*Journal of Exposure Science and Environmental Epidemiology* (2010) 20, 579–588

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# Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels

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It has been suggested that prenatal exposure to some organochlorine compounds (OCs) may adversely affect thyroid function and may, therefore, impair neurodevelopment. The main aim of this study was to examine the relationship of cord serum levels of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*4,4′*-DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*4,4′*-DDE), β-hexachlorocyclohexane (β-HCH), hexachlorobenzene (HCB), four individual polychlorobiphenyl (PCB) congeners (118, 138, 153, and 180), and their sum, with neonatal thyroid stimulating hormone (TSH) levels in blood samples in a mother–infant cohort in Valencia, Spain. This study included 453 infants born between 2004 and 2006. We measured OC concentrations in umbilical cord serum and TSH in blood of newborns shortly after birth. Associations between neonatal TSH levels and prenatal OC exposure adjusted for covariates were assessed using multivariate linear regression analyses. Neonatal TSH levels tended to be higher in newborns with β-HCH levels in umbilical cord above 90th percentile (104 ng/g lipid) than in those with levels below the median (34 ng/g lipid), with an adjusted increment in neonatal TSH levels of 21% (95% confidence interval = −3, 51; $P = 0.09$). No statistically significant association was found between the remaining OCs and TSH at birth. Prenatal exposure to β-HCH may affect neonatal thyroid hormone status and its function in neurological development.
*Journal of Exposure Science and Environmental Epidemiology* (2010) **20,** 579–588; doi:10.1038/jes.2009.47; published online 26 August 2009

**Keywords:** *DDTs, hexachlorobenzene, hexachlorocyclohexane, polychlorobiphenyls, thyroid stimulating hormone, umbilical cord serum.*

## Introduction

Organochlorine compounds (OCs) are synthetic chemicals that are highly resistant to degradation, bioaccumulate in the food chain, and may represent a human health hazard (United Nations Environment Programme, 2007). Some OCs, including polychlorobiphenyls (PCBs), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*4,4′*-DDT), and its main metabolite 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*4,4′*-DDE), have been banned or restricted for many years in developed countries, but can still be detected in human samples (Carrizo et al., 2006; Lopez-Espinosa et al., 2008), largely because of dietary exposure (Herrera et al., 1996).

Accumulation of OCs in fat tissue during the life of the mothers may be an important source of exposure for offspring during gestation and through breast feeding (Waliszewski et al., 2001). These lipophilic and low-molecular-weight substances can pass through the placenta (Waliszewski et al., 2001; Soechitram et al., 2004) and may interfere with development and functions of the fetus. Epidemiological studies suggest that prenatal exposure to PCBs (Jacobson and Jacobson, 2003) and OC pesticides (Eskenazi et al., 2006; Ribas-Fito et al., 2007) may have deleterious effects on neurodevelopment, which is consistent with reports of the impact of these substances on neurobehavioral function in animals (Eriksson et al., 1992; Schantz and Widholm, 2001). Thyroid hormone disruption has been proposed as a potential mechanism of action for the

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Received 4 May 2009; accepted 14 July 2009; published online 26 August 2009

neurodevelopment effects of some OCs (Porterfield, 2000), as thyroid hormones are important regulators of brain development during fetal and neonatal periods (Dussault and Ruel, 1987). Thyroid hormones are involved in neuronal migration and proliferation (Lavado-Autric et al., 2003) and in synaptogenesis and myelinization (Rice and Barone, 2000). Slight differences in the concentration of thyroid hormones during pregnancy or after delivery may lead to impaired neurocognitive development in children (Pop et al., 2003; Alvarez-Pedrerol et al., 2007b).

Some epidemiological studies have suggested that thyroid hormone levels may be disrupted by prenatal or perinatal exposure to PCBs (Maervoet et al., 2007) or to other OCs (Chevrier et al., 2008; Lopez-Espinosa et al., 2009). Various mechanisms of action have been proposed to explain the potential association between thyroid hormones and some OCs, including inhibition of the function of the thyroid stimulating hormone (TSH) receptor, competitive binding to thyroid hormone transport proteins, or alteration of the excretion or clearance of thyroid hormones (Boas et al., 2006).

The aim of this study was to assess the association between prenatal OC exposure and TSH levels at birth in a mother–infant cohort in Valencia, Spain.

## Methods

### *Population and Study Design*

We drew the study sample from a cohort established in Valencia as part of the INMA (Environment and Childhood) study, a population-based cohort study in different regions of Spain on prenatal environmental exposures in relation to growth, development, and health from early fetal life until childhood (Ribas-Fito et al., 2006). The Hospital Ethics Committee of La Fe Hospital (Valencia) approved the research protocol and all mothers gave written informed consent before inclusion at the first trimester of pregnancy. Inclusion criteria were age $\geqslant$16 years, singleton pregnancy, enrollment at 10–13 weeks of gestation, and delivery scheduled at the reference hospital. Exclusion criteria were assisted conception, chronic hypertension, and communication handicap. From February 2004 to June 2005, 840 of the 1563 eligible women were included in the study after they signed the informed consent form (participation rate 54%). Fifteen women were recruited in October 2003 for a pilot study and are also included in the analysis. Among these women, 787 were followed to delivery (28 withdrew, 5 were lost to follow-up, 6 had induced abortion, 25 had spontaneous abortion, and 4 fetal deaths occurred). Deliveries took place between May 2004 and February 2006.

Out of these 787 women, the cord blood sample at delivery was missing in 288, the lipid analysis was missing in 24, and neonatal TSH measurements were missing in 2 cases. Sixteen neonates were also excluded because of maternal intake of thyroid hormone supplements or anti-thyroid hormone drugs, and four because neonatal TSH was measured before 2 days after delivery. Therefore, the final study sample comprised 453 neonates (Figure 1).

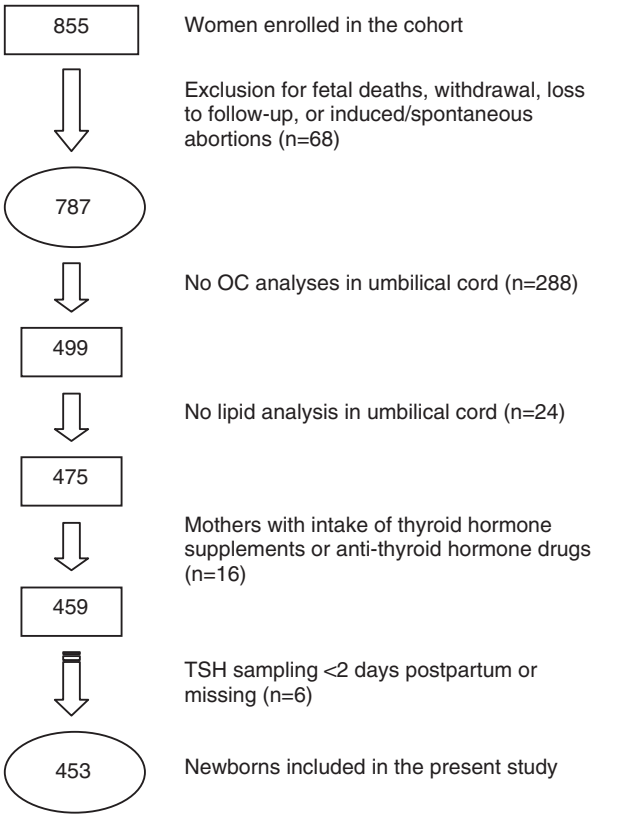


**Figure 1.** Scheme of study population, INMA-Valencia cohort, 2004–2006. OCs: organochlorine compounds; TSH: thyroid stimulating hormone.

Maternal and newborn characteristics of this sample were comparable to those of the rest of the cohort (Table 1), except for a significantly higher probability of missing umbilical cord serum samples from mothers with rural residence (distant from hospital), those with earlier miscarriages, and those with low –birth weight. However, neonatal TSH levels did not differ between the final study sample and the remainder of the enrolled cohort ($P = 0.98$).

### *OC Analyses and Lipid Determination*

We determined the concentrations of seven individual PCB congeners (IUPAC numbers: 28, 52, 101, 118, 138, 153, and 180), HCB, pentachlorobenzene, $\alpha$-HCH, $\beta$-HCH, $\gamma$-HCH, $\delta$-HCH, *4,4′*-DDT, *2,4′*-DDT, and their metabolites *4,4′*-DDE, *2,4′*-DDE, *4,4′*-DDD, *2,4′*-DDD in umbilical cord serum. The laboratory analytical methods and quality control procedures were described elsewhere (Carrizo et al., 2006; Lopez-Espinosa et al., 2009). Briefly, OC concentrations were determined by gas chromatography with electron

**Table 1.** Characteristics of the study population, INMA-Valencia cohort, 2004–2006.

| | Newborn not included ($n=334$) | Newborn included ($n=453$) |
|---|---|---|
| *Maternal variables (%)* | | |
| Born in Spain | 88.3 | 88.1 |
| Rural residence | 9.3 | 3.8** |
| Age $\geqslant$30 years | 52.4 | 55.6 |
| Pre-pregnancy BMI $>26\,kg/m^2$ | 23.4 | 24.5 |
| Primiparous | 54.2 | 55.6 |
| Up to primary studies | 31.1 | 36.0 |
| Working in pregnancy | 82.0 | 83.2 |
| Smoking in pregnancy | 23.9 | 22.2 |
| Alcohol in pregnancy | 37.6 | 37.6 |
| *Newborn variables (%)* | | |
| Male | 50.6 | 54.7 |
| Birth weight $<2500\,g$ | 8.4 | 3.8** |
| Gestational age $<37$ weeks | 7.2 | 5.1 |
| SGA | 13.5 | 9.9 |
| Previous miscarriages | 28.7 | 20.8** |

**$P<0.05$ ($P$-value from $\chi^2$ test).
BMI, body mass index; SGA, small weight for gestational age.

capture detection using an Agilent 6890N GC with a Micro-ECD (Agilent Technologies, Palo Alto, CA, USA). Percent recoveries ranged from 70% to 130% and limits of detections (LODs) from 0.002 to 0.07 ng/ml depending on the OCs.

We determined the total cholesterol and triglycerides by means of enzymatic methods, calculating total serum lipid concentrations using the method of Phillips et al. (1989).

### *Thyroid Hormone Analysis*

The laboratory analytical methods for measuring TSH at birth were described elsewhere (Barona-Vilar et al., 2008). Briefly, neonatal TSH levels were measured in a heel-prick blood sample spotted on filter paper, which is routinely obtained shortly after birth for the national hypothyroidism screening program. In preterm newborns, samples were collected weekly in the hospital until term age, considering the neonatal TSH value of the last gathered sample in our analyses. TSH was measured by using time-resolved sandwich fluoroimmunoassay (AutoDELFIA, Perkin Elmer/Wallac, Turku, Finland). LOD was 0.2 mIU/l. The inter-assay coefficients of variance were 9%, 9.5%, and 6.7% for low, medium, and high concentrations of TSH, respectively. Sensitivity and specificity were 100% and 99%, respectively.

### *Other Covariates*

The mothers completed two questionnaires at enrolment during the first (10–13 weeks) and third trimester (28–32 weeks) of pregnancy, about socio-demographic characteristics and life style variables. The questionnaires were administered by in-person interviews. Covariates considered for inclusion in the models were country of birth, area of residence, maternal age, pre-pregnancy body mass index (BMI), pregnancy weight gain, parity, education, working status (during the third trimester), socio-economic status, cohabitant, smoking habit, and alcohol and caffeine consumption. Both BMI and weight gain were classified according to Institute of Medicine Guidelines (Abrams et al., 2000). We applied a widely used Spanish adaptation of the British classification system (Domingo-Salvany et al., 2000) to define socio-economic status according to current or most recent occupation of the mothers or, for those without employment outside the home, according to the husband's occupation. Active prenatal smoking was defined as smoking at least one cigarette per day during the third trimester. Information on anthropometrical measurements at birth was obtained from the hospital delivery and medical records, and these variables were also considered as covariates. Birth weight (g) was measured by the midwife at delivery. The measurement was standardized for gestational age by using the residuals method. Gestational age was established on the basis of the date of the last menstrual period self-reported by the mothers. However, an early ultrasound of the crown-rump length was also available and used for gestational dating when the difference with the last menstrual period was equal to or greater than 7 days. This occurred in 11.9% of the cases. Small weight for gestational age (SGA) was defined as birth weight below the 10th percentile based on growth reference charts for gestational age by sex for a Spanish population (Carrascosa et al., 2004).

### *Statistical Analysis*

We determined the relationships between neonatal TSH levels and OCs found in umbilical cord serum with detection frequency $>50\%$ (*4,4′*-DDT, *4,4′*-DDE, HCB, $\beta$-HCH, PCBs 118, 138, 153, and 180, and sum of these four PCBs). Neonatal TSH was $\log_{10}$ transformed to improve the normality and was treated as a continuous variable. We used one-way analysis of variance to examine covariates according to TSH levels. The linearity of the relationship between TSH and OC levels was evaluated by using adjusted general additive models, comparing models with OC levels in a linear and non-linear manner (a cubic smoothing spline with 2, 3, and 4 degrees of freedom) by likelihood ratio test (LRT). In general, no significant improvement in the model (LRT $P>0.05$) was obtained with non-linear models. However, the graphical examination provided some evidence of a non-linear shape for most studied OCs, in which a different slope for higher levels ($\sim$90th percentile) of the contaminants were observed. Hence, OC levels were examined as a continuous $\log_{10}$-transformed variable and as a categorical variable ($<$50th, 50th–90th, and $>$90th percentiles). Parameter estimates of the regression models were expressed as percentage of change in neonatal TSH levels associated with

a 10-fold increase in exposure (when OC levels were considered as continuous variables) or with a change from the percentile of reference (<50th) to the other percentiles (50th–90th or >90th percentiles).

We used unadjusted and adjusted linear regression analyses. Multivariate models were adjusted for variables associated with TSH levels in bivariate analyses at a significance level of $P \leqslant 0.20$, sequentially excluding non-significant variables from the model after a backward procedure, using the F test for the change in $R^2$. In this model, potential confounders for OCs were also retained if the OC coefficient changed by >10% when they were dropped. Age at time of sampling was considered as a continuous variable after log transformation, because the adjusted $R^2$ value was higher than when considered as a categorical variable. The remaining covariates were categorized (Table 2). OCs were examined separately to compare results with earlier studies, replacing values below the LOD

**Table 2.** Characteristics of pregnant women and their newborns according to neonatal TSH levels ($n = 453$), INMA-Valencia cohort, 2004–2006.

| Variables | *n* (%) | TSH (mIU/l) | |
|---|---|---|---|
| | | GM (GSD) | *P* |
| Mothers | | | |
| *Country of birth* | | | *0.41* |
| Spain | 399 (88.1) | 1.27 (2.01) | |
| Latin America | 40 (9.2) | 1.49 (1.87) | |
| Rest of Europe | 12 (2.7) | 1.24 (2.45) | |
| *Residence* | | | *0.14* |
| Urban | 46 (10.1) | 1.27 (2.08) | |
| Metropolitan | 225 (49.7) | 1.34 (2.03) | |
| Semi-urban | 165 (36.4) | 1.20 (1.95) | |
| Rural | 17 (3.8) | 1.71 (1.85) | |
| *Age* | | | *0.16* |
| <25 years | 53 (11.7) | 1.16 (2.04) | |
| 25–29 years | 148 (32.7) | 1.30 (1.86) | |
| 30–34 years | 174 (38.4) | 1.39 (2.04) | |
| ≥35years | 78 (17.2) | 1.16 (2.15) | |
| *Pre-pregnancy BMI* | | | *0.72* |
| <19.8 kg/m$^2$ | 64 (14.2) | 1.36 (2.09) | |
| 19.8–26 kg/m$^2$ | 277 (61.3) | 1.29 (1.99) | |
| >26 kg/m$^2$ | 111 (24.5) | 1.25 (2.00) | |
| *Weight gain* | | | *0.33* |
| Low (kg) | 111 (24.9) | 1.29 (2.20) | |
| Normal (kg) | 170 (38.1) | 1.35 (1.92) | |
| High (kg) | 165 (37.0) | 1.20 (1.96) | |
| *Parity* | | | *0.39* |
| Primiparous | 252 (55.6) | 1.25 (1.99) | |
| Multiparous | 201 (44.4) | 1.33 (2.02) | |
| *Education* | | | *0.89* |
| Incomplete | 13 (2.9) | 1.33 (2.03) | |
| Primary | 150 (33.1) | 1.34 (2.06) | |
| Secondary | 187 (41.3) | 1.26 (1.95) | |
| University | 103 (22.7) | 1.27 (2.04) | |
| *Working status*[a] | | | *0.77* |
| Housewife | 65 (14.4) | 1.32 (2.08) | |
| Unemployed | 98 (21.8) | 1.33 (1.95) | |
| Student | 5 (1.1) | 0.96 (2.72) | |
| Employed | 282 (62.7) | 1.28 (1.98) | |
| *Socio-economic status* | | | *0.63* |
| IV + V (lowest) | 276 (60.9) | 1.32 (2.00) | |
| III | 105 (23.2) | 1.24 (2.10) | |
| I + II (highest) | 72 (15.9) | 1.25 (1.88) | |
| *Cohabitant* | | | *0.53* |
| Baby's father | 443 (97.8) | 1.29 (1.99) | |
| Others | 10 (2.2) | 1.48 (2.58) | |
| *Smoking* | | | *0.03*** |
| No | 350 (77.8) | 1.25 (2.00) | |
| Yes | 100 (22.2) | 1.48 (1.96) | |

**Table 2.** *Continued*

| Variables | *n* (%) | TSH (mIU/l) | |
|---|---|---|---|
| | | GM (GSD) | *P* |
| *Alcohol intake* | | | *0.86* |
| 0 g/day | 281 (62.4) | 1.29 (2.02) | |
| 0–1 g/day | 120 (26.7) | 1.28 (1.95) | |
| >1 g/day | 49 (10.9) | 1.36 (2.06) | |
| *Caffeine intake* | | | *0.63* |
| 0–100 mg/day | 324 (72.0) | 1.28 (2.01) | |
| >100–200 mg/day | 97 (21.6) | 1.37 (2.04) | |
| >200 mg/day | 29 (6.4) | 1.23 (1.83) | |
| Newborns | | | |
| *Sex* | | | *0.03*** |
| Male | 248 (54.7) | 1.38 (1.98) | |
| Female | 205 (45.3) | 1.19 (2.02) | |
| *Low birth weight* | | | *0.20* |
| <2500 g | 17 (3.8) | 1.74 (2.62) | |
| ≥2500 g | 436 (96.2) | 1.27 (1.98) | |
| *Prematurity* | | | *0.76* |
| <37 weeks | 23 (5.1) | 1.23 (2.27) | |
| ≥37 weeks | 430 (94.9) | 1.29 (1.99) | |
| *SGA* | | | *0.03*** |
| Yes | 45 (9.9) | 1.59 (2.13) | |
| No | 408 (90.1) | 1.26 (1.98) | |
| *Time of TSH sampling* | | | *0.01*** |
| 2 days | 182 (40.3) | 1.44 (1.99) | |
| 3 days | 163 (36.1) | 1.27 (2.01) | |
| 4 days | 48 (10.6) | 1.07 (1.89) | |
| ≥5 days | 59 (13.0) | 1.11 (2.02) | |

***P*<0.05 (*P*-value from ANOVA test).

BMI, body mass index; GM, geometric mean; GSD, geometric standard deviation; SGA, small weight for gestational age; TSH, thyroid stimulating hormone.

[a]Working status at third trimester.

for OCs with 1/2 LOD in the continuous analysis. OC values were lipid adjusted for the calculation of means and medians, dividing serum residue levels by total serum lipid concentrations, but considering serum lipid as a separate term in the regression models with OCs as continuous variables. Models excluding OC outliers (>3 standard deviation (SD) from mean) were re-run. As no major differences were found, the results are presented with all data included. The normality and homoscedasticity of regression residuals were also assessed. STATA version 9 statistical software package (Stata Corporation, College Station, TX, USA) was used for the data analyses.

## Results

### *Study Population*

Table 2 shows characteristics of pregnant women and their newborns according to neonatal TSH levels ($n=453$). Briefly, mean maternal age was 30 years (range = 16–43 yrs). Approximately 11% of the women were from other countries. Mothers who smoked during pregnancy (22.2%) had infants with higher TSH levels (Table 2).

The mean birth weight and length of the newborns were 3286 g (range = 1200–4600 g) and 50 cm (38–56 cm), respectively, and the mean gestational age was 40 weeks (30–42 wks). TSH levels were higher in male newborns, newborns with SGA, and in samples collected soon after birth (Table 2). Finally, the median age of neonates at the time of the heel prick was 3 days.

### *TSH Status in Newborns and Concentrations of OCs in Umbilical Cord Serum*

Table 3 shows the geometric means (GM), 95% confidence intervals (CI), and 50th and 90th percentiles of OC content in umbilical cord serum (ng/g lipid) and neonatal blood TSH levels (mIU/l).

The levels of PCBs increased with congener chlorination. Thus, PCB 118 was found in 72%, PCB 138 in 86%, PCB 153 in 95%, and PCB 180 in 92%. GM for the sum of four PCBs was 0.33 ng/ml (131 ng/g lipid). Among the other OCs, *4,4′*-DDE was the most frequently detected in cord samples (99.8% detected, GM = 0.49 ng/ml, or 197 ng/g

**Table 3.** Residues of OCs (ng/g lipid) in umbilical cord serum and neonatal TSH (mIU/l) levels ($n = 453$), INMA-Valencia cohort, 2004–2006.

| Residues | ⩾LOD | GM | 95% CI | P50th | P90th |
|---|---|---|---|---|---|
| *4,4′*-DDT[a] | 55.1 | 8.0 | 7.0, 9.3 | 8.4 | 61 |
| *4,4′*-DDE[a] | 99.8 | 197 | 181, 213 | 187 | 551 |
| HCB[a] | 92.5 | 75 | 68, 82 | 87 | 225 |
| *β*-HCH[a] | 78.4 | 20 | 17, 23 | 34 | 104 |
| PCB 118[a] | 71.5 | 19 | 18, 21 | 23 | 68 |
| PCB 138[a] | 86.1 | 29 | 26, 31 | 34 | 69 |
| PCB 153[a] | 95.1 | 40 | 37, 43 | 46 | 87 |
| PCB 180[a] | 91.8 | 27 | 25, 30 | 33 | 61 |
| ΣPCBs[a] | — | 131 | 123, 139 | 144 | 266 |
| TSH[b] | 100 | 1.3 | 1.2, 1.4 | 1.4 | 3.2 |

*4,4′*-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; *4,4′*-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; *β*-HCH, beta-hexachlorocyclohexane; CI, confidence interval; GM, geometric mean; HCB, hexachlorobenzene; LOD, limit of detection; OC, organochlorine compounds; P, percentile; PCBs, polychlorobiphenyls; ΣPCBs, sum of four PCBs (PCB 118, 138, 153, 180); TSH, thyroid stimulating hormone.
[a]Expressed on a lipid basis (ng/g lipid).
[b]Expressed in mIU/l.

lipid). Finally, the median neonatal TSH was 1.4 mIU/l (range = 0.2–8.3 mIU/l).

### *Association between Neonatal TSH Levels and OCs in Umbilical Cord Serum*

Table 4 shows the association between neonatal TSH levels in blood and umbilical cord serum OC concentrations in the unadjusted and adjusted analyses. After adjustment for covariates, newborns with higher (>90th percentile) prenatal exposure to *β*-HCH had an increment in neonatal TSH levels of 21% (95% CI = −3.0, 50.8; $P = 0.09$) in comparison to babies with lower (<50th percentile) exposure to this compound (GM = 0.39 ng/ml or 151 ng/g lipid *versus* 0.01 ng/ml or 5.9 ng/g lipid). Median *β*-HCH levels for the whole population and those with levels higher than 90th percentile were 0.08 ng/ml (34 ng/g lipid) and 0.33 ng/ml (131 ng/g lipid), respectively.

## Discussion

TSH levels tended to be higher in newborns with greater *β*-HCH levels in umbilical cord than in those with levels below the median ($P = 0.09$). No statistically significant association was found between the remaining OCs and neonatal TSH.

In this study, *β*-HCH was detected in 78.4% of samples at a median level of 0.08 ng/ml (34 ng/g lipid). *β*-HCH is an isomer of the pesticide formulation of HCH, which was widely used from the 1940s, but whose commercial use in most countries has been banned or restricted for two or more decades (Breivik et al., 1999). The strongest insecticide properties of HCH derive from the *γ*-isomer (lindane). Some uses of lindane were authorized in Spain until recently (PNA Convenio Estocolmo y Reglamento 850/2004, 2007). Lindane may be transformed into other isomers such as *β*-HCH (Walker et al., 1999), which has high physical and metabolic stability due the positions of its chlorine atoms, explaining its environmental and biological persistence (Willet et al., 1998).

Authors have suggested that *β*-HCH may cause central nervous system, reproductive, and endocrine damage (Willet et al., 1998). Its endocrine disruptor ability has been shown *in vitro* (Steinmetz et al., 1996), but there have been few studies on the thyroid-disrupting effects of *β*-HCH in human beings (Mazhitova et al., 1998; Ribas-Fito et al., 2003; Alvarez-Pedrerol et al., 2007a, 2008). In this study, a tendency to an association between high prenatal *β*-HCH levels and greater neonatal TSH levels was found, consistent with the findings of earlier studies by the multi-center INMA project in other Spanish areas (Ribas-Fito et al., 2003; Alvarez-Pedrerol et al., 2008). A positive association between *β*-HCH in cord serum and neonatal TSH levels was found in a cohort from Northeastern Spain (Ribas-Fito et al., 2003), who showed higher *β*-HCH levels (median = 0.5 ng/ml) than those detected in this study (median = 0.08 ng/ml). In fact, the babies in our series with umbilical cord serum *β*-HCH levels >90th percentile (median = 0.3 ng/ml) also showed a higher neonatal TSH *versus* babies with lower prenatal exposure to *β*-HCH (median = 0.02 ng/ml), similar to the above findings, although the association did not reach significance. A relationship between cord serum *β*-HCH and postpartum TSH levels was also recently reported in a cohort from the Mediterranean island of Menorca (Alvarez-Pedrerol et al., 2008), in which babies with TSH⩾10 mIU/l showed similar *β*-HCH levels (GM = 0.5 ng/ml) to the infants in this study with higher exposure to this compound (GM = 0.4 ng/ml). Finally, the Menorcan study found that serum *β*-HCH levels (GM = 0.2 ng/ml) in preschoolers (4 years) were associated with lower total triiodothyronine (TT3), but not with higher TSH or free thyroxine (FT4) levels (Alvarez-Pedrerol et al., 2007a). Other than these reports, few data have been published on prenatal *β*-HCH exposure and thyroid hormone status, and they showed no association between *β*-HCH concentrations and thyroid hormone levels (Mazhitova et al., 1998). With regard to *β*-HCH umbilical cord levels, our results (median: 0.08 ng/ml or 34 ng/g) are lower than levels found by Torres-Arreola et al. (2003) in Mexico (54.25 ng/g) and Ataniyazova et al. (2001) in Uzbekistan (2060 ng/l), and higher (3.38 ng/g) than levels found in China (Tan et al., 2009).

We found no association between PCB levels in umbilical cord serum and newborn TSH levels, in common with earlier studies (Fiolet et al., 1997; Ribas-Fito et al., 2003; Otake et al., 2007; Dallaire et al., 2008), but unlike some other reports (Koopman-Esseboom et al., 1994; Chevrier et al.,

**Table 4.** Unadjusted and adjusted regression analyses between OC concentrations in umbilical cord serum and neonatal TSH levels ($n = 453$), INMA-Valencia cohort, 2004–2006.

| Residues | TSH | | | | | |
|---|---|---|---|---|---|---|
| | Unadjusted regression analyses | | | Adjusted regression analyses[a] | | |
| | % change[b] | 95% CI | *P* | % change[b] | 95% CI | *P* |
| *4,4′-DDT* | *−0.6*[c] | *−9.7, 9.3* | *0.89* | *−0.7*[c] | *−9.6, 9.2* | *0.89* |
| <P50th | R[d] | | | R[d] | | |
| P50th–P90th | 0.2[d] | −12.6, 14.9 | 0.97 | −0.9[d] | −13.4, 13.5 | 0.90 |
| >P90th | −1.0[d] | −20.7, 23.5 | 0.93 | −1.9[d] | −21.3, 22.3 | 0.86 |
| *4,4′-DDE* | *−2.8* | *−17.9, 15.1* | *0.74* | *−1.0* | *−16.7, 17.7* | *0.91* |
| <P50th | R | | | R | | |
| P50th–P90th | 5.2 | −8.2, 20.6 | 0.46 | 5.4 | −8.0, 20.8 | 0.45 |
| >P90th | −5.1 | −23.9, 18.4 | 0.64 | −5.2 | −24.4, 18.9 | 0.64 |
| *HCB* | *1.5* | *−12.2, 17.3* | *0.84* | *−2.8* | *−16.4, 13.1* | *0.72* |
| <P50th | R | | | R | | |
| P50th–P90th | 6.6 | −7.0, 22.2 | 0.36 | 4.9 | −8.9, 20.7 | 0.51 |
| >P90th | 9.5 | −12.2, 36.6 | 0.42 | 6.6 | −15.0, 33.7 | 0.58 |
| *β-HCH* | *2.0* | *−7.3, 12.2* | *0.69* | *1.4* | *−8.1, 11.8* | *0.79* |
| <P50th | R | | | R | | |
| P50th–P90th | −3.5 | −15.8, 10.6 | 0.61 | −4.2 | −16.6, 10.1 | 0.55 |
| >P90th | 21.8 | −2.3, 51.8 | 0.08* | 21.0 | −3.0, 50.8 | 0.09* |
| *PCB 118* | *0.3* | *−13.6, 16.6* | *0.96* | *−2.1* | *−15.7, 13.8* | *0.78* |
| <P50th | R | | | R | | |
| P50th–P90th | −2.6 | −15.0, 11.7 | 0.71 | −3.7 | −16.0, 10.4 | 0.59 |
| >P90th | −3.6 | −22.6, 20.0 | 0.74 | −5.5 | −24.0, 17.4 | 0.61 |
| *PCB 138* | *7.8* | *−9.6, 28.6* | *0.40* | *5.3* | *−12.2, 26.3* | *0.58* |
| <P50th | R | | | R | | |
| P50th–P90th | 4.8 | −8.5, 20.2 | 0.50 | 3.6 | −9.9, 19.2 | 0.62 |
| >P90th | 0.9 | −19.1, 25.9 | 0.94 | 0.9 | −19.3, 26.1 | 0.94 |
| *PCB 153* | *−3.4* | *−20.3, 17.1* | *0.73* | *−11.1* | *−27.8, 9.5* | *0.27* |
| <P50th | R | | | R | | |
| P50th–P90th | 9.8 | −4.2, 25.9 | 0.18 | 7.1 | −7.1, 23.5 | 0.35 |
| >P90th | −8.3 | −26.3, 14.1 | 0.44 | −11.8 | −29.8, 10.7 | 0.28 |
| *PCB 180* | *−3.8* | *−19.3, 14.8* | *0.67* | *−12.2* | *−27.5, 6.4* | *0.19* |
| <P50th | R | | | R | | |
| P50th–P90th | 7.0 | −6.6, 22.7 | 0.33 | 4.4 | −9.4, 20.4 | 0.55 |
| >P90th | −2.4 | −21.6, 21.6 | 0.83 | −5.4 | −24.7, 19.0 | 0.64 |
| *ΣPCBs* | *1.5* | *−19.1, 27.3* | *0.90* | *−5.3* | *−25.4, 20.2* | *0.65* |
| <P50th | R | | | R | | |
| P50th–P90th | 10.5 | −3.6, 26.6 | 0.15 | 7.5 | −6.4, 23.5 | 0.31 |
| >P90th | 4.3 | −16.4, 30.1 | 0.71 | 1.3 | −19.0, 26.8 | 0.91 |

*$P < 0.10$.

*4,4′*-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; *4,4′*-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; *β*-HCH, beta-hexachlorocyclohexane; CI, confidence interval; HCB, hexachlorobenzene; OC, organochlorine compounds; P, percentile; PCBs, polychlorobiphenyls; R, reference; ΣPCBs, sum of PCB congeners 118, 138, 153, 180; TSH, thyroid stimulating hormone.

[a]Adjusted for maternal age, age at time of sampling, prematurity, small weight for gestational age, prenatal smoking at third trimester of pregnancy, and sex.

[b]Percentage of change in neonatal TSH levels (mIU/l) associated with a 10-fold increase in exposure or with a change from the reference to current category.

[c]Regression analyses with OC as continuous variables ($\log_{10}$ transformed).

[d]Regression analyses with OC as categorical variables (<P50, 50–90, >90).

2007; Alvarez-Pedrerol et al., 2008). This discrepancy in results may be explained by differences in PCB levels, in the matrix used for exposure quantification, in the specific congeners selected for measurement, in the sample size, or in the statistical analysis, including strategies to control for other covariates.

We found no association among HCB, *4,4′*-DDT, or *4,4′*-DDE and neonatal TSH, in agreement with earlier investigations (Dewailly et al., 1993; Ribas-Fito et al., 2003; Alvarez-Pedrerol et al., 2008). However, an association was earlier reported in the present cohort between maternal *4,4′*-DDE levels and maternal TSH levels at 12 weeks of pregnancy (Lopez-Espinosa et al., 2009).

Thyroid function has a crucial function in brain maturation and development in human beings (Dussault and Ruel, 1987). Some studies on newborns with congenital hypothyroidism found an association between thyroid hormone levels at birth and impairment of neurodevelopment (Weber et al., 2000). However, there is little information on the role of thyroid function in brain development of infants whose abnormality in neonatal thyroid function is transient or mild (Rose et al., 2006). Nevertheless, some studies suggested that subtle changes in thyroid hormone homeostasis may affect the development of the central nervous system, as growth and development in the fetus and during childhood is highly dependent on normal levels of thyroid hormones (Dussault and Ruel, 1987). One study of preschool children found that TSH levels in the upper quartile of the normal range were related to impairment of cognitive function and attention behavior (Alvarez-Pedrerol et al., 2007b). Moreover, thyroid hormone disruption has been proposed as a potential mechanism of action for the neurodevelopment effects of certain OCs (Porterfield, 2000). We have traced only one study that addressed this issue in relation to $\beta$-HCH; the authors found an adverse association between prenatal exposure to this compound and neonatal neurodevelopment, suggesting that the ability of $\beta$-HCH to alter thyroid hormones may be responsible for the action (Fenster et al., 2007).

Our study has some limitations. One of them is the participation rate (54%). A descending trend in participation rates has been described, especially in relation to studies in which biological specimens are collected (Morton et al., 2006). Although low participation itself does not necessarily compromise the internal validity of a study, these studies may be more vulnerable to self-selection bias than those with a high participation (Morton et al., 2006). However, an earlier study of our cohort found no differences in age participants and non-participants, although working was more frequent among participants (Rodriguez et al., 2006). A further limitation is that a complete dataset was not available for the entire cohort (453 *versus* 787), which may introduce a potential bias. This study found differences between study participants and non-participants in area of residence, birth weight, and earlier miscarriages. A relationship has been reported between altered thyroid hormone levels and the risk of low –birth weight (LaFranchi, 1999), and these reproductive outcomes have also been associated with higher OC exposure (Longnecker et al., 2001; Venners et al., 2005; Halldorsson et al., 2008). Therefore, any selection bias caused by the loss of these cases would be more likely to underestimate the associations between OCs and neonatal TSH. On the other hand, the TSH levels observed did not differ from those measured in neonates of the cohort who were not included in our study. Furthermore, a descriptive study (Barona-Vilar et al., 2008) on congenital hypothyroidism screening data from Valencia during the period 2004–2006 also showed a distribution of TSH values similar to that observed in our study (median = 1.23 *versus* 1.4 mIU/l in this study), indicating that our study sample might be representative of the general population in our area. However, the fact that TSH levels were all within the normal range precluded the possibility of assessing the association between OC exposure and abnormal neonatal TSH values. A further possible limitation is that we have data on concentrations of TSH at birth, but not on concentrations of total T3 (TT3), free T3 (FT3), TT4, or FT4. Finally, subjects would have been simultaneously exposed to a huge variety of chemicals that may have synergistic or cumulative effects.

Study strengths are that trained interviewers prospectively collected our data, and we considered all known important covariates in our analysis. Moreover, we lost few participants between recruitment and delivery. Our sample size was also larger than in many studies on prenatal exposure to OCs and thyroid hormone levels, allowing consideration of the effect of a large number of covariates. Thus, the similar effect estimates found in unadjusted and adjusted models indicate that residual covariates had little effect on results. Our findings were consistent with those of earlier Spanish cohorts in which OC levels were measured in the same laboratory and neonatal TSH levels were assessed using the same technique (part of routine early screening for hypothyroidism in Spanish hospitals), supporting the validity of this study.

In conclusion, although cord blood levels of most of the studied OCs were not associated with TSH at birth in our sample, our results suggest that prenatal exposure to high levels of $\beta$-HCH may alter thyroid status, as earlier reported. This study, therefore, contributes to our understanding of the limited available data on the association between thyroid hormone levels and prenatal OC exposure. This investigation forms part of a cohort study in which thyroid hormone and OC levels have been measured at different stages of pregnancy in the mother and at birth and will be followed in the children during their later life. This approach will allow us to determine possible associations between prenatal OC exposure and thyroid hormone levels at different time

windows of exposure and to evaluate their impact on the neurodevelopment of these children.

## Acknowledgements

We are grateful to mothers who participated in the study and to the Obstetrics and Data Processing Departments of La Fe Hospital, Valencia. We also thank Richard Davies for editorial assistance. The study was funded by grants from Spanish Ministry of Health and Ministry of Science and Innovation (INMA G03/176, FIS 03/1615, FIS 04/1112, FIS 04/1509, FIS 06/1213, Programa Juan de la Cierva-FSE) and Conselleria of Sanitat, Generalitat Valenciana (030/2008, 036/2008).

## Conflict of interest

The authors declare no conflict of interest.

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