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# Associations between blood persistent organic pollutants and 25-hydroxyvitamin D3 in pregnancy

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ABSTRACT

Persistent organic pollutants (POPs) are suggested to contribute to lower vitamin D levels; however, studies in humans are scarce and have never focused on pregnancy, a susceptibility period for vitamin D deficiency. We investigated whether serum levels of POPs were associated with circulating 25-hydroxyvitamin D3 [25(OH)D3] concentration in pregnancy. Cross-sectional associations of serum concentrations of eight POPs with plasma 25(OH)D3 concentration were analyzed in 2031 pregnant women participating in the Spanish population-based cohort INfancia y Medio Ambiente (INMA) Project. Serum concentrations of POPs were measured by gas chromatography and plasma 25(OH)D3 concentration was measured by high-performance liquid chromatography in pregnancy (mean 13.3 ± 1.5 weeks of gestation). Multivariable regression models were performed to assess the relationship between blood concentrations of POPs and 25(OH)D3. An inverse linear relationship was found between serum concentration of PCB180 and circulating 25(OH)D3. Multivariate linear regression models showed higher PCB180 levels to be associated with lower 25(OH)D3 concentration: quartile Q4 vs. quartile Q1, coefficient = −1.59, 95% CI −3.27, 0.08, p trend = 0.060. A non-monotonic inverse relationship was found between the sum of predominant PCB congeners (PCB 180, 153 and 138) and 25(OH)D3 concentration: coefficient (95% CI) for quartile Q2 vs. Q1 [−0.50 (−1.94, 0.94)], quartile Q3 vs. Q1 [−1.56 (−3.11, −0.02)] and quartile Q4 vs. Q1 [−1.21 (−2.80, 0.38)], p trend = 0.081. No significant associations were found between circulating 25(OH)D3 and serum levels of *p,p′*-DDE, *p,p′*-DDT, HCB, and ß-HCH. Our results suggest that the background exposure to PCBs may result in lower 25(OH)D3 concentration in pregnant women.



## 1. Introduction

Persistent organic pollutants (POPs) include a variety of toxic chemicals such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and organochlorine (OC) pesticides, which have common properties like resistance to environmental degradation, lipophilicity, and bioaccumulation in living organisms and the environment (National Research Council, 2006). Although the use of most POPs was severely restricted or banned during the 1970s, ubiquitous exposure to these toxic compounds of human populations including pregnant women and newborns is reported (Bergonzi et al., 2009;

*Abbreviations:* 25(OH)D3, 25-hydroxyvitamin D3; BMI, body mass index; CI, confidence interval; GAM, generalized linear model; HCB, hexachlorobenzene; ß-HCH, beta-hexachlorocyclohexane; IQR, interquantile range; LOD, limit of detection; OC, organochlorine; PCB, polychlorinated biphenyl; POP, persistent organic pollutant; *p,p′*-DDE, dichlorodiphenyldichloroethene; *p,p′*-DDT, dichlorodiphenyltrichloroethane.

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Ibarluzea et al., 2011; Woodruff et al., 2011). POPs circulate in the blood and come into contact with other body compartments, which increases susceptibility for adverse health effects including hormonal and immunological effects, impaired bone-tissue homeostasis, poor cognitive and psychomotor development, and higher risk of obesity, type 2 diabetes and cardiovascular diseases (Grandjean and Landrigan, 2006; Hodgson et al., 2008; Lee et al., 2006, 2011).

POPs and their persistent biotransformation products may disrupt endocrine systems. Gonadal steroids, thyroid hormones, and lipid soluble vitamins including vitamins A, D and E have been used as biomarkers to study POP-induced effects in diverse wildlife studies (Alvarez-Lloret et al., 2009; Routti et al., 2005, 2008), as well as in human studies including pregnant women (Alvarez-Pedrerol et al., 2009; Hagmar, 2003). Field and animal studies have reported associations between exposure to POPs and lower levels of circulating 25-hydroxyvitamin D [25(OH)D]. Lilienthal et al. (2000) reported a dose-dependent reduction in serum concentrations of vitamin D related to a PCB-mixture exposure in rats. Similarly, Routti et al. (2008) have reported circulating 1,25(OH)2D levels inversely correlated with hepatic POPs in the gray seals. In addition, embryonic exposure to PCBs may induce changes in the expression of the vitamin D receptor in the zebrafish (Ju et al., 2012). These results have suggested that exposure to POPs could impair metabolism of vitamin D.

Studies of exposure to POPs as a possible cause of vitamin D deficiency in humans are scarce. To date only one study has evaluated the relationship between exposure to OC pesticides and circulating vitamin D concentration in humans (Yang et al., 2012). Yang et al. (2012) reported significant inverse associations between serum concentrations of several OC pesticides, *p,p′*-DDT, *p,p′*-DDE, and ß-hexachlorocyclohexane, and serum 25(OH)D levels among the U.S. general population. Pregnant and lactating women are identified as vulnerable groups of vitamin D deficiency, which is present in ~20–85% of pregnant women in developed countries (Mulligan et al., 2010; Vandevijvere et al., 2012). In addition, the fetus and the newborn are highly dependent of the vitamin D status of their mother. An increasing epidemiological evidence shows that vitamin D deficiency in pregnancy is associated with multiple maternal, neonatal and infant adverse health outcomes (Christesen et al., 2012; Morales et al., 2012a,b; Nassar et al., 2011; Thorne-Lyman and Fawzi, 2012).

Studies that investigate the relationship of blood levels of POPs with circulating 25(OH)D concentration in pregnancy, a susceptibility period for vitamin D deficiency, are lacking. We aimed to investigate whether serum concentrations of several POPs were associated with plasma circulating 25(OH)D3 concentration in a population-based cohort of pregnant women.

## 2. Material and methods

### 2.1. Design and study population

Data come from participants of the INMA-INfancia y Medio Ambiente-(Environment and Childhood) Project (www.proyectoinma.org), a prospective population-based pregnant cohort study in Spain (Guxens et al., 2012). In brief, between November 2003 and February 2008 a total of 2150 women who fulfilled the inclusion criteria (≥16 years of age, intention to deliver at the reference hospital, no problems of communication, singleton pregnancy, and no assisted conception) were recruited during the first pre-natal visit in three areas of study: Valencia (39°N latitude, n = 855), Sabadell (41°N latitude, n = 657), and Gipuzkoa (42°N latitude, n = 638). Overall, circulating 25(OH)D3 concentrations in pregnancy were determined in 2031 participants considered to be eligible for the present study (94% of the recruited women). The study was approved by the Ethical Committees of the centres involved in the study and participants provided written informed consent.

### 2.2. Measurement assessment of plasma 25(OH)D3

A single blood specimen was drawn between week 8th and 27th of pregnancy (mean 13.3 ± 1.5 weeks of gestation). Samples were processed immediately and stored from −70 to −80 ° C until analysis. Plasma concentrations of 25(OH)D3 were quantified by high performance liquid chromatography (HPLC) method by using a BioRAD kit according to "Clinical and Laboratory Standard Institute" (NCCLS) protocols (BIO-RAD Laboratories, 2003). Detection limit was 5 ng/ml and inter-assay coefficient of variation was 4.5%. The assay was validated by "German Programmes of External Evaluation of Quality" (DGKL-RFB-Referencezinstutuk fur Bionalytik), and results were satisfactory in 100% of the cases.

### 2.3. Analysis of serum concentrations of POPs

The following POPs: *p,p′*-DDT (dichlorodiphenyltrichloroethane), *p,p′*-DDE (dichlorodiphenyldichloroethene), HCB (hexachlorobenzene), ß-HCH (hexachlorocyclohexane), and PCB (polychlorinated biphenyls) congeners 28, 118, 138, 153 and 180 were measured in serum of pregnant women. Serum samples were stored in crystal tubes at −80 °C and analyzed with gas chromatography using methods described elsewhere (Goñi et al., 2007; Grimalt et al., 2010; Vizcaino et al., 2010). Samples collected in Gipuzkoa and Sabadell areas were analyzed at the Gipuzkoa Basque Government Laboratory following a method previously described (Goñi et al., 2007), namely solid phase extraction of 500 μl of sera on C18 plates, purification by adsorption on silica/sulphuric acid columns, quantification by gas chromatography using an electron capture detector (ECD), and confirmation by gas chromatography with a mass spectrometry detector. Repeatability (short term precision), measured as relative standard deviation, was determined by spiking human serum at two levels: 0.15 and 1.5 ng/mL for PCBs and 0.3 and 3.0 ng/mL for organochlorine pesticides (n = 8 per level) and varied from 1% to 11%, depending on both spiking level and compound. In each batch of samples, two blanks were included as well as a duplicate of a control serum and of NIST Standard Reference Material (SRM) 1589a (National Institute of Standards and Technology, Gaithersburg, MD, USA). The results of control serum and SRM 1589a were monitored in control charts for ten months. Within laboratory reproducibility (long term precision) was evaluated from these results. The overall relative standard deviation (precision) of the analytical techniques was <15% for POPs.

Samples collected in Valencia were analyzed at the Centro Superior de Investigaciones Científicas (Barcelona). The analytical laboratory methods and quality control procedures have been described elsewhere (Grimalt et al., 2010). One ml of serum was spiked with the surrogate standards of tetrabromobenzene (TBB) and PCB209 and vortex stirred for 30 s at 2000 rpm. n-Hexane (3 ml) was added, followed by 2 ml of concentrated sulphuric acid. After reaction, the mixture was stirred for 30 s and the supernatant n-hexane phase was separated by means of centrifugation. The remaining sulphuric acid solution was re-extracted with 2 ml of n-hexane twice, each time with 30 s of stirring and subsequent centrifugation. The combined n-hexane extracts (7 ml) were then cleaned with 2 ml of sulphuric acid, stirred for 30 s, and centrifuged. Next, the n-hexane phase was separated and reduced to a small volume under a gentle nitrogen stream. The extract was transferred to gas chromatography (GC) vials by rinsing four times with 25 μl of isooctane and PCB142 was added as the injection standard. POP concentrations were determined with the aid of GC with electron capture detection (GC-ECD) on an Agilent 6890 N GC with a Micro-ECD (Agilent Technologies, Palo Alto, CA, USA). Selected samples were analyzed by means of NICI GC–MS with a GC from Agilent Technologies 6890A (USA) coupled to an MS detector 5973 N for confirmation of the qualitative and quantitative results. POP identification was based on retention time and mass spectral data. Quantification was

performed making reference to linear calibration lines and corrected with regard to the surrogate and injection standards. Procedural blanks were included with each sample batch. Limits of detection (LOD) and quantification (LOQ) were calculated from blanks (LOD = mean of all blanks + 3 sd, LOQ = mean + 10 sd). When the compound was absent from the blanks, the limits were determined from instrument limits of detection using injections of dilutions of standards. Recovery percentages ranged between 70% and 130%; detection limits were between 0.001 and 0.03 ng/ml, depending on the POP. Reference materials obtained from the Arctic Monitoring and Assessment Program (AMAP) were used to assess precision and accuracy. Precision, measured as relative standard deviation, was <14% for all the compounds.

Both laboratories were in compliance with the AMAP Ring Test Proficiency Program for persistent organic pollutants in human serum (Centre de Toxicologie, Institut National de Santé Publique du Québec) and performed satisfactorily in repeated international intercalibration exercises within the AMAP. Limits of detection (LOD) were 0.071 ng/ml for Sabadell and Gipuzkoa samples and between 0.010 and 0.071 ng/ml for Valencia sera. PCB28 is not described as more than 99% of participants had levels below the limit of detection. Total cholesterol and triglycerides were determined with the aid of enzymatic methods in both laboratories, calculating total serum lipid concentrations with the method developed by Philips (Phillips et al., 1989), and final measurements of POP concentrations are expressed in ng/g lipid. Correlations between adjusted and unadjusted lipid values were high (0.97 for *p,p′*-DDE and 0.95 for ΣPCBs).

### 2.4. Other variables

The following were considered a priori potential confounding factors because of their possible associations with circulating 25(OH)D3 concentration and serum concentrations of POPs: age, country of origin, gestational age and month at blood sampling, parity, social class, education level, pre-pregnancy body mass index (BMI), fish intake and vitamin D supplement use during first trimester of pregnancy, smoking and alcohol consumption in pregnancy, and physical activity during pregnancy. Questionnaires during the first trimester of pregnancy obtained information about parity (0 vs. 1 or more), age, country of birth (Spanish vs. foreign), social class (occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system) (I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled), education level (primary or less, secondary, university degree), pre-pregnancy BMI based on measured height at recruitment and pre-pregnancy self-reported weight (kilograms per square meter, $kg/m^2$) (underweight [<18.5], normal weight [18.5–24.99], overweight/obese [≥25]), and self-reported physical activity (inactive/low active, intermediate active and strong active). Information on fish type (large fatty, small fatty and lean fish) intake (times per week) and vitamin D supplement use (daily intake amount) was obtained through food frequency questionnaires. Information on smoking (no vs. yes) and alcohol consumption during pregnancy (no vs. yes, defined as consumption of alcohol beverages at least 1 time/month) was collected through questionnaires during the third trimester. All questionnaires were administered face-to-face by trained interviewers.

### 2.5. Statistical analysis

To increase efficiency and minimize selection bias we used multivariate multiple imputation to impute missing variables for participants considered to be eligible (women having information on circulating 25(OH)D3 concentration, n = 2031), including exposures (blood concentrations of POPs), covariables, and potential predictors of missing data in the imputation equations (see Supplemental material Table S1). Multiple imputation of missing values using chained equations (van Buuren et al., 1999) was carried out to account for the 2031 women. Twenty completed data sets were generated and analysed separately, and the results were combined using Rubin's rules (Royston, 2004). Distributions in imputed datasets and those observed are showed in Supplemental material Table S2. Analyses were also repeated restricting to those with complete data on all variables used in any analyses—that is, 'complete case' analysis. Analysis based on complete cases may suffer more chance variation, and under the missing at random (MAR) assumption multiple imputation increases efficiency and reduces biases that may arise in complete cases analyses (Sterne et al., 2009). Thus, multiple imputation results are presented in the main article and the complete case analysis results are presented in the supplementary information file (see Supplemental material Table S3).

Differences in baseline characteristics of participants across categories of circulating 25(OH)D3 concentrations were compared by using chi-2 tests for categorical variables, analysis of variance for continuous variables with normal distribution, and Kruskal–Wallis tests for variables with skewed distributions. Because distributions were skewed, geometric means and 95% confidence intervals were used to describe serum levels of POPs.

To adjust for month at blood collection, we used "deseasonalization" of 25(OH)D3 concentrations. Seasonality of 25(OH)D3 was tested by fitting the data to a sine function with a period of 12 months in a nonlinear regression cosinor model (Barnett and Dobson, 2010). Then, the predicted 25(OH)D3 concentrations based on month at blood collection for each subject, derived from the sinusoidal model, were subtracted from the actual observed value. Subsequently, the overall mean was added and the resulting deseasonalized 25(OH)D3 concentrations were analyzed (Morales et al., 2012a). Linear dose–response relationship between circulating 25(OH)D3 concentrations during pregnancy and serum concentrations of POPs was assessed by using adjusted generalized additive models (GAM) by graphical examination and likelihood ratio (Hastie and Tibshirani, 1990). To control the association between circulating 25(OH)D3 concentration and serum POPs for the confounding variables both linear regression models and log-binomial regression models were fitted. Multivariable linear regression analysis was performed between continuous values of 25(OH)D3 and POP levels introduced in the regression models as quartiles and associations were measured with the regression coefficients and their corresponding 95% confidence intervals (CI). In addition, log-binomial regression models were fitted to estimate the adjusted association between categorical values of 25(OH)D3 divided into tertiles and POP levels log2 transformed to achieve a normal distribution. We used log-binomial regressions, rather than logistic regression, which is appropriate for cohort studies to derive relative risk (and not odds ratio). The measure of association was the relative risk and their corresponding 95% CI that yields the relative risk change in 25(OH)D3 for each doubling of the dose in the corresponding POP compound levels.

All variables significantly related with circulating 25(OH)D3 concentration or POPs in the bivariate models ($P < 0.2$) were included in the multivariable models and retained only if they had an at least marginally significant association ($P < 0.1$) or modified the coefficient of 25(OH)D3 concentration by at least 5%. Adjustment variables were area of study, country of origin (Spanish vs. foreign), age (continuous), parity (0 vs. 1 or more), pre-pregnancy BMI (continuous), social class, education level, smoking (no vs. yes), alcohol consumption (no vs. yes), gestational age at blood sampling (continuous) and self-reported physical activity. Further adjustment for fish intake and vitamin D supplement use did not change the estimates. As POP background exposure can differ depending on the area of study, country of origin, age and body mass index supplementary stratified analysis were performed to see if there were consistent patterns. Analyses were conducted by using Stata software, version 11.1 (Stata-Corp, College Station, TX)

and R statistical package version 2.13.0 (R Project for Statistical Computing, http://www.r-project.org).

## 3. Results

The mean age of participating women was 31.7 (±4.1) years, and overall 91% of women were born in Spain. Most of the women were primiparous (55%) and 26% were overweight or obese before pregnancy (Table 1). Twenty-seven percent of mothers had a low educational level (primary or less) and 21% were from high social class. A total of 18% of women reported to smoke during first trimester of pregnancy and 10.0% alcohol consumption during pregnancy.

The median circulating 25(OH)D concentration in the study sample of pregnant women was 30.2 ng/mL (interquartile range,

**Table 1**
Distribution of main characteristics of the study population by circulating 25(OH)D3 concentration (n = 2031), the INMA Project.

| | | | | Tertiles of 25(OH)D3 (ng/mL) | | | |
|---|---|---|---|---|---|---|---|
| | N with data | Median (IQR) | p | <25.2 | 25.2–34.9 | >34.9 | p |
| Area of study (median, IQR) | 2031 | | | | | | |
| Valencia | 777 | 32.1 (24.3–38.7) | <0.001 | 31.6 | 39.6 | 43.6 | 0.001 |
| Sabadell | 642 | 29.2 (20.6–37.0) | | 34.5 | 30.6 | 29.7 | |
| Gipuzkoa | 612 | 28.7 (22.0–36.9) | | 33.9 | 29.8 | 26.7 | |
| Season at blood sampling | 2031 | | | | | | |
| Winter | 460 | 26.6 (18.2–33.8) | <0.001 | 32.3 | 21.1 | 14.6 | <0.001 |
| Spring | 505 | 27.7 (20.2–36.3) | | 31.7 | 21.3 | 21.7 | |
| Summer | 545 | 34.8 (28.7–42.4) | | 11.6 | 28.9 | 39.9 | |
| Fall | 521 | 30.7 (23.5–36.8) | | 24.4 | 28.7 | 23.8 | |
| Gestational age at blood sampling (weeks), mean, (sd) | 2025 | 13.3 (1.5) | | 13.4 (1.5) | 13.2 (1.4) | 13.2 (1.6) | 0.007 |
| Age at child's birth (years), mean (sd) | 1852 | 31.7 (4.1) | | 31.3 (4.3) | 31.7 (4.0) | 32.2 (4.2) | 0.002 |
| Country of origin | 2027 | | | | | | |
| Spanish | 1836 | 30.4 (22.9–37.5) | 0.303 | 89.5 | 91.9 | 90.2 | 0.299 |
| Foreign | 191 | 28.8 (20.3–38.3) | | 10.5 | 8.1 | 9.8 | |
| Parity | 2022 | | | | | | |
| 0 | 1108 | 29.7 (21.8–37.2) | 0.021 | 59.2 | 53.3 | 51.9 | 0.018 |
| 1 or more | 914 | 30.9 (23.8–37.9) | | 40.8 | 46.7 | 48.1 | |
| Social class | 2026 | | | | | | |
| I/II Managers/technicians | 424 | 31.7 (24.6–39.3) | <0.001 | 17.3 | 21.3 | 24.1 | 0.001 |
| III Skilled manual/nonmanual | 546 | 31.0 (23.0–38.2) | | 24.9 | 26.8 | 29.1 | |
| IV/V Semi-skilled/unskilled | 1056 | 29.2 (21.3–36.4) | | 57.8 | 51.8 | 46.8 | |
| Education level | 2021 | | | | | | |
| Primary or less | 540 | 29.9 (21.4–37.1) | 0.012 | 27.2 | 27.5 | 25.4 | 0.211 |
| Secondary | 822 | 29.6 (22.6–37.1) | | 42.5 | 40.9 | 38.6 | |
| University degree | 659 | 31.5 (23.7–38.6) | | 30.2 | 31.5 | 36.0 | |
| Pre-pregnancy BMI (kg/m$^2$) | 2023 | | | | | | |
| <18.5 | 98 | 28.1 (21.0–37.6) | 0.042 | 5.7 | 4.4 | 4.4 | 0.483 |
| 18.5–24.99 | 1401 | 30.7 (23.1–38.0) | | 67.1 | 69.3 | 71.3 | |
| > = 25 | 524 | 29.2 (21.7–36.6) | | 27.2 | 26.3 | 24.3 | |
| Smoking 1st trimester pregnancy | 1911 | | | | | | |
| No | 1558 | 30.7 (22.9–37.6) | 0.008 | 77.9 | 82.3 | 84.3 | 0.012 |
| Yes | 353 | 28.9 (20.2–36.8) | | 22.1 | 17.7 | 15.7 | |
| Alcohol consumption in pregnancy | 1904 | | | | | | |
| No | 1717 | 30.0 (22.5–37.3) | 0.002 | 92.5 | 90.9 | 87.2 | 0.005 |
| Yes | 187 | 33.1 (25.0–41.0) | | 7.5 | 9.1 | 12.8 | |
| Physical activity in pregnancy (self-reported) | 1890 | | | | | | |
| Inactive/low active | 805 | 29.4 (22.1–36.8) | 0.071 | 43.7 | 44.1 | 40.0 | 0.415 |
| Moderate active | 727 | 30.4 (22.7–37.6) | | 39.0 | 36.9 | 39.5 | |
| Strong active | 358 | 31.3 (23.5–38.6) | | 17.3 | 19.0 | 20.5 | |
| Large fatty fish. 1st trimester | 2013 | | | | | | |
| 0 times/week | 1006 | 29.5 (21.9–37.3) | 0.049 | 53.2 | 48.7 | 48.1 | 0.381 |
| 0–1 times/week | 855 | 31.1 (23.3–37.9) | | 39.6 | 43.5 | 44.3 | |
| >1 times/week | 152 | 30.9 (22.7–38.5) | | 7.2 | 7.8 | 7.6 | |
| Small fatty fish. 1st trimester | 2013 | | | | | | |
| 0 times/week | 858 | 30.0 (22.5–37.5) | 0.651 | 43.9 | 42.9 | 41.1 | 0.797 |
| 0–1 times/week | 949 | 30.7 (23.0–37.6) | | 45.9 | 46.5 | 49.0 | |
| >1 times/week | 206 | 29.7 (23.1–37.9) | | 10.1 | 10.6 | 9.9 | |
| Lean fish. 1st trimester | 2013 | | | | | | |
| 0–2 times/week | 632 | 30.1 (22.7–37.3) | 0.526 | 31.7 | 30.8 | 31.7 | 0.933 |
| 2–3 times/week | 302 | 30.9 (23.5–38.7) | | 14.1 | 15.5 | 15.4 | |
| >3 times/week | 1079 | 30.3 (22.7–37.5) | | 54.2 | 53.7 | 52.9 | |
| Vitamin D from supplements 1st trimester | 2031 | | | | | | |
| No | 1661 | 28.8 (21.3–36.8) | <0.001 | 92.1 | 79.3 | 74.0 | <0.001 |
| <10 mcg | 338 | 34.1 (28.2–40.5) | | 7.6 | 19.1 | 23.2 | |
| > = 10 mcg | 32 | 36.7 (31.3–42.9) | | 0.3 | 1.6 | 2.8 | |
| Vitamin D from diet 1st trimester (tertiles) | 2013 | | | | | | |
| <2.05 mcg | 671 | 29.5 (22.6–37.1) | 0.094 | 35.5 | 33.7 | 30.8 | 0.230 |
| 2.05–3.15 mcg | 671 | 30.7 (23.7–37.8) | | 30.4 | 34.5 | 35.1 | |
| >3.15 mcg | 671 | 30.7 (21.4–38.2) | | 34.1 | 31.8 | 34.1 | |
| Total vitamin D intake (diet + supplements) 1st trimester (tertiles) | 2013 | | | | | | |
| <2.04 mcg | 671 | 30.9 (23.3–37.6) | 0.699 | 31.1 | 34.7 | 34.1 | 0.480 |
| 2.04–3.21 mcg | 671 | 29.8 (23.1–37.5) | | 33.5 | 34.0 | 32.4 | |
| >3.21 mcg | 671 | 30.5 (21.0–37.8) | | 35.4 | 31.2 | 33.5 | |

Values are percentages for categorical variables and mean (sd) for continuous variables.

22.7–37.6 ng/mL). Concentrations of circulating 25(OH)D3 differed among areas of study, with women from the Valencia area showing the highest concentrations and those from the Gipuzkoa area the lowest concentrations (Table 1). Increased circulating 25(OH)D3 concentration was found among older participants, non-primiparous women, those reporting moderate alcohol consumption and those having vitamin D supplements in pregnancy. On the contrary, decreased 25(OH)D3 concentration was found among participants with lower social class and education level, smokers and those overweight/obese before pregnancy.

*p,p′*-DDE, HCB, ß-HCH and PCBs congeners 138, 153 and 180 were detectable in more than 70% of pregnant women (Table 2). The highest serum concentrations (ng/g lipid) were found for *p,p′*-DDE and PCBs 138, 153 and 180, the PCB cogener 153 being the most abundant. Overall, serum concentrations of PCBs were higher in pregnant women from the Gipuzkoa area and *p,p′*-DDE, *p,p′*-DDT and HCB levels among those from the Valencia area. Correlations between different POPs were: 0.30 (*p,p′*-DDE and ΣPCBs), 0.16 (*p,p′*-DDT and ΣPCBs), 0.43 (HCB and ΣPCB), and 0.13 (ß-HCH and ΣPCB). Correlations between PCB118 and PCB congeners 138, 153 and 180 ranged between 0.25 and 0.33. Strongest correlations were found between most abundant PCBs congeners 138, 153 and 180 ranging from 0.83 to 0.91.

When we fitted generalized additive models, among 8 examined POPs we found an inverse linear relationship between serum levels of PCB180 and circulating of 25(OH)D3 concentration (p for gain = 0.31) and a non-monotonic inverse relationship with ΣPCBs (p for gain <0.01) (Fig. 1). Multivariate linear regression models showed higher PCB180 levels to be associated with lower 25(OH)D3 concentration: quartile Q4 vs. quartile Q1, coefficient = −1.59, 95% CI −3.27, 0.08, test for trend = 0.060 (Table 3). Although a negative relationship was also observed between 25(OH)D3 concentration and levels of PCB congeners 118, 153 and 138 no statistical significance was achieved (all p value trends > 0.2) (Table 3). A non-monotonic inverse relationship was found between ΣPCBs and 25(OH)D3 concentration: coefficient (95% CI) for quartile Q2 vs. Q1 [−0.50 (−1.94, 0.94)], quartile Q3 vs Q1 [−1.56 (−3.11, −0.02)] and quartile Q4 vs. Q1 [−1.21 (−2.80, 0.38)], test for trend = 0.081. No significant associations were found between plasma 25(OH)D3 and serum concentrations of OC compounds *p,p′*-DDE, *p,p′*-DDT, HCB, and ß-HCH (Table 3).

In log-binomial regression models we found that a doubling concentration of serum PCB180 and ΣPCBs increased the risk of having lower circulating concentration of 25(OH)D3 (see Supplemental material Table S4). As compared with participants in the upper tertile of 25(OH)D3 (>34.7 ng/mL) a doubling serum concentration of PCB180 and ΣPCBs were associated with a higher risk of having lower 25(OH)D3 concentration (<34.7 ng/mL): relative risk (RR) 1.04 (p = 0.017) and 1.03 (p = 0.040), respectively.

Stratified analysis by area of study showed inverse association between serum concentration of PCB180 and circulating 25(OH)D3 in

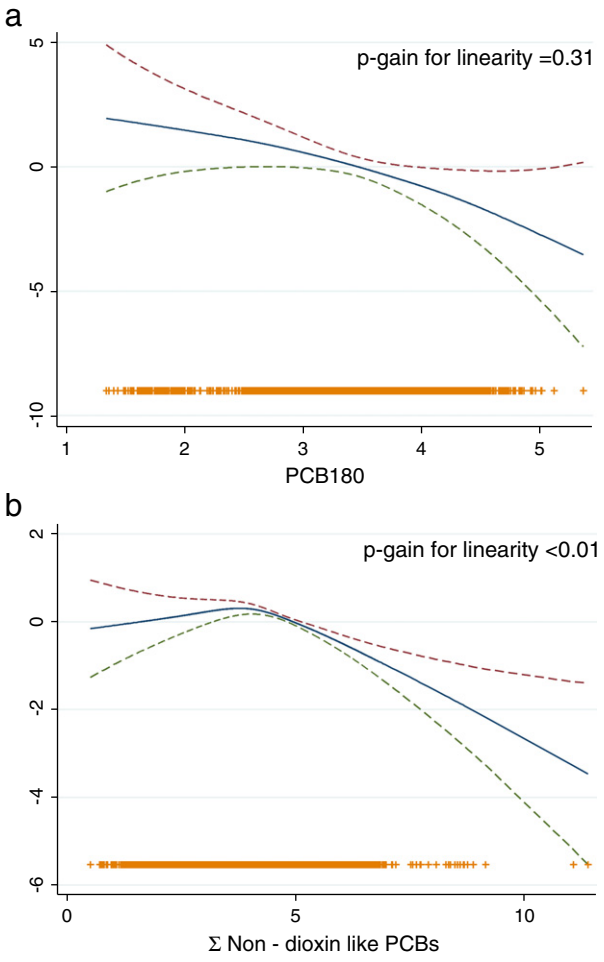


**Fig. 1.** The relation (and 95% confidence levels) of serum concentration of a) PCB180 and b) ΣPCBs with circulating 25(OH)D3* in pregnant women a) (n = 1541), the INMA Project. *Deseasonalized maternal 25(OH)D3 concentrations based on month at blood collection for each subject derived from the sinusoidal model. General additive models adjusted for area of study, country of origin, age, parity, pre-pregnancy body mass index, social class, education level, smoking, alcohol consumption, gestational age at blood sampling and self-reported physical activity. The symbols (+) on the X-axis indicate serum concentrations of PCB180 or ΣPCBs observations (log-transformed).

**Table 2**
Percentage of samples above the limit of detection (LOD) and serum concentrations[a] (ng/g lipid) of persistent organic pollutants (geometric mean, 95% CI), overall and by area of study, the INMA Project.

| | N with data | % > LOD | All | Valencia | Sabadell | Gipuzkoa |
|---|---|---|---|---|---|---|
| PCBs | | | | | | |
| PCB118 | 1574 | 24.9 | 7.6 (7.4–7.8) | 16.3 (15.0–17.6) | 6.0 (5.9–6.1) | 6.3 (6.2–6.5) |
| PCB180 | 1698 | 91.6 | 28.8 (27.8–29.8) | 35.0 (33.3–36.9) | 20.2 (19.1–21.3) | 35.7 (33.9–37.7) |
| PCB153 | 1700 | 95.9 | 40.9 (39.6–42.2) | 46.6 (44.2–49.1) | 30.6 (29.0–32.2) | 49.9 (47.3–52.7) |
| PCB138 | 1667 | 88.4 | 24.8 (23.9–25.7) | 36.0 (34.0–38.1) | 16.4 (15.6–17.3) | 29.1 (27.5–30.8) |
| ΣPCBs[b] | 1657 | 87.0 | 97.4 (94.2–100.7) | 125.6 (119.6–131.9) | 68.3 (65.0–71.8) | 117.7 (111.2–124.6) |
| OCs | | | | | | |
| *p,p′*-DDE | 1727 | 99.4 | 127.2 (122.1–132.6) | 187.0 (173.6–201.4) | 125.6 (117.6–134.1) | 94.7 (88.7–101.0) |
| *p,p′*-DDT | 1702 | 17.9 | 6.3 (6.1–6.5) | 6.8 (6.0–7.6) | 6.1 (5.9–6.4) | 6.1 (5.9–6.2) |
| HCB | 1673 | 92.5 | 41.3 (39.6–43.1) | 74.7 (69.7–80.2) | 35.3 (33.0–37.7) | 31.8 (29.9–33.9) |
| ß-HCH | 1325 | 71.6 | 26.5 (25.4–27.6) | 27.8 (26.0–29.8) | 30.2 (28.4–32.2) | 19.6 (18.0–21.3) |

LOD: limit of detection; OCs: organochlorines; *p,p′*-DDE: dichlorodiphenyldichloroethene; *p,p′*-DDT: dichlorodiphenyltrichloroethane; GM: geometric mean; HCB: hexachlorobenzene; ß-HCH: beta-hexachlorocyclohexane; PCB: polychlorinated biphenyl.

[a] Values calculated after samples with non-detectable levels were set at a value of half the detection limit of the corresponding POP, before correction for lipids.

[b] ΣPCB congeners 138, 153 and 180.

**Table 3**
Adjusted associations between serum concentrations of persistent organic pollutants (POP) and circulating 25(OH)D3[a] among eligible pregnant women with missing data imputed by multivariate multiple imputation (n = 2031), the INMA Project.

| POP | 25(OH)D3 continuous | | | |
|---|---|---|---|---|
| (ng/g lipid) | Coef. | (95% CI) | p value | p for trend |
| PCB118 | | | | |
| Q1 | Ref. | | | 0.317 |
| Q2 | 0.25 | (−1.57, 2.07) | 0.789 | |
| Q3 | −0.37 | (−2.08, 1.33) | 0.668 | |
| Q4 | −0.81 | (−2.48, 0.85) | 0.339 | |
| PCB180 | | | | |
| Q1 | Ref. | | | |
| Q2 | −0.85 | (−2.32, 0.62) | 0.257 | 0.060 |
| Q3 | −1.34 | (−2.93, 0.25) | 0.099 | |
| Q4 | −1.59 | (−3.27, 0.08) | 0.063 | |
| PCB153 | | | | |
| Q1 | Ref. | | | |
| Q2 | −0.34 | (−1.76, 1.08) | 0.637 | 0.339 |
| Q3 | −0.74 | (−2.25, 0.78) | 0.340 | |
| Q4 | −0.69 | (−2.24, 0.86) | 0.381 | |
| PCB138 | | | | |
| Q1 | Ref. | | | |
| Q2 | 0.77 | (−0.63, 2.18) | 0.282 | 0.249 |
| Q3 | −0.92 | (−2.44, 0.60) | 0.234 | |
| Q4 | −0.43 | (−2.01, 1.15) | 0.593 | |
| ΣPCBs[b] | | | | |
| Q1 | Ref. | | | |
| Q2 | −0.50 | (−1.94, 0.94) | 0.497 | 0.081 |
| Q3 | −1.56 | (−3.11, −0.02) | 0.048 | |
| Q4 | −1.21 | (−2.80, 0.38) | 0.134 | |
| *p,p′*-DDE | | | | |
| Q1 | Ref. | | | |
| Q2 | −0.10 | (−1.48, 1.27) | 0.884 | 0.920 |
| Q3 | −0.27 | (−1.68, 1.14) | 0.704 | |
| Q4 | −0.01 | (−1.59, 1.58) | 0.993 | |
| *p,p′*-DDT | | | | |
| Q1 | Ref. | | | |
| Q2 | 0.14 | (−1.79, 2.07) | 0.888 | 0.941 |
| Q3 | −0.07 | (−1.76, 1.62) | 0.938 | |
| Q4 | 0.16 | (−1.53, 1.85) | 0.853 | |
| HCB | | | | |
| Q1 | Ref. | | | |
| Q2 | 1.09 | (0–.31, 2.50) | 0.128 | 0.658 |
| Q3 | 0.02 | (−1.49, 1.52) | 0.983 | |
| Q4 | −0.08 | (−1.76, 1.60) | 0.926 | |
| ß-HCH | | | | |
| Q1 | Ref. | | | |
| Q2 | −0.46 | (−1.94, 1.02) | 0.542 | 0.982 |
| Q3 | −0.43 | (−1.95, 1.08) | 0.578 | |
| Q4 | −0.05 | (−1.68, 1.57) | 0.948 | |

All models adjusted for area of study, country of origin, age, parity, pre-pregnancy body mass index, social class, education level, smoking, alcohol consumption, gestational age at blood sampling and self-reported physical activity.
CI: confidence interval; *p,p′*-DDE: dichlorodiphenyldichloroethene; *p,p′*-DDT: dichlorodiphenyltrichloroethane; GM: geometric mean; HCB: hexachlorobenzene; ß-HCH: beta-hexachlorocyclohexane; PCB: polychlorinated biphenyl.
[a] Deseasonalized 25(OH)D3 concentration.
[b] ΣPCB congeners 180, 153 and 138.

Valencia and Sabadell areas, but not in the Gipuzkoa area although evidence of an interaction was not observed (Table 4). Inverse associations were restricted to women born in Spain (p for interaction 0.035). Consistent inverse associations were observed among women with normoweight or overweight/obese before pregnancy.

## 4. Discussion

We found an inverse association between serum levels of PCB180 and circulating 25(OH)D3 concentration among 2031 pregnant women of a population-based cohort study. In addition, a non-monotonic inverse dose–response was found for ΣPCBs. No significant associations were found between circulating 25(OH)D3 concentration and other examined PCB congeners and OC pesticides including *p,p′*-DDE, *p,p′*-DDT, HCB, ß-HCH. To our knowledge this is the first study in humans linking exposure to POPs and vitamin D status in pregnancy, a susceptibility period for vitamin D deficiency. Our results showed that 4% and 3% of cases of vitamin D insufficiency in pregnancy (concentration <34.7 ng/ml) would be due to higher background exposure to PCB180 and ΣPCBs, respectively. These results have important clinical and public health implications indicating that if blood concentrations of PCB180 an ΣPCBs would be reduced to half value of the current concentrations in Spanish pregnant women the proportion of pregnant women with blood vitamin D concentrations considered as adequate (≥34.7 ng/ml) would increase in 4% and 3%, respectively.

To date, only one previous study has investigated the relationship between blood concentrations of POPs and 25(OH)D in humans (Yang et al., 2012), and no study has never focused on pregnancy. In contrast to our results, in a subsamples of 1275 subjects of the US general population participating in the National Health and Examination Survey (NHANES) 2003–2004 Yang et al. reported significant inverse associations between serum concentrations of several OC pesticides, *p,p′*-DDT, *p,p′*-DDE, and ß-HCH, and 25(OH)D levels, but lack of association with PCDDs, PCDFs, and PCBs (Yang et al., 2012). Whenever there were significant associations, the decreases of serum 25(OH)D levels were particularly prominent in the last category of OC pesticides, belonging to the highest 10% of study subjects. Differences between populations investigated in both studies difficult the comparisons. However, remarkable differences in blood concentrations of OC pesticides and PCBs exist between the US general population and participants in the present study, which could explain the different results between both studies. The geometric mean of *p,p′*-DDE and PCB180 for pregnant women in the INMA study were 127.2 and 28.8 ng/g lipid, respectively, whereas for the US population ≥20 years participating in the NHANES 2003–2004 the corresponding values were 268 and 19.2 ng/g lipid, respectively, and in females values were 241 and 14.2 ng/g lipid, respectively (Centers for Disease Control and Prevention, 2009). Although results from the complete case analysis showed that subjects in the upper quartile of *p,p′*-DDT tended to have lower 25(OH)D3 concentration, results after multiple imputation procedure, graphic results from GAM (data not shown), and log-binomial regression models did not support this association. Low ability to detect *p,p′*-DDT in blood samples of participants in the present study limit the conclusions that can be drown on the relationship between *p,p′*-DDT and circulating 25(OH)D3 concentration in pregnant women. Studies on populations with a higher background exposure to *p,p′*-DDT are needed to disentangle its actual effects on circulating 25(OH)D3 concentration.

Nevertheless, our findings on the inverse relationship between PCB180 and ΣPCBs with circulating 25(OH)D3 concentration are in accordance with previous results observed in animal studies investigating the effects of PCBs on serum concentrations of the vitamin D3 metabolites. Lilienthal et al. (2000) have reported that exposure to a reconstituted PCB mixture reflecting the congener pattern in human breast milk resulted in dose-dependent reductions in serum concentrations of the steroids 25(OH)D3 and 1-25(OH)2D3 in rats dams and offspring. Similarly, Alvarez-Lloret et al. (2009) showed that treatment with PCB126 significantly altered the chemical composition of vertebral bone mineral composition at the molecular level and a significant decrease in 25(OH)D serum levels in Sprague–Dawley rats. Moreover, embryonic exposure to PCBs has been found to induce changes in the expression of the vitamin D receptor in the zebrafish (Ju et al., 2012).

Potential mechanisms could explain the inverse association between blood concentrations of PCBs and 25(OH)D3. The inactive precursor vitamin D3, or cholecalciferol, is synthesized in the skin from 7-dehydrocholesterol under the action of ultraviolet B light. Cholecalciferol is a secosteroid that is structurally similar to steroids as testosterone, cholesterol and cortisol. In the liver cholecalciferol

**Table 4**
Associations[a] (relative risk, RR, and 95% confidence interval) between serum concentration of PCB180 and circulating 25(OH)D3 concentration stratified by area of study, country of origin, age or pre-pregnancy body mass index (n = 2031), the INMA Project.

| | 25(OH)D3 (ng/mL) | | | | |
|---|---|---|---|---|---|
| | >34.7 (n = 677) | ≤34.7 (n = 1354) | | | |
| | | RR | (95% CI) | p value | p for interaction |
| Area of study | | | | | |
| Valencia (n = 777) | Ref. | 1.04 | (0.99–1.09) | 0.127 | |
| Sabadell (n = 642) | Ref. | 1.04 | (0.99–1.08) | 0.077 | 0.949 |
| Gipuzkoa (n = 612) | Ref. | 0.98 | (0.91–1.05) | 0.616 | 0.289 |
| Country of origin | | | | | |
| Spanish (n = 1840) | Ref. | 1.06 | (1.02–1.11) | 0.002 | 0.021 |
| Foreign (n = 191) | Ref. | 0.99 | (0.94–1.04) | 0.646 | |
| Age (median) | | | | | |
| <median (n = 1016) | Ref. | 1.03 | (0.99–1.06) | 0.107 | 0.990 |
| > = median (n = 1015) | Ref. | 1.02 | (0.96–1.08) | 0.506 | |
| Pre-pregnancy BMI (kg/m$^2$) | | | | | |
| 18.5–24.99 (n = 1404) | Ref. | 1.04 | (1.00–1.08) | 0.047 | 0.918 |
| > = 25 (n = 528) | Ref. | 1.03 | (0.98–1.09) | 0.244 | |

Adjusted for area of study, country of origin, age, parity, pre-pregnancy body mass index, social class, education level, smoking, alcohol consumption, gestational age at blood sampling and self-reported physical activity.
[a] Estimates from log-binomial regression models per each doubling of the dose (log-transformed).

is hydroxylated to become calcifediol (25-hydroxyvitamin D3) (DeLuca, 2004), the form most measured in the blood to assess vitamin D status. A second hydroxylation in the kidney will result into the active form of vitamin D3, 1,25-hydroxyvitamin D3 (or calcitriol). Both hydroxylation steps are catalyzed by cytochrome P450-containing enzymes, the 25-hydroxylase (CYP27) and the 1alfa-hydroxylase, which has been referred to CYP27B1 (Jones et al., 1998). Many isoforms of this group of enzymes, including those that catalyze the metabolism of sex steroid, have been reported to be influenced by PCBs (Haake-McMillan and Safe, 1991; Safe, 1990). Hence, it is plausible that OH-containing metabolites of PCBs could disrupt cholecalciferol hydroxylation in the liver resulting in a reduced circulating concentration of 25(OH)D3.

The large sample size and the population-based design of the study strength the findings of the present report. After multiple imputation procedure 94% of our initial population was included in the present study, which minimizes the selection bias. However, the study has several limitations. Cross-sectional analysis could result in reverse causality, however we do not know a potential mechanism whereby 25(OH)D3 could alter serum concentrations of POPs. A single 25(OH)D3 measurement per woman was available that could not reflect long-term status during the entire pregnancy. However, dealing with misclassification of estimated long term vitamin D exposure by month of blood draw was accounted estimating deseasonalized 25(OH)D3 concentration based on a sinusoidal model providing a more accuracy measurement of the 25(OH)D3 concentrations during a whole year. Furthermore, it has been reported that a single 25(OH)D serum measurement may provide a reasonably representative measure of vitamin D for adults (Sonderman et al., 2012). Low detectability in blood samples of *p,p′*-DDT and PCB118 (less 25% of participants have detectable concentrations) limits the conclusion that can be drawn from results for these POPs. Although we could adjust for a wide range of potential confounders, lack of information on time of outdoor activity during pregnancy may result in some residual confounding. Finally, we acknowledge that marginally significant associations found in the present study could be due to chance.

## 5. Conclusion

We found that the background exposure to some PCBs was associated with lower circulating 25(OH)D3 concentration in pregnant women. Other POPs examined were not associated with circulating 25(OH)D3 concentration in pregnancy. Chemical exposure to POPs as a possible cause of vitamin D deficiency in pregnancy should be considered in future studies. If associations reported here were confirmed, efforts to reduce body burden of POPs in pregnant women may be helpful to increase vitamin D levels and prevent vitamin D deficiency-related pregnancy and offspring outcomes.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.03.011.

## References

Alvarez-Lloret P, Lind PM, Nyberg I, Orberg J, Rodríguez-Navarro AB. Effects of 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) on vertebral bone mineralization and on thyroxin and vitamin D levels in Sprague–Dawley rats. Toxicol Lett 2009;187:63–8.
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 2009;43:7909–15.
B.I.O.-R.A.D. Laboratories GmbH. 25(OH)-vitamin D3 by HPLC. Munchen, Germany: Instruction manual; 2003.
Barnett AG, Dobson AJ. Analysing Seasonal Health Data. Berlin, Heidelberg: Springer; 2010.
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.
Centers for Disease Control, Prevention. Fourth Report on Human Exposure to Environmental Chemicals. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2009 [Available at http://www.cdc.gov/exposurereport/ [accessed 9 September 2012]].

Christesen HT, Falkenberg T, Lamont RF, Jørgensen JS. The impact of vitamin D on pregnancy: a systematic review. Acta Obstet Gynecol Scand 2012. http://dx.doi.org/10.1111/aogs.12000.
DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80:1689S–896S.
Goñi F, López R, Etxeandia A, Millán E, Amiano P. High throughput method for the determination of organochlorine pesticides and polychlorinated biphenyls in human serum. J Chromatogr B Analyt Technol Biomed Life Sci 2007;852:15–21.
Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet 2006;368:2167–78.
Grimalt JO, Howsam M, Carrizo D, Otero R, de Marchi MRR, Vizcaino E. Integrated analysis of halogenated organic pollutants in sub-millilitre volumes of venous and umbilical cord blood sera. Anal Bioanal Chem 2010;396:2265–72.
Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, et al. Cohort Profile: the INMA–INfancia y Medio Ambiente—(Environment and Childhood) Project. Int J Epidemiol 2012;41:930–40.
Haake-McMillan JM, Safe SH. Neonatal exposure to Aroclor 1254: effects on adult hepatic testosterone hydroxylase activities. Xenobiotica 1991;21:481–9.
Hagmar L. Polychlorinated biphenyls and thyroid status in humans: a review. Thyroid 2003;13:1021–8.
Hastie T, Tibshirani R. Generalized additive models. New York, NY: Chapman & Hall; 1990.
Hodgson S, Thomas L, Fattore E, Lind PM, Alfven T, Hellström L, et al. Bone mineral density changes in relation to environmental PCB exposure. Environ Health Perspect 2008;116:1162–6.
Ibarluzea J, Alvarez-Pedrerol M, Guxens M, Marina LS, Basterrechea M, Lertxundi A, et al. Sociodemographic, reproductive and dietary predictors of organochlorine compounds levels in pregnant women in Spain. Chemosphere 2011;82:114–20.
Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev 1998;78:1193–231.
Ju L, Tang K, Guo XR, Yang Y, Zhu GZ, Lou Y. Effects of embryonic exposure to polychlorinated biphenyls on zebrafish skeletal development. Mol Med Rep 2012;5:1227–31.
Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, et al. A strong dose–response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999–2002. Diabetes Care 2006;29:1638–44.
Lee DH, Steffes MW, Sjödin A, Jones RS, Needham LL, Jacobs Jr DR. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One 2011;6:e15977.
Lilienthal H, Fastabend A, Hany J, Kaya H, Roth-Härer A, Dunemann L, et al. Reduced levels of 1,25-dihydroxyvitamin D(3) in rat dams and offspring after exposure to a reconstituted PCB mixture. Toxicol Sci 2000;57:292–301.
Morales E, Guxens M, Llop S, Rodríguez-Bernal CL, Tardón A, Riaño I, et al. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics 2012a;130:e913–20.
Morales E, Romieu I, Guerra S, Ballester F, Rebagliato M, Vioque J, et al. Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. Epidemiology 2012b;23:64–71.
Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D deficiency in pregnancy and lactation. Am J Obstet Gynecol 2010;202:429.e1–9.
Nassar N, Halligan GH, Roberts CL, Morris JM, Ashton AW. Systematic review of first-trimester vitamin D normative levels and outcomes of pregnancy. Am J Obstet Gynecol 2011;205:208.e1–7.
National Research Council, Committee on Human Biomonitoring for Environmental Toxicants. Human Biomonitoring for Environmental Chemicals. Washington, DC: The National Academies Press; 2006 [Available: http://www.nap.edu/catalog/11700.html/ [accessed 9 September 2012]].
Phillips DL, Pirkle JL, Burse VW, Bernert Jr JT, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 1989;18:495–500.
Routti H, Nyman M, Bäckman C, Koistinen J, Helle E. Accumulation of dietary organochlorines and vitamins in Baltic seals. Mar Environ Res 2005;60:267–87.
Routti H, Nyman M, Jenssen BM, Bäckman C, Koistinen J, Gabrielsen GW. Bone-related effects of contaminants in seals may be associated with vitamin D and thyroid hormones. Environ Toxicol Chem 2008;27:873–80.
Royston P. Multiple imputation of missing values. Stata J 2004;4:227–41.
Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 1990;21:51–88.
Sonderman JS, Munro HM, Blot WJ, Signorello LB. Reproducibility of serum 25-hydroxyvitamin d and vitamin d-binding protein levels over time in a prospective cohort study of black and white adults. Am J Epidemiol 2012;176:615–21.
Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009;338:b2393.
Thorne-Lyman A, Fawzi WW. Vitamin D during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis. Paediatr Perinat Epidemiol 2012;26:75–90.
van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. Stat Med 1999;18:681–94.
Vandevijvere S, Amsalkhir S, Van Oyen H, Moreno-Reyes R. High prevalence of vitamin d deficiency in pregnant women: a national cross-sectional survey. PLoS One 2012;7:e43868.
Vizcaino E, Grimalt JO, Lopez-Espinosa MJ, Llop S, Rebagliato M, Ballester F. Maternal origin and other determinants of cord serum organochlorine compound concentrations in infants from the general population. Environ Sci Technol 2010;44:6488–95.
Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ Health Perspect 2011;119:878–85.
Yang JH, Lee YM, Bae SG, Jacobs Jr DR, Lee DH. Associations between organochlorine pesticides and vitamin D deficiency in the U.S. population. PLoS One 2012;7:e30093.