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Abbreviations:

BMI: body mass index; DAP: dialkylphosphate; DE: diethylphosphate metabolites; DEP: diethylphosphate; DETP: diethylthiophosphate; DEDTP: diethyldithiophosphate; DM: dimethylphosphate; DMP: dimethylphosphate; DMTP: dimethylthiophosphate; DMDTP: dimethyldithiophosphate; DDE: dichlorodiphenyl dichloroethylene; DDT: dichlorodiphenyl trichloroethane; FC: free cholesterol; HCB: hexachlorobenzene; βHCH: beta-hexachlorocyclohexane; LOD: limit of detection; LOQ: limit of quantification; OP: organophosphate pesticides; PCB: polychlorinated biphenyls; PL: phospholipid; PON1: paraoxonase 1; POP: persistent organic pollutants; TC: total cholesterol; T2D: type 2 diabetes; TG: triglyceride

Abstract

Background: Experimental evidence suggests that developmental exposure to persistent organic pollutants (POP) and to some non persistent pesticides may disrupt metabolic regulation of glucose metabolism and insulin secretion, and thereby contribute to the current epidemic of obesity and metabolic disorders. Quasi experimental situations of undernutrition *in utero* have provided some information. However, the evidence in humans concerning the role of the prenatal environment in these disorders is contradictory, and little is known about long-term outcomes, such as type 2 diabetes, of prenatal exposure.

Objectives: Our aim was to evaluate the effects of prenatal exposure to POP and organophosphate pesticides on fetal markers of glucose metabolism in a sample of newborns from the Pelagie mother-child cohort in Brittany (France).

Methods: Dialkylphosphate (DAP) metabolites of organophosphate pesticides were measured in maternal urine collected at the beginning of pregnancy. Cord blood was assayed for polychlorinated biphenyl congener 153 (PCB153), p,p'-dichlorodiphenyl dichloroethene (DDE) and other POP. Insulin and adiponectin were determined in cord blood serum (n = 268).

Results: A decrease in adiponectin and insulin levels was observed with increasing levels of DDE , but only in girls and not boys. Adiponectin levels were not related to the concentrations of other POP or DAP metabolites. Decreasing insulin levels were observed with increasing PCB153 concentrations. Insulin levels increased with DAP urinary levels. Additional adjustment for BMI z-score at birth modified some of these relations.

Conclusions: Our observations bring support for a potential role of organophosphate pesticides and POP in alterations to glucose metabolism observable at birth.

Keywords: persistent organic pollutants, organophosphates, adiponectin, insulin, cord blood, glucose metabolism markers

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All participants provided informed written consent and the appropriate ethics committees approved the study (Ethical board for human participation in research study on health, CCTIRS and the National commission on informatics and liberty, CNIL).

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1. Introduction

The prevalence of obesity has increased steadily worldwide, including in France where its prevalence among adults in 2012 was 15% (Eschwege et al., 2015). The involvement of the perinatal environment in the pathogenesis of obesity and diabetes has been demonstrated by follow-up of the Dutch Hunger Winter: individuals who suffered undernutrition *in utero* and were subsequently born with a low birth weight were around six times more likely to develop type 2 diabetes (T2D) by age 64 than individuals with the highest birth weight (Bouret et al., 2015).

There is growing experimental evidence for a role of so-called "environmental obesogens" in this epidemic, especially when exposure occurs during developmental period. Maternal smoking has been implicated, and perinatal exposure to persistent organic pollutants (POP), phthalates, metals, pesticides, or chemicals with estrogenic or endocrine-disrupting properties may induce abnormalities in the metabolic regulation of glucose metabolism, insulin secretion and lipogenesis; these environmental exposures may thereby contribute to the increasing prevalence of metabolic disorders (Heindel et al., 2009; La Merill et al., 2011; Thayer et al., 2012; de Cock et al., 2014).

Not all these environmental exposures have been the object of studies in humans. In a large meta-analysis of low-level prenatal exposure to POP among 12 European birth cohorts, exposure to polychlorinated biphenyl congener 153 (PCB153) was found to be linearly associated with lower birth weight, whereas no association was found with *p,p'*-dichlorodiphenyl dichloroethene (DDE), the main metabolite of dichlorodiphenyl trichloroethane (DDT) (Govarts et al., 2012; Casas et al., 2015). Recent reviews of studies investigating the consequences of such exposure on child growth have had difficulties in identifying even the general direction of the associations (de Cock et al., 2014; Tang-Péronard 2011). The recent pooled analysis of infant growth in seven European cohorts concluded that prenatal exposure to DDE was associated with increased infant growth (weight-for-age z-score) whereas postnatal PCB153 was associated with decreased growth (Iszatt et al., 2015).

A US National Toxicology Program Workshop convened in January 2011 evaluated that the evidence was sufficient to conclude that there is an association between the risk of T2D and certain POP, more specifically *trans*-nonachlor, DDT/DDE, dioxins/dioxin-like chemicals, some polychlorinated biphenyls (PCB) and Agent Orange, but not sufficient to establish causality (Thayer et al., 2012; Taylor et al., 2013; Lee et al., 2014). Possible associations were observed for other POP but with fewer relevant studies; no formal meta-analysis has been conducted and most of the studies reviewed were cross-sectional or prospective studies starting at adult ages.

A small number of epidemiological studies have suggested an association between prenatal exposure to organophosphate pesticides (OP) and poor fetal growth outcomes in interaction with maternal or fetal genetic susceptibility (low activity of the paraoxonase PON1 enzyme) (Berkowitz et al., 2004; Wolff et al., 2007; Harley et al., 2011). However, there is still no consensus about the consequences of OP exposure for fetal growth (Mink et al., 2012). No human data is available for child growth, obesity or the risk of T2D following prenatal exposure to OP, although evidence from animal studies suggests that there may be effects (Thayer et al., 2012; Slotkin et al., 2011).

Longitudinal epidemiological studies of potential links between prenatal exposure to environmental agents and long-term body weight and type 2 diabetes outcomes are logistically difficult. The analysis of the concentration of fetal markers at birth is an alternative approach to assessing the impact of environmental agents on glucose metabolism. High cord insulin levels, related at birth with low gestational age, are associated with the persistence of hyperinsulinemia in early childhood, which is a reliable marker of insulin resistance (Wang G et al., 2014).

Adiponectin is produced by adipose tissue and modulates insulin sensitivity and inflammation in adults (Kadowaki et al., 2005). In adults (Li et al., 2009), children and adolescents (Cruz et al. 2004; Gilardini et al. 2006; Shaibi et al. 2007), adiponectin concentrations are low in subjects with high body mass index (BMI), metabolic syndrome or type 2 diabetes.

A number of studies of healthy newborns have reported positive relationships between cord levels of adiponectin and birth weight or birth length (Sivan et al., 2003; Chan et al., 2004; Kotani et al., 2004; Tsai et al., 2004; Mantzoros et al., 2004), or have found low levels of adiponectin in low birth weight, preterm or small-for-gestational age newborns (Martos-Moreno et al., 2009; Mazaki-Tovi et al., 2011); however, others found no association between adiponectin and birth weight (Lindsay et al., 2003; Bozzola et al., 2010). Adiponectin levels at birth are moderately correlated with values at older ages (Hibino et al., 2009; Volberg et al., 2013). However, little is known about the predictive value of adiponectin levels at birth for obesity-related outcomes at later ages. A positive association has been found between the adiponectin level at birth and central adiposity at age 3 (Mantzoros et al., 2009) or BMI gain from birth to 3 years (Nakano et al., 2012); by contrast, adiponectin at birth has also been found to be inversely associated with weight gain in the first 6 months (Mantzoros et al., 2009), or with BMI and weight gain at one year (Mazaki-Tovi et al., 2011).

Our aim was to determine prenatal exposure to organophosphateorganophosphate pesticides, organochlorine pesticides and PCB by assaying maternal and fetal fluids, and assess the associations with two markers of glucose metabolism at birth. In the absence of a measure of glucose at birth, we selected adiponectin, in addition to insulin, despite the fact that its predictive value at birth for obesity-related outcomes at later ages is not well established. Its concentration is positively correlated with insulin sensitivity (Finucane et al., 2009) and it is considered as a reliable marker of insulin resistance in humans.
This study was based on the mother-child PELAGIE cohort set up in the general population of the Brittany region.

2. Methods

2.1 Population and data collection

The PELAGIE cohort included 3,421 pregnant women from Brittany from 2002 to February 2006. Gynecologists, obstetricians, and ultrasonographers recruited women during consultations in early pregnancy (before the 19th week of gestation), and obtained written informed consent. Women completed a questionnaire at home concerning family, social and demographic characteristics, diet, and lifestyle.

Women were asked to return the questionnaire by mail, along with a first-morning-void urine sample that they collected and transferred into two vials containing nitric acid (to inhibit bacterial proliferation). Samples were mailed to the study laboratory (INSERM U1085, Rennes, France) in a prestamped package at ambient temperature, with routine delivery taking 1 to 3 days. On receipt, samples were frozen and stored at –20°C. At the time of delivery, cord blood samples were collected from 2,138 (62%) women of the cohort. Cord blood samples were centrifuged immediately at the maternity unit and samples (clot and serum) were stored at -80°C in our lab. The main reasons for missing samples of umbilical cord blood may have been oversight or emergency delivery. Midwives and pediatricians at the maternity units provided study staff with medical information about the pregnancy, delivery, and neonatal health for 3,399 women.

A random sample of 601 births (18%) was selected from the cohort of liveborn singletons (n=3,322). Urine and cord blood samples were available for 579 (96%) and 394 (66%) women, respectively. Correlations with hormone levels in cord blood could be assessed for 283 newborns for whom a cord blood serum sample was still available after chemical analysis of persistent organic pollutants. We restricted our analysis to the 268 of these 283 cases with mothers with no diabetes.

2.2 Exposure assessment

2.2.1 Prenatal exposure to organophosphate pesticides

One urine sample from each mother collected at the beginning of pregnancy was analyzed for six nonspecific dialkylphosphate (DAP) metabolites of numerous OP insecticides (diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP)). Chemical analysis of 10-mL urine samples was carried out by the LABOCEA Institute (Plouzané, France) by solid-phase extraction (Symbiosis Prospekt II type, HySphere C18 HD cartridge; Spark Holland, The Netherlands) and liquid chromatography (Alliance Waters, Separations Module 2690; Waters, Saint-Quentin-en-Yvelines, France) with a Synergi Fusion RP C18 column (250 × 2 mm, 4 μm) and triple quadrupole mass spectrometry (LC-MS/MS, Quattro Ultima, Micromass/Waters, Saint-Quentin-en-Yvelines, France).

The limits of quantification (LOQ) for the chemical analyses of maternal urine samples were 1.25, 1.7, 0.02, 0.2, 1, and 0.45 µg/L forDEP, DETP, DEDTP, DMP, DMTP, and DMDTP, respectively, and the coefficients of variation at LOQ were 19, 19, 20, 17, 19 and 20%, respectively. Metabolite concentrations were converted from micrograms per liter to the corresponding molar concentrations (nanomoles per liter). Concentrations were summed to obtain overall concentrations of diethylphosphate metabolites (DE; sum of DEP, DETP, and DEDTP), dimethylphosphate metabolites (DM; sum of DMP, DMTP, and DMDTP), and dialkylphosphate metabolites (DAP; sum of DM and DE). To calculate these sums, censored values (i.e., those <LOQ) were replaced by 0 because of the large variations in LOQ values between metabolites.

2.2.2 Prenatal exposure to organochlorine pesticides and PCBs

The concentrations in cord blood of nine PCBs (congeners 118, 138, 153, 170, 180, 183, 187, 194 and 203) and three frequently detected organochlorine pesticides (beta-hexachlorocyclohexane [βHCH], DDE, hexachlorobenzene [HCB]) were determined by the Centre de Toxicologie de Québec of the National Institute of Public Health of Québec by GC-MS with limit of detection (LOD) of 0.01 to 0.02 µg/L (more details are provided in Supplemental File 1).

Concentrations are reported as wet weight (micrograms per liter). Total cholesterol (TC), free cholesterol (FC), triglyceride (TG), and phospholipid (PL) concentrations were also measured in these samples by an enzymatic method (in g/L); the total lipid concentration could therefore be calculated as 1.677*(TC-FC)+FC+TG+PL (Patterson et al., 1991).

PCB concentrations are highly correlated, so we studied the congener 153 as a marker of the total exposure to PCB, the congener 187 for its estrogenic activity, and the sum of congeners 118, 138 and 170, for their anti-estrogenic activity (Wolff et al., 1997). We summed the concentrations of the PCB 118, 138, and 170 expressed on a molar basis with censored value (i.e., <LOD) replaced by LOD/2 (because all PCB had the same LOD).

2.3 Hormone levels determination in cord blood serum

Hormone levels were determined at the Laboratory of Hormonology of Rennes University Hospital in cord blood serum for 268 newborns for whom cord serum was still available after chemical analyses of persistent organic pollutants.

Adiponectin was determined by the ELISA assay from R&D Systems (Abingdon, UK). Insulin was determined using the bi-Insulin IRMA from CisBio International (Gif sur Yvette, France). The intra- and inter-assay coefficients of variation were < 4.7 and <3.8% or <7.9 and <7.5% for adiponectin and insulin, respectively.

2.4 Statistical analysis

The concentration of adiponectin was normally distributed and was used untransformed; the distribution of insulin was log-transformed to approximate normality. Outliers, defined as concentrations greater/lower than 3 standard errors of the hormone's normalized distribution, were excluded.

We focused on the pollutants detected in at least 75% of the samples, and also PCB187 and DE metabolites (to obtain a more complete picture of DAP metabolites) (Table 2). Associations between each fetal hormone and biomarker concentration, considered as a categorical variable (according to quartiles for most compounds except PCB187 and DE metabolites which were classified into three categories), were estimated using multivariate linear regression. Adjustment factors were all variables possibly related to variations of hormonal concentrations or to the measurement of pesticides concentrations (based on *a priori* knowledge), including maternal age (continuous), maternal pre-pregnancy body mass index (BMI: <25, ≥ 25 kg/m^2), maternal educational level (primary/secondary, graduated from secondary, post-secondary), maternal smoking (non smoker, stopped smoking at the beginning of the pregnancy, still smoker at inclusion), parity (0, 1, at least 2), maternal high blood pressure (yes, no), newborn sex (boy, girl) and concentration of maternal urinary creatinine (µg/l) for non persistent pollutants (continuous) or total lipids (µg/l) in cord blood for persistent pollutants (continuous).

For analyses of adiponectin, we also adjusted for level of hemolysis in the cord blood sample (no hemolysis, moderate hemolysis, substantial hemolysis) because hormonal concentrations vary according to extent of hemolysis. For insulin analysis, we adjusted for hemolysis (no hemolysis, moderate hemolysis) and samples with substantial hemolysis (n=16) were excluded because most of the insulin concentrations in the corresponding samples were below the LOD.

Interactions with newborn sex were tested, and stratified results are presented when p <10%. The p-values for trend were calculated with the categorical exposure variables expressed as continuous variables in the regression model (i.e., 0, 1, 2, 3).

Weight and adiposity at birth may lie on the causal pathway between prenatal exposure and hormone concentrations in cord blood. We chose BMI z-score as a marker of adiposity at birth (de Cunto et al., 2014). The regression models, additionally adjusted for gestational age and BMI z-score at birth determined according to WHO reference data (http://www.who.int/childgrowth/en/; de Onis et al., 2007) were rerun as a sensitivity analysis.

To take into account the correlations between POP for the results reported with a single-pollutant approach, we also ran a multipollutant model. We chose not to include highly correlated pollutants (rho[Spearman]>0.70), because linear models may perform poorly with highly correlated data.

Second, we conducted restricted cubic spline regressions adjusted for the same covariates as described above. We used the SAS macro %RCS_Reg which produced graphical representations and statistical tests for both the overall association and the non-linear components of the association (Desquilbet et al., 2010). Levels of exposures were log-transformed and censored values were imputed using a simple distribution-based imputation method (proc Lifereg SAS; Jin et al., 2011). Statistical analyses were performed with SAS software (SAS/STAT version 9.3; SAS Institute Inc., Cary, NC).

3. Results

Sociodemographic and medical characteristics of mothers and newborns are presented in Table 1. A large percentage of the mothers had had university education (65%). Fourteen percent were overweight at the beginning of pregnancy. Only 15% declared smoking during pregnancy and less than 2% reported regular alcohol consumption. Only 3% of the newborns were preterm.

DAP metabolites were quantified in nearly all maternal urine samples (92%), with a median concentration of 38.8 nmol/L, mainly constituted of DM metabolites (Table 2). Organochlorine pesticides (β-HCH, DDE, HCB) and selected PCBs were detected in 74% (for PCB187) to 100 % (for PCB153) of the cord blood samples; DDE had the highest median concentration for a single compound (0.19 µg/L), followed by PCB153 (0.11 µg/L) and β-HCH (0.04 µg/L). As expected, DAP and DM concentrations were strongly correlated with each other (r=0.94), and less strongly with DE metabolites. The three classes of PCBs (PCB153, PCB187, sum of PCB118, 138, 170) were correlated (r>0.84), each also being strongly correlated (r>0.90) with the sum of the nine PCB congeners. The three organochlorine pesticides were moderately correlated with each other (r=0.47 to 0.67), and slightly more with PCB153 (r=0.55 to 0.70).

The sociodemographic and medical characteristics, and the DAP metabolite and POP concentrations of the 268 mother-child pairs included in this analysis were similar to those in the total random subcohort of 601 pairs for which urinary and cord blood biomarkers were available (Suppl Table 1).

Two outliers were excluded from the analysis of adiponectin concentrations. Mean concentrations of adiponectin were higher among girls (Suppl Table 2). Insulin was positively correlated with z-score of BMI at birth in both sexes and higher among girls of overweight mothers. Adiponectin was lower among babies born preterm in both sexes and negatively correlated with maternal pre-pregnancy BMI among girls (Suppl Table 3),

A non linear increase in insulin levels was observed in association with concentrations of DAP metabolites, stronger with DM metabolites (Table 3 and Figure 1). These associations were reinforced by additional adjustment for BMI z-score at birth (Suppl Table 4). No association was found with adiponectin levels.

We observed a decrease in insulin levels with increasing concentrations of each class of PCBs and with β-HCH at low concentration (0.029-0.040 µg/l); there were no such associations for adiponectin (Table 4 and Figure 2).

There was a significant interaction with sex in the pattern of associations with β-HCH (not apparent on the spline model) and with DDE. The trends for lower insulin and adiponectin levels associated with higher DDE concentration were observed exclusively among girls (Suppl Table 5 and Suppl Figure 1). Additional adjustment for BMI z-score at birth led to the attenuation of the associations detected for insulin, and in the case of PCBs, they were no longer statistically significant. The associations observed for adiponectin remained virtually unchanged (Suppl Table 6).
When PCB153, β-HCH and DDE were entered simultaneously into the model (the correlation between HCB and PCB153 was 0.70 and we decided not to include HCB), the association detected between higher PCB153 and lower insulin concentrations was still present; also, there was a significant decrease in adiponectin concentration with low exposure. The association between DDE and low insulin and adiponectin levels was still observed among girls (Suppl Table 7

4. Discussion

Our study shows associations between prenatal exposure to POP and to OP compounds and both adiponectin and insulin concentrations at birth suggesting a potential influence of exposure to these compounds at exposure levels currently observed in the general population, on glucose metabolism during the fetal life.

We observe a decrease in adiponectin levels with increasing levels of DDE in cord blood. Low adipokine levels at birth have been reported among preterm and small-for-gestational age newborns (Martos-Moreno et al., 2009). However, the strength of the association we observed between DDE and adiponectin concentrations was not modified by adjustment for birth weight z-score, suggesting the DDE has a direct effect on glucose metabolism during gestation independent of fetal growth. This is in agreement with epidemiological observations of an absence of association between prenatal exposure to DDE and birth weight (Casas et al., 2015), and an association with increased infant growth (Iszatt et al., 2015). We observed this association only among girls. Sex-specific associations with prenatal exposure to DDE have previously been reported for child growth, in studies that presented separate results by sex. In a Spanish birth cohort, prenatal DDE exposure was associated with overweight at age 6.5 especially among girls (Valvi et al., 2012).

Similarly, in the Faroe Islands, obesity development at age 7 was associated with prenatal DDE exposure only among girls born to overweight mothers (Tang-Péronard et al., 2014). In adult women in Michigan, BMI was positively associated with prenatal exposure to DDE (Karmaus et al., 2009).

We observe a decrease in insulin levels with increasing levels of PCB153; however, this association however did not hold after adjustment for birth weight z-score, which can be interpreted as resulting from PCB153 reducing fetal growth. This is consistent with epidemiological observations of birth weight and infant growth both decreasing with increasing levels of prenatal PCB153 (Casas et al., 2015; Iszatt et al., 2015). On the other hand, the decrease in insulin levels with increasing levels of DDE among girls, and among newborns with low concentrations of beta-HCH in cord blood, were not modified by adjustment for birth weight z-score; this suggests that the association between environmental exposure and insulin concentration is independent of changes in fetal growth (Holness et al., 2000). In experiments with mice, perinatal DDT exposure led to an impairment of thermogenesis, a decrease in energy expenditure, and an impairment of glucose and lipid metabolism (La Merrill et al., 2014).

In our study, insulin levels at birth increased with the DAP concentration in maternal urine (a relationship not modified by adjustment for birth weight z-score). This is in agreement with clinical observations and animal experiments showing that OPs cause hyperglycemia, which induces a concomitant increase of insulin secretion (Lasram et al., 2014). Early-life exposure of rats to chlorpyrifos leads to hyperinsulinemia but with normal glucose levels, suggestive of insulin resistance (Slotkin et al., 2011). Several recent reviews have considered mitochondria as pesticide, particularly OP, targets (Lee et al., 2014): the toxicity of OP may be the consequence of dysfunction of mitochondrial oxidative phosphorylation, leading to impairment of cellular energy metabolism, anti-oxidant defense and calcium uptake. The resulting release of inflammatory cytokines may be responsible for compensatory hyper-insulinism and the impairment of glucose homeostasis (Karami-Mohajeri et al., 2013; Baltazar et al. 2014)

Our study is based on a modest-sized sample, and uses a limited number of fetal markers. Children included in the survey were a subsample of the whole cohort and did not show characteristics different from those the initial cohort. Adiponectin and insulin concentrations were determined using standard methods available in clinical practice and expected factors of variation were present. Hemolysis had to be taken into account because it appears to influence circulating levels of both adiponectin and insulin. The main strength of this study is the biological measures of several contaminants, sometimes early in pregnancy, in parallel with measures of hormone levels at birth. Transplacental transfer of POP is well documented and determination of POP concentration in cord blood is a reliable method for evaluating fetal exposure to these contaminants (Viczaino et al., 2014). OP are characterized by a short biological half-life: once absorbed they are rapidly metabolized and excreted from the body. Their presence in amniotic fluid (Bradman et al., 2003) suggests that fetuses too are exposed to these chemicals. Urinary DAP are non specific metabolites of most organophosphate pesticides: assaying DAP is thus a simple, integrated, sensitive and noninvasive method for assessing OP exposure (Needham et al., 2005). However, large intraindividual variability in metabolite levels in spot urine samples taken during pregnancy has been demonstrated (Bradman et al., 2005). Consequently, repeated urine assays may provide a more accurate characterization of OP prenatal exposure. However despite the limitations, the efficacy of this method for detecting developmental neurotoxicity of OP has been demonstrated in several birth cohorts, including one study where similar relations between cognitive scores at age 7 and DAP measured earlier or later during pregnancy were found (Bouchard et al., 2011).

We took into account a number of known determinants of hormone levels and tried to accommodate for mixtures of POP. Nevertheless, our approach may not have completely excluded the joint action of co-exposures in our effect estimates.

5. Conclusions

Our findings suggest that prenatal exposure to persistent pesticides and PCB may have consequences for adiponectin and insulin levels at birth, and therefore, possibly, long-lasting effects on glucose metabolism. Our observations are coherent with experimental, clinical and epidemiological observations of hormonal modifications induced by altered fetal and infant growth. Observed changes are small but our study suggests early metabolic consequences of prenatal exposure to OP pesticides. As similar results have not been previously reported, these findings need to be confirmed by further analyses in other populations.

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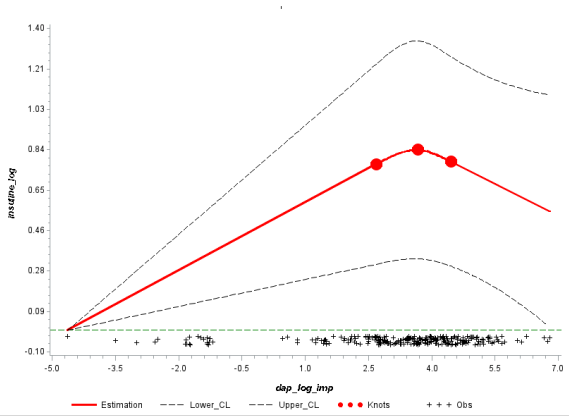
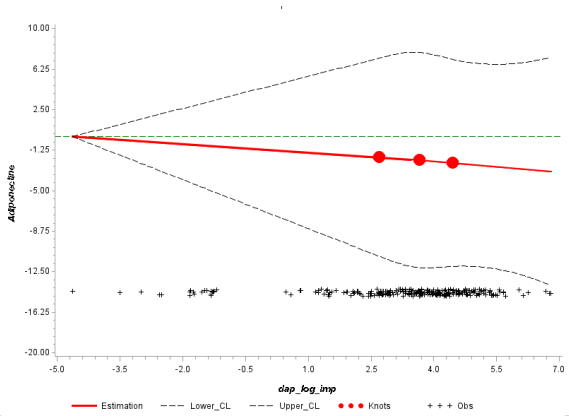
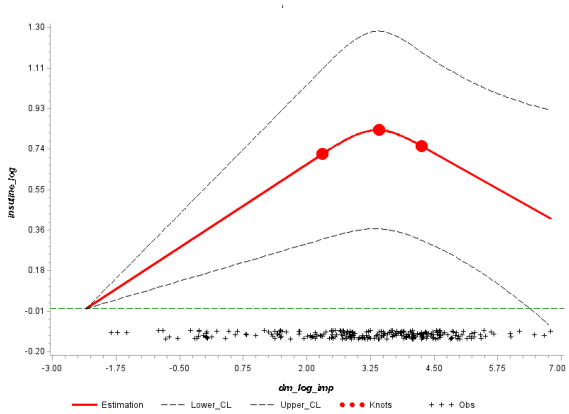
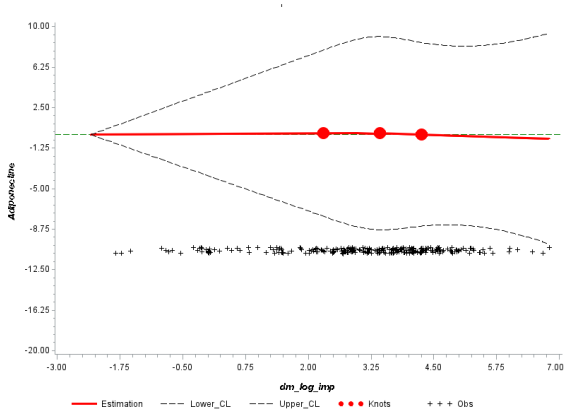
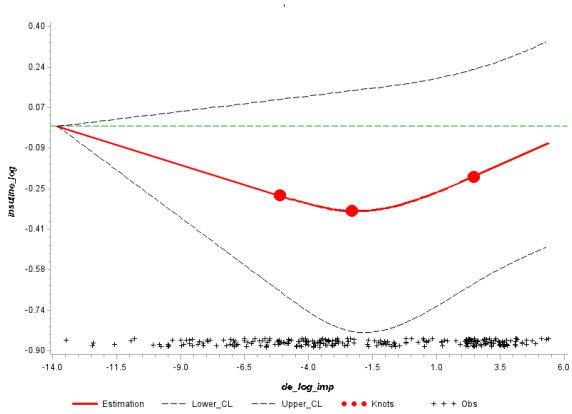
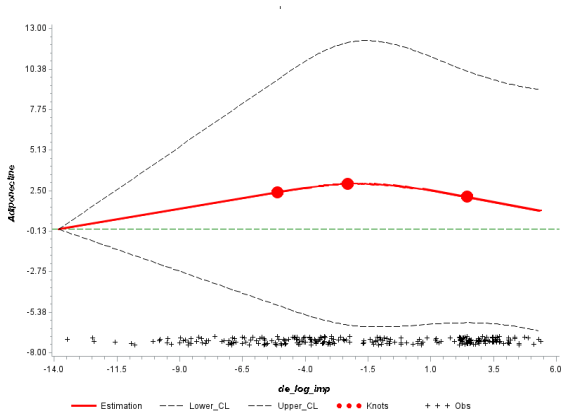
Figure 1. Restricted cubic splines between prenatal exposure to organophosphate pesticides and levels of insulin and adiponectin

	Log(Insulin) [a, b]	Adiponectin [a]
Log(DAP)		
	p-overall=0.005 p nonlinear component=0.03	p-overall=0.84 p nonlinear component=0.97
Log(DM)		
	p-overall=0.002 p nonlinear component=0.004	p-overall=0.99 p nonlinear component=0.93
Log(DE)		
	p-overall=0.24 p nonlinear component=0.09	p-overall=0.80 p nonlinear component=0.51

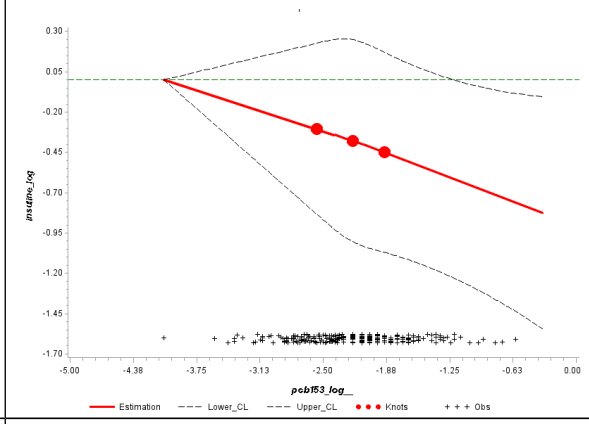
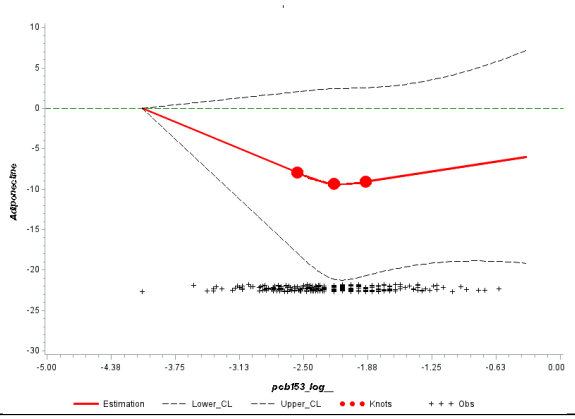
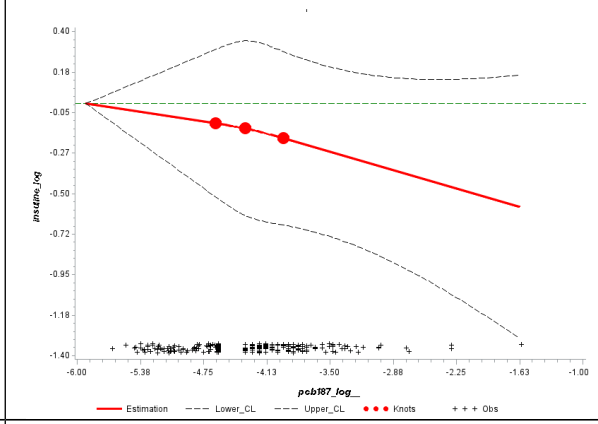
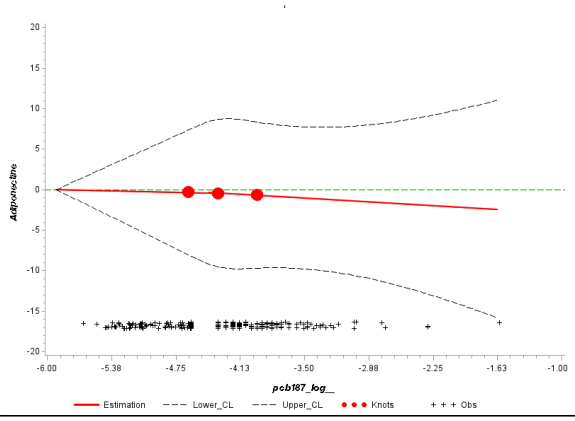
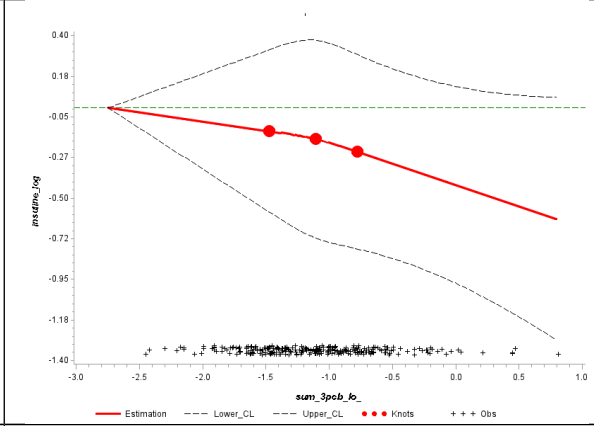
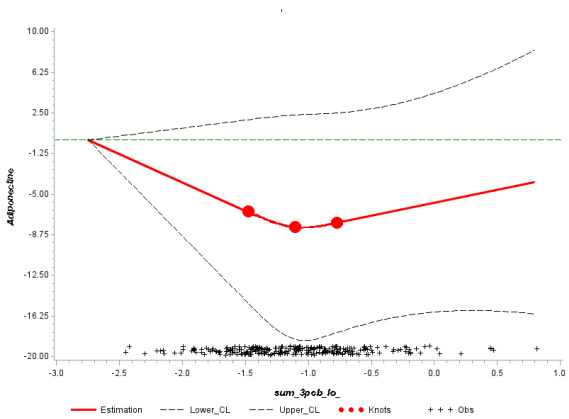
CI, confidence interval; DAP, dialkylphosphate; DE, diethylphosphate; DM, dimethylphosphate.

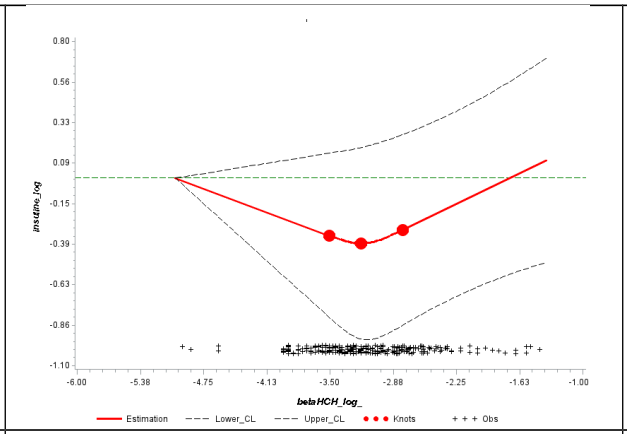
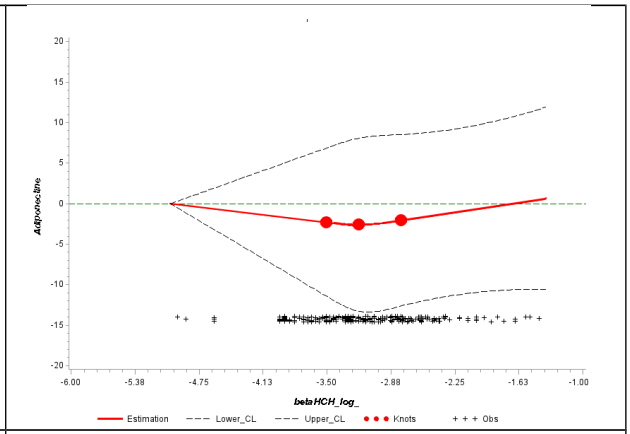
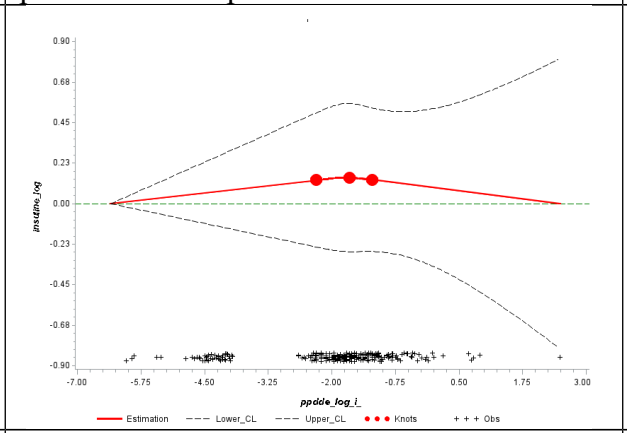
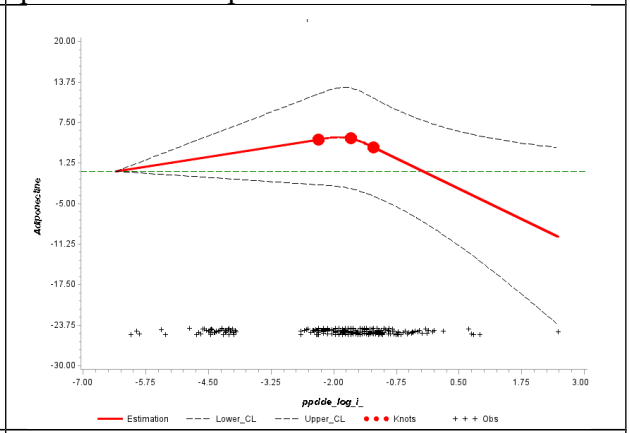
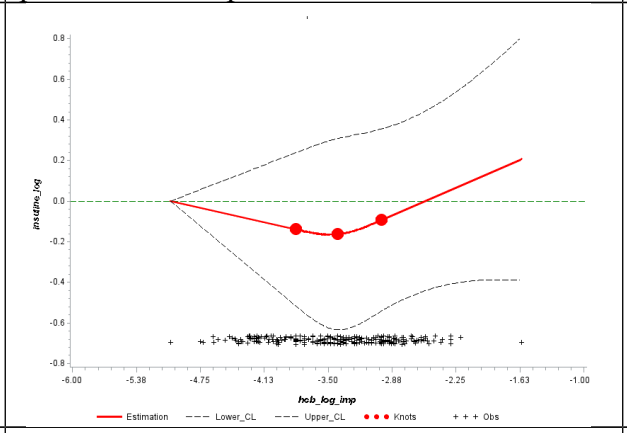
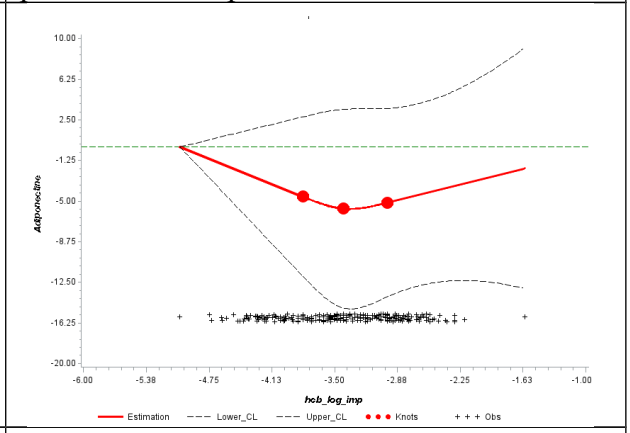
Adjusted dose-response association between organophosphate pesticides (log-scale, nmol/l) and insulin (log-scale, μU/ml) and adiponectin (μg/ml). Organophosphate pesticides were coded using restricted cubic spline functions with three knots located at the 25th, 50th, and 75th percentiles of the distribution. Y-axis represents the difference in hormone levels between individuals with any value of organophosphate pesticides with individuals with low exposure (ie, minimal value of the distribution)..

[a] Adjusted for creatinine (continuous), hemolysis (3 categories), maternal age (continuous), body mass index (2 categories), educational level (3 categories), smoking status (3 categories), parity (3 categories), high blood pressure (2 categories), and sex (2 categories)

[b] Excluding serum with substantial hemolysis (n=16)

Figure 2. Restricted cubic splines between prenatal exposure to persistent organic pollutants and levels of insulin and adiponectin

	Log(Insulin) [a, b]	Adiponectin [a]
Log(PCB153)		
	p-overall=0.08 p nonlinear component=0.89	p-overall=0.30 p nonlinear component=0.18
Log(PCB187)		
	p-overall=0.31 p nonlinear component=0.76	p-overall=0.94 p nonlinear component=0.92
Log(Sum of PCB 118, 138, 170)		
	p-overall=0.19 p nonlinear component=0.65	p-overall=0.31 p nonlinear component=0.17

Log(BetaHCH)		
	p-overall=0.10 p nonlinear component=0.04	p-overall=0.74 p nonlinear component=0.47
Log(ppDDE)		
	p-overall=0.78 p nonlinear component=0.60	p-overall=0.08 p nonlinear component=0.02
Log(HCB)		
	p-overall=0.50 p nonlinear component=0.27	p-overall=0.48 p nonlinear component=0.29

HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; PCB, PCB, polychlorinated biphenyl; p-p′-DDE, p-p′-dichlorodiphenyldichloroethylene.

Adjusted dose-response association between persistent organic pollutants (log-scale, µg/l) and insulin (log-scale, µU/ml) and adiponectin (µg/ml).

Persistent organic pollutants were coded using restricted cubic spline functions with three knots located at the 25th, 50th, and 75th percentiles of the distribution. Y-axis represents the difference in hormone levels between individuals with any value of the persistent organic pollutant with individuals with low exposure (ie, minimal value of the distribution).

[a] Adjusted for total lipid (continuous), hemolysis (2 categories), maternal age (continuous), body mass index (2 categories), education (3 categories), smoking status (3 categories), parity (3 categories), high blood pressure (2 categories), and sex (2 categories)

[b] Excluding serum with substantial hemolysis (n=16)

Table 1. Maternal and newborn characteristics (N=268)

	N (%) or median (Q1 ; Q3)
Maternal characteristics	
Maternal age (years)	
< 25	31 (11.6)
25-30	108 (40.3)
30-35	99 (36.9)
≥ 35	30 (11.2)
Continuous	29.9 (27.2 ; 32.5)
Education level	
Primary/secondary	45 (16.8)
Graduated from secondary school	48 (17.9)
Post-secondary	175 (65.3)
Body mass index (kg/m²)	
< 18.5	23 (8.6)
18.5-25	208 (77.6)
≥ 25	37 (13.8)
Continuous	21.4 (19.9 ; 23.4)
Smoking status	
No smoker	194 (72.5)
Stop smoking at the beginning of the pregnancy	30 (11.4)
Smoker at inclusion	40 (15.2)
Missing	4

Alcohol consumption	
Never	225 (85.9)
Occasionally	33 (12.6)
Regularly (once a day)	4 (1.5)
Missing	6
Parity	
0	114 (42.5)
1	101 (37.7)
≥ 2	53 (19.8)
High blood pressure	
No	257 (95.9)
Yes	11 (4.1)
Newborn characteristics	
Sex	
Boy	132 (49.2)
Girl	136 (50.8)
Gestational age (weeks)	40 (39 ; 40)
Preterm birth (<37 weeks)	8 (3.0)
Birth weight (g)	3370 (3100 ; 3670)
Z-score of birth weight	0.15 (-0.55 ; 0.75)
Z-score of birth bmi	0.25 (-0.42 ; 0.82)

Table 2. Exposure biomarkers levels measured in maternal urine (N=254) and in cord blood samples (N=267).

			Urine/Cord blood concentration				Creatinine/Lipid-Adjusted concentration			
	LOQ	N (%) > LOQ	p25	Median	p75	Max	p25	Median	p75	Max
Urinary biomarkers			nmol/L				nmol/g creatinine			
Dialkyl phosphates	0.30	234 (92.1)	14.669	38.835	85.649	895.653	12.619	38.236	88.438	1573.380
Dimethyl phosphates	1.50	229 (90.2)	10.024	30.664	70.861	895.653	10.145	30.605	70.970	1456.233
Diethyl phosphates	0.10	127 (50.0)	<LOQ	0.107	11.588	216.34	<LOQ	0.112	10.940	194.129
Cord blood biomarkers			µg/L				µg/g lipids[a]			
PCB 153	0.01	267 (100.0)	0.077	0.110	0.150	0.730	0.019	0.028	0.046	0.332
PCB 187	0.01	197 (73.8)	<LOD	0.013	0.019	0.200	<LOD	0.003	0.005	0.091
Sum of PCBs[b]	0.01	267 (100.0)	0.229	0.331	0.460	2.253	0.056	0.087	0.139	1.024
Beta HCH	0.01	264 (98.9)	0.030	0.041	0.062	0.260	0.007	0.011	0.018	0.108
pp'DDE	0.02	220 (82.4)	0.100	0.190	0.300	12.000	0.001	0.046	0.084	1.042
Hexachlorobenzene	0.02	211 (79.0)	0.022	0.033	0.051	0.200	0.004	0.008	0.015	0.083

HCH, hexachlorocyclohexane; LOD, limit of detection; LOQ, limit of quantification; PCB, polychlorinated biphenyl; p-p′-DDE, p-p′-dichlorodiphenyldichloroethylene.

[a] 6 missing values of total lipid level

[b] Sum of congeners 118, 138, and 170. Concentrations below the LOD were replaced by LOD/2 (n=5, n=0, and n=14 respectively); expressed in nmol

Table 3. Prenatal exposure to organophosphate pesticides, and insulin and adiponectin concentrations

			Log(Insulin) [b]			Adiponectin
	N	LSmeans (μU/ml)	Beta (95% CI) [a]	N	LSmeans (μg/ml)	Beta (95% CI) [a]
DAP (nmol/l)			P-trend=0.12			P-trend=0.73
≤ 14.67	60	3.67	Ref.	64	35.79	Ref.
14.67 – 38.84	59	4.59	0.22 (-0.04 ; 0.50)	62	34.87	-0.92 (-5.14 ; 5.11)
38.84 – 85.65	59	4.56	0.22 (-0.05 ; 0.48)	62	37.64	1.84 (-3.23 ; 6.92)
> 85.65	61	4.62	0.23 (-0.04 ; 0.50)	64	35.78	-0.01 (-6.00 ; 4.15)
Interaction with sex			P=0.69			P=0.34
DM (nmol/l)			P-trend=0.13			P-trend=0.50
≤ 10.024	60	3.53	Ref.	64	35.60	Ref.
10.024 – 30.66	59	5.02	**0.35 (0.09 ; 0.62)**	63	34.78	-0.82 (-5.87 ; 4.22)
30.66 – 70.86	61	4.53	0.25 (-0.01 ; 0.51)	62	37.70	2.10 (-2.94 ; 7.15)
> 70.86	59	4.55	0.25 (-0.01 ; 0.52)	63	36.38	0.78 (-4.34 ; 5.91)
Interaction with sex			P=0.14			P=0.87
DE (nmol/l)			P-trend=0.19			P-trend=0.89
≤0.10	121	4.04	Ref.	126	35.81	Ref.
0.10 – 11.59	60	4.29	0.06 (-0.17 ; 0.29)	63	37.67	1.86 (-2.48 ; 6.20)
> 11.59	58	4.74	0.16 (-0.08 ; 0.39)	63	35.09	-0.72 (-5.16 ; 3.72)
Interaction with sex			P=0.62			P=0.11

CI, confidence interval; DAP, dialkylphosphate; DE, diethylphosphate; DM, dimethylphosphate; LSmeans, least-squares means

[a] Adjusted for creatinine (continuous), hemolysis (3 categories), maternal age (continuous), body mass index (2 categories), educational level (3 categories), smoking status (3 categories), parity (3 categories), high blood pressure (2 categories), and sex (2 categories)

[b] Excluding serum with substantial hemolysis (n=16)

Table 4. Prenatal exposure to persistent organic pollutants and insulin and adiponectin concentrations

			Log(Insulin) [b]			Adiponectin
	N	LSmeans (μU/ml)	Beta (95% CI) [a]	N	LSmeans (μg/ml)	Beta (95% CI) [a]
PCB153 (ug/l)			P-trend=0.05			P-trend=0.29
≤0.077	63	4.48	Ref.	67	38.29	Ref.
0.077 – 0.111	74	4.76	0.05 (-0.19 ; 0.30)	75	34.65	-3.64 (-8.41 ; 1.13)
0.111 – 0.150	57	4.10	-0.09 (-0.37 ; 0.18)	60	35.41	-2.88 (-8.14 ; 2.38)
>0.150	57	3.42	-0.27 (-0.56 ; 0.02)	63	35.03	-3.25 (-8.74 ; 2.24)
Interaction with sex			P=0.74			P=0.59
PCB187 (ug/l)			P-trend=0.06			P-trend=0.17
≤ 0.010	122	4.66	Ref.	126	37.67	Ref.
0.010 – 0.018	67	4.22	-0.10 (-0.32 ; 0.13)	70	35.71	-1.97 (-6.37 ; 2.44)
> 0.018	62	3.67	-0.24 (-0.48 ; 0.01)	69	34.48	-3.19 (-7.89 ; 1.51)
Interaction with sex			P=0.77			P=0.35
Sum of PCBs 118, 138, 170 (ug/l)			P-trend=0.31			P-trend=0.52
≤0.223	63	4.36	Ref.	67	38.24	Ref.
0.223 – 0.331	66	4.65	0.06 (-0.19 ; 0.32)	66	33.96	-3.26 (-8.18 ; 1.67)
0.331 – 0.461	62	4.49	0.01 (-0.26; 0.27)	66	35.36	-1.99 (-7.03 ; 3.05)
>0.461	60	3.78	-0.14 (-0.42 ; 0.14)	66	36.18	-2.20 (-7.62 ; 3.22)
Interaction with sex			P=0.96			P=0.32
BetaHCH (ug/l)			P-trend=0.59			P-trend=0.81
≤0.029	60	5.21	Ref.	64	35.77	Ref.
0.029 – 0.040	65	4.22	**-0.38 (-0.64 ; -0.12)**	67	36.88	1.11 (-3.94 ; 6.17)
0.040 – 0.061	64	3.56	-0.21 (-0.47 ; 0.05)	66	36.03	0.27 (-4.84 ; 5.37)
>0.061	62	5.21	-0.16 (-0.44 ; 0.12)	68	36.79	1.02 (-4.27 ; 6.31)
Interaction with sex			P=0.98			P=0.03
pp'DDE (ug/l)			P-trend=0.34			P-trend=0.27

≤ 0.100	62	4.53	Ref.	67	36.26	Ref.
0.100 – 0.180	63	4.35	-0.04 (-0.30 ; 0.22)	64	39.55	3.30 (-1.64 ; 8.24)
0.180 – 0.290	60	4.35	-0.04 (-0.30 ; 0.23)	66	38.10	1.84 (-3.12 ; 6.80)
> 0.290	66	4.53	-0.13 (-0.39 ; 0.13)	68	33.78	-2.48 (-7.44 ; 2.49)
Interaction with sex			P=0.74			P=0.04
Hexachlorobenzene (ug/l)			P-trend=0.71			P-trend=0.73
≤ 0.022	65	4.35	Ref.	69	37.33	Ref.
0.022 – 0.033	61	3.71	-0.16 (-0.42 ; 0.09)	65	35.40	-1.93 (-6.78 ; 2.91)
0.033 – 0.051	63	4.71	0.08 (-0.18 ; 0.33)	64	35.94	-1.40 (-6.35 ; 3.55)
> 0.051	62	4.26	-0.03 (-0.29 ; 0.24)	67	36.30	-1.03 (-6.01 ; 3.95)
Interaction with sex			P=0.97			P=0.45

CI, confidence interval; HCH, hexachlorocyclohexane; LSmeans, least-squares means; PCB, PCB, polychlorinated biphenyl; p-p′-DDE, p-p′-dichlorodiphenyldichloroethylene.

[a] Adjusted for total lipid (continuous), hemolysis (2 categories), maternal age (continuous), body mass index (2 categories), education (3 categories), smoking status (3 categories), parity (3 categories), high blood pressure (2 categories), and sex (2 categories)

[b] Excluding serum with substantial hemolysis (n=16)