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Abstract

Background: Several studies have reported decreases in birth size associated to organochlorine compound (OC) exposure but uncertainties remain regarding the critical windows of prenatal exposure and the effects on fetal body segments.

Objective: To examine the relationship between prenatal OC concentrations and fetal anthropometry.

Methods: We measured 4,4'-dichlorodiphenyldichloroethylene (4,4'-DDE), hexachlorobenzene (HCB) and polychlorinated biphenyl (PCB) congeners (138, 153 and 180) in 2369 maternal and 1140 cord serum samples in four Spanish cohorts (2003-2008). We used linear mixed models to obtain longitudinal growth curves for estimated fetal weight (EFW), abdominal circumference (AC), biparietal diameter (BPD), and femur length (FL) adjusted by parental and fetal characteristics. Standard deviation (SD) scores of growth at 0–12, 12–20, and 20–34 weeks of gestation, and size at gestational week 34 were calculated for the four parameters. We studied the association between OCs and the fetal outcomes by cohort-specific linear models and subsequent meta-analyses.

Results: PCBs were associated to a reduction in AC up to mid-pregnancy, and BPD and FL from gestational week 20 onwards. An inverse association was also found between HCB and AC growth in early pregnancy. The reduction of these parameters ranged from -4% to -2% for a doubling in the OC concentrations. No association between 4,4'-DDE and fetal growth was observed.

Conclusions: To our knowledge, this is the first study to report an association between prenatal exposure to some PCBs and HCB and fetal growth: AC during the first two trimesters of pregnancy, and BPD and FL later in pregnancy.

Introduction

Fetal growth is an important indicator of child health since its impairment may be associated with poor neurodevelopment (Richards et al. 2002), and with chronic diseases in adulthood (Barker 2007). It has been hypothesized that some organochlorine compounds (OCs) can cross the placenta (Vizcaino et al. 2014) and may interfere with fetal development and growth (Windham and Fenster 2008).

To date, most studies on prenatal exposure to OCs and fetal growth have used anthropometric measures at birth (El Majidi et al. 2012; Govarts et al. 2012) and, to a lesser extent, outcomes such as being small-for-gestational-age (Ribas-Fito et al. 2002) as proxy measures of *in utero* growth. A limitation with these approaches is that fetal growth can only be assessed after delivery and they do not allow different patterns of development to be examined throughout pregnancy. Thus, growth-retarded fetuses and healthy but constitutionally small ones may have the same birth weight (Gardosi 2004). In addition, it has been suggested that birth weight only poorly reflects fetal growth during the first two trimesters of pregnancy (Gardosi 2004). Finally, assessment of birth size does not fully capture the time during gestation in which fetal growth failures begin or the onset of transient effects that may occur during intrauterine life. Therefore, the study of the effects of these contaminants on fetal growth using longitudinal ultrasound measurements may be useful to identify specific prenatal periods of vulnerability to OC exposure, and especially the age at which fetal growth failure may begin.

Within the Spanish INMA (Environment and Childhood) Project, we aimed to examine the relationship between maternal and cord concentrations of 4,4'-dichlorodiphenyldichloroethylene

(4,4'-DDE), hexachlorobenzene (HCB), and three polychlorinated biphenyls (PCBs) (138, 153, and 180) and fetal growth using serial ultrasound measurements at 12, 20 and 34 weeks of pregnancy, and taking into account the individual growth potential of the fetus.

Material and Methods

Study design and population

The INMA Project is a multicenter population-based mother-and-child cohort study established in different areas of Spain following a common protocol (Guxens et al. 2012). This study included the cohorts of Asturias, Gipuzkoa, Sabadell, and Valencia. The Hospital Ethics Committees of each region approved the research protocol.

A total of 2644 eligible women (≥ 16 years, singleton pregnancy, enrollment at 10–13 weeks of gestation, non-assisted conception, delivery scheduled at the reference hospital, and no communication impairment) were recruited in the first trimester of pregnancy and gave their written informed consent prior to inclusion (2003-2008). After excluding the women who withdrew from the study, were lost to follow-up, or had induced or spontaneous abortions or fetal deaths, 2506 (95%) women were followed up to delivery (May 2004-August 2008). In the present study, the sample size was 2407 mothers and their newborns (Table 1) with at least two valid ultrasounds and OC determinations in maternal ($n=2369$) and/or cord ($n=1140$) serum. The maternal and newborn characteristics of this study sample were comparable to those of the rest of the cohort (data not shown).

OC exposure assessment

OC concentrations were measured in maternal serum samples of the four cohorts and in umbilical cord serum samples of three cohorts (Asturias, Gipuzkoa, and Valencia). Samples collected in Gipuzkoa and Sabadell were analyzed at the Basque Government's Public Health Laboratory in San Sebastian (limit of detection (LOD) of 0.071 ng/mL for all the OCs) and samples from Asturias and Valencia were analyzed at the Barcelona Institute of Environmental Assessment and Water Research (LODs of OCs between 0.010 and 0.035 ng/mL), as previously described (Goni et al. 2007; Vizcaino et al. 2010). OC concentrations were determined by gas chromatography with electron capture detection and confirmation by gas chromatography coupled to a mass spectrometer detector. Both laboratories complied with the Arctic Monitoring and Assessment Program for persistent organic pollutants in human serum (Centre de Toxicologie, Institut National de Santé Publique du Québec). In this study, we present the results of those OCs with a detection frequency >80% (4,4'-DDE, HCB, and PCBs 138, 153, and 180). Values below the LOD were replaced by values within the range [0, LOD] based on a multiple imputation procedure (see the Statistical analysis section).

Total cholesterol and triglycerides were determined by means of enzymatic techniques, and total serum lipid concentrations were calculated (Phillips et al. 1989). Means (standard deviations [SDs]) of total lipid contents in maternal and cord serum were 6.02 (1.10) and 2.53 (0.67) mg/mL, respectively.

Fetal ultrasonography

Ultrasound examinations were routinely scheduled for gestational weeks 12, 20 and 34, and were performed by obstetricians specialized in conducting this type of examinations at the respective hospitals. We also had access to the records of any other ultrasound scan performed on the women during pregnancy, which allowed us to obtain from 2 to 8 valid ultrasound measurements per subject between the 7th and 42nd weeks of gestation. Of a total of 2478 women providing ultrasound data, 164 (6.6%) had two examinations, 2035 (82.1%) had three, 235 (9.5%) had four, and 44 (1.8%) had five or more. Thus, a total of 7602 scans were used to build longitudinal growth curves for fetal parameters (Iniguez et al. 2013).

Gestational age was based on the self-reported date of the last menstrual period, but it was estimated using an early crown-rump length measurement if the self-reported and estimated dates differed by ≥ 7 days (Westerway et al. 2000). Women for whom this difference exceeded 3 weeks were removed from the study to avoid possible bias ($n=18$).

Fetal measures were abdominal circumference (AC), biparietal diameter (BPD), and femur length (FL). Data outside the range of the mean ± 4 SDs for each gestational age were also eliminated to avoid the influence of extreme values ($n=5$, 8, and 8 for AC, FL and BPD, respectively). Additionally, estimated fetal weight (EFW) was calculated using the Hadlock algorithm (Hadlock et al. 1985). We used linear mixed models (Pinheiro and Bates 2000) separately in each cohort to obtain longitudinal growth curves for the four fetal parameters. Our aim was to discriminate between small fetuses (related to the size of the general population) and reduced growth (related with the characteristics of the fetus itself) (Mamelle et al. 2001).

Therefore, models were adjusted for those available covariates related to the constitutional potential of the fetus (Mamelle et al. 2001). Fetal growth modeling is briefly described below and in greater detail in the Supplemental Material, Detailed modeling procedure, Table S1, and Figures S1-S4.

The full model parameterization for each fetal parameter in each cohort is:

$$Y_{ij}^{(\lambda)} = X_{ij}\beta + Z_{ij}b_i + \varepsilon_{ij} \quad [1]$$

where:

- $Y_{ij}^{(\lambda)}$ is a Box-Cox transformation of the fetal parameter value Y_{ij} in the i^{th} fetus at time T_j , suggested in Gurrin et al. (2001) and Royston and Altman (1994), in order to obtain normality and linearity;
- $X_{ij} = [1, p(T_{ij}), C_i^1, \dots, C_i^P, T_{ij} \times C_i^1, \dots, T_{ij} \times C_i^P]$ is the fixed-effects regressor matrix for the i^{th} fetus at time T_j . $p(T_{ij})$ represents a polynomial of entire order until 3 in T_j or a low-order fractional polynomial, described by Royston and Altman (1994). (C_i^1, \dots, C_i^P) is the subset of the covariates considered for the i^{th} fetus: maternal and paternal height, maternal and paternal weight or body mass index (BMI), maternal age, parity, country of origin, and fetal sex. Finally, $(T_{ij} \times C_i^1, \dots, T_{ij} \times C_i^P)$ represent the interactions of each covariate with the time at measurement;
- β is the vector of fixed coefficients to be estimated;
- $Z_{ij} = [1, T_{ij}]$ is the random-effects regressor matrix for the i^{th} fetus at time T_j ;

- b_i is the vector of random effects which is estimated for each fetus, and whose distribution across the fetal population is assumed to be bivariate normal, $b_i = (b_{0i}, b_{1i}) \propto N(0, D)$;

- ε_{ij} is the within-subject error, $\varepsilon_i = (\varepsilon_{i1}, \dots, \varepsilon_{iN}) \propto N(0, \sigma^2 \Lambda_i)$;

In our models the standard assumption of $\Lambda_i = I$ was relaxed in order to allow for 1) heteroscedasticity, and 2) autocorrelation modeling of within-subject errors. This was performed in the following way:

$$\Lambda_i(j, j) = g(T_{ij}, C_i, M_i, \delta) \quad [2]$$

$$\Lambda_i(j, k) = f(d_{jk}, \phi) \quad [3]$$

where:

- $g(x)$ is a function, assigning different variances to each value x , according to a parameter δ . Time (T), biological covariates (C), and a series of dichotomous variables (M_i^1, \dots, M_i^Q) tagging pregnancies with at least two consecutive ultrasounds performed too close together in time were tested as variables explaining heteroscedasticity. M^j were often included in the models;

- $f(x)$ is a function modeling the auto-correlation structure within subjects. Different models of decays with distance were tested but in our models an exponential variogram was always selected. This model represents an exponential decay in the correlation between observations with the difference in time between them, that is, $f(d_{jk}, \phi) = 1 - \exp(-d_{jk}/\phi)$. $d_{jk} = |T_{ij} - T_{ik}|$ and ϕ parameter to be estimated.

The selection criterion to select the covariates included in X, Z as well as explaining heteroscedasticity for within-subject errors, was p -value<0.05 (conditional F-test for fixed effects; LR-test for random effects).

Fetal growth curves provided predictions for weeks 12, 20 and 34, and were used to calculate unconditional and conditional SD:

$$z_{ij} = \frac{Z_{ij} - E[Z_{ij}]}{\text{Var}[Z_{ij}]} \quad [4]$$

$$z_{2|1} = \frac{Z_2 - \mu_{2|1}}{\sigma_{2|1}^2} \quad [5]$$

where $\mu_{2|1} = E[Z_2 | Z_1] = \mu_2 + \frac{\sigma_{12}^2}{\sigma_1^2}(Z_1 - \mu_1)$, and $\sigma_{2|1}^2 = \text{Var}[Z_2 | Z_1] = \sigma_2^2 - \frac{\sigma_{12}^2}{\sigma_1^2}$.

Unconditional scores (4) describe the size of a fetus and were calculated at 12, 20 and 34 weeks of gestation. Conditional SD scores (5), that is, the standardization of the response at time T_2 , according to the observed value at time T_1 , evaluate the growth in the interval T_1 – T_2 , and were calculated for the intervals 12–20 and 20–34 weeks of gestation.

Other covariates

During the first and third trimesters of pregnancy, women completed two detailed in-person questionnaires on anthropometric, socio-demographic and life style characteristics, as well as two semi-quantitative food frequency questionnaires, as further described elsewhere (Llop et al. 2010). We considered the following maternal variables: age (years), height (cm), pre-pregnancy

BMI (in Kg/m²), weekly gestational weight gain (GWG) from week 12 to delivery (low, recommended, and high) in accordance with the recommendations of the Institute of Medicine (IOM 2009), country of birth (Spain, Latin America, and other), area of residence (rural and urban), education (up to primary, secondary, and university), employment during pregnancy (yes and no), socio-economic status according to the most privileged occupation of the mother or father during pregnancy (class I: managerial jobs, senior technical staff, and commercial managers; class II: skilled non-manual workers; and class III: manual workers) (Domingo-Salvany et al. 2000), parity (0 and ≥ 1 children), tobacco consumption at week 12 of pregnancy (yes and no), passive smoking at home, workplace, or leisure areas/restaurants (yes and no), season of last menstrual period, and intake of vegetables (g/day), fruit (g/day), seafood (including three variables: lean, oily fish, and other seafood in g/day), total energy (kcal/day), and beverages containing alcohol (yes and no). We also considered paternal height (cm) and BMI (Kg/m²), and sex of the fetus.

Statistical analysis

For descriptive purposes, we present numbers and percentages for categorical variables, and means and SDs for continuous variables. Percentiles (P) 25, P50 and P75 are presented for OCs. We evaluated placental transfer by calculating P25, P50, and P75 of the ratios of maternal and umbilical cord OC concentrations, and Pearson's partial correlations between the compounds (log₂-transformed) adjusted by cohort. We also used Pearson's correlations adjusted by cohort to describe pairwise relationships between log₂(OC) measured in the same matrix. Contaminant concentrations are expressed as wet-weight concentrations (in ng/mL) and in ng/g lipid for descriptive analyses.

We conducted multiple linear regression analyses to analyze the association between maternal and cord serum OC concentrations and SD scores of fetal growth measurements at weeks 0–12, 12–20, 20–34, and size at 34 weeks of gestation. We used wet-weight OC concentrations with adjustment for maternal or cord lipid levels as a separate term to minimize potential biases due to lipid standardization (Schisterman et al. 2005), after log₂-transformation of OC (log₂(OC)) and lipid concentrations to account for right skewed distributions. In order to identify predictor and confounder variables, we conducted linear regression analyses to determine covariates associated with fetal growth outcomes and tobit regression to study the relationship between covariates and log₂(OC) concentrations accounting for the left-censoring of the data at the LOD (Lubin et al. 2004). The adjustment for covariates was performed in line with the following procedure. Firstly, we included in basal models any variable associated with each outcome in bivariate analyses at a significance level of p -value<0.20. Then, we sequentially excluded variables not related at p -value<0.10 using the F test and following a backward procedure. Later, we also added any potential confounder (included in the section of covariates) of OCs to the models if the OC coefficient changed by >10% when it was added. Moreover, we included in all the models, regardless of their statistical significance, the variables: cohort, total serum lipid levels, and maternal age, BMI, and country of birth, since these four latter were strongly associated with OCs in previous studies (Ibarluzea et al. 2011;Llop et al. 2010;Vizcaino et al. 2010). GWG was also included in some models (Govarts et al. 2014;Verner et al. 2013;Vizcaino et al. 2014). Specifically, GWG was included in models of cord OCs and outcomes measured from week 12 onwards since GWG was calculated from week 12 to delivery. We assessed the normality and

homoscedasticity of regression residuals, excluding extreme outliers (studentized residuals ≥ 4) or highly influential observations (Cook's distance > 0.5) from the main analyses.

Final models were stratified by cohort to account for the possible heterogeneity of the association between exposure and response variables. Final coefficients and their 95% confidence intervals (CIs) were estimated using meta-analysis. Heterogeneity was quantified with the I^2 statistic (Higgins et al. 2003) and the random-effects model was used when levels were above 50%. Parameter estimates were expressed as the percentage of change in the outcome with respect to the mean, and its 95% CI associated with a doubling in the OC concentrations.

We used multiple imputation with chained equations (Sterne et al. 2009) to deal with missing values in covariates and with values $< \text{LOD}$ in exposure variables (avoiding the fixed imputation of $\text{LOD}/2$ by assuming a log-normal distribution of OCs and conditioning the imputation to the range $[0, \text{LOD}]$). We generated a total of 50 complete datasets by using the *mice* package for R (Van Buuren and Groothuis-Oudshoorn 2011), and estimates on each dataset were combined using Rubin's rules for multiple imputation (Little and Rubin 2002). To impute OC values $< \text{LOD}$, we defined an additional function for bootstrap multiple imputation of interval censored variables (Lubin et al. 2004). Information on the multiple imputation procedure is available in the Supplemental Material, Details on multiple imputation (MI) modeling, and in Table S2. All results in the present work (including the description of OC concentrations, the Pearson's correlation analyses, and the final cohort-specific adjusted models) were based on pooled estimates from a multiple imputed dataset.

We carried out different sensitivity analyses to evaluate the robustness of the results. Firstly, we compared models using multiple imputation (the main analysis of our study) with the complete case analysis (models restricted to subjects with complete data in covariates and replacing OC values <LOD with 1/2LOD value). In models of multiple imputation, we conducted a multi-pollutant analysis that simultaneously included the OCs showing an association with fetal growth in the present analysis. We also run an analysis excluding GWG. Finally, we also investigated differences by sex by including the interaction of this variable with the contaminants in the main analyses.

We used the statistical software R.3.1.1 (R Core Team 2014). Where associations are referred to as statistically or marginally significant associations, this implies a p -value <0.05 or <0.1, respectively.

Results

Study population characteristics

Table 1 shows the characteristics of the study population. Mean maternal age was 31 (range: 16–43) years, 56% were primiparous, and 32% were smokers at the beginning of pregnancy.

OC concentrations

Wet-weight concentrations of OCs in maternal and cord serum samples are shown in Table 2. We used individual total lipid values to calculate OC concentrations on a lipid content basis. Maternal medians for 4,4'-DDE, HCB, as well as for PCBs 138, 153, and 180, were 141, 50, 29, 48, and 34 ng/g lipid, respectively. Respective cord medians were 154, 61, 34, 48, and 34 ng/g lipid (data not shown).

Maternal and cord serum OC correlations and placental transfer of contaminants

Three out of the four participant cohorts (Asturias, Gipuzkoa, and Valencia) had information on both maternal and cord serum OC concentrations. Table 3 shows the Pearson's correlation coefficients between maternal and cord $\log_2(\text{OC})$ concentrations expressed in ng/mL as well as in ng/g lipid (range: 0.38 to 0.77, p -value<0.001 in all cases). Maternal serum concentrations (ng/mL) of DDE, HCB, PCB 138, 153 and 180 averaged 2.46, 2.11, 2.41, 2.55, and 2.79 times those of cord serum, respectively. Ratios were close to 1 when OC concentrations were lipid-adjusted (Table 3). Supplemental Material, Table S3 shows the Pearson's correlations between OCs in the same matrix (p -value<0.001 in all cases) being similar using the wet-weight and the lipid-adjusted concentrations. Correlations were lower between DDE and other OCs (ranges: 0.12–0.27 and 0.31–0.39 in maternal and cord serum, respectively), and higher between HCB and PCBs or between PCB congeners (ranges: 0.53–0.88 and 0.54–0.82 in maternal and cord serum, respectively).

OC exposure and ultrasound measurements of fetal growth

In Figure 1, the adjusted regression analyses are shown for the relationship between $\log_2(\text{OC})$ concentrations measured in either maternal or cord serum samples and SD scores of fetal growth or size (% of change; 95% CI, for a doubling in OCs).

Between 0 and 12 weeks of pregnancy, there were not statistically significant associations between OC concentrations and fetal outcomes except in the case of cord HCB (-2.3%; 95% CI: -4.4, -0.2%) or cord PCB 138 (-2.6%; 95% CI: -5.1, -0.1%) and AC growth. Marginally

significant inverse associations (p -value <0.10) were also found between cord HCB and EFW and between cord PCB 138 and the rest of fetal outcomes.

During the period between 12 and 20 weeks of gestation, we did not find any statistically significant associations between OC concentrations and growth of BPD or FL. Regarding AC growth, patterns were similar for maternal and cord serum (Figure 1), but only statistical significant for cord PCB 138 (-3.3%; 95% CI: -5.8, -0.8%) and PCB 180 (-3.7%; 95% CI: -6.5, -0.9%). Marginally significant inverse associations were also found between AC and the rest of OCs measured in cord (4,4'-DDE, HCB, and PCB 153). Associations for EFW growth did not reach statistical significance but were marginal with maternal PCBs 138 and 153.

During the period between 20 and 34 weeks of pregnancy, negative associations were observed for all the fetal outcomes and PCBs measured in either maternal or cord serum. Significantly so for maternal PCB 138 and EFW growth (-2.1%; 95% CI: -4.2, -0.1%), maternal PCBs and FL growth (PCB 138: -2.8%; 95% CI: -4.9, -0.8%; PCB 153: -3.8%; 95% CI: -6.0, -1.6%; and PCB 180: -3.0%; 95% CI: -5.3, -0.7%), and cord PCBs and BPD growth (PCB 138: -2.8%; 95% CI: -5.3, -0.3%; and PCB 153: -3.1%; 95% CI: -6.0, -0.2%).

Patterns of associations on growth parameters were coherent with those observed on size at 34 weeks, but only some associations were statistically significant (Figure 1). Negative associations between cord PCBs 138 and 153 and growth in BPD between 20 and 34 weeks of gestation were also apparent for the same exposures and BPD size at 34 weeks, though the association with PCB 153 was only marginally significant. All three PCBs measured in maternal serum were associated with significantly lower FL growth between 20 and 34 weeks of gestation and smaller

FL size at 34 weeks. Although AC growth was significantly lower in association with cord HCB and PCB 138 at 0–12 weeks, and with cord PCBs 138 and 180 at 12–20 weeks, AC size at week 34 was not clearly associated with either exposure. No significant associations between DDE and fetal growth were observed during pregnancy.

The estimates of the main analysis (i.e. multiple imputation) were similar to those of the complete case analysis (i.e. restricted to data with no missing information in covariates and OC values <LOD replaced with 1/2LOD value), the multi-pollutant analysis (i.e. main analysis including other OCs), or the main analysis excluding GWG (Figure 2 and Supplemental Material, Figures S5-S7). The main differences were found in the multi-pollutant analysis with wider CIs. There were no consistent interactions with sex (data not shown), and only two interactions were statistically significant. Specifically, cord HCB and FL growth between the 12 and 20 weeks of pregnancy (females: -1.9%; 95% CI: -4.9, 1.0%, males: 2.3%; 95% CI: -0.4, 5.0%, p -interaction=0.03) and maternal 4,4'-DDE and AC growth between the 20 and 34 weeks of gestation (females: 1.5%; 95% CI: -0.6, 3.5%, males: -1.2%; 95% CI: -2.9, 0.6%, p -interaction=0.05).

Discussion

To the best of our knowledge, this is the first study to consider specific fetal body segments that could be affected by exposure to OCs during different critical exposure windows. Increases in PCB concentrations were related to reductions in AC up to mid-pregnancy, and to decreases in fetal BPD and FL from gestational week 20 onwards. We also found an inverse association between HCB and AC during the first trimester of pregnancy. The estimated mean difference in

these fetal parameters ranged from -4% to -2% for a doubling in the OC concentrations. The magnitudes of the estimates in the multiple imputation analysis were similar to those of the complete case analysis, as well as those of the multiple imputation analysis after adding other OCs or excluding GWG. Clear results suggesting a differential effect between sexes were not found.

No associations between serum DDE concentrations and ultrasound measurements were found. No previous studies using fetal anthropometry measures are available for comparison with our results but controversy exists about birth size. While associations were reported in some studies (Lopez-Espinosa et al. 2011; Wolff et al. 2007), others found little or no evidence of associations with DDE exposure (Govarts et al. 2012; Sagiv et al. 2007).

Cord serum HCB concentrations were inversely associated with AC in early pregnancy. Although this fetal measure and birth weight are not directly comparable, a marginally significant decrease in birth weight associated to cord HCB concentrations was reported in newborns from the INMA-Valencia cohort (Lopez-Espinosa et al. 2011). Additionally, a non-statistically significant inverse association between birth weight and maternal HCB concentrations was reported in another previous INMA study (Basterrechea et al. 2014).

Some PCBs measured in cord serum were negatively associated with AC. Specifically, this was the case with PCB 138 up to the second trimester and PCB 180 at 12–20 weeks of pregnancy. In spite of the limitations of comparability between fetal and birth outcomes, a systematic analysis of 20 epidemiological studies on PCBs reported insufficient evidence of an association with birth weight <2.500 g (El Majidi et al. 2012). Conversely, an inverse linear exposure–response

relationship between birth weight and cord PCB 153 was reported in a meta-analysis conducted in 12 European cohorts, which include the children in the present study (Casas et al. 2014;Govarts et al. 2012). In a second analysis controlling for GWG, the strength of the association was reduced, although a statistically significant reduction in birth weight was still observed (Govarts et al. 2014). In the present study, maternal serum PCB 138, 153, and 180 concentrations were associated with lower FL growth from 20–34 weeks and smaller FL size at 34 weeks. Cord serum PCB 138 and 153 concentrations were associated with lower BPD growth from 20–34 weeks and smaller BPD size at 34 weeks (with the latter significant only for PCB 138). A marginally significant reduction in birth length associated with cord PCB 153 concentrations was previously found in the INMA-Valencia cohort (Lopez-Espinosa et al. 2011). Conversely, associations with birth length or head circumference were not found in other studies (Sagiv et al. 2007;Wolff et al. 2007).

Although the biological mechanisms underlying the effects of OCs on fetal growth are not well established, these compounds can disrupt the endocrine system, which is involved in fetal development (Bourguignon and Parent 2010). Thus, thyroid hormones play an important role in somatic growth, and the differentiation and functioning of many tissues during development (Blazer et al. 2003), and some studies have suggested the existence of an association between altered thyroid levels during pregnancy and exposure to some OCs (Alvarez-Pedrerol et al. 2009;Lopez-Espinosa et al. 2009). OCs may also impede placental functions and contribute to fetal growth impairment. Thus, exposure to some OCs has been associated to placental vascular and trophoblastic lesions in animals studies (Backlin et al. 1998) and alterations of the placental

transport of calcium and other nutrients in humans (Hamel et al. 2003; Tsuji et al. 2013) which are essential for fetal development.

Several shortcomings of the present study warrant cautious interpretation of the findings until more studies are available. Since multiple estimates were derived, results should be taken with caution because some statistically significant associations could result from chance. The estimates of the coefficients and their confidence intervals should be taken as a global picture of the pattern of the relations between the variables involved in the study (Rothman 1990). Secondly, the criteria of inclusion may have imposed some selection and an underrepresentation of pregnant women with increased risk of adverse pregnancy outcomes. However, the aim of these commonly used criteria is to obtain a more homogeneous population and reduce the confounding potential. Another weakness is the possible selection bias between women included in or excluded from the present study, yet the differences in the main study variables observed between both groups were not significant. Although the OC concentrations were measured in two different laboratories with different LODs, both participated in the same monitoring and assessment program for persistent organic pollutants in human serum to verify their analytical results and to ensure the comparability of their data. In addition, random-effects meta-analysis was used to address heterogeneity resulting from the use of different laboratories and other factors that could differ among the cohorts.

One of the major strengths of this work with respect to previous studies on birth size is the repeated measurements of fetal anthropometry, which allowed us to study associations between OCs and growth in different stages of pregnancy. We accurately assessed fetal growth by means

of a longitudinal analysis, adjusting for parental and fetal characteristics, in order to compare the expected versus real growth of each fetus. The use of individualized standards is expected to reduce misclassification by identifying constitutionally small babies and those with restricted growth (Gardosi 2004). Secondly, the use of repeated measurements of fetal anthropometry allowed us to study associations between these growth parameters and OCs in different stages of pregnancy, and thereby identify critical periods within gestation. Thirdly, unlike most previous studies that have relied on a single blood measurement of exposure, we have information on OC exposure at the beginning of pregnancy and at delivery. Finally, other strengths of the present study are the large sample size, its prospective design, the low rate of participant drop-out between recruitment and delivery, detailed information on many potential confounders from early pregnancy, and the use of multiple imputation to deal with undetected values in the exposure variables and missing values in the covariates (Sterne et al. 2009).

Conclusions

In conclusion, PCB exposure may decrease fetal AC growth during the first two trimesters of pregnancy, and fetal growth of BPD and FL from mid-pregnancy onwards. A transient association between HCB and AC in early pregnancy was also found. The reduction of these parameters ranged from -4% to -2% for a doubling in the OC levels. No statistically significant association between DDE and fetal growth was observed. Ultrasound measurements constitute a promising tool to examine how early prenatal OC exposure may affect fetal growth and more studies are needed.

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Table 1. Study population. The INMA Project, 2003-2008 (Spain).

Variables	Population (n=2407)
Maternal characteristics	
Age (years): Mean±SD	31±4.3
Height (cm): Mean±SD	163±6.2
BMI ^a (Kg/m ²): Mean±SD	24±4.4
Adequate GWG ^b : n(%)	890(38)
Born in Spain: n(%)	2209(92)
Primary studies: n(%)	588(25)
Working in pregnancy: n(%)	2008(83)
Lowest social class: n(%)	1045(43)
Rural residence: n(%)	141(6)
Primiparous: n(%)	1347(56)
Smoking ^c : n(%)	739(32)
Passive smoking: n(%)	1452(62)
Alcohol intake: n(%)	299(13)
Paternal characteristics	
Height (cm): Mean±SD	176±7.0
BMI (Kg/m ²): Mean±SD	26±3.4
Child characteristics	
Sex (male): n(%)	1245(52)

BMI: body mass index; GWG: gestational weight gain.

^a Pre-pregnancy BMI.

^b Adequate GWG during the 2nd and 3rd trimester according to the pre-pregnancy BMI: 0.44-0.58, 0.35-0.50, 0.23-0.33, and 0.17-0.27 Kg/week for underweight, normal, overweight, and obese women, respectively (IOM 2009).

^c At week 12 of pregnancy.

Table 2. Percentage \geq LOD and median (P25, P75) of OCs (ng/mL). The INMA Project, 2003-2008 (Spain).

Variable	4,4'-DDE	HCB	PCB 138	PCB 153	PCB 180
Maternal serum					
Overall $\% \geq$LOD	99.2	93.2	90.9	96.2	93.6
Overall (n=2369)	0.83 (0.49, 1.56)	0.29 (0.16, 0.51)	0.17 (0.11, 0.25)	0.28 (0.19, 0.40)	0.20 (0.13, 0.30)
Asturias (n=450)	1.39 (0.80, 2.41)	0.37 (0.22, 0.58)	0.20 (0.14, 0.29)	0.34 (0.24, 0.45)	0.24 (0.16, 0.34)
Gipuzkoa (n=596)	0.54 (0.35, 0.84)	0.20 (0.12, 0.32)	0.18 (0.13, 0.26)	0.30 (0.21, 0.43)	0.22 (0.14, 0.34)
Sabadell (n=594)	0.71 (0.43, 1.16)	0.23 (0.13, 0.38)	0.11 (0.07, 0.16)	0.20 (0.14, 0.28)	0.14 (0.09, 0.20)
Valencia (n=729)	1.09 (0.64, 1.90)	0.43 (0.21, 0.69)	0.20 (0.13, 0.28)	0.30 (0.21, 0.41)	0.22 (0.15, 0.31)
Umbilical cord serum					
Overall $\% \geq$LOD	98.3	87.7	81.8	91.6	86.3
Overall (n=1140)	0.38 (0.23, 0.67)	0.16 (0.09, 0.27)	0.08 (<LOD, 0.12)	0.12 (0.08, 0.17)	0.08 (<LOD, 0.12)
Asturias (n=318)	0.47 (0.25, 0.85)	0.13 (0.09, 0.22)	0.08 (0.05, 0.12)	0.13 (0.09, 0.18)	0.07 (0.04, 0.10)
Gipuzkoa (n=324)	0.24 (0.15, 0.37)	0.11 (<LOD, 0.17)	0.08 (<LOD, 0.12)	0.12 (0.08, 0.17)	0.10 (<LOD, 0.13)
Valencia (n=498)	0.46 (0.30, 0.78)	0.22 (0.13, 0.36)	0.09 (0.05, 0.12)	0.11 (0.08, 0.16)	0.08 (0.05, 0.11)

DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; LOD: limit of detection; OC: organochlorine compound; P: percentile; PCB: polychlorinated biphenyl

Table 3. Concentration ratios and Pearson's correlations of OCs in maternal and umbilical cord serum (n=1102). The INMA Project, 2003-2008 (Spain).

OC	Expressed in ng/mL		Expressed in ng/g lipid	
	Ratio ^a : C _m /C _{uc}	Pearson ^b	Ratio ^a : C _m /C _{uc}	Pearson ^b
	Median (P25, P75)	Coef.	Median (P25, P75)	Coef.
4,4'-DDE	2.46 (1.88, 3.21)	0.77	1.04 (0.76, 1.46)	0.75
HCB	2.11 (1.49, 2.94)	0.57	0.89 (0.56, 1.36)	0.54
PCB 138	2.41 (1.69, 3.28)	0.41	1.01 (0.67, 1.47)	0.38
PCB 153	2.55 (1.93, 3.45)	0.49	1.11 (0.79, 1.52)	0.47
PCB 180	2.79 (1.96, 4.07)	0.56	1.19 (0.79, 1.79)	0.55

DDE: dichlorodiphenyldichloroethylene; HCB: hexachlorobenzene; OC: organochlorine compound; P: percentile; PCB: polychlorinated biphenyl.

Both maternal and cord concentrations were available for three out of the four cohorts (Asturias, Gipuzkoa and Valencia).

^aRatio: C_m/C_{uc}: ratio of maternal/cord concentrations in raw scale.

^bPearson's correlation coefficient between maternal and cord OC concentrations in log scale (adjusted by cohort). *p*-values <0.001 in all the correlations.

Figure Legends

Figure 1. Associations between OC concentrations and fetal growth measurements. The INMA Project, 2003-2008 (Spain). AC: abdominal circumference; BPD: biparietal diameter; DDE: dichlorodiphenyldichloroethylene; EFW: estimated fetal weight; FL: femur length; HCB: hexachlorobenzene; OC: organochlorine compound; PCB: polychlorinated biphenyl. Adjusted linear regression models between $\log_2(\text{OC})$ concentrations and fetal growth measurements. Meta-analysis of results from multiple imputation. Results expressed as %change in fetal measurements associated with a doubling in OC concentrations. * p -value<0.05; ** p -value<0.01.

Figure 2. Sensitivity analysis of the association between OC concentrations and fetal size at 34 weeks of gestation. The INMA Project, 2003-2008 (Spain)

AC: abdominal circumference; BPD: biparietal diameter; DDE: dichlorodiphenyldichloroethylene; EFW: estimated fetal weight; FL: femur length; GWG: gestational weight gain; HCB: hexachlorobenzene; OC: organochlorine compound; PCB: polychlorinated biphenyl.

Adjusted linear regression models between $\log_2(\text{OC})$ concentrations and fetal growth measurements. Meta-analysis of results from multiple imputation. Results expressed as %change in fetal measurements associated with a doubling in OC concentrations.

Main analysis: results from multiple imputation; Complete case: analysis excluding cases with missing values in covariates and fixed imputation of LOD/2 for OC values <LOD; Multi-pollutant: main analysis including the OCs showing an association with fetal growth in the present analysis, i.e. models of 4,4'-DDE were additionally adjusted for \sum PCBs and HCB, models of HCB were adjusted for \sum PCBs, and models of PCBs were adjusted for HCB; Excluding GWG: analysis excluding gestational weight gain. ^aGWG was not included in models of maternal OCs and outcomes measured at week 12 since GWG was calculated from week 12 to delivery.

Figure 1.



