disease history were recruited before delivery from five university hospitals located in four cities of Korea, i.e., Seoul, Anyang, Ansan, and Jeju, from February to December 2011, as a part of the CHECK (Children's Health and Environmental Chemicals in Korea) Panel. Details about the demographic characteristics of the participating women were shown in Chapter 1. On arrival at the hospital for delivery (within 1 day before delivery), maternal blood samples were collected, separated for serum on site, and stored in polypropylene cryovials at −20 °C until analysis. Breast milk samples were collected from the same women at 7, 15 or 30 days after delivery, in polypropylene tubes following a detailed instruction with pictorial guide. The breast milk samples were then frozen and transported on ice to the laboratory. Samples were stored in the laboratory at −70 °C until further analysis. Among 138 recruited mothers collected for blood serum at delivery, 87 mothers provided breast milk samples but only 82 samples were analyzed for adipokines due to the limited amount of the samples. Most of breast milk samples were collected at the 15th day after delivery (n=74), and one and seven samples were collected at days 7 and 30 after delivery, respectively.

One-on-one interview with participating pregnant women was conducted at the time of enrollment. Demographic characteristics such as age, body weight, and height, medical history, or pregnancy related records were obtained from this interview.

Institutional Review Boards of School of Public Health, Seoul National University, and all participating university hospitals (Soonchunhyang University Seoul Hospital, Hallym University Sacred Heart Hospital, Korea University

Ansan Hospital, and Jeju National University Hospital) approved the study. The informed written consents were obtained from the participating women. All samples and data were processed blind.

Chemical analysis

Maternal serum samples were processed and measured for 19 PCB congeners, 19 PBDEs congeners, and 19 OCPs, as shown in a previous studies (Chapter 2 and 3). For POPs quantification, ^{13}C-labeled 8 PCBs (EC9605-SS; Wellington Laboratories, Guelph, ON, Canada), ^{13}C-labelled 6 PBDEs (MBDE-MXE; Wellington) and ^{13}C-labeled 19 OCPs (ES-5349-L; Cambridge Isotope Laboratories, Andover, MA, USA) were used as surrogate internal standards.

The experimental procedures of analysis of OCPs, PCBs and PBDEs in serum were optimized with some modifications of previous studies (Dmitrovic et al., 2002; Kang et al., 2008). In brief, serum samples (2 mL) were fortified with formic acid and Milli-Q water for protein denaturation, after ^{13}C-labeled OCPs, PCBs and PBDEs were spiked. The samples were extracted by SPE using Sep-Pak C_{18} SPE cartridge, which was pre-washed with MeOH and conditioned with Milli-Q water. The extracted cartridge was rinsed with Milli-Q water and subsequently dried. A Sep-Pak Plus NH_2 cartridge, pre-washed with 6 mL of hexane, was connected to the lower end of the C_{18} cartridge. Eight milliliter of hexane was passed through the combined NH_2-C_{18} cartridges and was collected. After removing C_{18} cartridge, 6 mL of 5% DCM in hexane was passed through NH_2 cartridge and was combined to a previous fraction. The pooled eluents were cleaned up onto a silica gel/florisil SPE cartridge, using 12mL of 50% DCM in hexane. The purified eluents were concentrated and dissolved in 100μL nonane for instrumental analysis. POPs concentrations were normalized by lipid weight of serum. Total lipid (mg/dL) was calculated from concentrations of total cholesterol and triglyceride consistently with Chapter 2 and 3.

A high-resolution gas chromatography interfaced with a high-resolution mass spectrometer (HRGC/HRMS; JMS 800D, JEOL, Tokyo, Japan) was used for the identification and quantification of OCPs, PCBs and PBDEs. Details of

instrumental parameters have been reported elsewhere (Moon et al., 2007; Moon et al., 2009). In brief, OCPs, PCBs and PBDEs were quantified using the isotope dilution method based on relative response factors of individual compounds. The HRMS was operated under positive EI mode, and ions were monitored by selected ion monitoring using molecular ions of target compounds. A DB5-MS (30 m length, 0.25 mm inner diameter, 0.25μm film thickness; J&W Scientific, Palo Alto, CA, USA) was used for the separation of OCPs and PCBs. A DB5-MS (15 m length, 0.25 mm inner diameter, 0.1 μm film thickness; J&W Scientific) was used for the separation of from tri- to heptaBDE congeners.

The recoveries of spiked ^{13}C-labeled compounds were 91 ± 10% (average± SD) for OCPs, 62 ± 5.5% for PCBs and 87 ± 13% for PBDEs. Solvents injected before and after the injection of standards showed negligible contamination or carryover. Procedural blanks (n = 10) were processed with every set of 15 serum samples to check laboratory contamination. Blanks did not contain quantifiable amounts of target contaminants. LOQ was calculated as 10 times the signal to noise ratio. The respective LOQs for OCPs, PCBs and PBDEs were from 0.7 to 1.7 ng/g lw, from 0.8 to 8.3 ng/g lw and from 0.2 to 0.8 ng/g lw. All the POP concentrations were adjusted to the lipid contents of serum.

Adipokines measurements

For adipokine measurement, 2 ml of all breast milk samples were thawed at room temperature, and then centrifuged at 10,000 rpm for 1 minute to separate fat layers. Only the liquid phase of the samples were used and diluted to within the assay concentration range. Commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Leptin DuoSet®, and Human Adiponectin/Acrp30 DuoSet®, R&D systems, Minneapolis, MN, USA) was applied. Analytical method was validated following the manufacturer's instructions. Recoveries were calculated by comparing to the spiked internal standard, and were 86 % and 95 % for leptin and adiponectin, respectively. Recoveries for linearity within spiked matrix, 2-, 4-, and 8-fold diluted matrix ranged between 88 and 92 % for leptin assay, and between 130 and 140 % for adiponectin assay. Standard curves were generated in 96 well plates with $R^2 > 0.99$ and $R^2 > 0.96$ for leptin and adiponectin measurements, respectively. All assays were performed in duplicate. Detection limits for leptin and adiponectin were 15.63 and 62.5 ng/L, respectively.

Statistical analysis

Nine chemicals which were detected > 60%, i.e., HCB, *β*-HCH, *p,p'*-DDE, *p,p'*- DDT, oxyCHD, tNCHD, BDE 47, CB 138, and CB 153, and the sum of each group (ΣPCB, ΣPBDE, ΣDDT, ΣCHD, ΣHCH), were used in statistical analysis. However, we did not differentiate *β*-HCH from ΣHCH, because most of the ΣHCH was β-HCH, and the statistical results were basically the same. There was possibility to decline of adipokine expression following increase of breast feeding duration, so we conducted statistical analysis with dataset from breast milk at 15 days postpartum only (n=74), to minimize potential variation of adipokine levels by duration of lactation. For chemicals that were detected ≥ 75% of the population, a proxy value, i.e., LOQ divided by square root 2, was used to replace the non-detects (Hornung and Reed, 1990). In addition, the subjects with non-detects for any of the target chemicals were excluded from the statistical analysis (n=24). Thus, final dataset for statistical analysis was limited to 50 subjects.

Multivariate analysis was conducted following natural log transformation to minimize the effect of a highly right-skewed data. To determine the associations between POPs and adipokine measurements, age (continuous) and pre-pregnancy BMI (categorical) were included as covariates in the models. We also evaluated group difference according to the quartiles of the serum POPs levels with ANCOVA to consider the possible non-monotonic dose-responses (Beausoleil et al., 2013; Lee et al., 2011; Vandenberg et al., 2012). Statistical significance was defined at $p < 0.05$. SAS 9.3 (SAS Institute, Cary, NC, USA) was used for statistical analyses.

4.3. Results

Characteristics and POPs exposure level of study population

The participating pregnant women were generally in early 30s (median 33 years of age) and approximately a half of the participating women were primiparae (Table 4-1). About 70 % of the participating women gave birth to babies by normal spontaneous vaginal delivery. Before pregnancy, most women (61%) were within normal BMI range with a median of 20.5 kg/m^2.

Among 57 POPs analyzed, nine chemicals were detected in > 60% of samples (Table 4-2). *p,p'*-DDE and CB 153 was detected in > 90% of the serum samples. ΣHCH, ΣDDT, and ΣCHD were detected at a median of 9.20 (interquartile range (IQR) of 5.82-13.3), 67.3 (47.8-92.7), and 3.8 ng/g lw (2.77-5.06), respectively. Median serum concentrations of ΣPCB and ΣPBDE were determined at 27.3 ng/g lw (16.2-34.7) and 2.20 ng/g lw (1.49-4.93), respectively.

Table 4-1. Demographic parameters of study population

Parameter		Basic characteristics (n=82)
Mothers		
Age (years)	Median (range)	33 (25-46)
Gestational age (days)	Median (range)	276 (261-293)
Parity [a]	N (%)	primipara: 42 (51) multipara: 40 (49)
Delivery mode [b]	N (%)	NSVD: 56 (68) C-section: 26 (32)
Pre-pregnancy BMI (kg/m^2) [c]	N (%)	Underweight: 10 (12) Normal range: 61 (76) Overweight: 10 (12)
Sampling time (weeks)	N (%)	< 1wks: 1 (1) 2 wks: 74 (90) 4 wks: 7 (9)
Infants		
Sex	N (%)	male: 39 (48) female: 43 (52)
Weight at birth (Kg)	Median (range)	3.29 (2.51-4.01)

[a] Parity is the number of pregnancies carried and a women who has given birth once before is referred to as a primipara and other is referred to as a multipara.
[b] NSVD: Normal spontaneous vaginal delivery, C-section: Caesarean section.
[c] BMI were reported with 81 cases because of 1 missing data.

Table 4-2. Serum POPs concentrations in Korean lactating mothers (n=82)

	n (% DF)	GM	Median	(IQR)
OCPs				
HCB	60 (73)	4.24	4.2	(1.18-11.1)
∑HCH	68 (83)	7.67	9.2	(5.82-13.29)
p,p'-DDE	80 (98)	57.57	62.2	(43.37-83.88)
p,p'-DDT	69 (84)	4.68	5.1	(3.35-8.04)
∑DDT	81 (99)	65.93	67.3	(47.81-92.72)
oxyCHD	62 (76)	1.30	1.6	(0.49-2.27)
tNCHD	65 (79)	1.73	2.0	(1.04-3)
∑CHD	73 (89)	3.66	3.8	(2.77-5.06)
PBDEs				
BDE 47	70 (85)	0.96	1.1	(0.59-2.05)
∑PBDE	73 (89)	2.60	2.2	(1.49-4.93)
PCBs				
CB 138	65 (79)	4.02	5.2	(1.9-6.78)
CB 153	76 (93)	8.42	9.4	(6.21-13.05)
∑PCB	77 (94)	24.67	27.3	(16.24-34.69)

DF: Detection frequency. GM: Geometric mean. Interquartile range (IQR): the 25^{th} and 75^{th} percentile values.

Only the compounds of which frequency of detection was > 60% were shown here. ΣPCB is the sum of all target PCB congeners (CB 18, 28, 33, 44, 52, 70, 101, 105, 118, 128, 138, 153, 170, 180, 187, 194, 195, 199 and 206), and ΣPBDE is the sum of all target PBDE congeners (BDE 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184 and 191). ΣHCH included *α-, β-, γ-* and *δ-*HCH, ΣDDT included *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD, *o,p′*-DDD, *p,p′*-DDT and *o,p′*-DDT, and ΣCHD included oxyCHD, trans-chlordane, cis-chlordane, tNCHD and cis-nonachlordane.

Adiponectin and leptin concentrations in breast milk

Leptin was detected with a median of 17.9 ng/L (range ND-3356 ng/L) in the breast milk samples analyzed in the present study (n=82). For adiponectin, the median breast milk level was 16.5 μg/L (range 2.6-91.0 μg/L, Table 4-3). A significant negative correlation was observed between pre-pregnancy BMI and breast milk leptin levels (Spearman's rho=-0.24, n=77, $p<0.05$). However no significant relationship was observed between maternal BMI and adiponectin levels.

Table 4-3. Concentrations of leptin and adiponectin in skimmed breast milk samples in Korea and other countries

Country	Study year	n	Sampling months	Leptin (ng/L)				Adiponectin (μg/L)				Reference
				GM	Median	AM±STD	Range	GM	Median	AM±STD	Range	
Germany	2000-2001	674	1-2	164	-	281±381	-	10.55	-	12.8±10.1	-	Weyermann et al. 2007
Spain	2003	28	1	-	-	156±39	ND-853	-	-	-	-	Miralles et al., 2006
USA	1998-2005	30	1-13	-	400	-	ND-2200	-	16.6	-	6.9-80.4	Martin et al. 2006
		199	1-8	-	-	-	-	-	17.7	-	4.2-87.9	
Mexico	1998-2005	37	1	-	-	-	-	-	11.7	-	6.2-39.8	Martin et al. 2006
USA	1998-2005	45	1-7	-	-	-	-	-	-	25.6±8.4	-	Woo et al. 2009
Mexico	1998-2005	277	1-7	-	-	-	-	-	-	23.7±8.6	-	Woo et al. 2009
Czech republic	2006-2008	72	1	-	-	200±30	-	-	-	22.0±0.6	-	Bronsky et al., 2011
		72	3	-	-	100±10	-	-	-	20.5±0.6	-	
		72	6	-	-	100±20	-	-	-	21.4±0.8	-	
Italy	-	46	< 6	-	-	-	-	-	9.99	-	-	Savino et al., 2012
Korea	2011-2012	82	0.5-1	36.3	17.9	209±568	ND-3356	16.5	16.5	18.3±10.5	2.6-91.0	This study
		74	0.5	34.7	17.9	194±565	ND-3356	16.3	17.3	18.2±10.9	2.6-91.0	
		7	1	38.5	17.0	320±680	10.4-1536	19.4	11.7	19.9±5.4	3.6-15.3	

GM: geometric mean. AM: arithmetic mean. STD: standard deviation. ND: non-detected.

Association between maternal serum POPs and breast milk adipokines concentrations

Among nine target POPs, oxyCHD, ΣCHD, BDE 47, and CB 153 concentrations showed significant negative associations with breast milk leptin levels (see Table 4-4). One interquartile range (IQR) increase of oxyCHD and ΣCHD was associated with 81% (95% confidence interval: -92 to -56%) and 36% (95% CI: -55 to -10%) decrease of leptin concentrations, respectively. With one IQR increase of BDE 47, 62% decrease of leptin (95% CI: -76 to -39%) was estimated. For CB 153, one IQR increase was associated with 42% decrease of leptin (95% CI: -65 to -4%).

Adiponectin concentrations in breast milk were positively associated with oxyCHD and BDE 47 levels in maternal serum, after being adjusted with age and pre-pregnancy BMI (Table 4-4). An IQR increase of oxyCHD was associated with 42 % increase (95% CI: 8 to 87%) of adiponectin, and an IQR increase of BDE 47 was associated with 19% increase (95% CI: 2 to 39%).

When the serum POPs levels were grouped in quartile ranges, and were associated with leptin, the same significant negative associations were observed for oxyCHD, ΣCHD, BDE 47, and CB 153 (data not shown). For adiponectin, significant differences with generally increasing trend were observed with quartiles of *p,p'*-DDT, tNCHD, and ΣPCB (Figure 4-1).

Associations between breast milk adipokines and serum POPs became stronger among the women who reported normal pre-pregnancy BMI (Table 4-4). The β coefficients for leptin and ΣCHD, and leptin and CB 153 among the women with normal pre-pregnancy BMI (n=36) were -0.891 and -0.869, respectively, which were greater than -0.739 and -0.744 determined for the all participating women (n=50). Among normal pre-prenancy BMI group, one IQR increase of ΣCHD or CB 153 resulted in an additional 6% decrease of leptin. In *p* values, the

relationships between adiponectin and POPs became more significant in the normal weight group. The association between ΣCHD and adiponectin became more significant among the normal pre-pregnancy BMI group ($\beta = 0.249$, $p<0.05$) compared to the all participating women ($\beta = 0.168$, $p<0.1$). Similarly, among the normal BMI group, CB 138 level showed a marginally significant association with adiponectin, while no significant association was detected in the all population. This observation means one IQR increase of CB 138 corresponds to additional 7 % increase of adiponectin among the normal BMI group, compared to the all participating women.

Table 4-4. Relationship between POPs concentrations in maternal serum (ng/g lw) and concentrations of adipokines in breast milk samples

	Leptin (ng/L)				Adiponectin (µg/L)			
	All		*BMI normal only*		All		*BMI normal only*	
	β (95% CI)		β (95% CI)		β (95% CI)		β (95% CI)	
OCPs								
∑HCH	-0.201	(-0.65, 0.25)	-0.627	(-1.41, 0.16)	0.062	(-0.07, 0.19)	0.129	(-0.14, 0.39)
p,p'-DDE	-0.396	(-1.11, 0.32)	-0.183	(-1.05, 0.68)	0.022	(-0.19, 0.24)	0.020	(-0.27, 0.31)
p,p'-DDT	-0.118	(-0.7, 0.46)	-0.011	(-0.65, 0.63)	0.018	(-0.16, 0.19)	0.021	(-0.19, 0.23)
∑DDT	-0.385	(-1.11, 0.34)	-0.164	(-1.04, 0.71)	0.019	(-0.20, 0.24)	0.016	(-0.27, 0.31)
OxyCHD	-1.089**	(-1.64, -0.53)	-1.154**	(-1.84, -0.47)	0.229**	(0.05, 0.41)	0.312**	(0.07, 0.55)
tNCHD	-0.260	(-0.79, 0.27)	-0.407	(-1.02, 0.20)	0.079	(-0.08, 0.24)	0.124	(-0.08, 0.33)
∑CHD	-0.739**	(-1.3, -0.18)	-0.891**	(-1.55, -0.23)	0.168*	(-0.00, 0.34)	0.249**	(0.02, 0.48)
HCB	-0.282	(-0.66, 0.10)	-0.265	(-0.72, 0.19)	0.012	(-0.10, 0.13)	0.023	(-0.13, 0.18)
PBDEs								
BDE 47	-0.773**	(-1.15, -0.39)	-0.864**	(-1.31, -0.42)	0.139**	(0.01, 0.26)	0.176**	(0.01, 0.34)
∑PBDE	-0.091	(-0.55, 0.36)	0.131	(-0.43, 0.69)	0.075	(-0.06, 0.21)	0.049	(-0.14, 0.23)
PCBs								
CB 138	-0.092	(-0.72, 0.54)	-0.115	(-0.84, 0.61)	0.118	(-0.07, 0.3)	0.198*	(-0.03, 0.43)
CB 153	-0.744**	(-1.43, -0.06)	-0.869**	(-1.63, -0.11)	0.120	(-0.09, 0.33)	0.151	(-0.11, 0.42)
∑PCB	-0.406	(-1.15, 0.33)	-0.693	(-1.57, 0.19)	0.115	(-0.1, 0.33)	0.231	(-0.06, 0.52)

Signs ** and * indicate statistical significance of regression parameter at p=0.05, and 0.1, respectively. All POPs concentrations and thyroid hormone levels were natural log–transformed. Results of association regarding adipokines were adjusted for age (continuous) and pre-pregnancy BMI (categorical). Chemicals that were detected >=75% of the population at concentrations greater than the limit of quantification, a proxy value of 'LOQ divided by square root 2' was used. For chemicals that were detected in <75% but >=60%, statistical analysis was conducted with detected values only. CI: confidence interval.

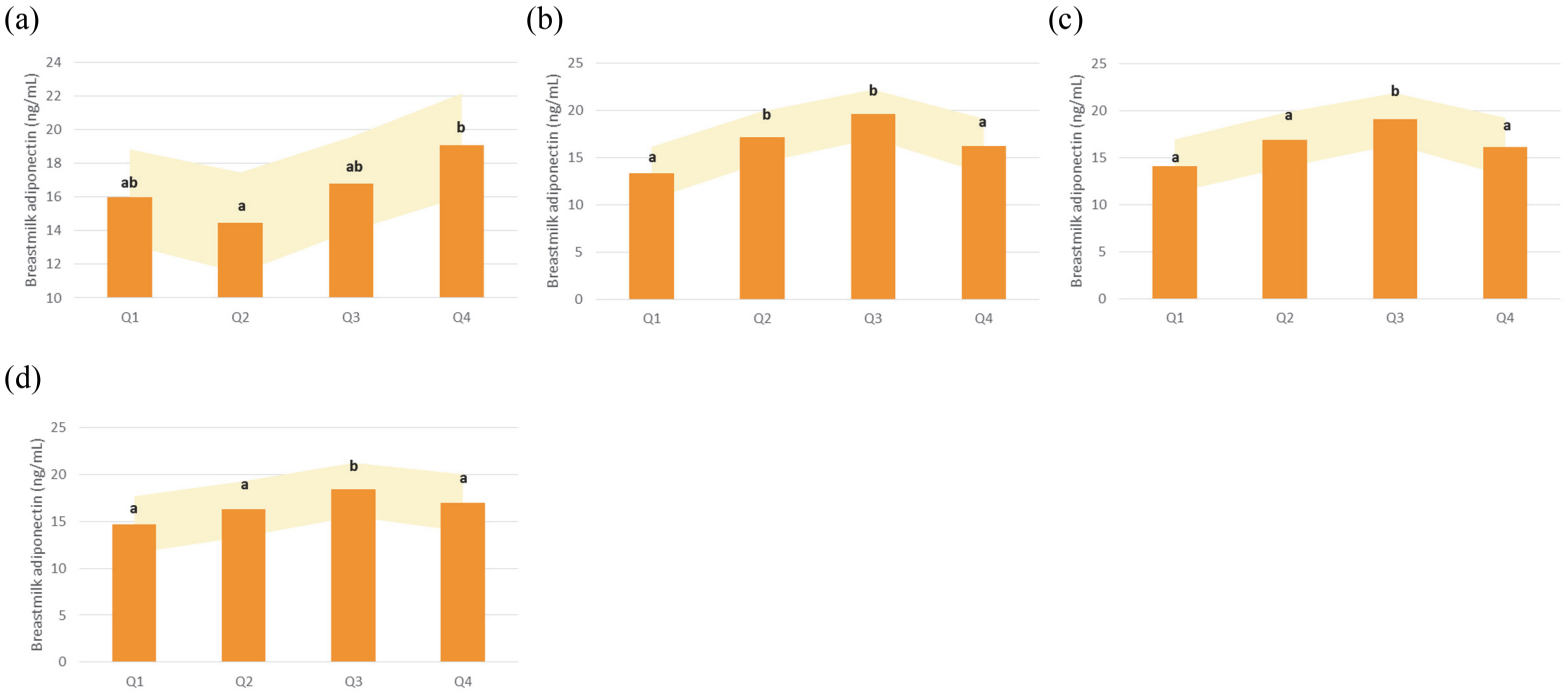

Figure 4-1. Levels of breast milk adiponectin concentrations according to quartiles of POPs: (a) *p,p'*-DDT, (b) ∑CHD, (c) BDE 47, and (d) ∑PCB. Bar and shade indicate estimated least square mean and 95% upper and lower confident limit (UCL/LCL) of least square mean, respectively. Different alphabets indicate significant difference at $p < 0.05$ in the LSMEANS in SAS. Adjusted for age, and pre-pregnancy BMI.

4.4. Discussion

Breast milk leptin and adiponectin levels of Korean mothers

To our knowledge, this is the first report of adiponectin and leptin concentrations among Korean lactating women. The measured mean levels and the range of breast milk adipokines in the present study are generally similar to those reported elsewhere (Table 4-3; Bronsky et al., 2011; Martin et al., 2006; Miralles et al., 2006; Savino et al., 2012; Weyermann et al., 2006; Woo et al., 2009). The geometric mean and median of leptin are generally lower than those reported elsewhere, indicating a highly right-skewed distribution of breast milk leptin concentrations. Consistently with previous studies, breast milk adiponectin levels are several times higher than leptin levels.

Association between maternal serum POPs and leptin concentrations in breast milk

Significant negative correlations between breast milk leptin and oxyCHD, ∑CHD, BDE 47, or CB 138 levels, observed after the adjustment of age and BMI, support the plausibility of association between POPs and obesity. Leptin plays a critical role in the metabolism of whole body, e.g., by increasing energy expenditure, and inhibiting food intake (Galic et al., 2010). For these reasons, decrease of leptin has been often associatd with increased risk of obesity. In an experimental study using rat under high fat diet, leptin given during the pregnancy and lactation successfully prevented obesity at 6 months of age (Pico et al., 2007). Human studies also demonstrated similar negative associations between breast milk leptin and the risk of obesity development later. A negative association was detected between leptin at 1 month of lactation and infant BMI at 18-24 months of age (Miralles et al., 2006). In a prospective cohort study, lower leptin levels in cord blood were associated with lower birth weight but with more pronounced weight gain in the first 6 months of life and higher BMI at 3 years of age (Boeke et al., 2013; Mantzoros et al., 2009).

Consistently with our observation, the same negative associations between leptin and POPs exposure were reported in two epidemiological studies (Table 4-5). CB 138, 180, and BDE 153 levels were negatively associated with serum and adipocyte leptin levels among obese adult population (Pereira-Fernandes et al., 2014). In 8-9-year-old boys, serum leptin concentration was also inversely correlated *β*-HCH and *p,p'*-DDE levels in serum (Burns et al., 2011). However, experimental studies suggested often the other direction of the association: Exposure to *p,p'*-DDE, oxyCHD, CB 153 showed positive association with leptin ((Howell and Mangum, 2011; Taxvig et al., 2012; Wahlang et al., 2013). However the *in vitro* experimental studies employing cell models often cannot reflect

complex regulatory feed-back mechanisms that take place *in vivo*. Two *in vivo* experimental studies employing mice (Male C57BL6/J) and rats (Sprague Dawley), the directions of association between POPs and leptin varied by the exposure duration: Longer term exposure to chlorinated POPs such as CB 153 (12 weeks) or Aroclor 1243 (30 days) were associated with increase of serum leptin, but shorter term exposure (15 day) to Aroclor 1243 led to decrease in serum leptin levels of the rodent models (Provost et al., 2007; Wahlang et al., 2013).

Intake of leptin via breast milk can be easily reflected in the circulation level of leptin in breast-fed infants (Ucar et al., 2000). Therefore higher breast milk leptin levels may help prevent obesity of the breastfed infants ((Boeke et al., 2013; Mantzoros et al., 2009; Miralles et al., 2006). In the present study, POPs body burden showed clear negative correlation with breast milk leptin levels, providing another evidence supporting the 'obesogen' hypothesis for POPs.

Association between maternal serum POPs and adiponectin concentrations in breast milk

Different from leptin, significant positive associations were observed between breast milk adiponectin and several POPs in serum, e.g., *p,p'*-DDT, oxyCHD, tNCHD, ∑CHD, BDE 47, CB 153, or ∑PCB (Table 4- 4, Figure 4-1). Our observations are generally supported by experimental studies. Exposure to *p,p'*-DDE, oxyCHD, or CB 153 showed positive associations with adiponectin levels in NIH3T3-L1 cell and 3T3-L1 adipocyte (Howell and Mangum, 2011; Taxvig et al., 2012). In rats, CB 153 exposure led to increased adiponectin levels with decreased peroxisome proliferator-activated receptor-α (PPAR-α) in serum (Wahlang et al., 2013).

However, among humans, the directions of the association varied. Turyk et al. (2012) reported positive associations between PBDEs including BDE 47 and adiponectin concentrations in the serum of general adult population (n=483). However, negative associations between total PCB and adiponectin in Korean adults with higher BMI value (Lim and Jee, 2014), and between CB 153 and adiponectin levels among obese women (n=27; Mullerova et al., 2008) were also reported. The reason for this inconsistent direction of associations is not clear. However it should be noted that the opposite direction of association between POPs and adiponectin was generally observed among adults with higher BMI, and therefore may not represent the true direction of the association among general population. The mechanisms underlying this phenomenon warrant further investigation.

Generally, elevated adiponectin can be interpreted as a compensational effort against increased adiposity, as in normal weight situation increased adiponectin would increase energy expenditure. In this context, clear positive association among the normal weight group of the present study population might

reflect feedback effort toward homeostasis. However, due to the limited sample size (n=36), the interpretation of this association warrants caution. More epidemiological confirmation and experimental supports are warranted.

Table 4-5. Association between adipokine levels and POPs body burden in human cross-sectional studies

Chemicals	Adiponectin	Leptin	Population	n	Country	Reference
CB 153	↓ serum		Obese women	27	Czech Republic	(1)
β-HCH, *p,p'*-DDE		↓ serum	8-9 yrs (boys)	350	Russia	(2)
∑PBDE	↑ serum		Adult	483	USA	(3)
CB 138, CB 180, BDE 153		↓ serum, adipocyte	Obese Adult	50	Belgium	(4)
∑PCB	↓ serum		Adult	98	Korea	(5)
p,p'-DDT, oxyCHD, tNCHD, ∑CHD, BDE 47, ∑PCB	↑ breast milk	↓ breast milk	Lactating mothers	78	Korea	This study

↓ and ↑ mean direction of association, and serum or adipocyte show media in which adipokine levels were measured. (1) Mullerova et al., 2008, (2) Burns et al., 2011, (3) Turyk et al., 2012, (4) Pereira-Fernandes et al., 2014, (5) Lim and Jee, 2014.

4.5. Summary and implications

To our knowledge, this is the first study which shows that several POPs exposure might influence the adipokine productions of mothers. Breast milk could serve as a non-invasive biological specimen for adipokine measurement. Our observations clearly show that at the current levels of exposure, several POPs are associated with altered adipokines such as leptin and adiponectin in breast milk.

The biological activity of orally ingested adipokines from human milk was addressed in previous studies, showing the presence of adiponectin receptor in fetal small intestine (Zhou et al., 2005) and the protective environment, i.e., low acidity, and limited gastic proteolysis in infant stomach (Henderson et al., 2011; Lonnerdal, 2003; Hamosh, 1996). In a mouse experiment, the highest molecular form of adiponectin was absorbed within 2 hours after administration into the stomach (Newburg et al., 2011). Also, breast milk adiponectin and leptin concentration were reflected to serum adiponectin and plasma leptin level of breast-fed infants (Newburg et al., 2011; Ucar et al., 2000).

As intake of adipokines through breastfeeding could contribute to their circulating levels in infants, alteration of breast milk adipokine by POPs exposure may have a significant public health implication among growing infants. Consequences of the alteration in breast milk leptin and adiponectin levels by POPs exposure, e.g. obesity in later stages of life, should warrant further follow-ups.

Chapter 5. Conclusion

The association between POPs exposure and thyroid hormones or adipokine levels among pregnant women or matching newborns was determined in a series of three studies. For this purpose, three groups of POPs (OCPs, PBDEs, and PCBs) and health effect markers (thyroid hormones and adipokines) were measured in CHECK Panel.

In the first part, the relationship between several POPs exposure and thyroid hormone concentrations was assessed (n=105). PCBs, BDE 47, DDTs or HCB were generally associated with the reduction of T3 or T4 and increase of TSH. Only a small extent of change of maternal thyroid hormone within the reference range has a significant impact on fetal development; thus, our findings on the significant disruption of thyroid hormones by the current levels of exposure to POPs in pregnant women should be emphasized.

In the second part, the associations between prenatal exposure to major POPs and thyroid hormone levels among newborn infants were investigated (n=104). Prenatal exposure to several POPs (BDE 47, BDE 99, *p,p′*-DDE, ΣCHD, or HCB) was found to be related to the decrease of T3 or T4 levels and the increase of TSH in cord serum and neonatal bloodspot both. In the analysis which explored the association between cord serum thyroid hormone and prenatal POPs exposure, adjustment of maternal thyroid hormone level was suggested as the one of the determinants of the fetal thyroid hormone levels.

In the third part, the relationship between several POPs levels in maternal serum and adipokine levels in breast milk was assessed (n=50). We found the significant influence of POPs exposure on the disturbance of adiponectin and leptin production in breast milk. This significant result is meaningful as the first

report on the association between breast milk leptin and adiponectin expression and POPs exposure. Also, it might provide another piece of evidence on the alteration of lipid metabolism effect of POPs at the currently occurring low-exposure levels, as well as on the possibility of POPs' functioning as 'obesogens'.

Overall, this study is unique in that we have simultaneously measured three groups of POPs and all five thyroid hormones in both maternal and fetal serum samples. However, this study has several limitations, namely: (1) multiple statistical testing and many predictors which might lead to the increase of type I error; (2) sample size that was not sufficient enough to gain statistical power; (3) there was still a possibility that we could not catch the effect of chemicals with low abundance, although POPs analysis was conducted following appropriate technical method and QA/QC. The third limitation can be overcome by the development of analyzing technology with significant improvement of detection limit. Also, possible low-dose effect of POPs should be further investigated. Due to chance with numerous regression model generations, false-positive research findings might rate. We tried to obtain complementary results with sensitivity analysis, and several kinds of simultaneous POPs measurements made it possible, although our sample size was not sufficient to have statistical power. To overcome the limitations identified above, a validation in a larger population sample, a follow-up study on the link between hormonal change in early life stage and adverse health outcomes in later life of stage to confirm the causality, and a prospective birth cohort should be conducted.

Thyroid hormones and adipokines are critical during gestation and early life stages. Thus, considering the importance of endocrine hormones at this stage and the effects of the interaction between thyroid hormones and adipokines concentration on obesity or weight loss, health implication of endocrine disruption

effects by low level POPs exposure deserves further follow-up investigations. Although several findings in this study were supported by previous experimental studies, they should be confirmed further in future work.

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국문 초록

산모-태아의 잔류성 유기오염물질 노출과 내분비계 교란 영향

서울대학교 대학원

보건학과 환경보건학 전공

김 선 미

현재 전 세계적으로 많은 잔류성 유기오염물질(persistent organic pollutants: POPs)은 그 독성과 생체축적성을 이유로 사용이 금지되었다. 그러나, 높은 잔류성 때문에 대부분의 POPs, 특히 유기염소계 농약(organochlorine pesticides: OCPs), 폴리염화비페닐(polychlorinated biphenyls: PCBs)류 물질들은 여전히 환경시료와 생체시료에서 빈번히 검출되고 있다. 또한, 새로운 POPs 중 난연제로 사용되고 있는 폴리브롬화디페닐에테르(polybrominated diphenyl ethers: PBDEs) 역시 최근까지의 사용과 축적성 때문에 검출이 자주 보고되고 있다. 이들 POPs의 알려진 독성들 중, 내분비계 교란은 호르몬 수준에서의 변화뿐 아니라 성장, 발달, 대사, 비만 등에까지 영향을 미칠 수 있기 때문에 최근 중요한 건강 영향 중 하나로 꼽힌다.

내분비계를 구성하는 호르몬들 중 갑상선호르몬과 아디포카인은 정상적인 발달과 에너지 대사를 위한 주요 호르몬이다. 특히 태아와 영유아기는 이들 호르몬의 변화와 내분비계 교란 영향에 매우 취약하다. 이 시기의 내분비계 교란은 평생에 걸쳐 영향을 미칠 수 있기 때문이다. 그러므로 산모, 태아 및 영유아 인구를 대상으로 POPs 노출에 따른 내분비계 교란 영향을 파악하는 것은 중요하다. 현재까지 이들 민감집단을 대상으로 한 연구들은 특정 물질군만을 대상으로 했거나, 제대혈만을 이용하여 제한적으로 파악되고 있으며, 이 때문에 역학연구를 통해 도출된 결과의 방향성도 반대로 나

타나기도 한다.

본 연구에서는 산모와 그 태아 인구를 대상으로 하여 POPs의 노출수준을 파악하고, POPs 노출에 따른 갑상선호르몬과 아디포카인의 변화 여부를 살펴보고자 하였다. 이를 위해 2011-2012년까지 알려진 직업적 노출을 배제한 일반인구 중 산모를 국내 5개 대학병원을 통해 모집하였다 (Children's Health and Environmental Chemicals of Korea Panel: CHECK Panel). 출산 시점의 산모 혈액과 제대혈, 출산후 15일과 30일째 되는 날의 모유 시료를 수집하였다. 혈액 시료에서는 POPs의 세 물질군으로 19종의 OCPs, 19종의 PCBs 이성체, 19종의 PBDEs 이성체를 각각 분석하였으며, 이 중 60% 이상의 검출률을 보인 개별 물질 및 이성체의 합(ΣPCB, ΣPBDE, ΣDDT, Σchlordane (CHD), Σhexachlorhexane (HCH))을 통계분석에 이용하였다. 전체 연구는 다음과 같이 세 부분으로 나누어 수행하였다.

첫 번째 연구에서는 산모 인구를 대상으로 주요 POPs 물질들의 노출수준과 갑상선호르몬 수준 간의 상관관계를 파악하였다. CHECK Panel 참가자 중 105명의 산모의 혈액 시료에서 대상 POPs 물질과 갑상선호르몬 5종 (free/ total T3, free/total T4, TSH)을 분석하였다. 분석 결과, CB 28, CB 52, CB 118과 같은 PCBs 물질들의 노출수준이 T3나 T4와 유의한 음의 상관관계를 보였으며, BDE 47도 음의 상관관계를 보였다. OCPs류 물질들 중에서는 총 DDT와 헥사클로로벤젠(hexachlorobenzene:HCB)의 농도가 T3 또는 T4의 감소와 유의한 상관관계를 보였다. 그러나 민감도분석 결과 BDE 47에 의한 교란 영향은 PCBs류 물질에 비해 약하게 나타났다. 본 연구에서 측정된 갑상선호르몬의 수준은 모두 정상범위 이내였으나, 직업적 노출군이 아닌 일반인구의 낮은 노출수준에서도 POPs의 갑상선호르몬 교란 가능성을 유의하게 나타내 보인 데 의의가 있다.

두 번째 연구에서는 신생아를 대상으로 주요 POPs 물질들의 노출수준과 갑상선호르몬 수준 간의 상관관계를 파악하였다(n=104). 제대혈 중 갑상선호르몬의 농도는 산모의 갑상선호르몬 수준에 의해 영향을 받을 수 있

기 때문에, 산모의 갑상선호르몬 수준을 다중회귀식의 보정변수로 추가로 포함하였다. 또한, 독립적인 갑상선호르몬의 수준을 반영할 수 있도록 신생아 혈액 중 갑상선호르몬 수준을 생후 2-3일 시기에 병원에서 시행하는 선천성대사이상검사 중 TSH의 측정결과를 활용하여 파악하였다. 상관분석 결과, 제대혈 중 BDE 47, BDE 99, ΣCHD, *p,p'*-디클로로디페닐디클로로에틸렌(*p,p'*-dichloro-diphenyldichloro-ethylene: *p,p'*-DDE)의 농도는 제대혈 및 신생아혈액 중 TSH의 농도와 양의 상관관계를 나타내었다. 동시에 *p,p'*-DDE와 HCB의 농도는 총T3와 T4 농도의 감소와 유의한 상관관계가 있었다. 모체혈액 중 *β*-헥사클로로헥산(*β*-hexachlorhexane: *β*-HCH), 총CHD, 총DDT, *p,p'*-DDE의 농도 역시 제대혈 및 신생아혈액 중 갑상선호르몬의 수준과 유의한 상관관계를 보였다. 이 연구의 결과는 일반인구 중에서도 태아기의 저농도 만성 POPs 노출이 내분비 교란을 일으킬 수 있는 가능성이 있음을 시사한다.

세 번째 연구에서는 산모의 POPs 노출수준과 모유 중 아디포카인 농도와의 상관관계를 파악하였다. 82명의 모유시료에서 아디포넥틴과 렙틴 2종의 아디포카인을 측정하였으며, 이 중 PCBs, PBDEs, CHDs, HCHs 그룹 내의 개별 물질 및 이성체가 모두 검출한계 이하 값을 보인 대상자를 제외하고 50명의 모유시료 결과를 이용하여 다중회귀분석 및 4분위수 그룹간 평균비교를 수행하였다. 산모혈액 중 옥시클로로데인(oxychlordane: oxyCHD), 총CHD, BDE 47, CB 138의 농도가 모유 중 렙틴과 음의 상관관계를 나타내었다. 모유 중 아디포넥틴의 수준과 *p,p'*-DDT, oxyCHD, 트랜스-노나클로로데인(trans-Nonachlordane: tNCHD), 총CHD, BDE 47, CB 153, 총PCB의 농도는 양의 상관관계를 보였다. 이 연구에서 나타난 방향성인 렙틴을 감소시키는 방향은 비만을 증가시킬 수 있는 요인이 되며, 이의 보상작용 또는 결과로 아디포넥틴이 증가했을 가능성이 있다. 이 연구는 우리나라의 모유 시료 중 아디포넥틴과 렙틴의 농도를 처음으로 보고한 연구이며, 또한 POPs에 의한 모유 중 아디포카인 발현의 교란 영향을 처음으로 보고

하는 연구이다.

종합적으로 산모-태아를 대상으로 한 세 단면연구를 통하여, 현재 일반인구의 POPs 노출수준이 갑상선호르몬과 에너지 및 지질 대사 호르몬에 유의한 영향을 줄 수 있음을 파악하였다. 특히 이 연구는 POPs 노출과 건강영향 연구를 위해 디자인된 우리나라의 첫 산모-태아 집단 대상 연구인 CHECK Panel 연구를 기반으로 하여, POPs의 고위험군, 민감집단에서의 위해영향을 파악하고, 사회 전반적으로 POPs를 줄여나가는 노력에 도움이 될 수 있을 것으로 기대된다. 본 연구에서 나타난 상관관계의 방향성은 기존에 보고된 실험연구의 결과들과 기전적으로 어느 정도 일치하는 편이지만, 인구집단을 대상으로 생물학적 의미를 갖기 위해서는 후속 연구가 수행되어야 할 것이다. 또한 태아와 영유아기 시기의 갑상선호르몬과 아디포카인이 갖는 중요성을 고려하여, 앞으로 전향적 출생 코호트에서의 추적연구, 더 큰 인구집단을 대상으로 한 연구결과의 확립 등이 필요할 것으로 생각된다.

주요어: 잔류성 유기오염물질(POPs), 유기염소계 농약(organochlorine pesticides: OCPs), 폴리염화비페닐(polychlorinated biphenyls: PCBs), 폴리브롬화디페닐에테르(polybrominated diphenyl ethers: PBDEs), 산모, 태아, 갑상선호르몬, 아디포넥틴, 렙틴, 모유, CHECK Panel

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