



Prenatal exposure to organochlorine pesticides and TSH status in newborns from Southern Spain

Carmen Freire^{a,*}, Maria-Jose Lopez-Espinosa^{a,b}, Marieta Fernández^a, Jose-Manuel Molina-Molina^a, Remedios Prada^a, Nicolas Olea^a

^a Laboratory of Medical Investigations, San Cecilio University Hospital, University of Granada, CIBER de Epidemiología y Salud Pública (CIBERESP), Av. Madrid s/n, 18071 Granada, Spain

^b Department of Social and Environmental Health Research, London School of Hygiene and Tropical Medicine, 15-17 Tavistock Place, WC1H 9SH London, UK

ARTICLE INFO

Article history:

Received 14 December 2010

Received in revised form 10 May 2011

Accepted 18 May 2011

Keywords:

Prenatal exposure

Male birth cohort

Organochlorine pesticides (OCPs)

Thyroid-stimulating hormone (TSH)

Endocrine disruption

ABSTRACT

Objective: To investigate the association between prenatal exposure to organochlorine pesticides (OCPs) and thyroid-stimulating hormone (TSH) levels in male newborns.

Methods: Exposure to 17 OCPs was analyzed in 220 placentas from a male birth cohort in Southern Spain, and TSH was measured in the umbilical cord blood. OCP concentrations were quantified by gas chromatography and mass spectrometry. Multivariate regression analysis was conducted to examine the association between pesticide exposure and neonatal TSH levels, adjusting for confounders.

Results: Newborn boys with higher exposure to endrin in placenta had higher odds of TSH cord blood levels ≥ 5 mU/L (80th percentile) (OR = 2.05; 95% CI = 1.01, 4.18; $p = 0.05$), whereas higher prenatal exposure to endosulfan-sulfate was associated with lower odds of TSH ≥ 5 mU/L (OR = 0.36; 95% CI = 0.17, 0.77; $p = 0.008$). A marginally significant negative association was found between TSH and hexachlorobenzene levels ($\beta = -0.15$; 95% CI = $-0.31, 0.02$; $p = 0.09$), and exposure to *p,p'*-DDE showed a marginally-significant higher odds of TSH ≥ 5 mU/L (OR = 1.32; 95% CI = 0.95, 1.83; $p = 0.09$). No association was found between TSH and the remaining pesticides.

Conclusions: Early exposure to certain environmental chemicals with endocrine-disruption activity may interfere with neonatal thyroid hormone status; however, the pattern of interference is not yet clearly elucidated.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Human exposure to environmental chemicals that disrupt endocrine homeostasis (endocrine disruptors [EDs]) has been mainly related to effects on sex hormones (estrogens and androgens). Thus, many organochlorine compounds (OCs) have been shown to display an estrogenic-like activity (DeCastro et al., 2006; Fernández et al., 2004). Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and dioxins/furans have also been demonstrated to influence thyroid hormone (TH) (thyroxine [T₄], triiodothyronine [T₃] and thyroid-stimulating hormone [TSH]) activity and metabolism in animal studies (Boas et al., 2006). With regard to OCPs, there are considerable animal data on the alteration of TH levels by exposure to *p,p'*-dichlorodiphenyldichloroethene (*p,p'*-DDE) and hexachlorobenzene (HCB) (ATSDR, 2002a, 2002b).

Abbreviations: OCPs, organochlorine pesticides; TSH, thyroid-stimulating hormone; ED, endocrine disruptor; OC, organochlorine compound; TH, thyroid hormone.

* Corresponding author. Laboratory of Medical Investigations, San Cecilio University Hospital, University of Granada, Av. Madrid s/n, 18071 Granada, Spain. Tel.: +34 958240758; fax: +34 958 249953.

E-mail addresses: cfreire@ugr.es (C. Freire), mjlopes@ugr.es (M.-J. Lopez-Espinosa), marieta@ugr.es (M. Fernández), molinajm@ugr.es (J.-M. Molina-Molina), reprama@hotmail.com (R. Prada), nolea@ugr.es (N. Olea).

Thyroid disruption may be caused by various mechanisms, since different chemicals interfere with the hypothalamic–pituitary–thyroid axis at distinct levels. Several OCs are structurally similar to THs and therefore have the potential to bind to proteins and receptors that characteristically bind THs, producing complex effects on TH signaling (Zoeller, 2007). In addition, changes in serum TH concentrations can be caused by chemicals that inhibit TH synthesis, release and metabolism (Boas et al., 2006).

Several studies have investigated associations between OC exposure levels in human tissues and TH status in adults (Langer et al., 2006) and infants (Alvarez-Pedrerol et al., 2008; Asawasinsopon et al., 2006; Lopez-Espinosa et al., 2010; Ribas-Fitó et al., 2003; Takser et al., 2005), with no conclusive results (Boas et al., 2006; Maervoet et al., 2007). Discrepancies in these findings might result from differences in study populations, sample sizes, matrices for exposure measurement (e.g., maternal venous blood, cord blood, breast milk), specimen collection time points and/or isomers. Investigations into the effects of early OC exposure on human thyroid function have largely studied PCBs and dioxins (Maervoet et al., 2007; Wang et al., 2005). Recent studies suggested that *p,p'*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane), *p,p'*-DDE, β -hexachlorocyclohexane (β -HCH) and HCB may also alter the thyroid system (Alvarez-Pedrerol et al., 2008, 2009a, 2009b; Takser et al., 2005). However, little is known about the disrupting

potential of OCPs other than DDT, HCB, or HCH, e.g., mirex, aldrin and dieldrin (Asawasinsopon et al., 2006; Takser et al., 2005).

THs play an important role during early human development and it has been speculated that some of the neurotoxic and reproductive effects of early exposure to environmental EDs may result from disruption of the thyroid system (Williams, 2008). Thus, studies in infants have focused on the association between THs and adverse health effects such as low birth weight, growth retardation and altered psychomotor and cognitive functions (Alvarez-Pedrerol et al., 2009a, 2009b; Freire et al., 2010b; Ribas-Fitó et al., 2007; Toft et al., 2004).

The production and use of most OCPs have been restricted or banned internationally since the 1970s, although some remain in legal use in various countries (Beard, 2006; PNA Convenio Estocolmo y Reglamento 850/2004., 2007). However, worldwide and intensive use of these compounds has led to widespread contamination of the environment and, due to their high persistence and bioaccumulation capacity, their presence is still detected in human tissues (Cerrillo et al., 2005; Lopez-Espinosa et al., 2007). Southern Spain has the largest area of intensive greenhouse agriculture in Europe and may constitute a special case of exposure to pesticides that have only recently been banned (in 2009), such as endosulfans (Cerrillo et al., 2005). In fact, Spain has been the main consumer of endosulfans in the European Union over the past few years (Endosulfan Preliminary Dossier, 2003).

Exposure to OCPs during human development may result in a permanent alteration of the endocrine system, and the fetus can be exposed to these compounds through the placenta. The present study aimed to investigate the relationship between cord blood TSH levels in a cohort of newborns from Granada, Southern Spain, and their prenatal exposure to a range of OCPs in placentas. This investigation is part of the INMA (Infancia y Medio Ambiente [Environment and Childhood]) Project, a prospective multi-center study in Spain (www.proyectoinma.org).

2. Methods

2.1. Subject recruitment

From 2000 to 2002, 700 eligible mother–son pairs registered at the San Cecilio University Hospital were enrolled at delivery, establishing the INMA-Granada male birth cohort, with the initial aim of investigating the relationship between chronic exposure to endocrine disrupting pesticides during pregnancy and urogenital malformations in newborn boys (Freire et al., 2010a; Lopez-Espinosa et al., 2007). Exclusion criteria were: maternal presence of serious chronic disease, such as diabetes, hypertension or thyroid disease; pregnancy complication that could affect growth or development; and non-residence in the hospital referral area (Fernández et al., 2007). The study was approved by the Institutional Ethical Committee of the Hospital, and signed informed consent was obtained from the eligible women who agreed to participate.

2.2. Laboratory analysis

2.2.1. Extraction and quantification of OCPs

A random sample of 308 placentas was selected from the cohort for chemical analysis. Placentas were collected at delivery and sent to the Laboratory of Medical Investigations for analysis. They were immediately coded, frozen, and stored at -86°C until processing (Lopez-Espinosa et al., 2007). Before analysis, half of the placenta was almost defrosted and then mechanically homogenized. Bioaccumulated compounds were extracted from samples by a previously described method (Fernández et al., 2007) with slight modifications. Briefly, 1.6 g of placenta homogenate was dissolved in hexane and eluted in a glass column filled with Alumine (Merck, Darmstadt, Germany). The eluate obtained was concentrated at reduced pressure under nitrogen stream

and then injected into the preparative high pressure liquid chromatography (HPLC). This previously described HPLC method allows us the separation of xenoestrogens from natural estrogens without destroying them. In addition, it has been demonstrated that the fraction collected in the first 11 min HPLC (α fraction) contains all the selected persistent chemicals (Fernández et al., 2007).

The presence of 17 OCPs (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD (dichlorodiphenyldichloroethane), *p,p'*-DDE, endosulfan-I and -II, endosulfandiols, -sulfate, -lactone, and -ether, aldrin, dieldrin, endrin, lindane, HCB, mirex, and methoxychlor) was determined by gas chromatography with electron-capture detection (Varian-3350 gas chromatograph plus ECD (63Ni)), using *p'*-dichlorobenzophenone as internal standard, and mass spectrometry. Standard solutions of organochlorine compounds were analyzed previously to determine retention times and calibration curves of these chemicals. The calibration linearity of all chemicals in pure and processed standards was >0.98 . The recovery of studied chemicals, measured by spiking placenta samples with pure chemicals, ranged from 84 to 102%. The reproducibility of the process was established by running 10 placenta samples 10 times. The limits of quantification (LQ) of the gas chromatograph for the studied chemicals ranged from 0.1 to 3.0 ng/mL. Chemical compounds were confirmed by GC and mass spectrometry (GC/MS) using a Saturn 2000 ion-trap mass spectrometer from Varian (Fernández et al., 2007; Lopez-Espinosa et al., 2007) in all the samples. Placental lipid content was gravimetrically quantified.

2.2.2. TSH determination

TSH was measured in a cord blood sample spotted on a filter paper (Schleicher & Schuell no. 2992), which was routinely obtained shortly after birth in our Hospital for the national neonatal hypothyroidism screening program (Cortés Castell et al., 2002). Data on neonatal TSH levels were gathered for 80% of the boys ($n=551$). Hormone concentrations were determined by using time-resolved sandwich fluoroimmunoassay (AutoDELFIA, Perkin Elmer/Wallac, Turku, Finland) at the Center for the Early Detection of Metabolopathies in Neonates in San Juan de Dios Hospital (Granada, Spain). The limit of detection (LD) was 0.01 mIU/L. A cord blood level ≥ 14 mIU/L is established in the Center's laboratory to trigger the protocol for the study and confirmation of neonatal hypothyroidism.

2.3. Covariates

Covariates considered in the statistical analysis are shown in Table 1. A post-delivery questionnaire was administered to gather data on: maternal age, alcohol consumption and cigarette smoking during pregnancy, parity, pre-pregnancy body mass index (BMI), maternal education, marital status, and area of residence (urban: city of Granada; metropolitan: towns of $>20,000$ inhabitants in city residential belt; sub-urban: towns of 10,000–20,000 inhabitants; rural: $<10,000$ inhabitants), among other reproductive and medical characteristics. Information on gestational age and anthropometric measurements at birth were obtained from medical records. We considered these covariates as potential confounders a priori.

Complete information on placental OCP concentrations, cord blood TSH levels and covariates were available for a subset of 220 subjects from the cohort. No differences in any study characteristics were found between this subset and the children without TSH measurements ($n=88$) (data not shown).

2.4. Statistical analysis

Descriptive analysis of continuous OCP concentrations was first conducted. For further analyses, values of those OCPs with a frequency of detection $\geq 50\%$ were transformed into natural logarithms and treated as continuous variables (for levels below the LD, we considered a value of half the LD); while concentrations of pesticides

Table 1

TSH levels (mU/L) measured in cord blood (n=220) by characteristics of study population from the INMA-Granada cohort, Southern Spain, 2000–2002.

	n (%)	TSH levels (mU/L)		
		GM	95% CI	p-value ^a
<i>Child variables</i>				
Small for gestational age				0.72
No	197 (89.5)	3.05	2.79; 3.34	
Yes	22 (10.5)	3.21	2.51; 4.11	
Gestational age (weeks) ^b	220 (100)	39.4	39.2; 39.6	0.76
Birth weight (g) ^b	220 (100)	3335	3277; 3394	0.26
<i>Maternal variables</i>				
Age at delivery (years) ^b	220 (100)	31.8	31.2; 32.5	0.44
Smoking during pregnancy				0.16
No	168 (76.4)	3.17	2.89; 3.48	
Yes	52 (23.6)	2.76	2.29; 3.33	
Alcohol consumption				0.12
No	196 (89.1)	3.15	2.90; 3.42	
Yes	20 (10.9)	2.52	1.71; 3.71	
Parity				0.14
Primiparous	110 (50)	3.27	2.91; 3.67	
Multiparous	110 (50)	2.88	2.56; 3.25	
Pre-pregnancy BMI (kg/m ²)				0.92
<19	13 (5.9)	3.20	2.41; 4.26	
19–24	142 (64.5)	3.06	2.74; 3.42	
25–29	51 (23.2)	3.07	2.66; 3.55	
≥30	14 (6.4)	3.04	1.97; 4.69	
Educational level				0.90
Up to primary school	115 (52.3)	3.05	2.70; 3.45	
Secondary school	69 (31.4)	3.03	2.60; 3.54	
University	36 (16.4)	3.20	2.73; 3.76	
Marital status				0.80
Stable partner	202 (91.8)	3.08	2.82; 3.36	
Without stable partner	18 (8.2)	2.97	2.32; 3.79	
Area of residence				0.51
Rural	50 (22.7)	2.81	2.28; 3.45	
Sub-urban	35 (15.9)	3.45	2.93; 4.06	
Metropolitan	108 (49.0)	3.10	2.75; 3.49	
Urban	27 (12.3)	2.99	2.35; 3.81	

TSH: thyroid-stimulating hormone; GM: geometric mean; CI: confidence interval.

^a p-value for simple linear regression, T-test or ANOVA.^b Continuous variables; BMI: body mass index.

detected in <50% of the placenta extracts were treated as dichotomous variables, using the LD as cut-off. TSH values were transformed into natural logarithms to improve the normality and were treated as a continuous variable. In a second analysis, TSH was dichotomized using as cutoff the value established by the World Health Organization for “moderately elevated neonatal concentrations” (5 mU/L) (WHO, 2007), which coincided with the 80th percentile (4.99 mU/L). We used one-way analysis of variance and the Student's *t*-test to examine the relationship of covariates with continuous TSH levels.

Linear and logistic regression analyses were performed to explore the unadjusted and adjusted association of OCP concentrations in placenta tissue samples with cord blood TSH levels. All multivariate models controlled for maternal age and gestational age (continuous), regardless of their statistical significance, based on reports in the literature (Chevrier et al., 2008; Maervoet et al., 2007; Takser et al., 2005). OCP concentrations were adjusted by g of placenta (ng/g) in the descriptive analysis, but the total placental lipid content was included as a separate term in the regression models for continuous OCPs because it was recently shown that lipid standardization models are highly prone to bias (Schisterman et al., 2005). Models were also adjusted for confounding factors, i.e., prenatal smoking (yes/no), alcohol consumption during pregnancy (yes/no), parity (primiparous/multiparous), birth weight (continuous) and maternal education (primary/secondary/university). Variables associated with TSH levels at a significance level of $p < 0.20$ in bivariate analysis or whose inclusion in the models changed OCP effect estimates by >10% were

Table 2

Organochlorine pesticide (OCP) concentrations measured in placenta (ng/g placenta), INMA-Granada cohort, Southern Spain, 2000–2002 (n=220).

OCPs	n (%≥LD)	GM ^a	95% CI ^a	P25	P50	P75	P95	Max.
<i>o,p'</i> -DDT	123 (55.9)	0.86	0.76; 0.97	<LD	0.50	0.73	3.18	6.98
<i>p,p'</i> -DDT	108 (49.1)	1.25	1.03; 1.52	<LD	<LD	0.91	7.47	51.2
<i>p,p'</i> -DDE	205 (93.2)	2.01	1.75; 2.30	0.84	1.74	3.37	11.9	65.7
<i>o,p'</i> -DDD	108 (49.1)	1.91	1.54; 2.37	<LD	<LD	1.86	15.1	35.3
∑ DDTs	215 (97.7)	4.16	3.61; 4.81	1.99	4.13	7.89	25.5	66.5
E-I	116 (52.7)	0.73	0.60; 0.88	<LD	0.25	0.58	5.11	29.8
E-II	69 (31.4)	1.37	1.14; 1.64	<LD	<LD	1.00	10.8	48.7
E-diol	116 (63.2)	2.10	1.07; 6.20	<LD	3.68	6.27	18.7	26.2
E-ether	110 (50.0)	0.23	0.20; 0.27	<LD	<LD	0.19	1.36	4.67
E-sulfate	109 (49.5)	0.93	0.75; 1.14	<LD	<LD	0.88	7.17	31.7
E-lactone	85 (38.6)	1.14	0.87; 1.50	<LD	<LD	0.79	17.2	40.3
∑ Endosulfans	211 (95.9)	4.02	3.35; 4.82	1.26	5.69	9.64	27.0	129
Aldrin	62 (28.2)	0.82	0.68; 0.99	<LD	<LD	0.50	4.41	7.86
Endrin	75 (34.1)	2.53	2.12; 3.01	<LD	<LD	1.50	10.8	59.1
Dieldrin	47 (21.4)	1.05	0.82; 1.34	<LD	<LD	<LD	8.73	10.0
Lindane	168 (76.4)	0.41	0.32; 0.53	0.12	0.25	0.47	1.84	6.66
HCB	94 (42.7)	1.02	0.84; 1.24	<LD	<LD	0.55	8.62	30.4
Methoxychlor	62 (28.2)	1.20	0.94; 1.52	<LD	<LD	0.50	10.0	22.0
Mirex	55 (25.0)	1.15	0.91; 1.47	<LD	<LD	0.44	8.72	22.3

LD: Limit of detection; GM: geometric mean; CI: confidence interval; P: percentile; Max.: maximum.

DDT: 1,1,1-trichloro-2,2-bis(4-chlorofenil)-etano; ∑ DDTs: sum of *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDD; E: endosulphan; ∑ Endosulfans: sum of E-I, E-II, E-diol, E-ether, E-sulfate, and E-lactone; HCB: hexachlorobenzene.^a Descriptive statistics for concentrations ≥ LD.

considered confounders. $p \leq 0.05$ was considered significant. SPSS version 16.0 (SPSS Inc., Chicago, IL, US) and STATA version 9.0 (Corporation, College Station, Texas) were used for the analyses.

3. Results

The mean age of the 220 mothers at delivery was 32 years (range = 16–46 years) and pre-pregnancy BMI was 24 kg/m² (17–40 kg/m²). Around 24% of women were smokers, 9% consumed alcohol during pregnancy, 50% were primiparous, 16% had university education, and only 8% did not have a stable partner. The mean birth weight was 3335 g (2070–4590 g), and the mean duration of gestation was 39 weeks (32–42 weeks); 10% of the boys were small for gestational age (SGA). All cord blood TSH levels were within normal laboratory reference values (<14 mU/L), with a geometric mean (GM) of 3.07 mU/L (95% confidence interval [CI] = 2.82, 3.34; range = 0.24–14.00 mU/L). Table 1 summarizes TSH levels by characteristics of the study population. Although differences did not reach statistical significance, cord blood TSH levels were higher among primiparous mothers ($p = 0.14$) and those smoking ($p = 0.16$) or consuming alcohol during pregnancy ($p = 0.12$).

Table 2 lists the percentages of detection, and GM, 95% CI, percentiles 25, 50, 75 and 95, and maximum levels \geq LD of the 17 OCPs quantified in the placenta samples. All samples were positive for at least one pesticide, with a mean of eight pesticides per placenta (range = 2–15 pesticides). *p,p'*-DDE was the most frequent pesticide (96.2%), followed by lindane (76.4%), endosulfan-diol (63.2%), *o,p'*-DDT (55.9%), endosulfan-I (52.7%), and endosulfan-ether (50.0%). The remaining OCPs studied were detected in <50% (21.4–49.5%) of placenta samples. Around 98% and 96% of the placenta samples, respectively, had detectable levels of at least one of the studied DDT and endosulfan isomers/metabolites. Unadjusted analysis with cate-

gorical TSH (≥ 5 mU/L) showed positive associations with endrin (OR = 2.32; 95% CI = 1.18, 4.55; $p = 0.01$) and *p,p'*-DDE (OR = 1.35; 95% CI = 0.99, 1.83; $p = 0.05$) and an inverse relation with endosulfan-sulfate (OR = 0.40; 95% CI = 0.20, 0.80; $p = 0.01$). No significant associations were found with continuous TSH levels (Table 3).

Table 4 shows the results of the adjusted analysis of the different OCP concentrations with cord blood TSH levels. Levels of TSH ≥ 5 mU/L remained significantly associated with endrin and endosulfan-sulfate placental concentrations after controlling for covariates, with a 2-fold higher odds (OR = 2.05; 95% CI = 1.01, 4.18; $p = 0.05$) in newborns exposed to concentrations of endrin \geq LD in utero, but a significantly lower odds of TSH ≥ 5 mU/L in newborns prenatally exposed to endosulfan-sulfate \geq LD. A higher odds of TSH ≥ 5 mU/L (OR = 1.32; 95% CI = 0.95, 1.83; $p = 0.09$) was observed with higher *p,p'*-DDE concentrations, although significance was not reached. With the exception of HCB, which showed a negative and marginally significant association ($\beta = -0.15$; 95% CI = 0.95, 1.83; $p = 0.09$), no relationship was found between OCP concentrations in placenta samples and continuous TSH levels.

4. Discussion

The relationship between exposure to placental OCPs and TSH levels was investigated in 220 male newborns from the general population. It was found that the risk of cord blood TSH ≥ 5 mU/L was higher in neonates prenatally exposed to greater concentrations of endrin but lower in those exposed to greater concentrations of endosulfan-sulfate. Marginally significant associations were found between higher TSH concentrations and higher *p,p'*-DDE and lower HCB. Exposure to OCPs during fetal development may exert some effects on thyroid status in neonates, as indicated in other studies

Table 3

Unadjusted association of TSH levels (mU/L) measured in cord blood with organochlorine pesticide (OCP) concentrations (ng/g placenta), INMA-Granada cohort, Southern Spain, 2000–2002 (n = 220).

OCPs	TSH ≥ 5 mU/L ^a			Continuous TSH ^b		
	OR	95% CI	p-value	β	95% CI	p-value
<i>o,p'</i> -DDT ^c	1.02	0.67; 1.55	0.92	0.04	-0.06; 0.15	0.43
<i>p,p'</i> -DDT ^d	0.83	0.43; 1.62	0.59	-0.08	-0.25; 0.08	0.32
<i>p,p'</i> -DDE ^c	1.35	0.99; 1.83	0.05	0.04	-0.03; 0.12	0.29
<i>o,p'</i> -DDD ^d	0.93	0.48; 1.81	0.84	-0.06	-0.23; 0.11	0.48
Σ DDTs ^c	1.28	0.94; 1.74	0.11	0.00	-0.08; 0.07	0.91
E-I ^c	0.96	0.71; 1.28	0.77	-0.03	-0.10; 0.04	0.45
E-II ^d	1.50	0.75; 2.99	0.25	0.01	-0.17; 0.19	0.91
E-diol ^c	1.17	0.92; 1.48	0.20	0.02	-0.04; 0.07	0.58
E-ether ^c	1.10	0.79; 1.53	0.58	0.00	-0.08; 0.09	0.94
E-sulfate ^d	0.40	0.20; 0.80	0.01	-0.14	-0.31; 0.02	0.09
E-lactone ^d	0.69	0.34; 1.39	0.30	-0.08	-0.25; 0.09	0.36
Σ Endosulfans ^c	1.07	0.86; 1.35	0.53	0.00	-0.06; 0.05	0.99
Aldrin ^d	1.09	0.53; 2.25	0.82	0.01	-0.17; 0.20	0.89
Endrin ^d	2.32	1.18; 4.55	0.01	0.08	-0.10; 0.25	0.39
Dieldrin ^d	1.30	0.60; 2.81	0.51	-0.02	-0.22; 0.18	0.85
Lindane ^c	1.01	0.67; 1.52	0.95	-0.04	-0.14; 0.07	0.50
HCB ^d	0.91	0.47; 1.78	0.78	-0.07	-0.24; 0.10	0.41
Methoxychlor ^d	1.61	0.80; 3.25	0.18	0.10	-0.08; 0.29	0.27
Mirex ^d	1.76	0.86; 3.60	0.12	0.18	-0.01; 0.37	0.07

OR: Odds ratio; CI: confidence interval; β : regression coefficient.

DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane; Σ DDTs: sum of *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDD; E: endosulfan; Σ Endosulfans: sum of E-I, E-II, E-diol, E-ether, E-sulfate, and E-lactone; HCB: hexachlorobenzene.

^a Simple logistic regression analysis with dichotomous TSH levels (≥ 5 mU/L) as dependent variable.

^b Simple linear regression analysis with log-transformed TSH levels as dependent variable.

^c Concentrations transformed into natural logarithm.

^d Categorical concentrations (\geq limit of detection).

Table 4

Adjusted association of organochlorine pesticide (OCP) concentrations (ng/g placenta) with neonatal TSH levels (mU/L) measured in cord blood, INMA-Granada cohort, Southern Spain, 2000–2002 (n = 220)^a.

OCPs	TSH ≥ 5 mU/L ^b			Continuous TSH ^c		
	OR	95% CI	p-value	β	95% CI	p-value
<i>o,p'</i> -DDT ^d	0.91	0.59; 1.42	0.69	0.04	-0.07; 0.14	0.49
<i>p,p'</i> -DDT ^e	0.79	0.39; 1.60	0.51	-1.0	-0.27; 0.07	0.26
<i>p,p'</i> -DDE ^d	1.32	0.95; 1.83	0.09	0.05	-0.26; 0.09	0.26
<i>o,p'</i> -DDD ^e	0.77	0.37; 1.58	0.48	-0.09	-0.03; 0.12	0.32
Σ DDTs ^d	1.17	0.84; 1.63	0.35	-0.02	-0.10; 0.06	0.57
E-I ^d	0.92	0.67; 1.25	0.58	-0.02	-0.10; 0.05	0.53
E-II ^e	1.50	0.71; 3.17	0.28	-0.04	-0.22; 0.14	0.68
E-diol ^d	1.09	0.85; 1.40	0.49	0.00	-0.05; 0.06	0.89
E-ether ^d	1.10	0.77; 1.56	0.60	-0.01	-0.10; 0.08	0.80
E-sulfate ^e	0.36	0.17; 0.77	0.008	-0.12	-0.29; 0.05	0.15
E-lactone ^e	0.80	0.38; 1.67	0.55	-0.05	-0.22; 0.12	0.58
Σ Endosulfans ^d	1.04	0.82; 1.31	0.75	-0.01	-0.06; 0.05	0.84
Aldrin ^e	0.94	0.43; 2.04	0.87	0.02	-0.16; 0.21	0.82
Endrin ^e	2.05	1.01; 4.18	0.05	0.03	-0.15; 0.20	0.76
Dieldrin ^e	1.28	0.57; 2.86	0.55	-0.01	-0.21; 0.20	0.93
Lindane ^d	0.92	0.59; 1.42	0.71	-0.03	-0.14; 0.07	0.53
HCB ^e	0.72	0.35; 1.47	0.36	-0.15	-0.31; 0.02	0.09
Methoxychlor ^e	1.72	0.81; 3.68	0.16	0.04	-0.15; 0.22	0.69
Mirex ^e	1.69	0.77; 3.69	0.19	0.12	-0.07; 0.32	0.22

OR: Odds ratio; CI: confidence interval; β : regression coefficient.

DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane; Σ DDTs: sum of *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, and *o,p'*-DDD; E: endosulfan; Σ Endosulfans: sum of E-I, E-II, E-diol, E-ether, E-sulfate, and E-lactone; HCB: hexachlorobenzene.

^a Association of OCP concentrations with TSH levels, adjusted for maternal age, gestational age, smoking and alcohol consumption during pregnancy, parity, birth weight, maternal education, and placenta lipid concentration.

^b Dichotomous TSH levels (≥ 5 mU/L) as dependent variable.

^c Log-transformed TSH levels as dependent variable.

^d Concentrations transformed into natural logarithm.

^e Categorical concentrations (\geq limit of detection).

(Alvarez-Pedrerol et al., 2008; Asawasinsopon et al., 2006; Lopez-Espinosa et al., 2010; Ribas-Fitó et al., 2003); however, there was not a consistent pattern in the association shown in the present study.

Very little information is available on the specific thyroid effects of early exposure to most OCPs. Although TH concentrations have been consistently associated with PCBs and dioxins (Hagmar, 2003; Maervoet et al., 2007), evidence on the effect of OCPs on TH levels in vulnerable groups (e.g., pregnant women and infants) has been controversial (Alvarez-Pedrerol et al., 2008; Asawasinsopon et al., 2006; Dallaire et al., 2008; Maervoet et al., 2007; Takser et al., 2005). Previous findings in a Spanish community highly exposed to HCB showed no significant association between this pesticide and TSH concentrations at birth but, in agreement with our results, a close-to-significant positive association between cord serum *p,p'*-DDE and infant TSH levels >10 mU/L; the earlier study also reported a positive relationship with β -HCH (Ribas-Fitó et al., 2003). Two more recent Spanish studies found a positive association with β -HCH but not with HCB or DDT (Alvarez-Pedrerol et al., 2008; Lopez-Espinosa et al., 2010). Asawasinsopon et al. (2006) found no relation between cord blood TSH and maternal or cord serum levels of DDT, dieldrin, heptachlor, HCB or lindane, and there have been other reports of no association between TSH levels and OCP concentrations (Dallaire et al., 2008; Maervoet et al., 2007; Takser et al., 2005). Higher serum TSH levels were related to higher *p,p'*-DDE exposure in Spanish pregnant women (Lopez-Espinosa et al., 2009), although TSH was not associated with maternal serum levels of DDTs and HCB (Chevrier et al., 2008). The present study found that neonatal TSH was related to placental endrin and endosulfan-sulfate. Limited data have been published on the effects of human exposure to these two pesticides on thyroid function (Dewailly et al., 1993). An unexpected finding was the negative relationship of TSH with endosulfan-sulfate and, although not significant, with HCB, since a higher exposure to OCPs might be expected to increase TSH levels due to a decrease in free or total T_4 neonatal levels (Maervoet et al., 2007; Takser et al., 2005).

Some mechanistic hypotheses can be proposed for the associations found in the present study. Thus, field and laboratory animal studies have demonstrated that DDT may be able to alter the production of THs and hormone availability at target tissues by competing with T_4 for binding to purified human transthyretin (Scollon et al., 2004; Sormo et al., 2005; Verreault et al., 2004). Transthyretin is the main plasma T_4 transporting protein in rats but may also play an important role in T_4 transportation in the human brain. DDT has also been shown to disrupt THs by increasing TH peripheral metabolism through an induction of hepatic microsomal enzymes, resulting in an increased excretion of plasma T_4 (Capen, 1994; Scollon et al., 2004). In contrast, an in vitro study found that neither *p,p'*-DDT nor *p,p'*-DDE interacted with transthyretin or thyroid-binding globulin (Cheek et al., 1999).

Few experimental data are available on the effects of endrin and endosulfan on the thyroid function. Interestingly, endrin was found to depress thyroid iodine uptake in various fish species, suggesting that it may compromise thyroid function by inhibiting TH synthesis and its intra-thyroidal storage, which can account for more than three months of TH reserves in humans (Bhattacharya et al., 1989; Singh and Singh, 1980). Evidence is emerging on the thyroid-disrupting effects of other pesticides, such as methoxychlor and endosulfan (Boas et al., 2006), but their mechanism of action has yet to be elucidated. Exposure to endosulfan was found to alter circulating TH levels in a study of fish, inhibiting T_4 production/secretion and enhancing T_4 clearance (Coimbra et al., 2005); low doses of endosulfan in the diet decreased plasma T_4 levels, restored T_3 levels, and depressed hepatic type I deiodinase activity at 21 days of exposure, but hepatic type III activity increased after 35 days of treatment. Contrary to expectations, a higher endosulfan dose did not alter TH plasma level or hepatic deiodinase activity in this study, suggesting that paradoxical results can be obtained depending on the dose and duration of exposure (Coimbra et al., 2005). Early exposure

to endosulfan-sulfate could lead to significant alterations in T_4 feedback to the hypothalamus, resulting in decreasing TSH levels at birth, as it has been reported for placental and cord plasma PCBs (Sandau et al., 2002; Wang et al., 2005).

Humans may be exposed to mixtures of OCPs and numerous other compounds, hampering the prediction of effects on TH levels. In the present series, all placentas had measurable concentrations of at least one of the 17 OCPs investigated, reflecting the ubiquity of exposure in the population, as previously reported (Lopez-Espinosa, 2006; Lopez-Espinosa et al., 2007). Chemicals may have different effects on the thyroid axis or act synergistically (Crofton et al., 2005). Therefore, alterations in TSH levels might result not only from prenatal exposure to endrin or endosulfan-sulfate but also from exposure to other OCPs, alone or in combination. It should be borne in mind that the INMA-Granada birth cohort is entirely composed of male infants, whose hormonal levels might have differed from those of females. Thus, Wang et al. (2005) described a gender difference in the sensitivity to EDs, reporting that prenatal exposure to dioxins – which have antiestrogenic activity – increased neonatal T_4 levels in female but not male infants.

A Spanish study of another INMA cohort (Alvarez-Pedrerol et al., 2007) showed that THs were related to cognitive function and attention behavior among preschoolers, suggesting that even small changes in TH levels may have a significant effect on brain function. Concentrations of DDT in cord serum were associated with a decrease in cognitive skills at preschool age in two other INMA cohorts (Ribas-Fitó et al., 2006). The present cohort is being followed up throughout childhood, and a recent longitudinal study showed that prenatal exposure to mirex (in 104 of these children) had a negative effect on cognitive development at the age of 4 years (Puertas et al., 2010). Although mirex did not show a significant effect on neonatal TSH status in the present study, a positive trend ($p=0.07$) was found with continuous TSH in the crude analysis and with $TSH \geq 5$ mU/L ($OR=1.69$; $p=0.19$) in the adjusted analysis. A further study has been recently conducted by our group into the impact of neonatal TSH levels on neurodevelopment at the age of 4 years (Freire et al., 2010b), which will be also investigated in the same cohort at the age of 9–10 years.

Other study strengths are that we examined the effects of a large number of covariates identified from the literature. Neonatal TSH levels were assessed using the same technique as in other Spanish studies (part of routine early screening for hypothyroidism in Spanish hospitals), supporting the validity of the present study. We measured prenatal exposure to OCPs in placenta samples, hampering comparison of our findings with previous reports on this issue. Nevertheless, a high correlation has been demonstrated among OC concentrations in maternal serum, cord serum and placenta (Bergonzi et al., 2009; Wang et al., 2004), and placental analysis offers the opportunity to measure the exposure of both mother and fetus to EDs without requiring an invasive procedure. A limitation of our study is that only concentrations of TSH were determined, and no data were available on total or free T_4/T_3 . However, TSH is considered a more sensitive indicator of thyroid disruption in comparison to T_4 or T_3 levels (The National Academy of Clinical Biochemistry, 2007).

5. Conclusions

This is the first report on the relationship between TSH status at birth and prenatal exposure to a wide range of OCPs as measured in the placenta. Although some associations were detected between OCP exposure and TSH cord blood levels, no clear pattern emerged on the influence of the compounds measured in placenta tissue on neonatal TH status. The impact of environmental chemicals on thyroid hormone levels and metabolism is a matter of growing concern, and further research is warranted to elucidate the causal

relationship between endocrine-disrupting chemicals and thyroid hormones.

Funding sources

This work was supported by the Consejería de Salud de la Junta de Andalucía [grant number P09-CTS-5488]; the Spanish Ministry of Health [grant number FIS07/0252]; and the European Commission [grant number CONTAMED-FP7-ENV-2007-1-212502 and EUS2008-03574].

Acknowledgments

The authors are grateful to Richard Davies for editorial assistance. The results would not have been achieved without the selfless collaboration of the study participants, the staff at the Department of Gynecology and Pediatrics of the San Cecilio University Hospital and at the Environmental Health Department of the Consejería de Salud de la Junta de Andalucía.

References

- Alvarez-Pedrerol M, Ribas-Fitó N, Torrent M, Julvez J, Ferrer C, Sunyer J. TSH concentration within the normal range is associated with cognitive function and ADHD symptoms in healthy preschoolers. *Clin Endocrinol* 2007;66:890–8.
- Alvarez-Pedrerol M, Ribas-Fitó N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, et al. Thyroid disruption at birth due to prenatal exposure to beta-hexachlorocyclohexane. *Environ Int* 2008;34:737–40.
- Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, et al. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. *Environ Sci Technol* 2009a;43:7909–15.
- Alvarez-Pedrerol M, Guxens M, Mendez M, Canet Y, Martorell R, Espada M, et al. Iodine levels and thyroid hormones in healthy pregnant women and birth weight of their offspring. *Eur J Endocrinol* 2009b;160:423–9.
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: a study from northern Thailand. *Environ Int* 2006;32:554–9.
- ATSDR. Toxicological profile for DDT, DDE and DDD. Atlanta, GA: Agency for Toxic Substances and Disease Registry; 2002a.
- ATSDR. Toxicological profile for hexachlorobenzene. Atlanta, GA: Agency for Toxic Substances and Disease Registry; 2002b.
- Beard J. DDT and human health (Australian Rural Health Research Collaboration). *Sci Total Environ* 2006;344:78–89.
- Bergonzi R, Specchia C, Dinolfo M, Tomasi C, De Palma G, Frusca T, et al. Distribution of persistent organochlorine pollutants in maternal and foetal tissues: data from an Italian polluted urban area. *Chemosphere* 2009;76:747–54.
- Bhattacharya T, Bhattacharya S, Ray AK, Dey S. Influence of industrial pollutants on thyroid function in *Channa punctatus* (Bloch). *Indian J Exp Biol* 1989;27:65–8.
- Boas M, Feldt-Rasmussen U, Skakkebaek NE, Main KM. Environmental chemicals and thyroid function. *Eur J Endocrinol* 2006;154:599–611.
- Capen CC. Mechanisms of chemical injury of thyroid gland. *Prog Clin Biol Res* 1994;387:173–91.
- Cerrillo I, Granada A, Lopez-Espinosa MJ, Olmos B, Jiménez M, Caño A, et al. Endosulfan and its metabolites in fertile women, placenta, cord blood, and human milk. *Environ Res* 2005;98:233–9.
- Coimbra AM, Reis-Henriques MA, Darras VM. Circulating thyroid hormone levels and iodothyronine deiodinase activities in Nile tilapia (*Oreochromis niloticus*) following dietary exposure to Endosulfan and Aroclor 1254. *Comp Biochem Physiol C Toxicol Pharmacol* 2005;141:8–14.
- Cortés Castell E, Chamorro Ureña F, Dulín Iñiguez E, Eguileor Gurtubai I, Espada Sáenz-Torre M, Remón Alvarez-Arenas J, et al. On behalf of the *Comisión de Errores Metabólicos Congénitos de la Sociedad Española de Bioquímica Clínica y Patología Molecular* (SEQC), 2002. Neonatal screening and collaboration among scientific institutions. *An Esp Pediatr* 2002;56:201–3.
- Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, Carchman RA, et al. Thyroid-hormone-disrupting chemicals: evidence for dose-dependent additivity or synergism. *Environ Health Perspect* 2005;113:1549–54.
- Cheek AO, Kow K, Chen J, McLachlan JA. Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Environ Health Perspect* 1999;107:273–8.
- Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *Am J Epidemiol* 2008;168:298–310.
- Dallaire R, Dewailly E, Ayotte P, Muckle G, Laliberté C, Bruneau S. Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in Québec, Canada. *Environ Res* 2008;108:387–92.
- DeCastro BR, Korrick SA, Spengler JD, Soto AM. Estrogenic activity of polychlorinated biphenyls present in human tissue and the environment. *Environ Sci Technol* 2006;40:2819–25.
- Dewailly E, Bruneau S, Ayotte P, Laliberté C, Gingras S, Belanger D, et al. Health status of birth of Inuit newborn prenatally exposed to organochlorines. *Chemosphere* 1993;27:359–66.
- Endosulfan Preliminary Dossier. Umweltbundesamt, Berlin, p. 55; 2003.
- Fernández MF, Rivas A, Olea-Serrano F, Cerrillo I, Molina-Molina JM, Araque P, Martínez-Vidal JL, Olea N. Assessment of total effective xenoestrogen burden in adipose tissue and identification of chemicals responsible for the combined estrogenic effect. *Anal Bioanal Chem* 2004;379:163–70.
- Fernández MF, Olmos B, Granada A, Lopez-Espinosa MJ, Molina-Molina JM, Fernández JM, et al. Human exposure to endocrine disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias. A nested case-control study. *Environ Health Perspect* 2007;115:8–14.
- Freire C, Ramos R, Puertas R, Lopez-Espinosa MJ, Julvez J, Aguilera I, et al. Association of traffic-related air pollution with cognitive development in children. *J Epidemiol Community Health* 2010a;64:223–8.
- Freire C, Ramos R, Amaya E, Fernández MF, Santiago-Fernández P, Lopez-Espinosa MJ, et al. Newborn TSH concentration and its association with cognitive development in healthy boys. *Eur J Endocrinol* 2010b;163:901–9.
- Hagmar L. Polychlorinated biphenyls and thyroid status in humans: a review. *Thyroid* 2003;13:1021–8.
- Langer P, Tajtaková M, Kocan A, Vlcek M, Petrik J, Chovancova J, et al. Multiple organochlorine pollution and the thyroid. *Endocr Regul* 2006;40:46–52.
- Lopez-Espinosa MJ. Mother-infant exposure to environmental chemical compounds with hormonal activity via the placenta [Doctoral Thesis]. Granada: Universidad de Granada; 2006.
- Lopez-Espinosa MJ, Granada A, Carreño J, Salvatierra MT, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from Southern Spain and some related factors. *Placenta* 2007;28:631–8.
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, et al. Association between thyroid hormone levels and 4,4'-DDE concentrations in pregnant women (Valencia, Spain). *Environ Res* 2009;109:479–85.
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Fuentes V, Garcia AM, Rebagliato M, et al. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. *J Expo Sci Environ Epidemiol* 2010;20:579–88.
- Maervoet J, Vermeir G, Covaci A, Nelen V, Baeyens W, Schepens P, et al. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. *Environ Health Perspect* 2007;115:1780–6.
- PNA Convenio Estocolmo y Reglamento 850/2004. Plan nacional de aplicación del convenio de estocolmo y el reglamento 850/2004, sobre contaminantes orgánicos persistentes. Subdirección general de calidad del aire y prevención de riesgos del ministerio de medio ambiente; 2007.
- Puertas R, Lopez-Espinosa MJ, Cruz F, Ramos R, Freire C, Pérez-García M, et al. Prenatal exposure to mirex impairs neurodevelopment at age of 4 years. *Neurotoxicology* 2010;31:154–60.
- Ribas-Fitó N, Sala M, Cardo E, Mazon C, De Muga ME, Verdú A, et al. Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns. *Occup Environ Med* 2003;60:301–3.
- Ribas-Fitó N, Torrent M, Carrizo D, Muñoz-Ortiz L, Julvez J, Grimalt JO, et al. In utero exposure to background concentrations of DDT and cognitive functioning among preschoolers. *Am J Epidemiol* 2006;164:955–62.
- Ribas-Fitó N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age. *Environ Health Perspect* 2007;115:447–50.
- Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec. *Environ Health Perspect* 2002;110:411–7.
- Singh H, Singh TP. Thyroid activity and TSH potency of the pituitary gland and blood serum in response to cythion and hexadrin treatment in the freshwater catfish, *Heteropneustes fossilis* (Bloch). *Environ Res* 1980;22:184–9.
- Scollon EJ, Carr JA, Cobb GP. The effect of flight, fasting and p, p'-DDT on thyroid hormones and corticosterone in Gambel's white-crowned sparrow, *Zonotrichia leucophrys gambelli*. *Comp Biochem Physiol C Toxicol Pharmacol* 2004;137:179–89.
- Schisterman EF, Whitcomb BW, Buck Louis GM, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. *Environ Health Perspect* 2005;113:853–7.
- Sormo EG, Jussi I, Jussi M, Braathen M, Skaare JU, Janssen BM. Thyroid hormone status in gray seal (*Halichoerus grypus*) pups from the Baltic Sea and the Atlantic Ocean in relation to organochlorine pollutants. *Environ Toxicol Chem* 2005;24:610–6.
- Takser L, Mergler D, Baldwin M, Smargiassi A, Lafond J. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. *Environ Health Perspect* 2005;113:1039–45.
- The National Academy of Clinical Biochemistry. Guía de consenso para el diagnóstico y seguimiento de la enfermedad tiroidea. *Acta Bioquímica Clínica Latinoamericana* 2007;41:87–119.
- Toft G, Hagmar L, Giwercman A, Bonde JP. Epidemiological evidence on reproductive effects of persistent organochlorines in humans. *Reprod Toxicol* 2004;19:5–26.
- Verreault J, Skaare JU, Janssen BM. Effects of organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous gulls, *Larus hyperboreus*. *Environ Health Perspect* 2004;112:532–7.
- Wang SL, Lin CY, Guo YL, Lin LY, Chou WL, Chang LW. Infant exposure to polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls (PCDD/Fs, PCBs)—correlation between prenatal and postnatal exposure. *Chemosphere* 2004;54:1459–73.

- Wang SL, Su PH, Jong SB, Guo YL, Chou WL, Papke O. In utero exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. *Environ Health Perspect* 2005;113:1645–50.
- Williams GR. Neurodevelopmental and neuropsychological actions of thyroid hormone. *J Neuroendocrinol* 2008;20:784–94.
- World Health Organization (WHO). Assessment of iodine deficiency disorders and monitoring their elimination: a guide for programme managers 3rd ed. 2007.
- Zoeller T. Environmental chemicals impacting the thyroid: targets and consequences. *Thyroid* 2007;17:811–7.