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**Thyroid Hormones in Pregnancy in Relation to Environmental Exposure to Organochlorine Compounds and Mercury.**

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**Abbreviations:**

$\beta$ -BHC:  $\beta$ -hexachlorocyclohexane; CNS: central nervous system; p,p'-DDE: p,p'-Dichlorodiphenyldichloroethylene; D1: 1 type deiodinase ; D2: 2 type deiodinase; D3: 3 type deiodinase; fT4: free thyroxin; GD: gestational day; GLM: general linear model; Hg: mercury; IHg: inorganic mercury; OC: organochlorine compound; PCBs: polychlorinated biphenyls; PCP: pentachlorophenol, TEQs: toxic equivalents; TH: thyroid hormones; THg: total mercury; TSH: thyroid stimulating hormone; TT3: total triiodothyronine; OHg: organic mercury; rT3: reverse triiodothyronine; 4'-HO-CB108: 4'-HO-2,3,3',4,5'-pentachlorobiphenyl.

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## ABSTRACT

Polychlorinated biphenyl compounds (PCBs), chlorinated pesticides and mercury (Hg) are global environmental contaminants that can disrupt the endocrine system in animals and humans. However, there is little evidence that they can interfere with endocrine status in pregnant women and neonates at low levels of exposure. The aim of this study was to examine thyroid hormone levels in pregnancy and in cord blood in relation to blood concentrations of organochlorine compounds (OCs) and Hg in healthy women recruited during pregnancy. There was a significant negative correlation between maternal total triiodothyronine (TT3) levels and three non coplanar congeners (CB-138, CB-153 and CB-180), two pesticides (p,p'-DDE and hexa-chlorobenzene), and inorganic Hg independently, without any other changes in thyroid status. No significant relationships were observed between OCs and cord serum thyroid hormones. Cord serum free thyroxin (fT4) was negatively correlated to inorganic Hg. These results suggest that even low levels of exposure, persistent environmental contaminants can interfere with thyroid status during pregnancy.

## INTRODUCTION

Adequate thyroid functioning during pregnancy is a known determinant of healthy pregnancy outcomes and successful brain development in the fetus (LaFranchi et al. 2005). Recent epidemiological studies have focused on sub-clinical maternal thyroid deficiency during pregnancy, particularly for hypothyroxinemia in early gestation, and its long term effects on psychomotor development of child (Pop et al. 2003). These effects could be mediated by impaired glucose metabolism in fetal brain during the critical period of neuroblast proliferation (Pickard et al. 1999). In addition, the trophoblast has a high binding capacity for T3 and it has been suggested that the placenta is a thyroid hormone dependent tissue (Kilby et al. 1998; Oki et al. 2004).

Experimental studies have shown that polychlorinated biphenyls (PCBs) and related chemicals decrease circulating TH during development (Donahue et al. 2004; Ulbrich et al. 2004; Zoeller et al. 2000). Pre- or postnatal exposition of humans or animals to PCBs can result in hormonal changes and neurodevelopmental deficits (Jacobson and Jacobson 2002, 2003; Vreugdenhil et al. 2002b; Vreugdenhil et al. 2002a; Vreugdenhil et al. 2004). In rats, Goldey et al. (1995) reported that ototoxic effects of PCBs were associated with decreased circulating TH following perinatal exposure (Goldey et al. 1995). And, it has been suggested that interference with endocrine systems, particularly the thyroid, could be one possible explanation for PCB-induced psychomotor delay observed in several cohort studies (Winneke et al. 2002).

Two classes of PCB metabolites are formed from PCB biotransformation: hydroxylated (HO-PCBs) and methyl sulfone PCBs. No data are available about human exposure to methyl sulfone

PCBs or their effects on thyroid status in experimental animals. However, most PCB congeners and hydroxylated PCBs, which disrupt thyroid hormone status, are transferred across the placenta to the fetus in concentrations resulting in levels of approximately 50 and 30%, respectively, of those in maternal plasma (Soechitram et al. 2004). Hydroxylated PCBs show high binding affinity for the serum thyroid hormone binding protein transthyretin, thus displacing the natural ligand, T4 (Cheek et al. 1999). PCBs as well as some other OCs such as hexa-chlorobenzene, are also known to increase the activity of hepatic drug-metabolizing enzymes, in particular UDP-glucuronosyltransferase, responsible for glucuronidation of T4 (Van Birgelen et al., 1995; van Raaij et al., 1993). *In vitro*, hydroxylated PCBs have low affinity for the human thyroid receptor, but do have a thyroid hormone-like affinity for the serum transport protein transthyretin (Cheek et al. 1999; Meerts et al. 2002) and inhibit the iodothyronine sulfotransferase activity (Schoor et al. 1998).

Chlorine substitution in the phenyl rings gives each PCB its own target and mechanism of toxicity. “Coplanarity” of PCB phenyl rings and “laterality” of chlorine atoms are important structural features that determine specific binding behavior with proteins and certain adverse responses in biological systems. There is evidence that coplanar PCB mutagenic toxicity is mediated through the Ah receptor (Safe 1994). Recently, it was reported that both, mono-ortho and non-coplanar types of PCB, and hydroxylated PCB metabolites may disrupt thyroid hormone status, in part, by affecting TR-mediated transcription, which may influence growth and development of TH target organs, particularly in the CNS (Iwasaki et al. 2002). Khan and Hansen (2003) suggest that non coplanar congeners interfere with the HPT-axis by producing a subnormal response of the pituitary and thyroid to TRH stimulation.

The developing fetus is particularly susceptible to thyrotoxic effects of PCBs and their metabolites. In rats, exposure to hydroxylated PCB 4'-HO-CB108 from gestational days (GD) 10 to 16 decreased maternal, foetal and neonatal plasma total T4 and free T4 in a dose dependant manner (Meerts et al. 2002). Chronic developmental exposure to Arochlor 1254 from GD6 to postpartum day 21 also reduces circulating levels of total T4 (Zoeller et al. 2000). At the same conditions of exposure, Goldey et al. (1995) observed decreased total T4 levels and a moderate reduction of T3 levels in offspring at high doses of exposure.

Other environmental pollutants, such as pesticides and mercury, may also disrupt thyroid function (Beard and Rawlings 1999; Ellingsen et al. 2000; Rathore et al. 2002; Watanabe et al. 1999). Long term workplace exposure to Hg interferes with thyroid metabolism by reducing of T4 deiodination (Ellingsen et al. 2000). In a community highly exposed to hexachlorobenzene, a significant positive association was found between this OC and TSH concentrations at birth (Ribas-Fito et al. 2003).

The general population is exposed to multiple environmental contaminants at relatively low doses, but few studies have reported thyroid status in pregnancy in relation to mixtures of environmental organic pollutants. Moreover, most studies report the sum of PCBs as an exposure measure, which can mask the specific effect of different groups of congeners with different mechanism of action. Thus, the objective of the present study was to examine the relation between exposure to potential endocrine disrupting chemicals (coplanar and non-coplanar PCBs, organochloride pesticide residues, and Hg) and thyroid status in pregnant women and the newborn.

## METHOD

### *Study Population*

The women participating in the study were recruited at first prenatal visit at the Centre for Local Community Services (part of the National Public Health System) in Southwest Québec. After signing a consent form, an interview administered questionnaire, which contained general socio-demographic data and information on residency, medical history, drinking and smoking habits and diet, was filled out and blood samples were obtained. Those who were recruited into the study during the 1<sup>st</sup> trimester (prior to the 13<sup>th</sup> week) provided a first sample at entry and a second during the 2<sup>nd</sup> trimester, while those who were recruited between the 14<sup>th</sup> and 24<sup>th</sup> week provided one sample prior to delivery. The first trimester sampling was performed before the first ultrasound examination. The gestational age at sampling was revised according to ultrasound data for 22 women, who provided two samples at second trimester. The study population was composed of 149 pregnant women, 101 of those gave birth at the participating hospital where mother's and cord blood samples and placental tissue were obtained at delivery. Two weeks following birth, a 2<sup>nd</sup> questionnaire was interview- administered. This second questionnaire included information on medical and obstetrical history, birth data, as well as smoking and drinking during pregnancy.

After verification, only 40 women had entered the study during the first 13 weeks of pregnancy and 109 entered at the 2<sup>nd</sup> semester. Thus, most data was available for the 2<sup>nd</sup> semester (n =149). At delivery, there were 101 mother's and 92 cord blood samples available for analyses. Thus, complete data throughout pregnancy were available for 38 women and for 101 from the 2<sup>nd</sup>

trimester and at delivery. Data for hormones and contaminants are missing for some women (n=2-4) due to insufficient quantity of blood or non respect of specimen storage protocol.

### *Biological sampling*

Blood samples for the first and the second trimesters were collected at the pregnant women's residence after night fasting while the third trimester sample and cord blood sample were taken at the hospital at delivery. Whole blood and serum samples were refrigerated at -20°C until contaminant and hormone determination (3-4 months).

### *PCBs, pesticides and Hg determination*

Laboratory analyses of PCB and chlorinated pesticides were performed by the Centre of Toxicology of Quebec by gas chromatography coupled with mass detection (GC-MS) using Chromatograph 6890 and mass detector 5973 from Agilent. Two milliliters of blood plasma were extracted using an ammonium sulfate/ethanol/hexane mixture, cleaned-up on Florisil columns, and taken to a final volume of 100 µL. Routine checks of accuracy and precision were performed using reference materials from National Institute of Standards and Technology. Also, periodic evaluations were carried out through participation in two external proficiency testing programs (Arctic Monitoring Assessment Program Ring test (Laboratoire de toxicologie humaine / INSPQ) and German Society of Occupational and Environmental Medicine, Erlangen, Germany). The detection limits were 0.02 µg/L for PCB congeners and chlorinated pesticides.

Cold Vapor Atomic Absorption Spectrometry was used to assess total Hg (THg) and inorganic Hg (IHg) using Pharmacia Instruments Mercury Monitor Model 100. Organic mercury (OHg)

was calculated as the difference between THg and IHg. Total Hg was determined using 500 µL of blood, digested with an equal volume of concentrated nitric acid. An aliquot of the digest was then introduced in the system's reaction chamber (containing a reducing solution of cadmium chloride and stannous chloride). Mercury vapor was generated and detected, and aqueous calibration was performed. The inorganic mercury fraction was determined using the same methodology except for the use of cadmium chloride, as part of the reactant mixture, which was omitted. Routine checks of accuracy and precision were performed using reference material from the Laboratoire de toxicology / INSPQ's interlaboratory comparison program. In addition, periodic evaluations were carried out through participation in the same program. The detection limit obtained was 2 nmol/L (0.2 µg/L). Variation coefficients (N~20, different days) at levels of 38 IHg and 82 OHg nmol/L were 4 % and 3.4 % respectively.

The detection limits were determined from the analyses of 10 actual samples, whose concentrations were between 4 to 10 times the estimated detection limit. The standard deviation of these 10 samples multiplied by 3 provided the detection limit, which was multiplied by 10 to provide the quantification limit.

#### *Lipid determination*

Total and free cholesterol (TC and FC), triglycerides (TG) and phospholipids (PL) were individually measured using enzymatic methods on the Technicon automatic analyzer (RA-500) as previously described (Moorjani et al. 1987). Plasma total lipids were calculated using the summation method: total lipids = 1.677 (TC-FC) + FC + TG + PL.

### *Thyroid hormone determination*

Thyroid hormones (TSH, total T3 (TT3), and free T4 (fT4)) were analyzed by radioimmunoassay at the Clinical Biochemistry Service of Saint-François d'Assise hospital (Quebec city, Canada) (Forest et al. 1998).

### *Statistical analysis*

All statistical analyses were performed using SAS version 8.12 (SAS Institute Inc. 1999). The log normally distributed data were log-transformed in order to use parametric tests. The STEPWISE procedure was used to test relationships between variables of interest and potential co-factors such as maternal age, smoking and alcohol consumption, child's gender and birth weight (for cord blood variables), gestational age at sampling, and total lipid concentrations. The relation between exposure variables and effect variables was examined by longitudinal repeated measure analysis (MIXED procedure) considering the within-subject effect and compound symmetry covariance structure. Relationships between cord blood exposure and effect variables were tested using ANCOVA (GLM procedure). Because a large number of samples with contaminant levels were below the detection limit, the cord blood exposure levels were coded in two levels: detected/undetected for selected congeners, and above/below median for summed variables. The CB-101 and CB-128 as well as trans-chlordane, cis-chlordane, and aldrin were excluded from statistical analysis because 100% were undetected values.

TH are involved in lipid metabolism and the reduction in their circulating level in hypothyroid subjects is associated with an atherogenic lipid profile (Al Tonsi et al. 2004). Therefore, in order

to take into consideration the effects of TH on blood lipid mobilisation, both, adjusted and unadjusted for lipid concentration analyses were performed. Two kinds of physiological sequences are possible in the tested relationship between lipophilic contaminants such as PCBs or pesticides, and TH: first, the hypothesized relation “ $\uparrow$  lipids  $\rightarrow$   $\uparrow$  blood PCBs  $\rightarrow$   $\downarrow$  thyroid hormone levels”, and second, an inverse relation “ $\downarrow$  TH  $\rightarrow$   $\uparrow$  lipids  $\rightarrow$   $\uparrow$  blood PCBs”. The comparison of two, adjusted and not adjusted for lipids, models can indicate whether lipids are a confounding factor in a hypothesized relation or an intermediate factor in the inverse link. The lipid concentration variables were introduced in linear models as fixed variables. The criterion for significance was set at  $p < 0.05$ .

In order to demonstrate the cumulative effect of studied pollutants, two groups of subjects following the degree of their exposure to 5 OCs significantly related to hormone levels, PCB 138, 153, 180, pp'-DDE,  $\beta$ -BHC and hexa-chlorobenzene, were defined. The “exposed” group includes women who have two or more pollutants higher than 75<sup>th</sup> percentile of distribution, “not exposed” group includes those with none or only one pollutant higher than 75<sup>th</sup> percentile.

## RESULTS

### *Population characteristics*

The women who gave birth (n=101) were aged of 27 years (range 15 – 39); 30% of women smoked during pregnancy and 8% consumed alcohol moderately (0.5-2 drink/week, 4-30g alcohol/week). During pregnancy, 11 women (10%) had gestational diabetes, 2 of those with pregnancy induced hypertension; 11 women had pregnancy induced hypertension without gestational diabetes, 2 of those with proteinuria. Five percent of births occurred before 37 weeks of pregnancy, the average of birth weight was 3.3 kg (range 1.9 – 5.0 kg), and 51 % of newborns were boys. The characteristics of women lost to follow up (n=48) were not significantly different from those given birth at participating maternity.

### *Hormone and contaminant levels during pregnancy and at birth*

The TH concentrations are given in Table 1, while Table 2 and 3 present the blood PCB congener and pesticide levels. We observed that TT3 and TSH levels increased during pregnancy, whereas fT4 levels decreased. TH levels in this pregnant women population are similar to reported data (de Escobar et al. 2004). The cord blood PCB concentrations were significantly lower than maternal blood, and, in most of samples, lower than detection limit level.

In general, unadjusted blood PCB congener concentrations appeared to increase during pregnancy. However, when adjusted for the increase of lipid mobilization during pregnancy, concentrations were similar throughout. In women with gestational diabetes, unadjusted PCB levels were significantly higher at delivery than in non diabetic women, but when adjusted for lipid levels, they were similar.

## ***Exposure and hormonal status***

### *Co-factors related to retained variables*

The relationships between variables of interest (maternal and cord blood TSH, TT3, fT4 levels, PCBs, pesticides, and mercury concentrations) were tested with respect to the following co-factors: maternal age, gestational age at sampling, cigarette smoking, alcohol use, birth weight, newborn gender, and plasma total lipid contents. Maternal age, gestational age at sampling, plasma total lipid content and cigarette smoking during pregnancy, were related to most maternal biochemical measures (data not shown) and were added in final mixed models. For cord blood measures, total lipid levels, maternal age, birth weight, gestational age at birth and cigarette smoking during pregnancy were associated with cord blood hormone levels and exposure variables.

### *Relationships between plasma PCBs, pesticides, and mercury concentrations and thyroid hormones levels in pregnant women*

Table 4 presents the results from mixed models including TSH, fT4, and TT3 levels during pregnancy in relation to plasma PCB concentrations. In both, adjusted and not adjusted for lipids models, only TT3 levels were strongly negatively related to PCB concentrations, especially to non-coplanar congeners (CB-138, CB-153, and CB-180). No relation was observed with the sum of mono-ortho-coplanar congeners. The CB-180 was positively correlated to TSH levels, but not to fT4 levels.

Concordant results were obtained when the correlation with plasma pesticides was examined. The hexa-chlorobenzene, cis-nanochlor, and p,p'-DDE concentrations were negatively related to TT3 levels in mothers in adjusted for lipids models. Blood IHg was also negatively related to

TT3 levels (Table 5). In addition, cis-nanochlor, when detected, was positively correlated to fT4 levels.

The Figure 1 illustrates the change of TT3 and fT4 levels during pregnancy by group of exposure to five pollutants which are significantly related to TT3 levels in previous analyses (CB-138, CB-153, CB-180, p,p'-DDE,  $\beta$ -BHC, and hexa-chlorobenzene). For the the women who had none or 1 of these pollutants higher than 75<sup>th</sup> percentile of distribution TT3 levels significantly increased from second trimester to delivery whereas for the women from the “exposed” group, TT3 levels decreased. Moreover, this relationship was much more significant than those from women classified according OC higher than 75<sup>th</sup> percentile, separately (data not shown).

*Relationships between cord blood plasma PCBs, pesticides, and mercury concentrations and cord blood hormones levels*

In general, the PCB congeners and pesticide residues in cord plasma were not significantly related to cord blood TH (data not shown). The cord blood organic Hg was not significantly related to hormone levels. Only cord blood inorganic Hg was negatively related to FT4 level (adjusted mean 16.5 pmol/L in subjects with undetected inorganic Hg versus 15.5 pmol/L in those with detected values; partial Spearman  $r=-0.26$ ,  $p=0.02$ ).

## DISCUSSION

Our results demonstrate a significant negative relationship between circulating total TT3 levels in pregnant women at low environmental doses of CB-138, CB-153, CB-180, inorganic mercury and two pesticides, pp'-DDE and hexa-chlorobenzene. In addition, only cis-nanochlor, in women having detected values, was related to both, increased fT4 and decreased TT3, during pregnancy. No other significant relation was observed in regard to fT4 or TSH levels. No association was observed between cord blood organic pollutant concentrations and TH levels, except for the negative correlation between inorganic Hg and fT4 in cord blood serum. The results from the Dutch cohort study show a decrease in maternal T3, T4 in pregnancy and infant TSH levels in relation to TEQs (toxic equivalents) of milk PCB dioxin-like and non coplanar congeners (Koopman-Esseboom et al. 1994). The same authors note that higher levels of maternal and cord blood plasma CB-118, CB-138, CB-153 and CB-180 congeners correlated significantly with higher plasma TSH levels in the infant, in the 2<sup>nd</sup> week after birth. Higher levels of 3 non coplanar congeners (CB-137, CB-138, and CB-153) in human milk also correlated significantly with higher TSH levels in umbilical blood plasma. In another study, which explored cord for blood TSH in relation to the same congeners (CB-118, CB-138, CB-153, CB-180), no relation between PCBs and TSH was found (Ribas-Fito et al. 2003), but other TH (T3, T4) were not measured. In the Steuerwald et al. (2000) study on the effects of exposure to methyl mercury on thyroid function at birth, no relation was found with mercury (Hg) levels, but cord blood resin-T3 uptake levels were negatively correlated to the sum of 3 non coplanar PCB congeners (CB-138, CB-153, and CB-180) in maternal blood sample. The lowering of resin-T3 uptake is one of indicators of primary or secondary hypothyroidism. Thus, thyroid binding globulin (TBG) levels rose in cord blood with increased maternal PCB exposure.

The lack of relationship in regard to cord blood TH in our study could be related to other biological factors such as iodine and selenium intake or circadian variation (Andersen et al. 2003; Beckett et al. 2005), which are likely to influence endogenous hormone homeostasis, as well as to the very low level of exposure in fetal tissues. Exposure levels to PCB congeners in this population were 3-45 times lower than in previous reported studies reviewed by Longnecker et al. (2003). In addition, fetal TGG and other binding proteins are low (Hume et al. 2004), that could preserve the fetus from toxic effects of chemicals which act on TH binding. Also, thyroid status can be disrupted by other factors, unmeasured in the present study, including environmental pollutants such as pentachlorophenol (PCP) or HO-PCBs, which are metabolites of hexa-chlorobenzene and PCBs, respectively, as was previously reported in another Quebec populations (Sandau et al. 2002). These authors reported negative correlations between cord plasma free T3 and T4, as well as TBG, with sum of PCP and HO-PCBs, but not with PCB congeners individually or the sum of PCBs. Curiously, the concentrations of PCBs and HO-PCBs were also negatively correlated to TSH in cord plasma. Although these correlations were highly significant, they were obtained from small sample of newborns (n=20) without any adjustment for confounding variables.

Similar to the present results, the selective effect of PCBs on T3 levels has been reported in fish eating women group (n=32), in which PCB 153 concentration was negatively related to TT3 levels (Hagmar et al. 2001). Osius et al.'s (1999) study of schoolchildren showed that PCB 138, 153 and 180 were negatively related to free T3 levels without any significant change in TSH or T4 concentrations; this relationship was significant only in girls. To our knowledge, these are the only two studies which have demonstrated a more pronounced effect on T3 than on T4 thyroid hormone. However, in physiological and pathological conditions the isolated reduction of T3

levels is rarely observed because there are effective compensatory mechanisms *via* T4 production.

The results of the present study indicate that blood lipid content is not a major confounding factor for the relationship between TH and OCs. Both, adjusted and unadjusted for lipids models revealed the same degree of significance for OCs exposure. Thus, the rise of lipids following the TH decrease is unlikely to be an intermediate factor of the observed relationships.

Although epidemiological studies can not explore precise mechanisms of observed statistical relationships, some mechanistic hypotheses can be proposed. The deiodination mechanism could be hypothesized to explain observed decrease in T3 levels in relation to exposure to OCs and Hg. As reviewed by Bianco et al. (2002), the T3 degradation by 3 type deiodinase (D3) which catalyzes the inner ring deiodination of T4 to reverse T3 (rT3) and of T3 to 3,3'-T2, represents an important pathway for the inactivation of TH. D3 shows substrate preference for T3 over T4, and is expressed at high levels in human placenta tissue (Huang et al. 2003). The overexpression of D3 called “consumptive hypothyroidism” and reported in infantile hemangiomas, is characterized by undetectable serum T4, and T3, and high rT3 levels. Our results could be related to direct or indirect induction of D3 activity or its increased expression, but we did not assess the free T3 or rT3 levels to confirm this hypothesis. This needs further experimental research at low levels of OCs exposure. In addition, an increase in placenta D3 activity in methyl Hg exposed mice, has been reported (Watanabe et al. 1999). Interestingly, the brain D3 activity was depressed in the fetuses from exposed dams. In our study, it is difficult however to explain the lack of association with cord serum T3 knowing that placenta D3 participates in fetal T3 degradation in humans (Santini et al. 1999).

Other types of deiodinases present in different tissues can contribute to T4 and T3 deiodination. In humans, 80% of circulating T3, the physiologically active form of thyroid hormone, is generated from peripheral deiodination of T4 by enzymatic action of 5'-monodeiodinase and 20% is derived from thyroidal secretion (Pilo et al. 1990). There are two types of 5'-monodeiodinase enzyme: the D1 is located at the plasma membrane and the D2 is associated with endoplasm reticulum. The T3 generated by D1 does not have direct access to nuclei but instead must first be exported into the plasma. Both D1 and D2 deiodinases contribute to plasma T3 content. The substrates for these enzymes are rT3 and T3 sulfate for D1, and T4 and rT3 for D2 (Bianco et al. 2002). However, serum T3 concentration remains normal in D1 or D2 deficient mice (Maia et al. 1995).

Several studies have explored the effect of OCs on D1 and D2 deiodinase activity. One study reported the depression of liver D1 activity in response to Arochlor 1242 and 1254 treatment in the chick embryo (Gould et al. 1999). Wade et al. (2002) examined the effect of subchronic exposure to complex mixture of persistent contaminants (16 OCs, lead and cadmium) on thyroid hormones in male rats and reported increased TSH level at the lowest level of exposure without any changes of T4 or T3. Moreover, the authors observed significant reduction in hepatic D1 activity and speculated that TSH increase could be related to pituitary D2 depression. There is a need for further investigations to explore the role of deiodinases in toxicity of environmental pollutants, such as PCBs and pesticides, in human.

In addition to deiodination, thyroid hormone is also importantly metabolized by conjugation of the phenolic hydroxyl group with glucuronic acid or sulfate (Leonard and Köhrle 1996). This

mechanism is also involved in OCs toxicity. For example, hexa-chlorobenzene was shown to decrease of total and free T4 levels in rats, without significant effect on T3 (Kleiman de Pisarev et al. 1990). It was shown that hexa-chlorobenzene decrease kidney and brown adipose tissue D1 activity after 15-21 days of exposure, but total body D1 activity was significantly increased. In addition, hexa-chlorobenzene increase the activity of hepatic T4 uridine diphosphoglucuronosyl transferase (UDPGT) in a time-dependent manner, without changes in T3-UDPGT (Alvarez et al. 2005). The same mechanism on T4-UDPGT was proposed to explain the decrease in T4 following PCB exposure (Barter and Klaassen 1994). We did not observe, however, any negative association between free T4 and OCs. Thus, we cannot consider an effect on the enzyme responsible for TH conjugation as a possible explanation of these results. We observed, however, a negative relationship between free T4 in cord blood serum and inorganic Hg. If this relationship was not due to chance, it may be related to inducing properties of inorganic Hg on UDPGT in renal tissue reported in mice (Tan et al. 1990). Moreover, workplace exposure to inorganic Hg was reported to be associated with increases in T4, rT3, and the T4/T3 ratio (Ellingsen et al. 2000) suggesting an inhibitory effect of Hg on deiodinase activity. In our study, inorganic Hg was associated with a decrease in maternal total T3 during pregnancy that could be due to effect of Hg on deiodinase activity. However, since the free T4 levels were not changed and free T3 was not determined, this explanation remains speculative.

Binding to TBG and/or to transthyretin, two major TH transporters in blood could be proposed as an alternative hypothesis to explain the observed negative relationship between OCs and total T3 levels during pregnancy. PCBs, especially non coplanar congeners, bear a structural resemblance to the endogenous thyroid hormones and have high affinity with thyroid hormone-binding proteins such as transthyretin (Chauhan et al. 2000). Also, hydroxylated PCB metabolites bound

to transthyretin with affinities similar to that of T4, but they have low affinity to TBG (Cheek et al. 1999). Alteration of thyroid hormone-binding capacity in serum is associated with variations in total thyroid hormone concentration. Diminished serum thyroid hormone values are observed in subjects with TBG deficiency. However, decreased concentration or affinity of transthyretin is not associated with variations in serum concentrations of thyroid hormones (Bartalena and Robbins 1992). Few data are available about affinities of PCBs and pesticides to bind to TBG.

There are substantial and important differences between humans and animals with respect to structural characteristics of deiodinase enzyme and thyroid economy. In both, rodents and human, deiodinases are selenocysteine-containing proteins and the presence of selenocysteine in the protein is critical for enzyme activity. However, the carboxy-terminal of D1 from rat liver was different from that of other species (Santini et al. 1992). Also, the rat has a much larger contribution of T3 secreted directly from the thyroid gland than in humans. It has been estimated that only approximately 20% of plasma T3 in humans comes from thyroïdal secretion, as opposed to about 40% in rats. It has also been estimated that D1 catalyzes about half of the daily extrathyroidal T3 production from T4 in the rat *versus* an estimate of 25% in human (Bianco et al. 2002). There is also heterogeneity in the transport of thyroid hormones between species. In humans, TH are primarily bound to TBG. The remainder is bound to less specific proteins, such as albumin and transthyretin. These three proteins transport more than 95% of TH (Barlow 1997; Bartalena and Robbins 1992). In growing rats, a significant difference is that TBG is not found between 2 and 7 months, the age range typically used in basic toxicology studies (Vranckx et al. 1994). In adult rats, TH are bound to the low affinity carriers albumin and transthyretin. As a consequence, the half-life of thyroid hormones in adult rats is shorter than in humans (Mc Clain 1995). These various interspecies differences imply a different predisposition of rats compared to

humans to perturbations of thyroid homeostasis by chemicals that influence thyroid status (Lans et al. 1994).

One of limitations of our study is the measure of total T3 and free T4 without free T3 and total T4 levels. The TT3 does not include rT3 and T3 sulfate levels which could help us to confirm the hypothesis that PCB, pesticide and mercury affect T4 or T3 deionization. Also, we are unable to show if the observed relationship is related to free T3 decrease or to T3 fraction binding to TBG. Moreover, it is difficult to distinguish the proper effect of each OC on TH due to their high collinearity (correlations between OC more than 0.60). However, their cumulative or synergistic effects can not be excluded considering the most important decrease of TT3 when it is correlated with more than one OC.

Thyroid status is frequently assessed during pregnancy, but limited routinely to measurements of TSH. Little data exists about the role of physiological changes in thyroid status in pregnant women and the effect of subtle T3 and T4 variations on women's health. One study suggests that low free T3 levels are associated with postpartum depression syndrome (Ijuin et al. 1998), but further investigations are needed to evaluate the long term consequences of subtle thyroid changes related to environmental exposure to persistent organic contaminants. In conclusion, the potential of to low dose exposure to OC mixtures to interfere with hormonal status during pregnancy warrants further investigations with complete assessment of thyroid status to confirm our results and to determine the short and long term consequences of these disturbances.

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**Table 1. Blood levels of hormones during pregnancy and at birth.**

	First trimester (n=40)		Second trimester (n=147 <sup>a</sup> )		At delivery (n=100)		Cord blood (n=92)	
	median	5 <sup>th</sup> -95 <sup>th</sup> percentiles	median	5 <sup>th</sup> -95 <sup>th</sup> percentiles	median	5 <sup>th</sup> -95 <sup>th</sup> percentiles	median	5 <sup>th</sup> -95 <sup>th</sup> percentiles
TSH, mIU/L	2.1	0.09 – 9.55	2.2	0.62 – 5.5	2.6	0.8 – 7.53	9.8	3.4 – 30.4
Free T4, pmol/L	14.3	11.5 – 18.7	12.8	10.2 – 15.8	11.6	8.7 – 15.05	16.1	12.8 – 19.6
Total T3, nmol/L	2.7	1.97 – 3.6	3.2	2.3 – 4.2	3.3	2.4 – 4.5	1.3	0.9 – 1.9

<sup>a</sup> in women which were sampled two time in second trimester only the second sample is included in this column

mIU=milliInternational Unit

**Table 2 Concentrations of plasma PCB congener, µg/L.**

Congener	Median and 5 <sup>th</sup> -95 <sup>th</sup> centiles or % of samples over detection limit value			
	I trimester (n=39)	II trimester (n=145)	At delivery (n=101)	Cord blood (n=92)
CB28	10%	28%	21%	2%
CB52	0%	2%	4%	1%
CB99	0.02 [nd-0.05]	0.02 [nd -0.05]	0.02 [nd -0.06]	6%
CB101	0%	0%	0%	0%
CB105	3%	12%	23%	8%
CB118	0.02 [nd -0.08]	0.03 [nd -0.08]	0.03 [nd -0.10]	33%
CB128	0%	0%	0%	0%
CB138	0.06 [0.02-0.18]	0.07 [0.03-0.20]	0.08 [0.03-0.25]	0.02 [nd -0.06]
CB153	0.07 [0.03-0.26]	0.08 [0.03-0.27]	0.09 [0.04-0.30]	0.02 [nd -0.08]
CB156	0.02 [nd -0.05]	0.02 [nd -0.05]	0.02 [nd -0.07]	37%
CB170	0.01 [nd -0.07]	0.02 [nd -0.07]	0.02 [nd -0.07]	7%
CB180	0.04 [0.02-0.14]	0.05 [0.02-0.17]	0.05 [0.02-0.19]	0.01 [nd -0.05]
CB183	8%	15%	27%	1%
CB187	0.02 [nd -0.06]	0.02 [nd -0.05]	0.02 [nd -0.06]	14%
Σ <i>mono-ortho</i> -coplanars (105, 118, 156)	0.06 [nd-0.14]	0.06 [nd-0.15]	0.07 [0.04-0.18]	0.04 [nd-0.07]
Σ PCB	0.33 [0.16-1.31]	0.35 [0.18-1.05]	0.39 [0.20-1.22]	0.16 [nd-0.35]

**Table 3 Blood mercury and plasma pesticides concentrations (µg/L).**

	Median and 5 <sup>th</sup> -95 <sup>th</sup> centiles or % of samples over detection limit value			
	I trimester (n=39)	II trimester (n=145)	At delivery (n=101)	Cord blood (n=92)
Total Hg	0.80 [0.40 – 2.20]	0.60 [nd – 2.0]	0.60 [nd – 1.2]	0.60 [nd – 1.6]
Organic Hg	0.40 [nd – 1.40]	0.20 [nd-1.20]	0.20 [nd-0.80]	0.30 [nd-1.30]
Trans-nanochlor	0.03 [nd -0.09]	0.04 [0.02-0.10]	0.05 [nd -0.15]	14%
Oxy-chlordane	0.02 [nd -0.06]	0.03 [0.02-0.07]	0.03 [0.02-0.08]	10%
Mirex	19%	15%	20%	1%
Hexa-chlorobenzene	0.04 [0.03-0.10]	0.06 [0.03-0.11]	0.06 [0.04-0.12]	0.02 [0.01-0.05]
DDT	0.01 [nd -0.04]	0.03 [nd -0.05]	0.04 [nd -0.07]	11%
pp'-DDE	0.38 [0.16-0.90]	0.43 [0.22-0.97]	0.47 [0.20-1.20]	0.16 [0.08-0.40]
Cis-nanochlor	0%	1%	20%	0%
Trans-chlordane	0%	0%	0%	0%
Cis-chlordane	0%	0%	0%	0%
β-BHC	0.03 [nd -0.05]	0.04 [nd -0.08]	0.05 [nd -0.09]	1%
Aldrin	0%	0%	0%	0%

**Table 4 Hormone levels and PCBs concentrations during pregnancy**

	TSH, mIU/L		Free T4, pmol/L		Total T3, nmol/L	
	Unadjusted	Adjusted <sup>a</sup>	Unadjusted	Adjusted <sup>a</sup>	Unadjusted	Adjusted <sup>a</sup>
<b>ΣPCB, µg/L</b>						
estimate	0.65	0.45	-0.08	0.49	-0.37	-0.47
DF	151	148	151	148	151	148
Type 3 F value	0.50	0.21	0.05	1.6	6.4*	9.6**
<b>Σmono-ortho-coplanars (CB105, CB118, CB156), µg/L</b>						
estimate	3.0	0.46	-2.6	2.7	-1.3	-2.1
DF	151	148	151	148	151	148
Type 3 F value	0.13	0.0	0.60	0.62	0.98	2.27
<b>CB138, µg/L</b>						
estimate	0.90	-0.55	-0.48	3.1	-2.1	-2.8
DF	151	148	151	148	151	148
Type 3 F value	0.03	0.01	0.05	2.1	7.2**	11.2**
<b>CB153, µg/L</b>						
estimate	-0.18	-0.93	0.57	2.5	-1.2	-1.5
DF	151	148	151	148	151	148
Type 3 F value	0.0	0.08	0.19	3.6	5.9*	8.6**
<b>CB180, µg/L</b>						
estimate	7.8	7.5	-1.4	-0.27	-1.2	-1.4
DF	151	148	151	148	151	148
Type 3 F value	5.3*	4.6*	1.1	0.04	6.0*	7.7**

<sup>a</sup>Adjustment for total lipid concentrations

Mixed model parameters for repeated measures adjusted for gestational age at sampling, maternal age, and cigarette smoking during pregnancy

\* p<0.05; \*\* p<0.01

**Table 5. Hormone levels, mercury and pesticides concentrations during pregnancy**

	TSH, mIU/L		Free T4, pmol/L		Total T3, nmol/L	
	Unadjusted	Adjusted <sup>a</sup>	Unadjusted	Adjusted <sup>a</sup>	Unadjusted	Adjusted <sup>a</sup>
Organic Hg, µg/L						
estimate	0.58		-0.18		0.05	
Type 3 F value	0.50		0.34		0.18	
Inorganic Hg, µg/L						
estimate	-0.41		-0.26		-0.27	
Type 3 F value	0.28		0.57		4.20*	
Trans-nanochlor, µg/L						
estimate	-0.66	-1.9	-5.3	-1.65	-0.50	-0.91
Type 3 F value	0.01	0.09	3.80	0.40	0.20	0.80
Oxy-chlordane, µg/L						
estimate	10.3	4.6	-7.8	3.9	-4.1	-4.6
Type 3 F value	0.46	0.08	1.53	0.37	2.96	3.15
Mirex, detected vs undetected value						
estimate	0.09	0.03	-0.11	0.13	0.07	0.08
Type 3 F value	0.01	0.0	0.14	0.19	0.36	0.41
Hexa-chlorobenzene, µg/L						
estimate	-5.4	-11.0	-2.3	8.1	-3.4	-5.2
Type 3 F value	0.20	0.82	0.20	2.91	3.83	7.51**

<sup>a</sup>Adjustment for total lipid concentrations

Mixed model parameters for repeated measures adjusted for gestational age at sampling, maternal age, and cigarette smoking during pregnancy (df=151 for unadjusted and df=148 for adjusted analyses)

\* p<0.05; \*\* p<0.01

**Table 5. Hormone levels, mercury and pesticides concentrations during pregnancy – cont'd**

DDT, µg/L						
estimate	-8.5	-14.2	-0.89	9.9	1.39	0.26
Type 3 F value	0.3	0.8	0.02	2.8	0.4	0.01
pp'-DDE, µg/L						
estimate	0.25	-0.06	-0.75	0.09	-0.37	-0.54
Type 3 F value	0.04	0.0	2.0	0.03	3.3	6.1*
Cis-nanochlor, detected vs undetected value						
estimate	0.67	0.41	0.33	0.74	-0.34	-0.35
Type 3 F value	0.36	0.13	0.73	3.92	5.33*	5.40*
β-BHC, µg/L						
estimate	-2.1	-5.4	2.3	8.8	-2.4	-3.4
Type 3 F value	0.04	0.2	0.3	4.2*	2.0	3.8

<sup>a</sup>Adjustment for total lipid concentrations

Mixed model parameters for repeated measures adjusted for gestational age at sampling, maternal age, and cigarette smoking during pregnancy (df=151 for unadjusted and df=148 for adjusted analyses)

\* p<0.05; \*\* p<0.01

**Figure 1.** Change in TT3 (black) and fT4 (grey) levels between second trimester and delivery in pregnant women by group of exposure

