Introduction

Childhood obesity has increased rapidly since the mid-1980s [World Health Organization/United Nations Environment Programme (WHO/UNEP) 2013]. Greater body mass index (BMI) in childhood is associated with future risk of obesity, cardiovascular disease, certain cancers and a range of other diseases (Baker et al. 2007; Han et al. 2010). There is emerging interest in the possibility that exposure to certain xenobiotic chemicals may be obesogenic (Grün and Blumberg 2009; Holtecamp 2012; La Merrill and Birnbaum 2011) and may change growth patterns and induce weight gain, obesity, and ultimately insulin resistance and type 2 diabetes (La Merrill et al. 2013). It has been suggested that potential effects of obesogens may be strongest when exposure occurs during pregnancy (Huang et al. 2007).

Thus far, the most consistent evidence of obesogenic effects in humans has been reported for gestational tobacco exposure (Oken et al. 2008; Thayer et al. 2012). Over the last two decades, a number of longitudinal epidemiological studies have studied the potential obesogenic effects of prenatal exposures to endocrine-disrupting chemicals (EDCs), and recent literature reviews have summarized these studies (La Merrill and Birnbaum 2011; Lee et al. 2014; Tang-Péronard et al. 2011; Wang et al. 2014; WHO/UNEP 2013). Most epidemiological studies have evaluated potential effects of single persistent organic pollutants, and most have focused on organochlorine compounds, with the most consistent evidence for obesogenic effects thus far reported for dichloro-diphenyldichloroethylene (DDE) (Delvaux et al. 2014; La Merrill and Birnbaum 2011; Lee et al. 2014; Valdi et al. 2014; Warner et al. 2013). Only a few studies have evaluated potential obesogenic effects of prenatal exposure to other groups of EDCs, including bisphenol A (BPA), phthalates, and heavy metals (Delvaux 2014; Gardner et al. 2013; Harley et al. 2013; Tian et al. 2009; Valdi et al. 2013). Recent international expert workshops (e.g., by the U.S. National Toxicology Program) have called for further epidemiological research to establish whether the obesogenic effects seen in animals are supported by evidence in humans (Thayer et al. 2012).

Until now, epidemiological studies on obesogenic effects of in utero exposure to EDCs have assessed the risks of single-pollutant exposures. Most human populations are exposed to mixtures of EDCs rather than to a single pollutant, and isolating the potential effects of one EDC exposure from another is difficult when exposures are correlated due to common sources (Sun et al. 2013). Only a few studies have evaluated the health effects of mixtures of EDCs (Braun et al. 2014; Grandjean et al. 2012; Lee et al. 2007, 2010; Lenters et al. 2015; Patel et al. 2010) and none have addressed obesogenic effects. With an increasing number of chemicals now proposed as suspected obesogens, there is a need to identify those most relevant for human obesity risk.

The aim of the current study is to use data on multiple chemical exposures measured in the INMA (“Infancia y Medio Ambiente” [Environment and Childhood] study) to evaluate the associations between biomarker concentration of 27 EDCs and child weight status at age 7 years.

Methods

Study population. Data from the Environment and Childhood Project (INMA) in Sabadell (Barcelona, Spain) were used. The study protocol has been described elsewhere (Guxens et al. 2012). Briefly, 657 women were enrolled during 2004–2006, in the first trimester of pregnancy during their first ultrasound visit at the public health center. Women were eligible for participation if they were > 16 years of age, had no communication problems, a singleton
pregnancy, and no assisted conception. Questionnaires were administered by trained interviewers during the first (around week 12) and third trimester (around week 32), at delivery, and at 14 months, 4 years, and 7 years after birth to assess maternal and child health status, sociodemographic characteristics, maternal reproductive history, and other characteristics. Ethical approval was obtained from the Clinical Research Ethical Committee of the Municipal Institute of Health Care, and informed consent was obtained from all subjects at each visit.

**Outcome assessment.** Weight (kilograms) and height (centimeters) of the children at approximately 7 years of age (range, 64–95 months) were measured by specially trained nurses; 470 children participated in this follow-up. Child weight and height were measured using standard protocols (without shoes and in light clothing). Age- and sex-specific body mass index (BMI) z-scores were calculated based on the WHO standard reference (de Onis et al. 2007, 2009). Overweight was defined as BMI z-score (zBMI) ≥ the 85th percentile.

**Chemical exposures.** Our analyses included 27 chemicals, suspected to be EDCs, previously measured in the cohort in biological samples collected during pregnancy or at birth. Specific analytical methods for each group of chemicals are described in their respective reference. Maternal urine was collected in the first and third trimesters of pregnancy and used to measure BPA (Casas et al. 2013), 10 phthalate metabolites (Valvi et al. 2015), and metal concentrations [arsenic (As), lead (Pb) and cadmium (Cd)] (Fort et al. 2014). Maternal blood was collected during the first trimester of pregnancy and used to measure organochlorine pesticides (DDE, hexachlorobenzene (HCB), and β-hexachlorocyclohexane (βHCH)) and polychlorinated biphenyls (PCBs) (Mendez et al. 2011). Cord blood was used to measure total mercury (Hg) concentration (Llop et al. 2012). Maternal colostrum samples collected at the hospital during the first 48–96 hr postpartum were used to measure polybrominated diphenyl ethers (PBDEs) (Gascon et al. 2012). PBDEs were measured in colostrum milk because its higher fat content enabled better detection rates than did cord blood. Further, colostrum levels reflect well the accumulation of maternal exposure during pregnancy (Gascon et al. 2012). To account for urine dilution, the urinary concentrations of phthalate metabolites, BPA, and metals were divided by the urinary concentrations of creatinine (concentrations are expressed in micrograms per gram creatinine for phthalates and BPA, and in nanograms per gram creatinine for metals). For each of these chemicals, the two adjusted urine measurements during pregnancy were averaged because of the high within-person variability characterizing these exposures. The serum concentration of the organochlorine pesticides and the PCBs were lipid-normalized in units of nanograms per gram serum lipid. The cord blood total mercury concentration was expressed in micrograms per liter. The colostrum concentrations of the PBDEs were also lipid-normalized in units of nanograms per gram colostrum lipid.

**Statistical analysis.** All chemical concentrations were log10 transformed to obtain normal distributions. Correlations between the transformed concentrations of the 27 EDCs in the original data sets were assessed by computing Pearson correlation coefficients. Linearity of the associations between EDC levels and zBMI were assessed in the original data set using generalized additive models (GAM) (data not shown). Because some of the EDCs demonstrated significant nonlinear associations (p for linearity < 0.1) with zBMI, we analyzed all exposure variables in categories defined by tertiles. For zBMI at age 7 years we fitted multiple linear regression models and reported beta coefficients with 95% confidence intervals (CIs). For overweight we fitted generalized linear models with Poisson family, log link, and robust variance to estimate relative risks (RRs) and 95% CIs.

There were missing values in the exposure variables ranging from 3% (organochlorine pesticides and PCBs) to 59.6% (PBDEs) of participants. Only 8.5% of the participants had complete data on all 27 exposure variables. Overall, 28.7% of exposure data values were missing (3,642 data points out of the 12,690 total values for 27 chemicals × 470 children) (see Supplemental Material, Figure S1). Therefore, to evaluate multiple pollutant exposures in one model, we used a multiple imputation approach to impute the missing exposure variables (Royston and White 2011). As recommended, to assess the missing at random (MAR) assumption, we first tested whether the likelihood of missing exposure data (missingness) was associated with either of the outcomes (t-test for the continues and chi-test for overweight) (data not shown). We then tested whether exposure missingness was associated with known values of confounders and covariates in our data set. To determine which of the variables to include as predictors in the imputation process, we evaluated correlation, t-test, or chi-square test with each one of the imputed variables, as appropriate (data not shown). We used imputation models that were more general than the analyses models and included the health outcomes, the variables related to the missingness, and auxiliary variables that were associated with the exposure (Azur et al. 2011; White et al. 2011). We imputed 100 data sets based on the recommendations of Graham (2009). Detailed information regarding the imputation process and a list of the variables used is provided in Supplemental Material, “Description of the Imputation Procedure.” The same approach was applied to impute missing values for the model covariates (missing data ranged from 0.2% (breastfeeding) to 33.6% (sedentary behavior at age 7 years)).

Potential confounders were selected based on a review of the literature on the determinants of our exposure variables and risk factors for increased BMI (Casas et al. 2013; Forns et al. 2013; Gascon et al. 2012; Llop et al. 2012; Valvi et al. 2013, 2014, 2015). All statistical models were adjusted for the same set of potential confounders: child’s sex (male, female), gestational age (continuous in weeks), birth weight (continuous, in grams), exact age at the time that the outcome was measured (continuous, in months), and maternal country of origin (Spain, non-Spain), maternal age at delivery (continuous, in years), maternal prepregnancy BMI (continuous, in kilograms per meter squared), maternal weight gain during pregnancy (low, recommended, or high) [Institute of Medicine (IOM) 2009], maternal social class [managers, technicians, and associate professionals (nonmanual); other nonmanual workers; and skilled, semiskilled, and unskilled manual workers], breastfeeding duration (less than or more than 16 weeks), and maternal smoking during pregnancy (nonsmoking, any smoking during pregnancy).

We first conducted single-pollutant models for each of the 27 EDCs separately to evaluate the associations between tertiles of exposures and child weight status at age 7 years. Crude and confounder-adjusted models were fitted using complete cases (i.e., removing missing values) and using imputed data sets. Results from multiply imputed data sets were combined using standard multiple imputation rules (White et al. 2011). We evaluated the confounding effect separately for each chemical and co-variable (data not shown).

To evaluate multiple chemical exposures simultaneously, we used principal component analysis. Principal-component analysis (PCA) reduces the number of correlated variables into a smaller number of artificial variables (factors) that capture most of the variance of the original variables while being uncorrelated with each other (Hatcher 1994). This allows the resulting factors to be included within the same model, reducing issues of multicollinearity. To apply PCA on the imputed data set, we first calculated an overall variance–covariance matrix based on within- and between-imputation covariance matrices, and this matrix was used to fit the PCA (Li et al. 1991). We chose to retain four factors based on the scree plot and the number of nontrivial factors (Brown 2009). We applied a Varimax rotation and calculated factor scores for each participant. These four factor scores were...
Previous studies have suggested that child sex, maternal smoking, maternal BMI, and maternal socioeconomic status may modify the associations between EDCs and later child weight status (Mendez et al. 2011; Thayer et al. 2012; Wang et al. 2014). We evaluated these potential effect modifiers in our study by including in the models the interaction term between the four PCA factors and the possible modifier. We also evaluated the associations between PCA factors and weight status stratified by the categories of the potential effect modifiers. We evaluated the p-values for the interaction terms and for the stratified analysis for each of the tertiles. Finally, as a sensitivity analysis we evaluated further adjustment for total daily caloric intake of the child during the last year and hours per day spent in sedentary activities, including time watching television, using computers, and playing video games (three categories: < 1 hr/day during week and < 2 hr/day during weekend; < 1 hr/day during week and 2–3 hr/day during weekend or 1–2 hr/day during week and < 2 hr/day during weekend or 1–2 hr/day during week and 2–3 hr/day during weekend; > 2 hr/day during week or > 3 hr/day during weekend).

Statistical significance was defined as p-value < 0.05. All analyses were performed using the statistical package STATA version 12.1 (StataCorp, College Station, TX, USA).

**Results**

Our analyses included 470 singleton children with available data on BMI at 7 years of age. Data on 27 EDCs exposure variables and demographic variables are presented in Table 1 and Table 2, respectively. The mean and the geometric mean (GM) concentrations of the 27 EDCs were generally similar in the original and the imputed data set (Table 1). The prevalence of overweight children at follow-up was 31.9% (n = 150) (Table 2). Mothers were predominantly of Spanish origin (91.7%), from lower socioeconomic class (44.5%), with a high prevalence of higher than recommended weight gain during pregnancy (38.9%), and a high smoking rate during pregnancy (27.2%) (Table 2).

There were minor differences in the imputed data set distributions compared with the original data set (Table 2). Correlation coefficients were generally weaker between EDCs from different chemical groups compared with those within groups (see Supplemental Material, Figure S2).

Single-pollutant, complete-case, adjusted models for the 27 EDCs exposures showed a statistically significant increase in zBMI with increasing exposure to HCB [adjusted (adj)] β tertile 3 vs. 1: 0.49; 95% CI: 0.16, 0.82], βHCH [adj β tertile 3 vs. 1: 0.37; 95% CI: 0.08, 0.82], PCB-138 (adj β tertile 3 vs. 1: 0.36; 95% CI: 0.04, 0.68), and PCB-180 (adj β tertile 3 vs. 1: 0.41; 95% CI: 0.05, 0.77) (Figure 1; see also Supplemental Material, Table S1). For DDE, tertile 3 estimates were increased compared with tertile 1 and nearly reached statistical significance (adj β tertile 3 vs. 1: 0.27; 95% CI: –0.02, 0.56). For 7OHMeOP [mono(4-methyl-7-hydroxoyctyl) phthalate], tertile 3 estimates were decreased compared to tertile 1 and nearly reached statistical significance (adj β tertile 3 vs. 1: –0.29; 95% CI: –0.59, 0.01).

Certain phthalates and certain PBDEs showed nonsignificant negative associations. For example, for PBDE-53 and PBDE-54 tertile 2 estimates were decreased compared with tertile 1 (adj β tertile 2 vs. 1: –0.31; 95% CI: –0.73, 0.11) (Figure 1; see also Supplemental Material, Table S1).

Adjusted estimates based on the imputed data set were very similar to those based on the complete case analyses (Figure 1; see also Supplemental Material, Table S1).

**Table 1. Concentrations and percentage of quantifiable and missing samples for the 27 EDCs in the original data set and the imputed data set (n = 470).**

| EDCs | < LOD | Missing | Complete case, original data set | Imputed data set |
|------|-------|---------|---------------------------------|-----------------|
| MEPa | 0 110 | 34 | 9379.9 | 605.4 (525.2, 685.7) | 379.5 |
| MnBPa | 0 110 | 5.8 | 835.7 | 46.1 (39.2, 53) | 32.4 |
| MBPa | 0 110 | 5.1 | 334.2 | 41.5 (37.4, 45.5) | 32.6 |
| MBBpa | 0 110 | 1.5 | 405.1 | 19.1 (15.7, 22.5) | 12.5 |
| 7OHMMoOPa | 81 122 | 0.4 | 343.5 | 3.4 (1.5, 5.4) | 1.7 |
| MECPpa | 0 110 | 7.7 | 718.9 | 51.6 (46.1, 57.1) | 40.8 |
| MEHHPa | 0 110 | 5.3 | 503.4 | 38.2 (33.5, 42.9) | 26.6 |
| MEHHPb | 0 110 | 4.1 | 378.3 | 27.8 (24.5, 31.1) | 21.5 |
| MEHPa | 0 110 | 1.8 | 266.9 | 14.6 (12.8, 16.3) | 11.2 |
| MCHPa | 0 182 | 14.4 | 1086.5 | 58.3 (49.7, 66.9) | 45.9 |
| BPAa | 0 97 | 0.3 | 69.4 | 3.7 (3.1, 4.2) | 2.7 |
| CDFa | 23 188 | 0.1 | 5.9 | 0.6 (0.5, 0.8) | 0.6 |
| Asa | 0 99 | 2.9 | 702.2 | 65.5 (57.7, 73.3) | 42.8 |
| Pb | 3 190 | 0.5 | 25.2 | 5.1 (4.5, 5.6) | 4.2 |
| Hg | 24 120 | 1.4 | 60 | 8.1 (7.5, 8.8) | 6.3 |
| DDE | 1 14 | 7.7 | 17263.4 | 236.4 (152.3, 320.5) | 128.3 |
| HCBd | 34 14 | 4.5 | 293 | 51.9 (48.1, 55.7) | 38.5 |
| βHCHd | 42 14 | 4.4 | 497.6 | 43.2 (47.4, 48.6) | 31.3 |
| PCB-138d | 100 14 | 4.5 | 98.1 | 20.4 (19.2, 21.5) | 16.9 |
| PCB-153d | 33 14 | 4.5 | 154.9 | 37.3 (35.4, 39.2) | 31.3 |
| PCB-180d | 75 14 | 3.9 | 119.7 | 25.2 (23.8, 26.6) | 20.7 |
| PBDE-47d | 67 280 | 0.1 | 1.5 | 0.7 (0.7, 1.2) | 0.5 |
| PBDE-99d | 58 280 | 0 | 8.9 | 0.6 (0.4, 0.7) | 0.3 |
| PBDE-100d | 39 280 | 0.3 | 3.2 | 0.4 (0.3, 0.4) | 0.2 |
| PBDE-153d | 24 280 | 0.1 | 12.2 | 1 (0.8, 1.1) | 0.7 |
| PBDE-184d | 25 280 | 0.1 | 12.2 | 1 (0.8, 1.1) | 0.7 |
| PBDE-208d | 37 280 | 0 | 5.3 | 1.1 (0.9, 1.3) | 0.8 |

Abbreviations: As, arsenic; βHCH, β-hexachlorocyclohexane; BPA, bisphenol A; Cd, cadmium; CI, confidence interval; DDE, dichlorodiphenylchloroethylene; EDCs, endocrine-disrupting chemicals; GM, geometric mean; HCB, hexachlorobenzene; Hg, mercury; LOD, limit of detection; MBBp, monobenzyl phthalate; MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MCBP, mono-2-ethylhexylphthalate; MEP, monooctyl phthalate; MBBp, mono-n-butyl phthalate; Pbb, lead; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; MCMHP, mono-2-carboxyhexyl phthalate; MECP, mono-2-ethyl-5-carboxypentyl phthalate; 7OHMMoOP, mono(4-methyl-7-hydroxyoctyl) phthalate.

First- and third-trimester urine (ng/g creatinine). First- and third-trimester urine (μg/L)
with tertile 1 of 0.63 (95% CI: 0.33, 1.19). Similar, nonsignificant negative associations were observed with zBMI for exposure to the phthalate factor (factor 2) in tertile 3 and tertile 2 compared with tertile 1. Exposure to the PBDE factor (factor 1) showed a nonsignificant decrease in the RRs for overweight (adj RR tertile 2 vs. 1: 0.61; 95% CI: 0.28, 1.34; adj RR tertile 3 vs. 1: 0.54; 95% CI: 0.25, 1.17). Similar, nonsignificant negative associations with zBMI were observed for exposure to the PBDE factor (factor 1) in tertile 3 and tertile 2 compared to tertile 1 (Table 3). Results from models including each single factor separately were similar to the results of the models simultaneously adjusting for all factors (Table 3).

There was no evidence for modification of the association between factors of exposure and child weight status by child’s sex, maternal prepregnancy BMI, maternal socioeconomic class and maternal smoking status (p-values for interaction > 0.2; data not shown). The results of the sensitivity analyses further adjusting for child total daily caloric intake and child sedentary behavior during the last year were also not different from our main analyses (data not shown).

Our results are largely consistent with the existing literature: We observed an increase in zBMI with increased prenatal exposure to the organochlorine compounds HCB, 

| Table 2. Characteristics (mean or percent) of 470 children and their mothers at child’s age 7 years, in the original and imputed data sets. |
|----------------------|-----------------|--------------------------|
| Characteristic       | Missing (n)     | Original data set        | Imputed data set |
|                      |                 | mean or percent (95% CI) | mean or percent (95% CI) |
| Child characteristics |                 |                          |                          |
| zBMI at age 7 years (mean) | 0 | 0.7 (0.6, 0.8) | 0.7 (0.6, 0.8) |
| Overweight at age 7 years (%) | 0 | 31.9 (27.7, 36.1) | 31.9 (27.7, 36.1) |
| Female sex (%) | 0 | 51.3 (46.7, 55.8) | 51.3 (46.7, 55.8) |
| Gestational age (weeks) (mean) | 7 | 39.7 (38.6, 39.9) | 39.7 (38.6, 39.9) |
| Birth weight (g) (mean) | 0 | 3261.9 (3223.8, 3299.9) | 3261.9 (3223.8, 3299.9) |
| Exact age at 7 years (months) (mean) | 0 | 81.8 (81.4, 82.2) | 81.8 (81.4, 82.2) |
| Any breastfeeding >16 weeks (%) | 2 | 68.4 (64.2, 72.6) | 68.3 (64.2, 72.5) |
| Child time spent watching TV or playing video games (hr/day) | 158 | 18.3 (14.2, 22.6) | 15.8 (12.1, 19.7) |
| < 1 during week and < 2 during weekend | 43.9 (38.4, 49.5) | 45.4 (40.0, 50.8) |
| < 1 during week and 2–3 during weekend, 1–2 during week and < 2 during weekend, 1–2 during week and 2–3 during weekend | 37.8 (32.4, 43.2) | 38.3 (33.7, 44) |
| Child daily total caloric intake | 58 | 1635.3 (1601.9, 1688.9) | 1641.9 (1608.2, 1675.6) |
| Maternal characteristics |                 |                          |                          |
| Age at delivery (years) (mean) | 1 | 31.8 (31.5, 32.2) | 31.8 (31.5, 32.2) |
| Prepregnancy BMI (kg/m²) (mean) | 0 | 23.8 (23.4, 24.2) | 23.8 (23.4, 24.2) |
| Maternal weight gain during pregnancy (IOM) (%) | 15 | Recommended | 42.9 (38.3, 47.4) | 43.8 (35.5, 47.6) |
| Lower than recommended | 18.2 (14.7, 21.8) | 18.3 (14.8, 21.9) |
| Higher than recommended | 38.9 (34.4, 43.4) | 38.7 (34.2, 43.1) |
| Country of origin, Spain (%) | 3 | 91.7 (89.1, 94.2) | 91.8 (89.1, 94.2) |
| Social class (ISCO-88 code) (%) | 0 | Professionals and managers (I, II) | 22.8 (19.2, 26.4) | 22.8 (19.2, 26.4) |
| Other nonmanuals (III) | 32.8 (28.5, 37) | 32.8 (28.5, 37) |
| Skilled, semiskilled, and unskilled manual (IV, V) | 44.5 (40.4, 49) | 44.5 (40.4, 49) |
| Smoking during pregnancy (%) | 6 | 27.2 (23.1, 31.2) | 27.3 (23.2, 31.3) |
| Daily total caloric intake during pregnancy (kcal/day) | 0 | 1641.9 (1608.2, 1675.6) | 1641.9 (1608.2, 1675.6) |

**Discussion**

To our knowledge, this study is the first to investigate the combined effects of pre- and perinatal exposure to 27 suspected EDCs on child weight status, using a multi-pollutant approach. Maternal serum concentrations of organochlorine compounds were related to weight status at age 7 years in single-pollutant and PCA, and these associations were robust to the adjustment for other EDCs exposures. A factor reflecting combined exposure to multiple phthalate metabolites showed weak evidence for an association with reduced BMI. Exposure to other EDCs, whether in single-pollutant or combined multi-pollutant analyses, showed no evidence for an association with childhood weight status.

Our results are consistent with the existing literature: We observed an increase in zBMI with increased prenatal exposure to the organochlorine compounds HCB, HCH, PCB-138, and PCB-180; an increase in overweight at age 7 years with exposure to HCB, HCH, PCB-138, and DDE; and increased z-scores and overweight with increased combined exposures to the PCA factor combining these compounds. Our findings for HCB are consistent with those of some
previous studies (Smink et al. 2008; Valvi et al. 2014), but others did not observe any associations (Delvaux et al. 2014; Mendez et al. 2011; Verhulst et al. 2009). Only one study evaluated prenatal exposure to \( \beta \)-HCH, and associations were positive but not statistically significant (Mendez et al. 2011). Results of the 13 studies that investigated PCBs were not consistent, and associations in some studies were shown to be modified by sex (Blanck et al. 2002; Delvaux et al. 2014; Gladen et al. 2000; Hertz-Picciotto et al. 2005; Jacobson et al. 2002; Delvaux et al. 2014; Gladen et al.

Thirteen of the 27 EDCs were investigated in this article. The associations were positive but not statistically significant (Mendez et al. 2011). Results of the 13 studies that investigated PCBs were not consistent, and associations in some studies were shown to be modified by sex (Blanck et al. 2002; Delvaux et al. 2014; Gladen et al. 2000; Hertz-Picciotto et al. 2005; Jacobson et al. 2002; Delvaux et al. 2014; Gladen et al.

Figure 1. Crude and adjusted associations [\( \beta \) coefficient (95% CI)] between maternal exposure to tertiles of 27 EDCs and child zBMI at age 7 years, single-pollutant models, for complete case and imputed data (\( n = 470 \)). Abbreviations: As, arsenic; BDE, polybrominated diphenyl ethers congeners; \( \beta \)-HCH, \( \beta \)-hexachlorocyclohexane; BPA, bisphenol A; Cd, cadmium; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; EDCs, endocrine-disrupting chemicals; HCB, hexachlorobenzene; Hg, Mercury; MBzP, monobenzyl phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHP, mono(2-ethyl-5-oxohexyl) phthalate; MEMP, mono(2-ethyl-5-carboxypentyl) phthalate; MCPP, mono(2-ethyl-5-carboxyhexyl) phthalate; PBS, polychlorinated biphenyl congener; zBMI, body mass index z-score.
Multi-pollutant EDCs and childhood weight

Overweight can be complex. Multiple comparison (Lampa et al. 2014), but their interpretations to be tested require further consideration; ecological aspects that are relevant to obesity according to their biological activity and toxicology. Semi-Bayesian models (Braun et al. 2014) the selection of exposure variables, such as between exposure and health outcomes in ecological methods that account for the association of results between the single-pollutant and PCA approach (Perneger 1998; Rothman 1990).

In our study there were missing values for many exposure variables. In epidemiological studies, the most common strategy for dealing with missing data is a complete-case analysis where participants with missing data on any variable are excluded from the analyses. This may introduce selection bias because the analysis sample no longer retains proportions of the original population. In addition, dropping observed values on some variables for a subject with missing values on other variables may lead to a loss of information. This issue is a special concern when dealing with many exposures (Basagaña et al. 2013). We used multiple imputation to address the missing data problem. This technique provides valid results under the MAR assumption (Desai et al. 2011a, 2011b; Hetjain 2011; Sterne et al. 2009; White et al. 2011). Given the large number of important variables included in the imputation process, we believe it is a fair assumption to consider that missingness was probably unrelated to the actual (unmeasured) exposure after conditioning on these covariates, and thus the MAR assumption held. Multiple imputation of missing values is still not common practice in epidemiological studies (Klebanoff and Cole 2008), and future studies evaluating multiple exposures may consider the technique to decrease bias and inaccuracy of estimates. We fitted the single-pollutant models separately in the original and imputed data sets and found no differences in effect estimates. This supported the evaluation of multi-pollutant models in the imputed data set.

In multi-pollutant studies, it is likely that different exposures are measured with different degrees of accuracy. Different levels of exposure misclassification may lead to different levels of bias in the effect estimates, limiting to some extent the conclusions that can be drawn from a comparison of effect sizes for different pollutants. In our study, for example, serum concentrations of organochlorines give a reliable estimate of long-term exposure because their half-lives are on the order of several years, whereas urine concentrations of nonpersistent EDCs (e.g., the phthalates and BPA) give an estimate of very short-term exposure because their half-lives are on the order of hours or days (WHO/UNEP 2013). Although we averaged the concentrations of this latter group of chemicals over two points during the pregnancy, thus giving a better approximation of average exposure, non-differential exposure misclassification may occur.

Table 3. Association between maternal exposure to tertiles of the four factors from principal-component analysis and BMI z-score or overweight at age 7 years based on single- and multiple-factor models (imputed data, n = 470).

| Exposure | Single-factor model (β (95% CI)) | Multiple-factor model (β (95% CI)) | Single-factor model (RR (95% CI)) | Multiple-factor model (RR (95% CI)) |
|----------|----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|
| Factor 1: PBDEs | Reference | Reference | Reference | Reference |
| 1 | -0.12 (-0.47, 0.23) | -0.15 (-0.50, 0.21) | 0.69 (0.33, 1.44) | 0.61 (0.28, 1.34) |
| 2 | -0.14 (-0.48, 0.20) | -0.19 (-0.54, 0.16) | 0.59 (0.29, 1.22) | 0.54 (0.25, 1.17) |
| Factor 2: phthalates | Reference | Reference | Reference | Reference |
| 1 | -0.15 (-0.44, 0.15) | -0.15 (-0.45, 0.15) | 0.49 (0.26, 0.94) | 0.49 (0.25, 0.96) |
| 2 | -0.10 (-0.38, 0.18) | -0.13 (-0.42, 0.17) | 0.66 (0.36, 1.19) | 0.63 (0.33, 1.19) |
| Factor 3: organochlorines | Reference | Reference | Reference | Reference |
| 1 | 0.10 (0.00, 0.20) | 0.12 (-0.19, 0.43) | 1.68 (0.85, 3.32) | 1.86 (0.92, 3.76) |
| 2 | 0.34 (0.00, 0.68) | 0.37 (0.03, 0.72) | 2.17 (1.05, 4.49) | 2.59 (1.19, 5.63) |
| Factor 4: MEP, As, Hg, BPA, PBDE-153, PBDE-154 | Reference | Reference | Reference | Reference |
| 1 | -0.05 (-0.36, 0.26) | -0.04 (-0.36, 0.28) | 0.96 (0.48, 1.93) | 0.99 (0.47, 2.11) |
| 2 | 0.06 (-0.27, 0.35) | 0.06 (-0.27, 0.38) | 0.89 (0.47, 1.67) | 0.95 (0.47, 1.90) |

All models were adjusted for child’s sex, gestational age, birth weight, exact age at the time that the outcome was measured (months), maternal country of origin, maternal age at delivery, maternal prepregnancy BMI, maternal weight gain during pregnancy, maternal social class, breastfeeding duration and maternal smoking during pregnancy. Multiple-factor models were also adjusted for all four factors in a single model.

*Factor 1 loaded with PBDEs: BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-209. *Factor 2 loaded with phthalates: MnBP, MBP, MBzP, 7OHMMeOP, MECCP, MEHHP, MEHP, MEOHP, MCMHP. *Factor 3 loaded with organochlorines: DDE, HCB, HCH, PCB-138, PCB-153, PCB-180. *Factor 4 loaded with MEP, As, Hg, BPA, PBDE-153, PBDE-154, and HCH; had negative loading values.
A strength of this study is that we were able to evaluate an extensive list of potential confounders, including sociodemographic, dietary, and physical activity–related factors in the mothers and children. Because our effect estimates in the single-pollutant analyses generally did not change between the crude and the adjusted models after inclusion of child and maternal characteristics, we conclude that these factors did not in fact have a large overall confounding effect and are unlikely to explain our findings. Even so, for each of the PCBs, maternal pre-pregnancy BMI changed the direction of effect estimates. This has been noted before in a previous analysis of our study population in earlier childhood (Mendez et al. 2011). We further examined the effect of several potential effect modifiers—child sex, maternal smoking, maternal BMI, and maternal socioeconomic status—because these have previously been reported to modify the associations between prenatal EDC exposure and later child weight status (Mendez et al. 2011; Thayer et al. 2012; Wang et al. 2014). However, we did not find any evidence for these variables to modify the effect of the single pollutants or the PCA-derived factors, and our results were relatively consistent in the different strata defined by these variables: boys and girls, smokers and nonsmokers, higher and lower social classes, and normal and overweight/obese mothers.

A limitation of this study is that we did not have data for other potential obesogens such as perfluorinated chemicals, polychlorinated dibenzodioxins (PCDDs), or organotins (La Merrill and Birnbaum 2011; Tang-Péronard et al. 2011). Evidence for obesogenic effects of these compounds comes mainly from experimental studies, with human studies so far available only for perfluorinated chemicals (Andersen et al. 2013; Halldorsson et al. 2013). We focused on potential obesogens that were already measured previously in our population. Although it would have given a more complete comparison of obesogenic effects of different chemicals, because no confounding effect was observed between the factors in our analysis, we consider it unlikely that the inclusion of data on these other groups of chemicals would have changed the current results.

Conclusions

In our study population, prenatal exposure to organochlorine compounds was associated with overweight in children at 7 years of age, and this association did not appear to be confounded by other EDC exposures. We recommend that other epidemiological studies consider multi-pollutant approaches together with single-pollutant approaches, especially when dealing with correlated exposures. Our findings for organochlorine exposures highlight the fact that it is difficult, if not impossible, to disentangle individual associations of highly correlated exposures; therefore public health action is needed to reduce exposure to mixtures of organochlorines as a whole.

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