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

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Polychlorinated biphenyls (PCBs), chlorinated pesticides, and mercury 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 during pregnancy and in cord blood in relation to blood concentrations of organochlorine compounds (OCs) and Hg in healthy women recruited during pregnancy. We found a significant negative correlation between maternal total triiodothyronine levels and three non-coplanar congeners (PCB-138, PCB-153, and PCB-180), three pesticides (*p,p′*-DDE, *cis*-nanochlor, and hexachlorobenzene), 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 was negatively correlated with inorganic Hg. These results suggest that at even low levels of exposure, persistent environmental contaminants can interfere with thyroid status during pregnancy. *Key words:* cord blood, environment, mercury, pesticide, polychlorinated biphenyls, pregnancy, thyroid. *Environ Health Perspect* 113:1039–1045 (2005). doi:10.1289/ehp.7685 available via *http://dx.doi.org/* [Online 24 May 2005]

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 epidemiologic studies have focused on subclinical maternal thyroid deficiency during pregnancy, particularly for hypothyroxinemia in early gestation, and its long-term effects on psychomotor development in children (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 triiodothyronine (T_3), and it has been suggested that the placenta is a thyroid hormone (TH)–dependent tissue (Kilby et al. 1998; Oki et al. 2004).

Experimental studies have shown that polychlorinated biphenyls (PCBs) and related chemicals decrease circulating THs during development (Donahue et al. 2004; Ulbrich and Stahlmann 2004; Zoeller et al. 2000). Prenatal 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. 2002a, 2002b, 2004). In rats, Goldey et al. (1995) reported that ototoxic effects of PCBs were associated with decreased circulating THs after perinatal exposure. It has also 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 (OH-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 TH 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 TH-binding protein transthyretin, thus displacing the natural ligand, thyroxin (T_4; Cheek et al. 1999). PCBs, as well as some other organochlorine compounds (OCs) such as hexachlorobenzene, are also known to increase the activity of hepatic drug-metabolizing enzymes, in particular, uridine diphospho-glucuronosyl transferase (UDPGT), responsible for glucuronidation of T_4 (Van Birgelen et al. 1995; van Raaij et al. 1993). *In vitro*, hydroxylated PCBs have a low affinity for the human thyroid receptor but do have a TH-like affinity for the serum transport protein transthyretin (Cheek et al. 1999; Meerts et al. 2002) and inhibit the iodothyronine sulfotransferase activity (Schuur 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 biologic systems. There is evidence that coplanar PCB mutagenic toxicity

is mediated through the aryl hydrocarbon (Ah) receptor (Safe 1994). Recently, it was reported that both mono-*ortho* and non-coplanar types of PCBs, and hydroxylated PCB metabolites may disrupt TH status, in part, by affecting thyroid hormone receptor (TR)-mediated transcription, which may influence growth and development of TH target organs, particularly in the central nervous system (Iwasaki et al. 2002). Khan and Hansen (2003) suggest that non-coplanar congeners interfere with the hypothalamo-pituitary-thyroid (HPT) axis by producing a subnormal response of the pituitary and thyroid to thyrotropin releasing hormone (TRH) stimulation.

The developing fetus is particularly susceptible to thyrotoxic effects of PCBs and their metabolites. In rats, exposure to hydroxylated PCB 4′-OH-CB-108 from gestational day (GD)10 to GD16 decreased maternal, fetal, and neonatal plasma total T_4 and free T_4 (fT_4) in a dose-dependent manner (Meerts et al. 2002). Chronic developmental exposure to Aroclor 1254 from GD6 to postpartum day 21 also reduces circulating levels of total T_4 (Zoeller et al. 2000). At the same conditions of exposure, Goldey et al. (1995) observed decreased total T_4 levels and a moderate reduction of T_3 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 T_4 deiodination (Ellingsen et al. 2000). In a community highly exposed to hexachlorobenzene, a significant positive association was found between this OC and concentrations of thyroid-stimulating

hormone (TSH) 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 (ΣPCB) 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.

Materials and Methods

Study population. The women participating in the study were recruited at their 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, each woman filled out an interview-administered questionnaire, which contained general sociodemographic data and information on residency, medical history, drinking and smoking habits, and diet, and blood samples were obtained. Those who were recruited into the study during the first trimester (before the 13th week) provided a first sample at entry and a second during the second trimester, whereas those who were recruited between the 14th and 24th weeks provided one sample before 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 the second trimester. The study population consisted of 149 pregnant women, 101 of whom gave birth at the participating hospital where maternal and cord blood samples and placental tissue were obtained at delivery. Two weeks after birth, a second questionnaire was interview administered. This second questionnaire included information on medical and obstetrical history, birth data, and 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 second trimester. Thus, most data were available for the second trimester (n = 149). At delivery, there were 101 maternal and 92 cord blood samples available for analyses. Thus, complete data throughout pregnancy were available for 38 women, and for 101 from the second trimester and at delivery. Data for hormones and contaminants are missing for some women (n = 2–4) because of insufficient quantity of blood or nonrespect of specimen storage protocol.

Biologic sampling. Blood samples for the first and second trimesters were collected at the pregnant women's residences after night fasting, whereas the third-trimester samples and cord blood samples 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 PCBs and chlorinated pesticides were performed by the Centre of Toxicology of Québec by gas chromatography coupled with mass detection using a chromatograph (model 6890) and mass detector (model 5973) from Agilent (Mississauga, Canada). Blood plasma (2 mL) was 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 the National Institute of Standards and Technology (Gaithersburg, MD, USA). Also, periodic evaluations were carried out through participation in two external proficiency testing programs [Artic Monitoring Assessment Program ring test; Laboratoire de toxicologie humaine/Institut national de santé publique (INSPQ) and the 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 a mercury monitor (model 100; Pharmacia Instruments, Piscataway, NJ, USA). Organic Hg (OHg) was calculated as the difference between THg and IHg. THg was determined using 500 µL 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). Hg vapor was generated and detected, and aqueous calibration was performed. The IHg fraction was determined using the same methodology except that cadmium chloride was omitted from the reactant mixture. Routine checks of accuracy and precision were performed using reference material from the Laboratoire de toxicologie humaine/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 nmol/L IHg and 82 nmol/L OHg were 4 and 3.4%, respectively.

The detection limits were determined from the analyses of 10 actual samples, whose concentrations were between 4 and 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, triglycerides, and phospholipids were individually measured using enzymatic methods on the Technicon automatic analyzer (model RA-500; Technicon, Cranesville, PA, USA) as previously described (Moorjani et al. 1987). Plasma total lipids were calculated using the summation method: total lipids = 1.677 (total cholesterol – free cholesterol) + free cholesterol + triglycerides + phospholipids.

Thyroid hormone determination. Thyroid hormones [TSH, total T_3 (TT_3), and fT_4] were analyzed by radioimmunoassay at the Clinical Biochemistry Service of Saint-François d'Assise hospital (Québec, Canada) (Forest et al. 1998).

Statistical analysis. All statistical analyses were performed using SAS (version 8.12; SAS Institute 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 cofactors such as maternal age, smoking and alcohol consumption, child's sex 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 analysis of covariance (general linear model procedure). Because a large number of samples had contaminant levels 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. PCB-101 and PCB-128

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

	First trimester (n = 40)		Second trimester (n = 147)[a]		At delivery (n = 100)		Cord blood (n = 92)	
	Median	5th–95th percentiles	Median	5th–95th percentiles	Median	5th–95th percentiles	Median	5th–95th 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
fT_4, 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
TT_3, 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

mIU, milli-international unit.
[a]For women who were sampled twice in the second trimester, only the second sample is included.

as well as *trans*-chlordane, *cis*-chlordane, and aldrin were excluded from statistical analysis because 100% were undetected values.

THs 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 THs on blood lipid mobilization, analyses both adjusted and unadjusted for lipid concentration were performed. Two kinds of physiologic sequences are possible in the tested relationship between lipophilic contaminants such as PCBs or pesticides, and THs: first, the hypothesized relation that increased lipids lead to increased blood PCBs, which lead to decreased TH levels; and second, an inverse relation where decreased THs lead to increased lipids, which lead to increased blood PCBs. The comparison of two models, adjusted and not lipid-adjusted, 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, we defined two groups of subjects based on the degree of their exposure to five OCs significantly related to hormone levels: PCB-138, PCB-153, PCB-180, p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE), and hexachlorobenzene. The exposed group included women who had two or more pollutant levels higher than the 75th percentile of distribution, and the nonexposed group included those with none or only one pollutant level higher than the 75th percentile.

Results

Population characteristics. The women who gave birth ($n = 101$) averaged 27 years of age (range, 15–39 years); 30% smoked during pregnancy, and 8% consumed alcohol moderately (0.5–2 drinks/week, 4–30 g of 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 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 giving birth at participating maternity hospitals.

Hormone and contaminant levels during pregnancy and at birth. The TH concentrations are shown in Table 1, and Tables 2 and 3 present the blood PCB congener and pesticide levels. We observed that TT_3 and TSH levels increased during pregnancy,

whereas fT_4 levels decreased. TH levels in this population of pregnant women are similar to data reported by de Escobar et al. (2004). The cord blood PCB concentrations were significantly lower than maternal blood and, in most 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 nondiabetic women, but when adjusted for lipid levels, they were similar.

Exposure and hormonal status: cofactors related to retained variables. The relationships between variables of interest (maternal and cord blood TSH, TT_3, fT_4, PCBs, pesticides, and Hg concentrations) were tested with respect to the following cofactors: maternal age, gestational age at sampling, cigarette smoking, alcohol use, birth weight, newborn's

sex, 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 TH levels and plasma PCBs, pesticides, and Hg concentrations in pregnant women. Table 4 presents the results from mixed models including TSH, fT_4, and TT_3 levels during pregnancy in relation to plasma PCB concentrations. In both lipid-adjusted and nonadjusted models, only TT_3 levels were strongly negatively related to PCB concentrations, especially to non-coplanar congeners (PCB-138, PCB-153, and PCB-180). No relation was observed with the sum of mono-*ortho* coplanar congeners (PCB-105, PCB-118, and PCB-156; Σmono-*ortho* coplanar PCBs). PCB-180 was

Table 2. Concentrations of plasma PCB congeners (μg/L) shown as median (5th–95th percentiles) or percent of samples above detection limit.

Congener	First trimester ($n = 39$)	Second trimester ($n = 145$)	At delivery ($n = 101$)	Cord blood ($n = 92$)
PCB-28	10%	28%	21%	2%
PCB-52	0%	2%	4%	1%
PCB-99	0.02 (ND–0.05)	0.02 (ND–0.05)	0.02 (ND–0.06)	6%
PCB-101	0%	0%	0%	0%
PCB-105	3%	12%	23%	8%
PCB-118	0.02 (ND–0.08)	0.03 (ND–0.08)	0.03 (ND–0.10)	33%
PCB-128	0%	0%	0%	0%
PCB-138	0.06 (0.02–0.18)	0.07 (0.03–0.20)	0.08 (0.03–0.25)	0.02 (ND–0.06)
PCB-153	0.07 (0.03–0.26)	0.08 (0.03–0.27)	0.09 (0.04–0.30)	0.02 (ND–0.08)
PCB-156	0.02 (ND–0.05)	0.02 (ND–0.05)	0.02 (ND–0.07)	37%
PCB-170	0.01 (ND–0.07)	0.02 (ND–0.07)	0.02 (ND–0.07)	7%
PCB-180	0.04 (0.02–0.14)	0.05 (0.02–0.17)	0.05 (0.02–0.19)	0.01 (ND–0.05)
PCB-183	8%	15%	27%	1%
PCB-187	0.02 (ND–0.06)	0.02 (ND–0.05)	0.02 (ND–0.06)	14%
ΣMono-*ortho* coplanar PCBs[a]	0.06 (ND–0.14)	0.06 (ND–0.15)	0.07 (0.04–0.18)	0.04 (ND–0.07)
Total PCBs	0.33 (0.16–1.31)	0.35 (0.18–1.05)	0.39 (0.20–1.22)	0.16 (ND–0.35)

ND, nondetectable.
[a]Sum of PCB-105, PCB-118, and PCB-156.

Table 3. Blood Hg and plasma pesticide concentrations (μg/L) shown as median (5th–95th percentiles) or percent of samples above detection limit.

	First trimester ($n = 39$)	Second trimester ($n = 145$)	At delivery ($n = 101$)	Cord blood ($n = 92$)
THg	0.80 (0.40–2.20)	0.60 (ND–2.0)	0.60 (ND–1.2)	0.60 (ND–1.6)
OHg	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%
Oxychlordane	0.02 (ND–0.06)	0.03 (0.02–0.07)	0.03 (0.02–0.08)	10%
Mirex	19%	15%	20%	1%
Hexachlorobenzene	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%
p,p'-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%

ND, nondetectable.

positively correlated with TSH levels but not with fT_4 levels.

Concordant results were obtained when the correlation with plasma pesticides was examined. Hexachlorobenzene, *cis*-nanochlor, and *p,p′*-DDE concentrations were negatively related to TT_3 levels in mothers in lipid-adjusted models. Blood IHg was also negatively related to TT_3 levels (Table 5). In addition, *cis*-nanochlor, when detected, was positively correlated with fT_4 levels.

Figure 1 illustrates the change of TT_3 and fT_4 levels during pregnancy by group of exposure to five pollutants that are significantly related to TT_3 levels in previous analyses (PCB-138, PCB-153, PCB-180, *p,p′*-DDE, and hexachlorobenzene). For the women in the nonexposed group (none or one of these pollutant levels higher than the 75th percentile of distribution), TT_3 levels significantly increased from the second trimester to delivery; for the women from the exposed group (two or more pollutant levels higher than the 75th percentile), TT_3 levels decreased. Moreover, this relationship was much more significant than those in women classified according to any OC level higher than 75th percentile, separately (data not shown).

Relationships between cord blood plasma PCB, pesticide, and Hg concentrations and cord blood hormone levels. In general, the PCB congeners and pesticide residues in cord plasma were not significantly related to cord blood THs (data not shown). The cord blood OHg was not significantly related to hormone levels. Only cord blood IHg was negatively related to fT_4 level (adjusted mean, 16.5 pmol/L in subjects with undetected IHg vs. 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 TT_3 levels in pregnant women at low environmental doses of PCB-138, PCB-153, PCB-180, IHg, and two pesticides, *p,p′*-DDE and hexachlorobenzene. In addition, only *cis*-nanochlor, in women having detected values, was related to both increased fT_4 and decreased TT_3, during pregnancy. No other significant relation was observed in regard to fT_4 or TSH levels. No association was observed between cord blood organic pollutant concentrations and TH levels, except for the negative correlation between IHg and fT_4 in cord blood serum. The results from the Dutch cohort study (Koopman-Esseboom et al. 1994) show a decrease in maternal T_3 and T_4 in pregnancy and in infant TSH levels in relation to toxic equivalents of milk PCB dioxin-like and noncoplanar congeners. These authors also noted that higher levels of maternal and cord blood plasma PCB-118, PCB-138, PCB-153, and

PCB-180 correlated significantly with higher plasma TSH levels in infants in the second week after birth. Higher levels of three non-coplanar congeners (PCB-137, PCB-138, and PCB-153) in human milk also correlated significantly with higher TSH levels in umbilical blood plasma. In another study, which investigated cord blood for TSH in relation to the same congeners (PCB-118, PCB-138, PCB-153, and PCB-180), no relation between PCBs and TSH was found (Ribas-Fito et al. 2003), but other THs (T_3, T_4) were not measured. In a study on the effects of exposure to methylmercury on thyroid function at birth, Steuerwald et al. (2000) found no relation with Hg levels, but cord blood resin-T_3 uptake levels were negatively correlated with the non-coplanar PCBs in maternal blood samples. The lowering of resin-T_3 uptake is one indicator 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 THs in our study could be related to other biologic 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 previously reported studies [reviewed by Longnecker et al. (2003)]. In addition, fetal TBG and other binding proteins are low (Hume et al. 2004), which could protect the fetus from toxic effects of chemicals that act on TH binding. Also, thyroid status can be disrupted by other factors not measured in the present study, including

environmental pollutants such as pentachlorophenol (PCP) or OH-PCBs, which are metabolites of hexachlorobenzene and PCBs, respectively, as was reported previously in another Québec population (Sandau et al. 2002). These authors reported negative correlations between cord plasma free T_3 and T_4, as well as TBG, with sum of PCP and OH-PCBs but not with PCB congeners individually or ΣPCB. Curiously, the concentrations of PCBs and OH-PCBs were also negatively correlated with TSH in cord plasma. Although these correlations were highly significant, they were obtained from a small sample of newborns ($n = 20$) without any adjustment for confounding variables.

Similar to the present results, the selective effect of PCBs on T_3 levels has been reported in women who eat fish (Hagmar et al. 2001) ($n = 32$); in that study, the PCB-153 concentration was negatively related to TT_3 levels. Osius et al.'s (1999) study of schoolchildren showed that PCB-138, PCB-153, and PCB-180 levels were negatively related to free T_3 levels without any significant change in TSH or T_4 concentrations; this relationship was significant only in girls. To our knowledge, these are the only two studies that have demonstrated a more pronounced effect on T_3 than on T_4. However, in physiologic and pathologic conditions the isolated reduction of T_3 levels is rarely observed because there are effective compensatory mechanisms via T_4 production.

The results of the present study indicate that blood lipid content is not a major confounding factor for the relationship between THs and OCs. Both lipid-adjusted and unadjusted models revealed the same degree of significance for OC exposure. Thus,

Table 4. Hormone levels and PCBs concentrations during pregnancy.

	TSH (mIU/L)		fT_4 (pmol/L)		TT_3 (nmol/L)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
ΣPCB (μg/L)						
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**
Σ*mono-ortho* coplanar PCBs[b] (μg/L)						
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
PCB-138 (μg/L)						
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**
PCB-153 (μg/L)						
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**
PCB-180 (μg/L)						
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**

df, degrees of freedom. Mixed model parameters for repeated measures adjusted for gestational age at sampling, maternal age, and cigarette smoking during pregnancy.
[a]Adjusted for total lipid concentrations. [b]Sum of PCB-105, PCB-118, and PCB-156. *$p < 0.05$. **$p < 0.01$.

the rise of lipids after the TH decrease is unlikely to be an intermediate factor of the observed relationships.

Although epidemiologic studies cannot explore precise mechanisms of observed statistical relationships, some mechanistic hypotheses can be proposed. The deiodination mechanism could be hypothesized to explain observed decrease in T_3 levels in relation to exposure to OCs and Hg. As reviewed by Bianco et al. (2002), the T_3 degradation by type 3 deiodinase (D3), which catalyzes the inner ring deiodination of T_4 to reverse T_3 (rT_3) and of T_3 to 3,3′-T2, represents an important pathway for the inactivation of THs. D3 shows substrate preference for T_3 over T_4 and is expressed at high levels in human placental tissue (Huang et al. 2003). The overexpression of D3, called "consumptive hypothyroidism" and reported in infantile hemangiomas, is characterized by undetectable serum T_4 and T_3 and high rT_3 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 T_3 or rT_3 levels to confirm this hypothesis. This needs further experimental research at low levels of OC exposure. In addition, an increase in placental D3 activity in methylmercury-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 T_3 given that placental D3 participates in fetal T_3 degradation in humans (Santini et al. 1999).

Other types of deiodinases present in different tissues can contribute to T_4 and T_3 deiodination. In humans, 80% of circulating T_3, the physiologically active form of TH, is generated from peripheral deiodination of T_4 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: D1 is located at the plasma membrane, and D2 is associated with endoplasmic reticulum. The T_3 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 T_3 content. The substrates for these enzymes are rT_3 and T_3 sulfate for D1, and T_4 and rT_3 for D2 (Bianco et al. 2002). However, serum T_3 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 Aroclor 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 THs in male rats and reported increased TSH levels at the lowest level of exposure without any changes in T_4 or T_3. Moreover, the authors observed significant reduction in hepatic D1 activity and speculated that the 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 humans.

In addition to deiodination, TH is also metabolized by conjugation of the phenolic hydroxyl group with glucuronic acid or sulfate (Leonard and Köhrle 1996). This mechanism is also involved in OC toxicity. For example, hexachlorobenzene was shown to decrease total and fT_4 levels in rats, without significant effect on T_3 (Kleiman de Pisarev et al. 1990). Hexachlorobenzene decreased kidney and brown adipose tissue D1 activity after 15–21 days of exposure, but total body D1 activity was significantly increased. In addition, hexachlorobenzene increased the activity of hepatic T_4 UDPGT in a time-dependent manner without changes in T_3-UDPGT (Alvarez et al. 2005). The same mechanism on T_4-UDPGT was proposed to explain the decrease in T_4 following PCB exposure (Barter and Klaassen 1994). However, we did not observe any negative association between fT_4 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 fT_4 in cord blood serum and IHg. If this relationship was not due to chance, it may be related to inducing properties of IHg on UDPGT in renal tissue reported in mice (Tan et al. 1990). Moreover, workplace exposure to IHg was reported to be associated with increases in T_4, rT_3, and the $T_4:T_3$ ratio (Ellingsen et al. 2000), suggesting an inhibitory effect of Hg on deiodinase activity. In our study, IHg was associated with a

Table 5. Hormone levels, Hg, and pesticides concentrations during pregnancy.

	TSH (mIU/L)		fT$_4$ (pmol/L)		TT$_3$ (nmol/L)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
OHg (µg/L)						
Estimate	0.58	—	−0.18	—	0.05	—
Type 3 F-value	0.50	—	0.34	—	0.18	—
IHg (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
Oxychlordane (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						
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
Hexachlorobenzene (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**
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
p,p′-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						
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

[a]Adjustment for total lipid concentrations: mixed model parameters for repeated measures adjusted for gestational age at sampling, maternal age, and cigarette smoking during pregnancy [degrees of freedom (df) = 151 for unadjusted and df = 148 for adjusted analyses]. *$p < 0.05$. **$p < 0.01$.
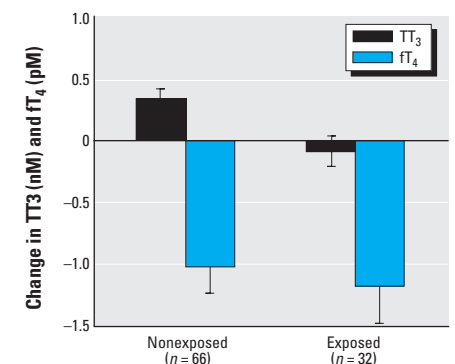
Figure 1. Change in TT$_3$ (nM) and fT$_4$ (pM) levels between second trimester and delivery in pregnant women by group of exposure, adjusted to gestational age at sampling. $p = 0.006$ for TT$_3$ but fT$_4$ was not statistically significant.

decrease in maternal TT_3 during pregnancy that could be due to an effect of Hg on deiodinase activity. However, because the fT_4 levels were not changed and free T_3 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 TT_3 levels during pregnancy. PCBs, especially non-coplanar congeners, bear a structural resemblance to the endogenous THs and have a high affinity for TH-binding proteins such as transthyretin (Chauhan et al. 2000). Also, hydroxylated PCB metabolites bound to transthyretin with affinities similar to that of T_4, but they have a low affinity for TBG (Cheek et al. 1999). Alteration of TH-binding capacity in serum is associated with variations in total TH concentration. Diminished serum TH values are observed in subjects with TBG deficiency. However, decreased concentration or affinity of transthyretin is not associated with variations in serum concentrations of THs (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 humans, 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 T_3 secreted directly from the thyroid gland than humans. It has been estimated that only approximately 20% of plasma T_3 in humans comes from thyroidal secretion, as opposed to about 40% in rats (Bianco et al. 2002). It has also been estimated that D1 catalyzes about half of the daily extrathyroidal T_3 production from T_4 in the rat versus an estimate of 25% in humans (Bianco et al. 2002). There is also heterogeneity in the transport of THs between species. In humans, THs 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 THs (Barlow 1997; Bartalena and Robbins 1992). In growing rats, a significant difference is that TBG is not found between 2 and 7 months of age, the age range typically used in basic toxicology studies (Vranckx et al. 1994). In adult rats, THs are bound to the low-affinity carriers albumin and transthyretin. As a consequence, the half-life of THs is shorter in adult rats than in humans (McClain 1995). These various interspecies differences imply a different predisposition of

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

One limitation of our study is the measure of TT_3 and fT_4 without free T_3 and total T_4 levels. The TT_3 does not include rT_3 and T_3 sulfate levels, which could help us to confirm the hypothesis that PCB, pesticides, and Hg affect T_4 or T_3 deiodination. Also, we are unable to show if the observed relationship is related to free T_3 decrease or to T_3 fraction binding to TBG. Moreover, it is difficult to distinguish the proper effect of each OC on THs because of their high collinearity (correlations between OCs > 0.60). However, their cumulative or synergistic effects can not be excluded considering the most important decrease of TT_3 when it is correlated with more than one OC.

Thyroid status is frequently assessed during pregnancy but limited routinely to measurements of TSH. Few data exist on the role of physiologic changes in thyroid status in pregnant women and the effect of subtle T_3 and T_4 variations on women's health. One study suggests that low free T_3 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 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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