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Exposure of pregnant women to persistent organic pollutants and cord sex hormone levels

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STUDY QUESTION: Is prenatal exposure to persistent organic pollutants (POPs) associated with variations of sex hormone levels in cord blood?

SUMMARY ANSWER: Prenatal exposure to a number of POPs is associated with a disruption of hormone levels in cord blood, with sex specificities.

WHAT IS KNOWN ALREADY: Epidemiological studies have reported disorders of reproductive health, in relation with POPs exposure during early life and the endocrine disruption properties of these chemicals have been suggested as possible mechanisms.

STUDY DESIGN, SIZE, DURATION: A subset of 282 mother-child pairs was selected from the prospective population-based PELAGIE birth cohort ($n = 3421$, 2002–2006, Brittany, France). Pregnant women were recruited before 19 weeks of gestation and followed until delivery.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Sex hormone levels including sex hormone-binding globulin (SHBG), estradiol (E2), total testosterone (T), free testosterone (fT = T/SHBG) and the aromatase index (AI = T/E2) were measured in 282 cord blood samples. Anti-Müllerian hormone (AMH) was measured in male newborns only. Pesticide concentrations of α-endosulfan, β-hexachlorocyclohexane (β-HCH), γ-HCH, dieldrin, pp′-dichlorodiphenyldichloroethylene (p,p′-DDE), hexachlorobenzene (HCB), heptachlor epoxide (HCE), as well as PCBs (congeners 153, 187 and the sum of anti-estrogenic PCBs 118, 138, and 170) and decabrominated diphenyl ether (BDE209) were also measured in cord blood. Associations between sex hormones and POPs exposure were explored using multiple linear regressions adjusted for potential confounders.

MAIN RESULTS AND THE ROLE OF CHANCE: High PCB levels were associated with an increase of SHBG (P-trend < 0.01) and AMH (P-trend < 0.05) and a decrease of fT (P-trend < 0.05) and AI (P-trend < 0.01). High pesticide levels, particularly α-endosulfan and HCE, were associated with an increase of SHBG ($P < 0.05$) and E2 ($P < 0.01$) and a decrease of fT ($P < 0.05$) and AI ($P < 0.01$). Several of these associations were stronger, or specific, among male or female newborns. The associations were not altered in the sensitivity analyses.

LIMITATIONS, REASONS FOR CAUTION: The study population was of relatively small sample size, and some compounds rarely detected in cord blood. The high level of correlation between POPs makes it difficult to identify the most contributing POPs. Hormone measurements were performed at birth (in cord blood) and may not adequately represent the infant endocrine system. Multiple statistical testing may have led to false-positive associations.

WIDER IMPLICATIONS OF THE FINDINGS: Our results are in discordance with those reported in the only published study of the kind but in accordance with studies about prenatal exposure to other endocrine disruptors such as phthalates. These findings may help understanding the pathways involved in adverse reproductive outcomes associated with POPs exposure.

STUDY FUNDING/COMPETING INTEREST(S): The PELAGIE cohort is funded by Inserm, French Ministry of Health, French Ministry of Labor, InVS, ANR, ANSES, and French Ministry of Ecology. None of the authors has any competing interest to declare.

Key words: steroids / hormones / polychlorinated biphenyls / organochlorine compounds / newborns / cohort studies / endocrine disruptors

Introduction

Persistent organic pollutants (POPs), such as organochlorine pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDE), are widespread and known endocrine disruptors (EDs). They are suspected to cause steroid-mediated health effects, and exposure during the period of intrauterine development is suspected to cause irreversible effects by influencing the functions of the endocrine and other physiological systems later in life (Grandjean *et al.*, 2008; WHO and UNEP, 2012).

ED chemicals can interfere with endocrine systems by different mechanisms of action. They can act directly, by mimicking (agonist) or blocking (antagonist) the action of natural hormones by binding to their natural receptors, or indirectly by altering hormone production, transport or regulation. In the case of steroid hormones, this indirect action can appear via a protein involved in hormone production (e.g. aromatase, which converts testosterone [T] to estradiol [E2]), via a carrier protein (e.g. sex hormone-binding globulin [SHBG], which has a high affinity for T and E2 transportation) or by affecting feedback regulations.

Actually, the estrogenic activity of organochlorine pesticides (e.g. dichlorodiphenyldichloroethylene [DDE], endosulfan) has been experimentally observed for quite some time (Kupfer, 1975), and it has been confirmed *in vitro* in human estrogen sensitive cells (Soto *et al.*, 1994). This activity has been shown to have a direct impact on the enzymes (aromatase) involved in steroidogenesis (Letcher *et al.*, 1999; Wójtowicz *et al.*, 2007). According to their structure, a weak estrogenic or anti-estrogenic activity has also been demonstrated for PCBs (especially Aroclor mixtures) both *in vivo*, with a uterotrophic assay in rats, and *in vitro* (Jansen *et al.*, 1993; Wolff *et al.*, 1997). Hypotheses of the mechanisms behind these effects potentially include estrogen receptor binding to hydroxylated metabolites, impact on aromatase activity (Woodhouse and Cooke, 2004) and, more recently, down-regulation of a WNT gene involved in the development of the female reproductive tract and subsequent adult function (Ma and Sassoon, 2006). PBDEs can also act on endocrine functions with anti-androgenic properties, as was recently shown in some (Stoker *et al.*, 2005; Lilienthal *et al.*, 2006) but not all studies (He *et al.*, 2008). This suggests congener-dependent effects of these compounds. Therefore, it is difficult to predict whether a compound or a mixture of compounds may or may not induce an adverse health effect.

Several animal studies have suggested that exposure to PCBs and DDE might induce adverse reproductive health outcomes that appear at all stages of life, including the modification of sex hormone levels or reproductive organs, early/late puberty onset, reduced fertility capacities or hormone-related cancers (breast, prostate) (WHO and UNEP, 2012). In humans, exposure to POPs (especially PCBs, organochlorine pesticides, dioxins and furans) has been associated with markers of reproductive functions such as genital malformations (McGlynn *et al.*, 2009), puberty onset (Toppari and Juul, 2010), fecundability (Chevrier *et al.*, 2013), semen quality or adult sex hormone levels (Meeker and Hauser, 2010), but evidence is still inconclusive. Most of these studies did not assess exposure during the critical windows of the developing systems (i.e. fetal and infant periods).

The modification of hormone levels at birth can reflect an alteration of the intrauterine development and be predictive of future pathologies (Barker *et al.*, 1993).

To our knowledge, only one study has investigated the sex-steroid hormone levels of newborns with regard to prenatal POP exposures (Cao *et al.*, 2008). More recently, associations between prenatal exposure to PCB and sex hormone levels among 14-year-old boys in the Faroe cohort were reported (Grandjean *et al.*, 2012).

During gestation, the placenta is a major endocrine organ, but it is not fully functional in regards to the mechanisms of steroidogenesis because it is devoid of the enzymatic complex necessary for the conversion of pregnenolone to androgens (which are themselves substrates for the synthesis of estrogens). Therefore, this step is performed by the fetal and maternal adrenal glands, and there is perfect cooperation between the placenta and the fetus leading to the concept of the fetoplacental unit.

The aim of this exploratory study was to investigate the possible effects of prenatal exposure to various POPs on cord blood levels of sex hormones, which represent the fetoplacental unit, among a French mother-child cohort (PELAGIE).

Materials and Methods

Study population

The study population came from the PELAGIE mother-child cohort, which enrolled 3421 pregnant women (participation rate of 80%), at 19th weeks or less of gestation, living in Brittany (France) between 2002 and 2006 (for more details, see Chevrier *et al.* (2013)). The women completed a questionnaire at home, and medical data were obtained by midwives and pediatricians for 99% of the women. At the time of delivery, cord blood samples were collected from 2138 (62%) women and were immediately centrifuged at the maternity hospital and stored at $-20^{\circ}C$ in our lab.

A random sample of 601 births (18%) was obtained from the live born singleton cohort ($n = 3322$) to perform chemical measurements. Among these newborns, cord blood samples were available for 396 (66%) newborns, but only 282 (47%) samples had sufficient serum volumes to perform both exposure and outcome assessments (see Supplementary Figure S1).

Ethical approval

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).

Chemical analyses of persistent organic pollutants in the cord blood serum

The levels of POPs were determined from the cord blood samples of the subcohort of participants at the Centre de Toxicologie de Québec of the National Institute of Public Health of Québec. Details about the 36 compounds measured and the GC/MS methods used (2 ml serum samples) were described elsewhere (Chevrier *et al.*, 2013) and in the Supplementary data, 'Chemical analyses of POP in cord blood serum samples' section.

Only compounds detected in more than 5% of the samples were retained for the present study. They included nine PCBs (congeners 118, 138, 153, 170, 180, 183, 187, 194 and 203), seven organochlorine pesticides (p,p′-DDE, β-hexachlorocyclohexane [βHCH], dieldrin, γ-hexachlorocyclohexane [γHCH], hexachlorobenzene [HCB], heptachlor epoxide [HCE], and α-endosulfan) and one BDE (congener 209). Because PCBs are highly correlated (see Supplementary Table SI), we chose to study the congener 153 as a marker of the total exposure to PCBs, the congener 187, which is suspected to have estrogenic activity, and the sum of congeners 118, 138 and 170, which are suspected to have anti-estrogenic activities (Wolff *et al.*, 1997).

The concentrations are reported as wet weight (units of micrograms per liter), and the limits of detection ranged from 0.01 μg/l for the majority of the compounds to 0.05 μg/l for BDE209. The total cholesterol (TC), free cholesterol (FC), triglyceride (TG) and phospholipid (PL) levels were also measured in these samples by an enzymatic method (in g/l), which allowed us to calculate the total lipid level as $1.677 \times (TC - FC) + FC + TG + PL$ (Patterson *et al.*, 1991).

Hormone level assessments in the cord blood serum

Assays were performed in the cord blood at the Hormonal Laboratory of the Hospital of Rennes (France). E2 and T levels were determined by chemiluminescent assays on the automated ADVIA-Centaur XP (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). SHBG and AMH were measured using the IRMA or ELISA kits from Beckman-Coulter, Villepinte, France. The intra- and inter-assay coefficients of variation were <8% and <9%, <6% and <9%, <6.1% and <8%, and <12.3% and <14.2% for E2, T, SHBG and AMH, respectively. Hemolysis was present in several samples, and the degree of hemolysis was ranked by visual inspection according to three levels (none, moderate or high).

In addition, we calculated the free testosterone index [$fT = (T \times 100)/SHBG$] and the aromatase index ($AI = T/E2$).

Statistical analysis

To evaluate possible nonlinear relations between POP exposures and hormones, we categorized exposure according to the frequency of detection: Pollutants that were detected in <50% of the samples were categorized into two classes, detected and undetected. Pollutants that were detected in 50–75% of the samples were categorized into three classes; either into terciles or the first class was undetected (<LOD) and the second and third classes less than or greater than the median concentration of the samples where the pollutant was detected—except for heptachlor epoxide which was detected in 51% of samples and was categorized into two classes, detected and undetected. Finally, pollutants that were detected in >75% of the samples were categorized into four classes (i.e. in quartiles).

To reach normality of the hormone level distributions, some variable transformations were used (log transformation for E2, SHBG and fT; inverse transformation for T), and some outliers, defined as ±4 SD, were excluded ($n = 2$ for E2, $n = 5$ for SHBG, $n = 6$ for T, $n = 4$ for AI, and $n = 3$ for fT).

The associations between each hormone and each pollutant were estimated using multivariate linear regressions. Tests for linear trends were performed in which each categorized exposure of interest was entered as an ordinal variable. Estimate coefficients and 95% confidence intervals are presented in figures (see Supplementary Tables SII and SIII for detailed results).

All the models were *a priori* adjusted for gestational age at birth (continuous), maternal age (continuous), parity (nulliparous; primiparous) and total lipids (continuous). We also adjusted for maternal pre-pregnancy body mass index (BMI; <25 versus ≥25 kg/m^2) when studying SHBG and fT. In addition, we explored whether hormone levels were impacted by sample characteristics (i.e. storage duration and hemolysis level), and we therefore adjusted all the models for hemolysis levels (none; moderate; high). Interactions with newborn sex were tested, and stratified analyses were systematically performed. Missing values of covariates were replaced by the mode ($n = 3$ for BMI) or the median ($n = 6$ for total lipids). The statistical analyses were performed using SAS software (SAS/STAT version 9.3; SAS Institute Inc., Cary, NC, USA).

Due to the semi-exploratory goal of our study (no evident expected results but statistical strategy guided by biological considerations), we did not proposed *P*-value corrections and accepted the possibility of false discoveries. All statistical tests were pre-specified in advance (not *post hoc*) and all results were reported as recommended (Rothman, 1990; Bender and Lange, 2001; Goeman and Solari, 2011).

Sensitivity analyses

Three sets of sensitivity analyses were performed. The first excluded 12 women with diseases during pregnancy (i.e. gestational diabetes or high blood pressure). The second used the full-adjusted models including, in addition to those mentioned above, the following covariates: smoking status during pregnancy (no; yes), birthweight (continuous), maternal educational level (<post-secondary; ≥post-secondary), maternal pre-pregnancy body mass index (<25; ≥25 kg/m^2), gestational diabetes (no; yes) and high blood pressure (no; yes). The missing values were replaced by the mode ($n = 4$ for smoking status, $n = 3$ for diabetes and $n = 1$ for high blood pressure).

The last set of sensitivity analyses was a multi-pollutant approach. POPs, which were associated with at least one hormone in the single-pollutant approach, were introduced simultaneously in the model. We chose not to include highly correlated pollutants (rho[Spearman] ≥ 0.70), assuming that linear models cannot disentangle associations in highly correlated data.

Results

Description of the study population

Table I shows the main characteristics of the pregnant women and their newborns. We can notice a highly educated population with a low prevalence of risk factors for adverse pregnancy outcomes (e.g. tobacco, obesity, pathologies of pregnancy). Most of the newborns were born at term, with a mean birthweight of 3379 g (SD = 470 g).

Characteristics associated with hormone levels

We observed significant variations in hormone levels associated with some maternal and newborn characteristics (data not shown): maternal age and parity were negatively correlated with E2 and SHBG and positively correlated with AI. Gestational age was negatively correlated with T, fT and SHBG, and BMI was associated with a decrease in SHBG and an increase in fT. There was no difference in hormone levels by newborn sex.

Table I Maternal and newborn characteristics ($N = 282$).

	N (%) or median (Q1; Q3)
Maternal characteristics	
Maternal age (years)	
<25	33 (11.7)
25–30	116 (41.1)
30–35	101 (35.8)
≥35	32 (11.4)
Continuous	29.8 (27.2; 32.5)
Education level	
Primary/secondary	48 (17.0)
Passed baccalaureate examination	49 (17.4)
Post-secondary	185 (65.6)
Maternal pre-pregnancy body mass index (kg/m^2)[a]	
<18.5	25 (9.0)
18.5–25	216 (77.4)
≥25	38 (13.6)
Continuous	21.5 (19.9; 23.4)
Smoking status[a]	
Non smoker	202 (72.7)
Stopped smoking at the beginning of the pregnancy	34 (12.2)
Smoker at inclusion	42 (15.1)
Alcohol consumption[a]	
Never	236 (85.5)
Occasionally	36 (13.0)
Regularly (once a day)	4 (1.5)
Parity	
0	122 (43.3)
1	105 (37.2)
≥2	55 (19.5)
Gestational high blood pressure[a]	
No	266 (94.7)
Yes	15 (5.3)
Gestational diabetes[a]	
No	267 (95.7)
Yes	12 (4.3)
Newborn characteristics	
Sex	
Boy	141 (50.0)
Girl	141 (50.0)
Gestational age (week)	40 (39; 40)
Birthweight (g)	3,395 (3,100; 3,680)
Z-score of birthweight	0.15 (−0.53; 0.77)
Testosterone (μg/l)	1.99 (1.69; 2.53)
Sex hormone-binding globulin (nmol/l)	33.6 (26.7; 40.8)
Free Testosterone	21.7 (16.3; 31.6)
Anti-Müllerian hormone (μg/l)	34.7 (23.9; 43.2)
Estradiol (μg/l)	6.14 (4.74; 11.63)
Aromatase Index	0.33 (0.24; 0.41)

[a]Missing data on body mass index ($n = 3$), smoking status ($n = 4$), alcohol consumption ($n = 6$), high blood pressure ($n = 1$) and diabetes ($n = 3$).

Description of POPs exposure

PCB153, PCB187, the sum of the anti-estrogenic PCBs, β-HCH, p,p′-DDE, and HCE were detected in most of the cord blood samples (75–100%) (see Table II). The highest median concentrations were observed for p,p′-DDE (0.19 μg/l) and PCB153 (0.11 μg/l). Several POP levels were highly correlated (rho[Spearman] > 0.7) (see Supplementary Table SI). The main characteristics associated with higher POP levels were older maternal age and an earlier year of inclusion. Higher levels of PCB were also observed among women with lower BMI and higher education level, whereas higher levels of organochlorine pesticides were observed among women with higher maternal BMI and nulliparous women.

POPs exposure and T, SHBG, fT, and AMH

Figure 1 shows the associations between POP exposures and T, SHBG, fT and AMH for male and female newborns combined and separately (except for AMH, which was only measured in males). The most common association observed when both sexes were combined was the significant increase in SHBG levels with several POPs, the strongest being for PCBs, followed by HCB, HCE and α-endosulfan. In parallel, we observed a significant decrease in fT levels with exposure to PCBs and HCB but not to HCE or α-endosulfan. We also found an increase in AMH levels with exposure to PCB187 and the sum of the anti-estrogenic PCBs among boys. No significant association was found for T levels.

When we examined the associations by newborn sex, we primarily observed similar associations in both sexes, but we also observed some specificities. The increase in SHBG and the decrease in fT associated with exposure to HCE were specific to male newborns, whereas the increase in SHBG associated with exposure to PCB187 was specific to female newborns (see Fig. 1). Among boys, we can additionally mention a significant decrease in T with exposure to BDE209 (see Fig. 1; inverse transformation of T leads to inverse interpretation).

POPs exposure and E2, and AI

Figure 2 shows the associations between POP exposures and E2 and AI levels for male and female newborns combined and separately. We observed a significant increase in E2 with exposure to α-endosulfan and HCE but not with exposure to PCBs, even if a slight increase in E2 could be suggested for the highest categories of exposure. When we explored the associations with AI levels, significant decreases were observed for PCBs, HCE and α-endosulfan.

From the analysis stratified by newborn sex, we can note that the increases in E2 observed with exposure to HCE and α-endosulfan and the decrease in AI with exposure to HCE were specific to female newborns (see Fig. 2).

Sensitivity analyses

The first two sets of sensitivity analyses only marginally modified the results with a slight decrease in statistical power but no variation in the estimates (data not shown). When we excluded women with diabetes or high blood pressure, we additionally observed a significant increase in AMH with exposure to p,p′-DDE (data not shown). Finally, additional models that included PCB153, HCE, α-endosulfan and BDE209 were tested simultaneously. Conclusions were similar to those from the single-pollutant approach except for the association between PCB153 and AI which was no longer significant (see Supplementary Tables SIV and SV).

Table II Persistent organic pollutants levels measured in cord blood samples ($N = 282$).

Cord blood biomarkers	LOD	N (%) > LOD	Cord blood concentration (μg/l)				Lipid-adjusted concentration (ng/g lipid)[a]			
			Median	p75	p90	Max	Median	p75	p90	Max
PCB 153	0.01	282 (100.0)	0.110	0.150	0.210	0.730	28.06	44.84	70.00	331.82
PCB 187	0.01	206 (73.1)	0.013	0.019	0.026	0.200	3.02	4.92	8.97	90.91
Sum of PCB[b]	0.01	282 (100.0)	0.115	0.164	0.214	0.825	30.42	49.86	76.80	375.00
p-p′-DDE	0.02	229 (81.2)	0.185	0.300	0.480	12.000	44.41	83.23	155.56	1137.93
β HCH	0.01	279 (98.9)	0.041	0.062	0.088	0.260	11.27	17.94	30.00	108.33
Hexachlorobenzene	0.02	219 (77.7)	0.033	0.051	0.067	0.200	8.14	15.00	23.68	83.33
Heptachlor epoxide	0.01	142 (50.5)	0.010	0.010	0.020	0.040	3.33	4.76	7.41	12.50
Dieldrin	0.01	45 (16.0)	<LOD	<LOD	0.022	0.081	<LOD	<LOD	5.88	44.12
γ HCH	0.01	33 (11.7)	<LOD	<LOD	0.011	0.140	<LOD	<LOD	5.65	17.95
α endosulfan	0.01	16 (5.7)	<LOD	<LOD	<LOD	0.046	<LOD	<LOD	<LOD	21.90
BDE 209	0.05	84 (32.3)	<LOD	0.075	0.200	1.200	<LOD	17.31	56.14	537.50

HCH, hexachlorocyclohexane; LOD, limit of detection; BDE, brominated diphenyl ether; p-p′-DDE, p-p′-dichlorodiphenyldichloroethylene.
[a]1 missing concentration for heptachlor epoxide, 1 for dieldrin, γ HCH and α endosulfan, 22 for BDE209 and 6 for total lipids level.
[b]Sum of congeners 118, 138, 170. Concentrations below the LOD for PCB118 ($n = 6$) and PCB170 ($n = 14$) were replaced by LOD/2.

We chose not to include HCB in the model due to the high level of correlation with PCBs (see Supplementary Table SI) but if we replace PCB153 by HCB, associations between HCB and hormone levels were somewhat weaker to those observed in the single-pollutant approach (data not shown).

Discussion

The most consistent findings observed were that higher prenatal exposures to several POPs were linked to higher levels of SHBG and lower levels of fT and AI measured in the cord blood. Increases in AMH—measured among male newborns only—were also observed with exposure to PCBs and suggested, sensitivity analyses, with p,p′-DDE. Most of the associations were observed in both sexes, but some POPs were specifically associated with hormonal levels in one sex, such as PCB187, HCE and α-endosulfan. No association was found with exposure to β-HCH, dieldrin or γ-HCH.

To our knowledge, only one study has explored the associations between prenatal exposure to PCBs (sum of congeners 28, 52, 101, 138, 153 and 180 measured in maternal blood) and sex hormones (T and E2 measured in cord blood) from the Duisburg cohort in Germany (Cao *et al.*, 2008). Contrary to our results, they observed a reduction of T among girls and a reduction of E2 among boys with similar levels of PCB exposure as that in our study (Cao *et al.*, 2008).

One study evaluated the impact of prenatal exposures to PCB and p,p′-DDE and reproductive hormone levels in 14-year-old boys (Grandjean *et al.*, 2012). They observed that an increase in prenatal PCB and p,p′-DDE exposures was associated with an increase in SHBG levels, a decrease in fT and a suggestive decrease in T. These results are in accordance with ours for PCB exposure but not for p,p′-DDE.

Some studies have evaluated the impact of prenatal exposures to ED compounds other than POPs on the sex hormones of newborns. They have been mainly concerned with prenatal exposure to phthalates, for which animal studies have shown anti-androgenic effects (Fisher, 2004). Main *et al.* (2006) explored the correlations between six phthalate monoesters measured in breast milk and the reproductive hormone levels of boys at 3 months of age. They observed patterns of modifications in hormone levels that were similar to ours: increasing levels of two phthalate monoesters were associated with increased levels of SHBG (positive correlations) and decreased levels of fT (negative correlations), but no association with T was present (Main *et al.*, 2006). Lin *et al.* (2011) also explored the correlations between several phthalate metabolites measured in maternal urine samples (third trimester of pregnancy) and reproductive hormones measured in cord blood. Among girls, they reported negative correlations with fT and the fT:E2 ratio but no correlation with E2. No association was observed among boys (Lin *et al.*, 2011). More recently, a study from the Sapporo cohort in Japan reported a decrease in AI with prenatal exposure to di(2-ethylhexyl) phthalates (Araki *et al.*, 2014).

Compared with other European birth cohorts, the median level of POP exposures in the PELAGIE cohort was similar for PCB153 (PELAGIE = 110 ng/l; combined cohorts = 140 ng/l) but was among the lowest for p,p′-DDE (PELAGIE = 180 ng/l; combined cohorts = 528 ng/l) (Govarts *et al.*, 2012). This lower level of exposure to p,p′-DDE could explain the lack of concordance of our results with those observed in the Faroes cohort (Grandjean *et al.*, 2012).

In our analysis, we adjusted for potential confounding factors based on biological considerations and those previously reported in the literature,

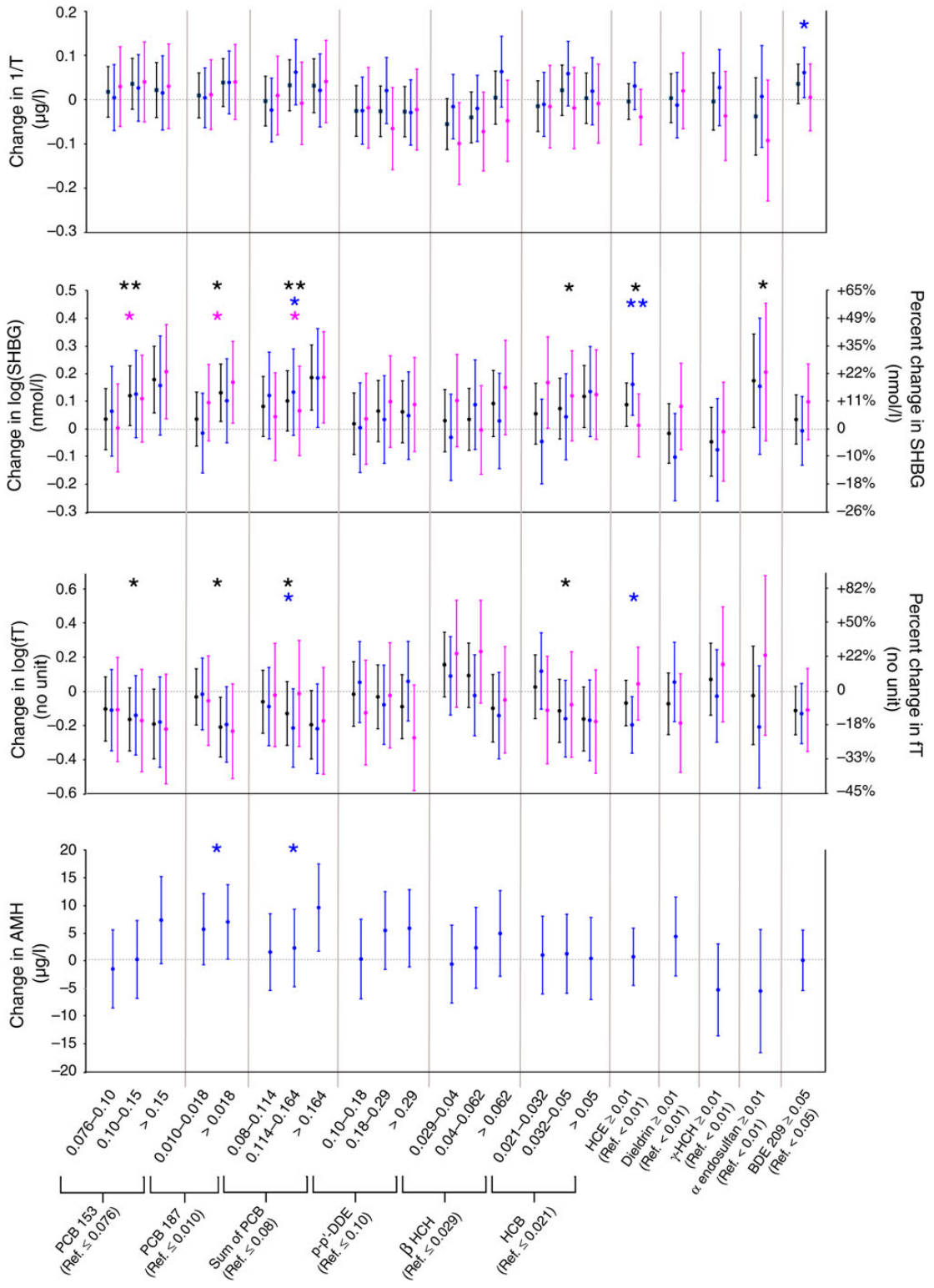

Figure 1 Estimate coefficient and 95% confidence interval between prenatal exposures to persistent organic pollutants (in µg/l) and testosterone (T), SHBG, free testosterone (fT), and AMH. Adjusted for gestational age (continuous), maternal age (continuous), total lipid level (continuous), parity (2 categories), and hemolysis level (3 categories). Additionally adjusted for body mass index (2 categories) for SHBG and fT. Both sexes in black ($n = 282$), boys in blue ($n = 141$), and girls in pink ($n = 141$). Levels of exposure were categorized according to the frequency of detection: in quartiles (>75% detected), in terciles (50–75% detected) or detected/undetected (<50% detected). DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCE, heptachlor epoxide; HCH, hexachlorocyclohexane; BDE, brominated diphenyl ether; PCB, polychlorinated biphenyl; *P-trend <0.05, **P-trend <0.01.

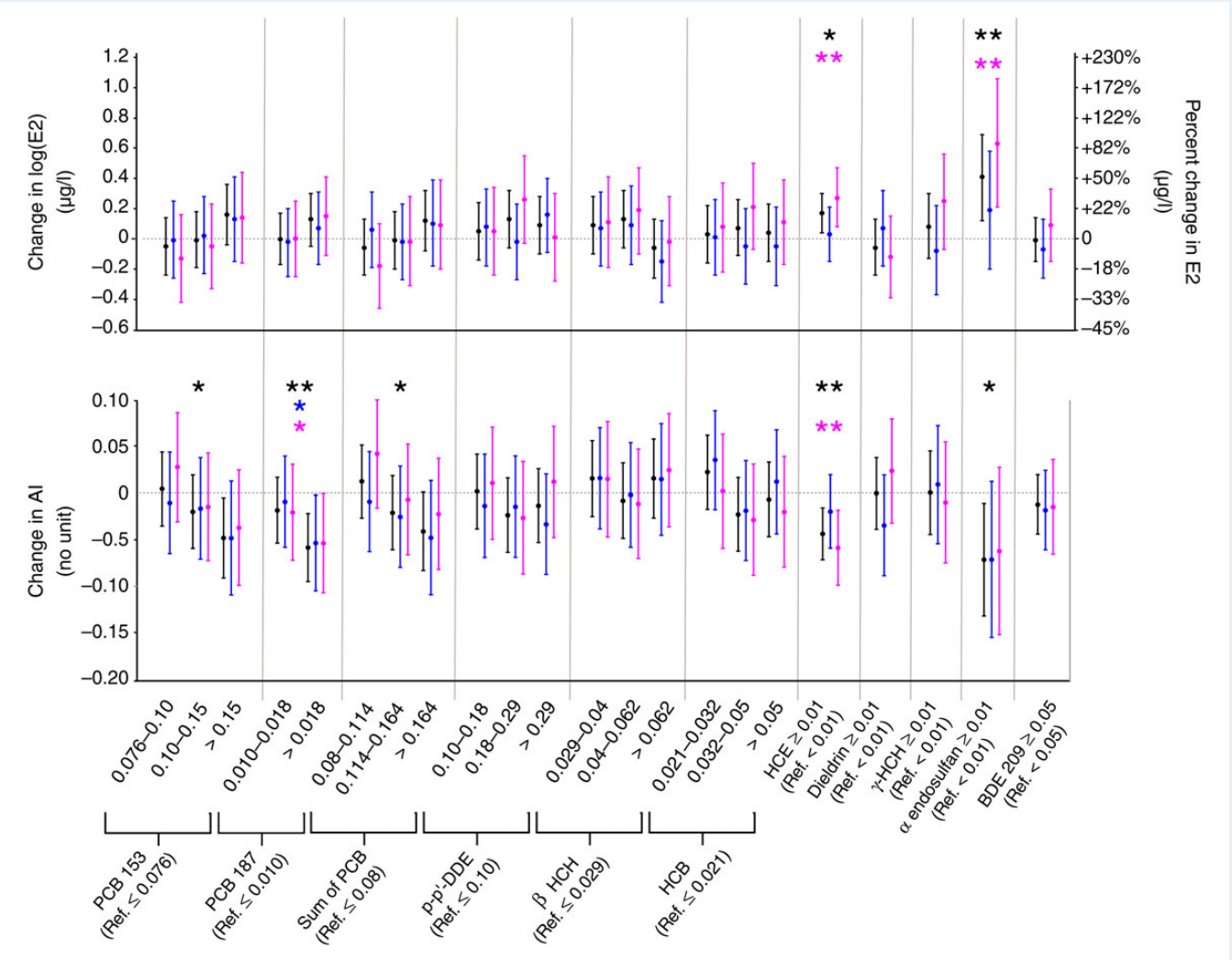

Figure 2 Estimate coefficient and 95% confidence interval between prenatal exposures to persistent organic pollutants (in µg/l) and estradiol (E2) and aromatase index (AI). Adjusted for gestational age (continuous), maternal age (continuous), total lipid level (continuous), parity (2 categories), and hemolysis level (3 categories). Both sexes in black ($n = 282$), boys in blue ($n = 141$), and girls in pink ($n = 141$). Levels of exposure were categorized according to the frequency of detection: in quartiles (>75% detected), in terciles (50–75% detected) or detected/undetected (<50% detected). DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCE, heptachlor epoxide; HCH, hexachlorocyclohexane; BDE, brominated diphenyl ether; PCB, polychlorinated biphenyl; *P-trend <0.05, **P-trend <0.01.

including gestational age, maternal age, parity and BMI (Troisi *et al.*, 2003; Keelan *et al.*, 2012; Thompson and Lampl, 2013; Hickey *et al.*, 2014). Variations in hormone levels with these selected factors were also observed from our sample. We chose not to adjust for birthweight in our first analysis because it could be on the causal pathway between POP exposures and steroid hormone levels. Finally, we did not take into account other potential confounding factors such as the type of labor or placental weight because of the large amount of missing data (>50%).

One weakness of our study is that the hormone measurements were performed at birth (cord blood) and may not adequately represent the infant endocrine system because the hypothalamic–pituitary–gonadal (HPG) axis is inhibited at the end of gestation, due to negative feedbacks controlled by placental hormones. After delivery, the HPG axis is reactivated until 6 months of age, with a peak of sex steroid concentrations at ~3 months of age, representing the 'mini-puberty' of infancy, and it is a recommended period for hormone measurements (Arbuckle *et al.*, 2008). The modifications in hormone levels observed in the cord blood can be interpreted as an alteration of the placental endocrine functions rather than an alteration in the HPG axis of the newborn (Hill *et al.*, 2014).

Despite the rather small sample size, the associations were robust because they were not altered in the sensitivity analysis, but we are aware that multiple testing could be a problem in our study and could lead to false positive discoveries. However, the goal of this study is more to provide clues to be confirmed in further studies rather than make definite conclusion. In this respect, and also because of the complexity of the data structure (i.e. high correlations, composite variables),

we made the decision not to control for multiple testing (Goeman and Solari, 2011).

E2 and T were measured with an immunoassay method, but the validity of the measurement could be questionable, particularly for T, because of possible cross-reacting substances (Hollier *et al.*, 2014). The comparison of the cord blood levels of T and E2 reported by several studies has shown lower concentrations of T with mass spectrometry than with an immunoassay, but the E2 levels did not differ dramatically between the two methods (Hollier *et al.*, 2014). The possible measurement error of the T levels could explain the lack of association with POP exposures in our study with T and may influence the associations with fT and AI.

We must acknowledge that the compounds α-endosulfan, HCE, γ-HCH, dieldrin, and BDE209 were rarely detected in our study and that interpretation of observations related to these compounds are only tentative. Circulating hormone levels are regulated by a number of cascade reactions and feedback loops and because of species-, tissues- and timing-specificity of endocrine signaling, it is difficult from our observational study to provide evidence about the mechanistic pathways involved in the observed associations. Nonetheless, the decrease in AI observed with several prenatal POP exposures and the increase in E2 observed among samples with detected values of α-endosulfan and HCE can be interpreted as the result of induction of the aromatase enzyme (in placental or fetal compartment). The variations in SHBG could be secondary to an increase in E2 levels (Rosner, 1990) or could reflect a direct effect of the compounds on SHBG synthesis by the fetal liver (potentially though the interaction with aryl hydrocarbon receptor) (Borlak *et al.*, 2002; Hammond, 2011). Finally, the increase in AMH levels with prenatal exposures to PCBs and, to a lesser extent, p,p′-DDE, and the decrease in T with BDE209, can be the consequence of an alteration of Sertoli and Leydig cells functions respectively (Josso *et al.*, 1993). All of these mechanistic hypotheses are only suggestive and we cannot exclude that other unmeasured hormones (steroids but also insulin, gonadotrophin or thyroid hormones) or binding proteins (albumin, transcortin) could also been involved in the observed associations.

Conclusion

Prenatal exposure to a number of POPs is associated with a disruption of hormone levels in cord blood with sex specificities. These findings may help understanding the pathways involved in disorders of human reproductive health associated with prenatal exposure to POPs. They support the necessity of a follow-up of puberty onset or adult reproductive functions in the PELAGIE cohort.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

C.W. contributed to statistical analyses, interpretation of data, and drafted the paper. A.D.-L. assisted in statistical analyses and interpretation of data. C.Ma. was responsible for hormone measurements. R.G. contributed to the interpretation of data. C.Mo. contributed to the initiation of the cohort study and acquisition of data. E.G. was responsible for pollutant measurements. N.B. contributed to the interpretation of results by evaluating potential biologic mechanisms. C.C. initiated the pollutant measurements and assisted in the paper drafting. S.C. initiated the cohort study, contributed to the interpretation of data and assisted in the paper drafting. All authors have revised the article critically and approved the final version.

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Conflict of interest

None declared.

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