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Effect of exposure to *p,p´*-DDE during the first half of pregnancy in the maternal thyroid profile of female residents in a Mexican floriculture area

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ABSTRACT

Background: Dichlorodiphenyldichloroethene (*p,p´*-DDE), the main metabolite of dichlorodiphenyltrichloroethane (DDT), has been associated with changes in human thyroid hormone levels. Maternal thyroid hormones are essential for adequate fetal neurodevelopment during the first half of pregnancy.
Objective: To evaluate the association between maternal *p,p´*-DDE concentration and the maternal thyroid profile during the first half of pregnancy.
Materials and Methods: We analyzed the information of 430 pregnant women from a Mexican floriculture area, with a gestational age ≤16 weeks. By questionnaire, we obtained sociodemographic, reproductive, and lifestyle, information. Serum concentrations of thyroid stimulating hormone (TSH), and total and free T3 and T4 were determined by means of Enzyme-Linked ImmunoSorbent Assay (ELISA). *p,p´*-DDE was analyzed by Gas Chromatography. The association between *p,p´*-DDE and thyroid profile was assessed through linear and logistic regression models.
Results: Thirty eight percent of women had *p,p´*-DDE levels below the Limit of Detection and 12.3% below the Limit of Quantification. Within the quantifiable range, median was 53.03 ng/g. TSH > 2.5 mIU/L was present in 9.3% of women; 47.7% had isolated hypothyroxinemia; 3.5% had subclinical hypothyroidism, and 5.8% had overt hypothyroidism. We observed a significant positive association between quantifiable *p,p´*-DDE and total T3 serum levels in comparison with those with concentrations below the Limit of Detection (β = 0.19; 95% CI = 0.06, 0.34). There were no significant associations with other hormones of the thyroid profile or with clinical diagnosis.
Conclusions: Our findings suggest that *p,p´*-DDE exposure, even at low concentrations, could disrupt thyroid homeostasis during pregnancy.

1. Introduction

Thyroid hormones (TH) play a basic role in human brain maturation; therefore, reduction in TH availability during critical periods of embryonic and fetal development has been associated with psychomotor delay during infancy. The fetal thyroid gland begins to concentrate iodine after week 11 of gestation and at the maturation of the fetal hypothalamus-hypophysis-thyroid axis efficiently secretes iodized hormones at approximately week 18 (Taylor and Lebovic, 2007; Cooper et al., 2007). Thus, during the first half of pregnancy, the fetus depends on the adequate maternal thyroid function for proper development of the nervous system.

Prenatal exposure to DDT and *p,p´*-DDE, its main metabolite, has been associated with impaired neurodevelopment in humans (Eskenazi et al., 2009; Torres-Sánchez et al., 2013). Because organochlorine (OC) compounds are considered endocrine disruptors, they may mimic or interfere the action of thyroid hormones (Kezios et al., 2013), so thyroid disruption has been one of the biological mechanisms proposed to explain their neurotoxic effect (Porterfield, 2000; Corlbon, 2004). Some studies conducted in animal models have shown that DDT and *p,p´*-DDE

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reduce the expression of deiodinase 2 (D2), compete for the transporter proteins of thyroid hormones (TransThyRetin [TTR] and Thyroid Binding Globuline [TBG]), induce hepatic enzymes of the CYP450 complex and glucuronyltransferase, and act on the thyroid hormone receptors located within the hypothalamus-hypophysis-thyroid axis, altering thyroid homeostasis (Liu et al., 2011, 2014).

In humans, the association between *p,p´*-DDE and the thyroid function has been studied to a lesser extent. Studies carried out in pregnant women are scarce, and show inconsistent results. Some authors report a negative association between *p,p´*-DDE levels and total Triiodothyronine (total T3) (Takser et al., 2005); others find a negative association with free Thyroxine (free T4) and a positive one with Thyroid Stimulating Hormone (TSH) (Lopez-Espinosa et al., 2009), whereas three other studies find no association (Chevrier et al., 2008; Alvarez-Pedrerol et al., 2009; Kim et al., 2013).

In Mexico, DDT was widely used in agriculture and in malaria control campaigns and other vector-borne diseases. Its use for the control of agricultural pests was restricted in 1991, and in 1999, for the control of malaria (Yáñez et al., 2002). However, due to its high persistence in the environment, recent studies have revealed the presence of *p,p´*-DDE in serum samples (Trejo-Acevedo et al., 2012), adipose tissue (Galván-Portillo et al., 2002; Waliszewski et al., 2004), umbilical cord blood (Barraza-Vázquez et al., 2008), and maternal milk (López Guzmán et al., 2006).

Estado de México, Mexico, is a geographical area where floriculture represents one of the main economic activities and DDT was used to control pests that affected the flower crops. In a previous study in male floriculture workers, conducted by our research group in this area, we found a positive association between *p,p´*-DDE serum levels and total T3 and total T4, and a non-significant TSH reduction (Blanco-Muñoz et al., 2016). To date, we do not have knowledge about studies in Mexico that have evaluated this topic in pregnant women. Given the relevance of the thyroid function during pregnancy, the objective of this study was to evaluate the association between serum *p,p´*-DDE concentrations and maternal thyroid profile in pregnant women residing in a floriculture area of Estado de México.

2. Materials and methods

2.1. Study design and population

Between June 2013 and December 2015, a pregnancy cohort study was assembled in a floriculture area of Estado de Mexico, Mexico. The main objective was to evaluate the effect of *p,p´*-DDE and organophosphate pesticides exposure during pregnancy on the occurrence of adverse reproductive outcomes, with a particular interest in maternal thyroid profile.

Pregnant women were identified at local health centers during their first prenatal visit or at the corresponding laboratory when pregnancy confirmation test performed. Study inclusion criteria were the following: women between the ages of 15 and 40 years; residents of the study area for ≥ 1 year; whose pregnancy was confirmed by means of the determination of human chorionic gonadotropin, and whose gestational age was ≤ 16 weeks. We excluded from the study women with antecedents of chronic diseases (cancer, cardiovascular diseases, nephropathies, and diabetes) or endocrine diseases (hypo- or hyperthyroidism, suprarenal pathology). Eligible women were informed of the study objectives and were invited to participate. A signed informed consent was obtained from them. The study was approved by the Ethics Committee of the National Institute of Public Health (Instituto Nacional de Salud Pública [INSP]) of Mexico.

From 635 pregnant eligible women, 480 (75.6%) accepted to participate in the study. The main reason for non-participation in the study was lack of time. This report corresponds to 430 pregnant women who had complete questionnaire information, and whose *p,p´*-DDE and TH results at baseline were available. None significant differences in relation to sociodemographic characteristics (age, marital status, occupation), number of pregnancies, habits (alcohol and tobacco consumption), and gestational age at study entry, were observed between these women and the remainder of the participants (data not shown).

2.2. Data collection

Baseline information was obtained by means of a structured questionnaire that included questions on the sociodemographic characteristics of the pregnant women (age, schooling, occupation, marital/cohabitant status, and residential address), reproductive history (number and outcomes of previous pregnancies), habits (alcohol, tobacco, and coffee consumption during their lifetime and during the current pregnancy), as well as information on the intake of vitamin supplements that contained iodine, from 3 months before pregnancy until the time of the interview. We also obtained information on age, schooling, and occupation of the pregnant woman's partner. All questionnaires were applied by trained personnel who were unaware of the study hypothesis and who also carried out the anthropometric measurements (weight and height) according to standardized procedures.

2.3. Blood and urine sample collection

On the same day that the interview was conducted, we obtained a blood sample (10 mL) collected in Vacutainer tubes; samples were centrifuged at 2500 rpm. From each participant, two serum aliquots were kept at –80 °C until its analysis; one of them in Eppendorf cryovials for thyroid hormones determination and another one in glass cryovials prewashed with hexane and covered with a Teflon top for *p,p´*-DDE determinations. Also, a spot urine sample was collected and maintained at –80 °C.

2.4. Laboratory determinations

2.4.1. Determination of p,p´-DDE

Serum concentrations of *p,p´*-DDE were determined at the Toxicology Laboratory of the Centro de Investigación y Estudios Avanzados (CINVESTAV), Instituto Politécnico Nacional (IPN), using Gas Chromatography with Electron Capture Detector (GC-ECD) (Agilent model 7890) and following the protocol recommended by the U.S. Environmental Protection Agency (1980) (US EPA 1980). The concentration of lipids (mg/dL) was assessed with the SPINREACT colorimetric kit. *p,p´*-DDE concentrations were reported in ng/mL in wet basis and in ng/g in lipid basis.

For every 10 samples, we analyzed one sample by duplicate and the coefficient of variation was < 10%. The recovery percentage of the samples enriched with 40 µL of aldrin (1×10^{-7} mg/mL) was maintained between 92.99% and 99.36%. Limit of Detection (LOD) and Limit of Quantification (LOQ, or traces) for the wet basis were 0.076 and 0.232 ng/mL, respectively, while for the lipid basis, these were 1.75 and 5.3 ng/g.

2.4.2. Determination of TSH and TH

Serum concentrations of TSH and of total and free T3 and T4 were determined by Enzyme-Linked ImmunoSorbent Assay (ELISA), using an automated immunoassay system (DRG International, Inc., USA). Reference values for the concentrations of thyroid hormones in the pregnant women were assessed according to the information provided in the commercial kits, as follows: a) TSH from 0.5 to 5.0 mlU/L; b) free T3 from 1.8 to 4.2 pg/mL and total T3 from 0.71 to 1.75 ng/mL, and c) free T4 from 0.76 to 2.24 ng/dL and total T4 from 4.71 to 23.7 µg/dL.

The intra-assay coefficients of variation of serum TSH, free T4, free T3, total T_4 and total T3 were: 3.36–3.88%, 3.2–10.9%, 3.1–4.9%, 3.9–4.3% and 3.2–9.6% respectively. The interassay coefficients of variation were: 3.3–9.1%, 7.9–10.8%, 10.2–13.1%, 2.4–4.5% and 1.4–10.3% respectively. The analytical sensitivity for TSH, free T4,

free T3 total T4 and total T3 were: 0.06 mIU/mL, 0.05 ng/dl, 0.05 pg/mL 0.5 μg/dl and 0.2 ng/mL respectively.

Measurements were performed at the Biology Laboratory of the Universidad Autónoma de Coahuila (UAC).

2.4.3. Determination of iodine in urine

In a subsample of 103 women we measured the urinary iodine concentration using an iodine-digesting ammonium persulfate method (Dunn et al., 1993). All samples were analyzed by duplicate and the coefficient of variation was < 5%. According to the criteria of the World Health Organization (WHO) for pregnant women, iodine deficiency was considered as a concentration < 150 μg/L (WHO, 2013).

Measurements were performed at the Nutrition Laboratory of the Universidad Autónoma del Estado de Hidalgo (UAEH).

2.5. Statistical analysis

We carried out a cross-sectional analysis of baseline data obtained from pregnant women. Descriptive statistics were used to describe the study population. Because a high percentage of the participants presented non-quantifiable (12.3%) or non-detectable (37.9%) *p,p´*-DDE levels, we stratified this variable in three categories: ≤LOD, > LOD but < LOQ, and ≥LOQ. In wet as well as in lipid basis, these categories included the same women.

Concentrations of total and free T3 and T4 showed a nearly normal distribution; thus, they were used untransformed in the statistical models. In contrast, TSH distribution was skewed to the right and therefore transformed into its natural logarithm. In addition, total and free T3 and T4 were categorized according to laboratory reference values. Because TSH levels decrease physiologically during pregnancy, as consequence of changes in thyroid homeostasis, we categorized them following the American Thyroid Association (ATA) criteria (Stagnaro-Green et al., 2011), which establishes a range of 0.1–2.5 mIU/L for pregnant women, and considers that concentrations > 2.5 mlU/L are suggestive of hypothyroidism. Isolated hypothyroxinemia was considered as TSH concentrations < 2.5 mlU/L accompanied by a free T4 concentration < 0.76 ng/dL. Subclinical hypothyroidism was considered in women having TSH concentrations > 2.5 mlU/L accompanied with a free T4 concentration ≥0.76 ng/dL. Overt hypothyroidism was considered with TSH concentrations > 2.5 mlU/L and a free T4 concentration < 0.76 ng/dL.

The associations between categorical *p,p´*-DDE serum concentrations with each thyroid hormone was estimated using independent linear regression models. To evaluate a dose–response relationship, categorical *p,p´*-DDE was also included in the statistical models as an ordinal variable (1: < LOD; 2: > LOD but < LOQ, and 3: ≥LOQ). A p value < 0.05 was considered as significant. The association between *p,p´*-DDE concentrations with TSH > 2.5 mIU/L(Yes or No), isolated hypothyroxinemia (Yes or No), subclinical hypothyroidism (Yes or No), and overt hypothyroidism (Yes or No), were evaluated using independent logistic regression models.

Because some authors have questioned the convenience of including serum lipid concentration as an adjustment variable in models evaluating the association between lipophilic chemicals and thyroid profile (Chevrier, 2013), for all estimations, we built two models: a) a model including *p,p´*-DDE variable in wet basis, without including total serum lipids as an independent adjustment variable, and b) a model in which *p,p´*-DDE in wet basis was included, and total serum lipids were included as a covariable.

Due to biological considerations, all final models were adjusted by maternal age (in years) and gestational age (in weeks). Other potential confounders evaluated included: Body Mass Index (BMI) [kg/m^2]); years of schooling; occupational pesticides exposure during current pregnancy (woman, partner or both); active smoking, categorized according to the consumption or non-consumption of tobacco at some time during the individual's life, including the current pregnancy (Yes or No); alcohol consumption, classified as never, consumed in the past, and consumed during the current pregnancy; consumption of at least one cup of coffee during current pregnancy (Yes or No); intake of multivitamin supplements that contained iodine from 3 before pregnancy until the time of the interview (Yes or No), and time of the day of the sample-taking. Only those variables that changed, by > 10%, the association between the *p,p´*-DDE and thyroid hormones were maintained in final models.

The analysis was performed with the Stata ver. 14 statistical software package (Stata Corp., TX, USA).

3. Results

3.1. Characteristics of the Study Population

Average gestational age at study entry was 10 ± 2.86 weeks (range: 4–16 weeks). Most women were young (24.0 ± 5.75 years), married or living in common law marriage (88%), and with average schooling of 9 years ± 2.58. At least one previous pregnancy was reported by 59% of women, and 37% had a history of adverse reproductive outcomes, mainly abortion (19%). Around 34% of women reported previous tobacco smoking, but only two smoked during the current pregnancy. Consumption of alcoholic beverages during the current pregnancy was reported by 5% of women, and around 50% consumed daily at least one cup of coffee. Eleven percent of women consumed supplements containing iodine from 3 months before pregnancy until interview. Few women were occupationally exposed to pesticides during the current pregnancy (1%); however, 38% of their partners had work activities related to pesticide exposure (Table 1).

Table 1
Selected characteristics of the study population.

Maternal characteristics	*N* = 430[a]
Age (years)	
Mean ± SD	24.00 ± 5.75
Marital status (%)	
Married/Common law	378 (87.91)
Educational level (years)	
Mean ± SD	9.16 ± 2.58
Parity (%)	
None	176 (40.93)
1	111 (25.81)
≥2	143 (33.25)
Adverse reproductive outcomes[b] (%)	
Yes	93 (36.61)
Body mass index (kg/m^2)[c]	
Mean ± SD	24.4 ± 4.37
History of active smoking (%)	
Yes	145 (33.72)
Alcohol consumption (%)	
Never	144 (33.49)
Before pregnancy	264 (61.39)
During pregnancy[c]	21 (4.88)
Coffee intake[c] (%)	
≥1 cup per day	218 (50.69)
Iodine supplementation[d] (%)	
Yes	46 (10.70)
Occupational exposure to pesticides[c](%)	
Only woman	5 (1.16)
Partner	165 (38.37)
Both	5 (1.16)

[a] Differences in *N* are due to missing values

[b] This information takes into account women with previous pregnancy(ies) (n = 254) and included previous abortion, miscarriages, low birth weight, prematurity, and congenital malformations.

[c] During pregnancy (≤16 gestational weeks)

[d] From three months before pregnancy until the interview. SD = Standard Deviation.

Table 2
Maternal p,p′-DDE concentrations and thyroid hormones during the first 16 weeks of pregnancy.

Variable	*N* = 430	(%)	Mean ± SD[a]	P^{b}_{10}	P^{b}_{50}	P^{b}_{90}
p,p′-DDE ng/mL[c]						
< 0.076[c]	163	37.9				
From 0.076 to < 0.232[d]	53	12.3				
≥0.232	214	49.8	2.81 ± 4.07	0.38	1.2	6.0
p,p′-DDE ng/g[d]						
< 1.75[c]	163	37.9				
From 1.75 to < 5.30[e]	53	12.3				
≥5.30	214	49.8	111.75 ± 140.20	19.02	53.03	282.59
TSH (mUI/L)			1.20 ± 1.25	0.21	0.85	2.43
< 0.1	7	1.6				
> 2.5	40	9.3				
Triiodothyronine (T3)						
Free			2.99 ± 2.47	2.12	2.67	3.55
< 1.8 (pg/mL)	31	7.2				
Total			1.41 ± 0.63	0.74	1.32	2.22
< 0.71 (ng/mL)	39	9.1				
Thyroxine (T4)						
Free			0.80 ± 0.35	0.58	0.75	0.97
< 0.76 (ng/mL)	230	53.5				
Total			6.04 ± 2.79	2.72	5.77	9.65
< 4.71 (μg/dL)	132	32.3				

[a] Arithmetic mean and Standard Deviation (SD)
[b] Percentiles of the distribution
[c] Geometric mean and SD for p,p′-DDE in wet basis = 1.63 ± 1.43 ng/mL and for p,p′-DDE in lipid basis = 63.62 ± 4.96 ng/g;
[d] Limit Of Detection (LOD);
[e] Limit Of Quantification (LOQ).

3.2. Serum p,p´-DDE, TH concentrations and urinary iodine concentrations

Table 2 shows maternal *p,p´*-DDE and thyroid hormone serum level distribution. Thirty eight percent of women had *p,p´*-DDE below LOD (0.076 ng/mL), and 12.3% had concentrations below LOQ (0.232 ng/mL). Within the quantifiable range, the median was 1.2 ng/mL in wet basis and 53.09 ng/g in lipid basis.

According to ATA criteria for TSH and free T4 concentrations, approximately 9% of women presented TSH serum concentrations > 2.5 mIU/L and 53.5% had free T4 concentrations < 0.76 ng/dL. Isolated hypothyroxinemia was identified in 205 women (47.6%), 15 (3.5%) had subclinical hypothyroidism, and 25 women (5.8%) had overt hypothyroidism. In a subsample of 103 pregnant women, the median urinary iodine concentration was 246 μg/L. Women who reported iodine supplements intake, had higher median urinary concentrations (285 μg/L) than those who did not (242 μg/L), p = 0.09, by Mann-Whitney test. Furthermore, 27 women (26.2%) had iodine concentrations < 150 μg/L (Data not shown in Tables).

3.3. Association between covariables of interest and thyroid hormones

Compared with never smokers, history of active smoking was marginally associated with a lower TSH concentration (β = −0.17; 95% CI = −0.36, 0.13; p < 0.10). Gestational age and BMI were positively associated with total T3 (β = 0.04; 95% CI = 0.02, 0.06, and β = 0.01; 95% CI = 0.002, 0.03, respectively). Gestational age was also positively associated with total T4 (β = 0.11; 95% CI = 0.04, 0.23). Women with two or more pregnancies had, on average, lower concentrations of free T4 (β = −0.07; 95% CI = −0.15, −0.01) than primiparous. Alcohol consumption before pregnancy and iodine supplementation were associated with lower free T3 (β = −0.23; 95% CI = −0.49, 0.04 and β = −0.36; 95% CI = −0.75, 0.04, respectively), whereas partners occupational exposure to pesticides during pregnancy increased free T3 serum levels (β = 0.25; 95% CI = −0.005, 0.50); however, all these association were marginally significant (p < 0.10). No other significant associations were observed (Table 3).

3.4. Association between p,p´-DDE and thyroid hormones

Women with quantifiable *p,p´*-DDE serum levels had significantly higher concentrations of total T3 (β = 0.19; 95% CI = 0.06, 0.34) than those having levels below LOD. Women with concentrations between LOD and LOQ also presented higher but no significant total T3 levels than the reference group (β = 0.04; 95% CI = −0.16, 0.24); however a significant dose-response gradient was observed (*p* for trend = 0.005). These results did not change when total serum lipid concentrations were included in the model (β = 0.20; 95% CI = 0.06, 0.33 and β = 0.04; 95% CI = −0.16, 0.25, respectively; *p* for trend = 0.004). *p,p´*-DDE levels were not associated with other thyroid hormones.(Table 4).

In the logistic regression models, after adjustment for potential confounders (age, gestational age and BMI), women with quantifiable *p,p´*-DDE levels had higher odds of having TSH > 2.5 mIU/mL than women with p,p′-DDE levels below LOD (OR = 1.73; 95% CI = 0.77, 3.85), but this association was not statistically significant. No association was observed with isolated hypothyroxinemia (OR = 1.00; 95% CI = 0.61, 1.66), with subclinical hypothyroidism (OR = 1.00; 95% CI = 0.31, 3.31), or with overt hypothyroidism (OR = 1.81; 95% CI = 0.68, 4.82). Similar results were obtained after adjustment for serum lipid levels (data not shown).

4. Discussion

Our results showed that *p,p´*-DDE, even at relatively low concentrations, was positively associated with serum maternal total T3 levels during the first half of the pregnancy, without significant associations with TSH or other thyroid profile hormones.

To our knowledge, this is the first study in Mexico that had evaluated the association between *p,p´*-DDE exposure and the thyroid profile in pregnant women. We observed low *p,p´*-DDE concentrations in our study population; this could be because the use of DDT for malaria control in Estado de México was lower than in other Mexican areas (Centro Nacional de Salud Ambiental y Vigilancia Epidemiológica, 2000), and the age of our participants, who were mainly young and had no occupational exposure, reducing the chances

Table 3
Crude association between selected maternal characteristics and thyroid hormone concentrations during the first 16 weeks of pregnancy.

Characteristics			Triiodothyronine (T3)				Thyroxine (T4)			
	TSH		Free		Total		Free		Total	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Age (years)	−0.008	−0.007, 0.02	0.002	−0.02,0.02	0.007	−0.002, 0.01	−0.002	−0.008,0.001	−0.001	−0.05, 0.03
Educational level (years)	0.20	−0.01, 0.05	−0.001	−0.05, 0.05	−0.02	−0.04, 0.01	−0.008	−0.04, 0.02	0.04	0.14, 0.06
Parity										
1	−0.20	−0.43, 0.01	0.001	−0.31, 0.31	0.11	−0.03, 0.23	0.03	−0.04, 0.11	0.15	−0.50, 0.82
≥2	−0.008	−0.21, 0.20	0.10	−0.19, 0.38	−0.03	−0.10,0.17	−0.07	−0.15, −0.01*	0.15	−0.46, 0.77
Gestational age (weeks)	−0.005	−0.03, 0.02	0.01	−0.03, 0.05	0.04	0.02, 0.06**	−0.01	−0.02, 0.01	0.11	0.04, 0.23**
Body mass index (kg/m^2)	0.001	−0.17, 0.02	0.001	−0.03, 0.03	0.01	0.002, 0.03*	−0.001	−0.01, 0.01	−0.01	−0.07, 0.05
History of active smoking Yes vs. No	−0.17	−0.36, 0.13	−0.24	−0.49, 0.02	0.01	−0.11, 0.14	0.01	−0.06, 0.07	0.03	−0.55, 0.56
Alcohol consumption										
Before pregnancy	−0.04	−0.24, 0.14	−0.23	−0.49, 0.04	0.04	−0.07, 0.17	−0.05	−0.12, 0.02	0.23	−0.32, 0.88
During pregnancy[a]	0.02	−0.48, 0.53	0.08	−0.61, 0.77	0.22	−0.11, 0.56	−0.10	−0.29, 0.08	0.60	−0.80, 2.08
Occupational exposure to pesticides[a]										
Only woman	−0.28	−1.12, 0.55	0.13	−1.15, 1.42	−0.06	−0.63, 0.49	−0.0.02	−0.33, 0.29	1.03	−1.44, 3.51
Partner	0.02	−0.15, 0.21	0.25	−0.005, 0.50	−0.07	−0.19, 0.05	−0.06	−0.13, 0.01	0.41	−0.13, 0.96
Both	−0.40	−1.24, 0.43	−0.05	−1.20, 1.10	0.10	−0.39, 0.73	−0.01	−0.31, 0.31	1.94	−0.53, 4.42
Iodine supplementation[b] Yes vs. No	−0.13	−0.42, 0.15	−0.36	−0.75, 0.04	−0.17	−0.01, 0.36	−0.20	−0.08, 0.13	−0.08	−0.77, 0.94

95% CI = 95% Confidence Interval.
[a] During pregnancy (≤16 gestational weeks);
[b] From three months before pregnancy until the interview.
* $p < 0.05$
** $p < 0.01$.

Table 4
Adjusted association between maternal p,p′-DDE serum levels and thyroid hormone concentrations during the first 16 weeks of pregnancy.

Organochlorine compound	TSH		Triiodothyronine (T3)				Thyroxine (T4)			
			Free		Total		Free		Total	
	β[a]	95% CI	β[a,b]	95% CI	β[a,c]	95% CI	β[a,d]	95% CI	β[a]	95% CI
p,p′-DDE (ng/mL)[e]										
0.076 to < 0.23	0.23	−0.07, 0.53	−0.04	−0.45, 0.37	0.04	−0.16, 0.24	0.04	−0.07, 0.14	−0.17	−1.03, 0.69
≥0.23	0.01	−0.18, 0.21	−0.13	−0.41, 0.14	0.19	0.06, 0.34	−0.05	−0.11, 0.02	0.13	−0.45, 0.70
p value for trend					**0.005**					
p,p′-DDE (ng/mL)[f]										
0.076 to < 0.23	0.25	−0.13, 0.64	−0.03	−0.43, 0.38	0.04	−0.16, 0.25	0.04	−0.06, 0.14	−0.15	−1.01, 0.70
≥0.23	0.14	−0.12, 0.40	−0.04	−0.32, 0.24	0.20	0.06, 0.33	−0.04	−0.11, 0.03	0.19	−0.39, 0.76
p value for trend					**0.004**					

[a] All models were adjusted by maternal and gestational age and the reference group was < Limit of Detection (LOD);
[b] Model was also adjusted for occupational exposure to pesticides during pregnancy;
[c] Model was also adjusted for Body Mass Index (BMI);
[d] Model was also adjusted for Body Mass Index (BMI) and occupational exposure to pesticides during pregnancy;
[e] p,p′-DDE wet basis;
[f] p,p′-DDE wet basis adjusted by serum lipids in the model.

of DDT exposure. Additionally, a study carried out in 2010 in Mexico City (Orta-García et al., 2014) observed a *p,p´*-DDE geometric mean (GM) of 8.90 ng/g which was lower than the GM found in our study; suggesting that *p,p´*-DDE concentrations have decreased in recent years in areas where malaria is not endemic.

Compared it with other studies on the subject (Table 5) our study reports a positive association with total T3, whereas Takser et al. in Quebec, Canada (2005), reported a negative association (β = −0.54; $p < 0.05$). Alvarez-Pedrerol et al. (2009) who studied the association between *p,p´*-DDE and thyroid hormones in two birth cohorts in Spain found a no statistically significant association between *p,p´*-DDE and total T3 (β = −1.8; $p > 0.05$), however using Generalized Additive Models, the figure showed a strong decline of total T3 when DDE levels were above 50 ng/gr for both independent cohorts The rest of the studies did not find significant associations (Lopez-Espinosa et al., 2009; Kim et al., 2013). Lopez-Espinosa et al. (2009) in Valencia, Spain, reported that the increase in *p,p´*-DDE concentration reduced free T4 concentration (β = −0.03; 95% CI 95% CI = −0.05; 0.00), and that pregnant women with higher levels of *p,p´*-DDE had higher odds of having TSH levels > 2.5 mIU/L (OR = 2.53; 95% CI = 1.36; 4.73). However, none of the other studies summarized in Table 5 (Takser et al., 2005; Chevrier et al., 2008; Alvarez-Pedrerol et al., 2009; Kim et al., 2013), including ours, observed an association with these hormones. In Seoul, South Korea, Kim et al. (2013) observed that *p,p´*-DDE plus *p,p´*-DDT levels was associated with a reduction of free T4; however, *p,p´*-DDE by itself was not associated with any of the thyroid hormones evaluated.

Comparability among studies was limited because the complete thyroid profile was only measured by Kim et al. (2013) and by our study group. Additionally, only Chevrier et al. (2008) and Lopez-Espinosa et al. (2009) presented data about the frequency of hormone concentrations outside the normal range, and only the latter evaluated the association between *p,p´*-DDE and TSH > 2.5 mIU/L. In the study of Chevrier et al. (2008), the frequency of women with TSH > 2.5 mIU/L was very low, and probably did not allow this type of analysis. In addition, the studies differ in the level of exposure *to p,p´*-DDE, gestational age at which samples were collected, and in the availability of information about iodine levels. Regarding exposure, nearly 50% of

Table 5
Comparison with previous studies that have evaluated the association between p,p′-DDE) exposure and thyroid profile during pregnancy.

Ref	Author, Country, Year	*N*	Sample recollection		p,p′-DDE levels	Thyroid profile					Thyroid profile alterations (%)
			Period	Gestational age		TSH	TT3	FT3	TT4	FT4	
-	Hernández-Mariano et al., Mexico, 2016	409	2013–2015	All women: ≤16 weeks (4–16)	Median[a] 1.20 ng/mL 53.03 ng/g Geometric mean [a] 63.62 ng/g	NA	↑	NA	NA	NA	TSH > 2.5 IUml/L: 9.3% T4 free < 0.76 ng/dl: 53.5%
17	Kim et al., Korea, 2013	138	2011	All women: Third trimester (a day before delivery)	Median 57.37 ng/g	NA	NA	NA	NA	NA	No information
18	Lopez-Espinoza et al., Spain, 2009	157	2003–2005	All women: 12 weeks ((10–13)	Median 176 ng/g	↑	NA	NE	NE	↓	TSH > 2.5 mUI/L: 11.5%
16	Alvarez–Pedrerol et al., Spain, 2009	1297	2006–2008	All women: 12 weeks (10–13)	Median Sabadell:112.2 ng/g Guipuzkoa:89.9 ng/g	NA	NA	NE	NE	NA	No information
9	Chevrier et al., U.S., 2008	334	1999–2000	26 week: 320 women Before delivery: 14 women	Geometric mean 1302 ng/g	NA	NE	NE	NA	NA	TSH > 2.5 mUI/L: 5.1% T4 free < 1.8 mUI/L: 2.64%
16	Takser et al., Canada, 2005	149	Not specified	1st trimester: 40 women 2nd trimester (14–24 weeks): 109 women	Median 0.43 ng/mL	NA	↓	NE	NE	NA	No information

↓: negative association;↑: positive association; TT3: total T3; FT3: free T3; TT4:total T4; FT4: free T4.
[a] Based on quantifiable values. NA: No Association; NE: Not Evaluated.

women in our study had *p,p´*-DDE concentrations below LOQ and/or LOD, and within the quantifiable range, values were in general terms lower than those in similar studies. In relation with gestational age, in our study all samples were taken during the first 16 weeks of pregnancy, whereas three of the previous studies obtained their samples well into the second half of pregnancy (Takser et al., 2005; Chevrier et al., 2008; Kim et al., 2013), and in the studies of Lopez-Espinosa et al. (2009) and Alvarez-Pedrerol et al. (2009), samples were collected between weeks 10 and 13. Urinary iodine concentrations were only considered by Alvarez-Pedrerol et al. (2009) and by Lopez-Espinosa et al. (2009), without confounding or modifying their results. In our study we were only able to determine urinary iodine in a subsample of pregnant women (103), founding that iodine deficiency had a low prevalence in the study area. The intake of iodine supplement did not confound the association between *p,p´*-DDE and thyroid hormones.

The small number of previous studies did not allow us to observe an effect pattern, however, the inconsistencies among studies suggest that the effects of *p,p´*-DDE on the thyroid profile during pregnancy could differ according to the range of exposure. This hypothesis was proposed by Langer et al. (2007), who found that OC compounds, specifically PCBs, at low concentrations are negatively associated with free T4 and total T3 levels, whereas elevated concentrations are positively associated. Likewise, the gestational period at which biological samples were taken is relevant because concentrations of thyroid hormones change along pregnancy; Abdelouahab et al. (2013), observed that exposure to polybromodiphenyl Ethers (PBDE), whose chemical structure and properties are similar to those of OC, was differentially associated with thyroid hormones depending on gestational age. Before week 20 of pregnancy, PBDE was positively associated with free T3 and T4 and negatively with total T3 and T4, whereas inverse associations with free T3 and total T4 were found when the evaluation was carried out at delivery. Finally, there can be differences in the characteristics of the populations studied, such as iodine status, exposure to other endocrine disruptors and immunological or genetic factors, which could modify or confound the observed results.

An important difference between our study population and the other studies is the high frequency of isolated hypothyroxinemia here reported. In Mexico, information about thyroid profile during pregnancy is scarce. A recent study including pregnant women without history of thyroid disease, reported that the prevalence of isolated hypothyroxinemia was 12.8%; however, the whole frequency of thyroid disorders was 46.7% (Cruz-Cruz et al., 2014); this suggests that prevalence of thyroid disorders during pregnancy in Mexico, could be greater than that reported by literature. In this regard, our findings may be explained by other potential thyroid disruptors, such as organophosphate and dithiocarbamate pesticides employed in floriculture, unfortunately we did not have available information about biomarkers to adjust for simultaneous exposures. On the other hand, given that iodine deficiency was not frequent in this population, and that autoimmune disease is the main cause of thyroid dysfunction in iodine-sufficient regions (Wier and Farley 2006), the identification of antithyroid antibodies, a parameter not evaluated in any of the previous studies, could contribute to explain the high frequency of isolated hypothyroxinemia that we observed.

Regarding the biological mechanisms involved in the thyroid disrupting effects of *p,p´*-DDE, the information was derived from studies carried out in male Sprague-Dawley rats, where exposure to *p,p´*-DDE reduced the concentrations of TTR, the main transporter protein of thyroid hormones in rodents, which translated into reductions of total and free T4 concentrations (Liu et al., 2011). Likewise, combined exposure to PCB153 and *p,p´*-DDE significantly reduced serum concentrations of TBG and TTR, suppressed the expression of type 2 deiodinase mRNA, which intervenes in the deiodination of T4 into T3, and induced the expression of hepatic enzymes UGT, CYP1A1, CYP2B1, and CYP3A1, which in turn increases T4 hepatic metabolism and biliary excretion (Liu et al., 2014). In general terms, these mechanisms, are compatible with a reduction of free T4 concentrations. However, a study conducted in male Wistar rats exposed for 6 weeks to low doses of DDT, showed an increase in the peripheral conversion of T4 into T3, with reduction of TSH levels and the thyroid gland morphological changes typical of iodine deficiency. The authors suggest that DDT would inhibit follicular iodine uptake, and that the increase in T3 could comprise an initial compensatory mechanism (Yaglova and Yaglov, 2014). This could contribute to explain the positive association between *p,p´*-DDE exposure and total T3 here reported; however, in the long term, this mechanism would lead to hypothyroidism after exhausting the mechanisms of compensation. Nevertheless, there are important differences in thyroid homeostasis between rodents and humans, such as only 20% of circulating T3 results from thyroid secretion in humans, while the remainder derives from the peripheral deiodination of T4. Additionally, TBG is the main transporter protein of thyroid hormones in humans, whereas in rodents, these proteins comprise TTR and albumin, which possess less affinity for thyroid hormones, therefore leading to the shortening of their half-life (Takser et al., 2005). These interspecies differences, added to the physiological modifications of the thyroid function that take place during human gestation, do not permit to extrapolate rodent's findings to pregnant women.

To adequate interpretation of our results we need to take in account some considerations. Although in the analyses we controlled for occupation of woman's partner and the woman's occupation, considered as “proxies” of exposure to other thyroid disruptor pesticides, we were not able to discard the existence of some degree of residual confusion. Likewise, we only adjusted for the intake of supplements that included iodine in their formulation as a proxy of the iodine status; the absence of information did not allow us to evaluate whether the association observed between *p,p´*-DDE and total T3 or the lack of association with other thyroid hormones is modified by iodine. Similarly to previous studies, the transversal approach of this analysis did not permit the establishment of the time sequence between exposure and effect. Thyroid hormones regulate lipid metabolism consequently, they may alter the serum concentration of lipophilic compounds, thus, the possibility of there being reverse causality between *p,p´*-DDE and total T3 cannot be completely discarded. It is noteworthy that for ongoing exposures such as OCs compounds, even longitudinal studies are subject to reverse causality, since the time ordering of exposure and outcome cannot always be firmly established (Chevrier, 2013). On the other hand, due to that half of the population presented *p,p´*-DDE concentrations below the LOQ/LOD, it was necessary to categorize this variable and, although the associations with total T3 demonstrate a dose-response relationship, the low exposure levels may have limited our capacity to find associations with T4 or TSH. Finally, TSH is a hormone that follows a circadian rhythm (Russell et al., 2008) and, although the biological samples were taken during the early morning hours, they were not collected at the same time, which could reduce comparability and could affect the assessment of the associations. For this reason, the time of sample-taking was a covariable included in the statistical analyses, although this did not confound the results observed and was not maintained in the final models.

Despite the limitations mentioned, we measured the complete thyroid profile in a relatively large sample of women, and our results show a positive and significant association between serum *p,p´*-DDE concentrations and total maternal T3. This suggests that *p,p´*-DDE acts as a thyroid gland disruptor in pregnant women. Although subtle, the modifications in the concentrations of thyroid hormones should be taken with caution, due to that small changes in the thyroid homeostasis of pregnant women could have consequences in very vulnerable populations such as that of fetuses (Kim et al., 2013). Epidemiological studies that have analyzed the effect of exposure to DDT metabolites on pregnant women are scarce and have exhibited inconsistent results; in addition, to our knowledge, the mechanisms by which of these compounds could act as thyroid disruptors in humans have not been elucidated. Therefore, there is a need to conduct more basic and

epidemiological investigations that include immunological information that allow to confirm or refute our findings and to address their biological and clinical relevance.

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Study approval

The research that produced this article followed the guidelines of the Declaration of Helsinki of the World Medical Association. Institutional review board approval was obtained from the Ethics Committee of Instituto Nacional de Salud Pública of México. The participation of subjects did not occur until after informed consent was obtained.

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References

Abdelouahab, N., Langlois, M.F., Lavoie, L., Corbin, F., Pasquier, J.C., Takser, L., 2013. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. Am. J. Epidemiol. 178, 701–713. http://dx.doi.org/10.1093/aje/kwt141.

Alvarez-Pedrerol, M., Guxens, M., Ibarluzea, J., Rebagliato, M., Rodriguez, A., Espada, M., Goñi, F., Basterrechea, M., Sunyer, J., 2009. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environ. Sci. Technol. 43 (20), 7909–7915.

Barraza-Vázquez, A., Borja-Aburto, V.H., Bassol-Mayagoitia, S., Monroy, A., Recio-Vega, R., 2008. Dichlorodiphenyldichloroethylene concentrations in umbilical cord of newborns and determinant maternal factors. J. Appl. Toxicol. 28, 27–34.

Blanco-Muñoz, J., Lacasaña, M., López-Flores, I., Rodríguez-Barranco, M., González-Alzaga, B., Bassol, S., Cebrian, M.E., López-Carrillo, L., Aguilar-Garduño, C., 2016 23. Association between organochlorine pesticide exposure and thyroid hormones in floriculture workers. Environ. Res. 150, 357–363. http://dx.doi.org/10.1016/j.envres.2016.05.054.

Centro Nacional de Salud Ambiental y Vigilancia Epidemiológica (CENSA), 2000. Situación de la Malaria y el uso de DDT en México. Diciembre, 2000.

Chevrier, J., 2013. Invited Commentary: maternal Plasma Plibrominated Diphenil Ehers and Thyroid Hormones-Challenges and Opportunities. Am. J. Epidemiol. 178, 714–719.

Chevrier, J., Eskenazi, B., Holland, N., Bradman, A., Barr, D.B., 2008. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. Am. J. Epidemiol. 168 (3), 298–310.

Colborn, T., 2004. Neurodevelopment and endocrine disruption. Environ. Health Perspect. 112 (9), 944–949.

Cooper, D.S., Greenspan, F.S., Landerson, P.L., 2007. La glándula tiroides. In: Martínez Moreno, M. (Ed.), Endocrinología Básica y Clínica. Manual Moderno, México, pp. 219–292.

Cruz-Cruz, E.A., Ramírez-Torres, A., Pimentel-Nieto, D., Roque Sánchez, A.M., 2014. Prevalence of clinical and subclinical hypothyroidism during pregnancy in a pregnant women population. Ginecol. Obstet. Mex. 82, 717–724.

Dunn, J.T., Crutchfield, H.E., Gutekunst, R., Dunn, A.D., 1993. Two Simple Methods for Measuring Iodine in Urine. Thyroid 3, 119–123.

Eskenazi, B., Chevrier, J., Rosas, L.G., Anderson, H.A., Bornman, M.S., Bouwman, H., Chen, A., et al., 2009. The Pine River statement: human health consequences of DDT use. Environ. Health Perspect. 117, 1359–1467.

FA, Wier, Farley, C.L., 2006. Clinical controversies in screening women for thyroid disorders during pregnancy. J. Midwifery Women's. Health 3, 152–158.

Galván-Portillo, M., Jiménez-Gutiérrez, C., Torres-Sánchez, L., López-Carrillo, L., 2002. Food consumption and adipose tissue DDT levels in Mexican women. Cad. Saude Publica 18, 447–452.

Kezios, K.L., Liu, X., Cirillo, P.M., Cohn, B.A., Kalantzi, O.I., 2013. Dichlorodiphenyltrichloroethane (DDT), DDT Metabolites and Pregnancy Outcomes. Reprod. Toxicol. 35, 156–164.

Kim, S., Park, J., Kim, H.J., Lee, J.J., Choi, G., Choi, S., et al., 2013. Association between several persistent organic pollutants and thyroid hormone levels in serum among the pregnant women of Korea. Environ. Int. 59, 442–448.

Langer, P., Kocan, A., Tajtáková, M., Rádiková, Z., Petrík, J., Koska, J., Ksinantová, L., et al., 2007. Possible effects of persistent organochlorinated pollutants cocktail on thyroid hormone levels and pituitary-thyroid interrelations. Chemosphere 70, 110–118.

Liu, C., Shi, Y., Li, H., Wang, Y., Yang, K., 2011. p,p′-DDE disturbs the homeostasis of thyroid hormones via thyroid hormone receptors, transthyretin, and hepatic enzymes. Horm. Metab. Res. 43, 391–396.

Liu, C., Ha, M., Li, L., Yang, K., 2014. PCB153 and p,p′-DDE disorder thyroid hormones via thyroglobulin, deiodinase 2, transthyretin, hepatic enzymes and receptors. Environ. Sci. Pollut. Res. Int. 21, 11361–11369. http://dx.doi.org/10.1007/s11356-014-3093-3.

Lopez-Espinosa, M.J., Vizcaino, E., Murcia, M., Lop, S., Espada, M., Seco, V., et al., 2009. Association between thyroid hormone levels and 4,4′-DDE concentrations in pregnant women (Valencia, Spain). Environ. Res. 128 (4), 479–485.

López Guzmán, D., Yañez Estrada, L., Athanasiadou, M., Bergman, A., Herrrera, C., Díaz Barriga, F., 2006. Determinación de los niveles de DDT, DDE y DDE-MESO2 en leche materna y sangre de poblaciones contaminadas por DDT en México. Acta Toxicol. Argent. 14, 34–36.

WHO, 2013. Urinary iodine concentrations for determining iodine status deficiency in populations. Vitamin and Mineral Nutrition Information System. World Health Organization, Geneva ⟨http://www.who.int/nutrition/vmnis/indicators/urinaryiodine⟩.

Orta-García, S., Pérez-Vázquez, F., González-Vega, C., Varela-Silva, J.A., Hernández-González, L., Pérez-Maldonado, I., 2014. Concentrations of persistent organic pollutants (POPs) in human blood samples from Mexico City, Mexico. Sci. Total Environ. 472, 496–501.

Porterfield, S.P., 2000. Thyroidal dysfunction and environmental chemicals–potential impact on brain development. Environ. Health Perspect. 108 (Suppl 3), 433–438.

Russell, W., Harrison, R.F., Smith, N., Darzy, K., Shalet, S., Weetman, A.P., Ross, R.J., 2008. Free Triiodothyronine has a distinct circadian rhythm that is delayed but parallels thyrotropin levels. J. Clin. Endocrinol. Metab. 93 (6), 2300–2306.

Stagnaro-Green, A., Abalovich, M., et al., 2011. Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum. Thyroid 21, 1081–1125. http://dx.doi.org/10.1089/thy.2011.0087.

Takser, L., Mergler, D., Baldwin, M., de Grosbois, S., Smargiassi, A., Lafond, J., 2005. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environ. Health Perspect. 113, 1039–1045.

Taylor, R.N., Lebovic. D.I., 2007. Endocrinología del embarazo. In Martínez Moreno M, editor. Endocrinología básica y clínica. México: Manual Moderno; pp. 671–692.

Torres-Sánchez, L., Schnaas, L., Rothenberg, S.J., Cebrián, M.E., Osorio-Valencia, E., 2013. Hernández Mdel, et al. Prenatal p,p´-DDE exposure and neurodevelopment among children 3.5-5 years of age. Environ. Health Perspect. 121 (2), 263–268.

Trejo-Acevedo, A., Rivero Pérez, N.E., Flores, R.R., Varela Silva, J.A., Pérez Maldonado, I.N., 2012. Assessment of the levels of persistent organic pollutants and 1-hydroxypyrene in blood and urine samples from Mexican. Bull. Environ. Contam. Toxicol. 88, 828–832.

U.S. Environmental Protection Agency, 1980. Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples. US Environmental Protection Agency, Washington, DC.

Waliszewski, S.M., Carvajal, O., Infanzón, R.M., Trujillo, P., Hart, M.M., 2004. Copartition ratios of persistent organochlorine pesticides between human adipose tissue and blood serum lipids. Bull. Environ. Contam. Toxicol. 73, 732–739.

Yaglova, N.V., Yaglov, V.V., 2014. Changes in thyroid status of rats after prolonged exposure to low dose dichlorodiphenyltrichloroethane. Bull. Exp. Biol. Med. 156, 760–762.

Yáñez, L., Ortiz-Pérez, D., Batres, L.E., et al., 2002. Levels of dichlorodiphenyltrichloroethane and deltamethrin in humans and environmental samples in malarious areas of Mexico. Environ. Res. 88, 174–181.