Prenatal organochlorine pesticide exposure and the disruption of steroids and reproductive hormones in cord blood: The Hokkaido Study

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Abstract

Certain organochlorine pesticides (OCPs) are designated as persistent organic pollutants and are regulated in many countries. The effects of OCPs on pediatric endocrinology are a concern; however, only limited data exist from human studies on maternal OCP exposure and its effects on infants' hormone levels. This study was conducted as part of the Hokkaido Study Sapporo Cohort, a prospective birth cohort study in Japan. Participants included 514 women who enrolled at 23–35 weeks of gestation between 2002 and 2005; maternal blood samples were collected in late pregnancy, and 29 OCPs were measured. Reproductive and steroid hormone levels in cord blood were also determined. Characteristics of mothers and their infants were obtained from self-administered questionnaires and medical records. Ultimately, 232 samples with both OCP and hormone data were analyzed. Fifteen of 29 investigated OCPs were detected in over 80% of the samples, with *p,p'*-dichlorodiphenyldichloroethylene showing the highest concentration (median value: 619 pg/g-wet). The association between OCPs and sex hormone levels varied by sex. Linear regression models after sex stratification showed that chlordanes, cis-hexachlorobenzene, heptachlor epoxide, Mirex, and toxaphenes in maternal blood were inversely associated with testosterone, cortisol, cortisone, sex hormone-binding globin, prolactin, and androstenedione-dehydroepiandrosterone (DHEA) and testosterone-androstenediones ratios among boys. Furthermore, these OCPs were positively correlated with DHEA, follicle stimulating hormone (FSH), and adrenal androgen-glucocorticoid and FSH-inhibin B ratios among boys. In categorical quartile models, testosterone and DHEA were inversely and positively associated with OCPs, respectively. Estradiol-testosterone and adrenal androgen-glucocorticoid ratios tended to increase with increasing OCP concentrations in the higher quartile, while the testosterone-androstenedione ratio tended to decrease. Sex hormone-binding globulin and prolactin showed an inverse association with OCPs. Among girls, the linear regression model showed that only *p,p'*-dichlorodiphenyltrichloroethane was inversely associated with the level of DHEA and the adrenal androgen-glucocorticoid ratio, but was positively associated with cortisone levels. However, no associations were observed using the quartile categorical model. These results suggest that prenatal exposure to OCPs disrupt reproductive hormones of fetuses in utero among boys, even at relatively low levels.

Key Words: Organochlorine pesticides; reproductive hormones; steroidal hormones; prenatal exposure; cord blood; birth cohort

Abbreviations:

CI, confidence interval

CYP11A1, cytochrome P450 family 11 subfamily A member 1

CYP17A1, cytochrome P450 family 17 subfamily A member 1

CYP19A1, cytochrome P450 family 19 subfamily A member 1

DDD, dichlorodiphenyldichloroethane

DDE, dichlorodiphenyldichloroethylene

DDT, dichlorodiphenyltrichloroethane

HCB, hexachlorobenzene

HCE, heptachlor epoxide

HCH, hexachlorocyclohexane

HSD17B1, hydroxysteroid 17-beta dehydrogenase 1

HSD3B1, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1

IRMA, immunoradiometric assay

DHEA, dehydroepiandrosterone

EIA, enzyme immunoassay

ELISA, enzyme-linked immunosorbent assay

FSH, follicle stimulation hormone

INSL3, insulin-like factor 3

LC-MSMS, liquid chromatography-tandem mass spectrometry

LH, luteinizing hormone

LSM, least square mean

OCP, organochlorine pesticides

SHBG, sex hormone-binding globulin

StAR, steroidogenic acute regulatory protein

1. Introduction

Organochlorine pesticides (OCPs) are chlorinated hydrocarbons used extensively in the 1940s for agriculture and pesticide control, and are now designated as persistent organic pollutants by the Stockholm Convention (http://chm.pops.int). Although the Stockholm Convention has issued an exemption for the production and public health use of dichlorodiphenyltrichloroethane (DDT) to control vector-borne diseases, most OCPs were banned in the United States, Europe, and many other countries in the early 1970s (WHO, 2012). The use of OCPs has been eliminated or restricted in Japan since the 1970's (Kanazawa et al. 2012). Although most OCPs have been prohibited for over 30 years, they are still detected in the environment and in human populations. According to Japanese monitoring data, the levels of DDT and its metabolites in water and sediment have decreased since 1990 and have consistently remained low since 2000; however, they are still detectable (Ministry of Environment, Japan 2006). Heptachlor epoxide (HCE), hexachlorocyclohexane (HCH), Mirex, Parlar-26, and Parlar-50 are also above detectable levels in water and sediments, even though the latter three have never been used in Japan (Ministry of Environment, Japan 2006).

The endocrine disrupting properties of OCPs are considered a health concern. In previous cross-sectional studies among adults, heptachlor and *o,p'*-DDT concentrations were associated with lower testosterone levels in men (Freire et al. 2014). In women, hexachlorobenzene (HCB), *p,p'*-DDT, *p,p'*-dichlorodiphenyldichloroethane (DDD), endosulfan, aldrin, and Mirex showed inverse associations with luteinizing hormone (LH) and follicle stimulation hormone (FSH) while showing positive associations with prolactin (Freire et al. 2014).

Maternal exposure to OCPs may affect fetal hormone levels. Sex steroid hormones including testosterone, progesterone, and estradiol exert their functions predominantly in the gonads, and dehydroepiandrosterone (DHEA) and androstenedione are activated to form androgens and estrogens that have important roles in sex differentiation and maturation (Labrie et al. 2001). Cortisol and cortisone are synthesized within the adrenal cortex, are involved in a wide range of physiological processes, and are essential for regulating and/or modulating homeostasis in metabolism, growth, neurodevelopment, and the immune system (Braun et al. 2013; Reynolds 2010). LH and FSH play critical roles in the development and regulation of numerous body functions via the hypothalamic-pituitary-gonadal (HPG) axis (Kuiri-Hänninen et al. 2014). Inhibin B and insulin-like factor-3 (INSL3) are major products secreted by the Leydig and Sertoli cells, respectively, and the establishment of sufficient numbers of these cells is critical for the production of sperms in adulthood (Ivell et al. 2013; Orth and Boehm 1990). In response to gonadotropins, testosterone (via LH signaling) and inhibin B together act to regulate the secretion of FSH; these constitute the major negative feedback signals that maintain the physiological function of the HPG axis (Carlson, 2009). However, only limited data exist regarding human studies on prenatal exposure to OCPs and their effects on steroids and reproductive hormone levels in offspring. There is only one study in France that found that prenatal α-endosulfan and HCE increase estradiol and sex hormone-binding globulin (SHBG), whereas these same agents reduce testosterone levels at birth (Warembourg et al. 2016).

We have previously reported that 21 of 29 tested OCPs were detected in maternal blood acquired between 2002 and 2005 in Japan (Kanazawa et al. 2012). The impact of relatively low levels of OCP exposure on hormones at birth has still not been well-investigated in epidemiological studies. In particular, the effects of OCPs other than DDTs are rarely investigated. Thus, we hypothesized that prenatal exposure to even relatively low levels of these agents may alter hormone levels in infants. To that end, the aim of this study was to examine the associations between prenatal OCP exposure and cord blood steroid and reproductive hormone levels.

2. Methods

2.1 Participants

This investigation was based on the Sapporo Cohort of the Hokkaido Study on Environment and Children's Health. Details of this study, including the population, data collection, sampling of the biological specimens, and contents of the administered questionnaire, were described previously (Kishi et al. 2017; Kishi et al. 2013; Kishi et al. 2011). Briefly, Japanese pregnant women who lived in Sapporo City or surrounding areas were recruited at 23–35 weeks of gestation between July 2002 and October 2005 at an obstetrics and gynecology hospital in Sapporo, Hokkaido, Japan. Among the 1796 eligible women approached, 25% were excluded because they were enrolled in the Japanese Cord Blood Bank or planned to deliver at another hospital. Ultimately, 514 pregnant women (28.6% of those approached) were enrolled in this study.

2.2 OCP measurement

Maternal blood samples were obtained at the time of patients' hospital examinations following recruitment (n=296). If a blood sample could not be obtained during pregnancy because of maternal anemia, a sample was collected during post-partum hospitalization within a week after delivery (n=130). All samples were stored at −80°C until analysis. OCPs in whole blood were measured by gas chromatography/high-resolution mass spectrometry and gas chromatography/negative-ion chemical-ionization mass spectrometry at IDEA Consultants, Inc. (Shizuoka, Japan) The 29 OCPs evaluated in this study were 5 chlordanes (*cis*-chlordane, *trans*-chlordane, *ci*s-nonachlor, *trans*-nonachlor, and oxychlordane), 6 DDTs (*o,p'*-DDT, *p,p'*-DDT, *o,p'*- DDE, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD), 3 'drins' (aldrin, dieldrin, and endrin), 3 heptachlors (heptachlors, *cis*- HCE, and *trans*-HCE), HCB, 4 HCH isomers (α-HCH, β-HCH, γ-HCH, and δ-HCH), Mirex, and 6 toxaphenes (Parlar-26, Parlar-41, Parlar-40, Parlar-44, Parlar-50, and Parlar-62). Details of the measurement methods have been described previously (Kanazawa et al. 2012).

2.3 Measurement of steroids and reproductive hormones

The methods used to measure steroids and reproductive hormones were described previously (Araki et al. 2017; Araki et al. 2014; Goudarzi et al. 2016). Briefly, the concentrations of 7 steroid hormones including progesterone, estradiol, testosterone, DHEA, androstenedione, cortisol, and cortisone in cord blood were measured using liquid chromatography-tandem mass spectrometry (LC-MSMS) (Yamashita et al. 2007a; Yamashita et al. 2007b). An immunoradiometric assay (IRMA) was used to measure the concentrations of LH, FSH, and prolactin (Spac-S LH Kit, Spac-S FSH Kit, and Spac-Prolactin Kit, respectively, TFB, Inc., Tokyo Japan). SHBG was also measured using IRMA-Count SHBG (Siemens, Berlin, Germany). Concentrations of inhibin B were measured by using an enzyme-linked immunosorbent assay (ELISA) (Inhibin B Gen ELISA, Beckman Coulter, Inc., CA, USA), while INSL3 was measured by using an enzyme immunoassay (EIA) (INSL3/RLF [human] EIA kit, Phoenix

Pharmaceutical., Inc., CA, USA). All hormone measurements were conducted at Aska Pharma Medical Co., Ltd (Kanagawa, Japan).

2.4 Questionnaires and medical records

The participants completed a self-administered questionnaire that extracted information on maternal age, education level, household income, maternal smoking and alcohol consumption during the first trimester, and medical history. Information at the time of delivery, including pre-pregnancy body mass index, pregnancy complications, gestational age, infant sex, parity, congenital anomalies such as hypospadias and cryptorchidism, and infant size was obtained from medical records (Kishi et al. 2013; Kishi et al. 2011).

2.5 Statistical analyses

Of the 514 participants, 10 were excluded from the study owing to miscarriage, stillbirth, relocation, or voluntary withdrawal prior to delivery. Among 426 maternal blood samples, 379 were of sufficient quantity for OCP analysis, while hormone measurements were obtained from 295 infant cord blood samples. Ultimately, 232 matched maternal serum and cord blood samples (for OCP and hormone levels measurements, respectively) were included in the statistical analysis.

Associations between maternal OCP concentrations and infant steroid hormone levels were examined for each OCP separately via linear regression analysis. In each model, the OCP was the independent variable while the hormone was the dependent variable. Initially, linear regression models for both sexes combined were constructed with the interaction terms of sex × OCP levels added in each model; this revealed significant differences between the sexes. Next, the models were applied following stratification by sex. OCP levels and the concentrations of steroid hormones were converted to a $\log_{10}$ scale because they were not normally distributed. Two-sided *P*-values <0.05 were considered statistically significant. Selected OCPs with *P*-values <0.1 in linear regression models were then categorized into concentration quartiles to examine dose-response relationships. The interquartile range for each OCP concentration and the least squares means (LSM) of log-transformed hormone levels were calculated and back-transformed. To calculate a *P*-value for the trend, linear contrast coefficients of −3, −1, +1, and +3 were assigned to the first, second, third, and fourth quartiles, respectively (Goudarzi et al., 2016; Itoh et al., 2016). *P*-values for trend <0.05 were considered statistically significant. The OCP levels in the first quartile were also compared to those in the second, third, and fourth quartiles using the Dunnett-Hsu method; *P*-values were adjusted using Bonferroni's correction ($P<0.0167$). When below their detection limits, the half values of these detection limits were used for both OCPs and hormones.

In addition to single hormone levels, we also examined the product-to-substrate ratios of hormones that are adjacent in the metabolic pathway to determine their enzyme activity indices (Hicks et al. 2014). For example, the estradiol-testosterone ratio represents the index of the cytochrome P450 family 19 subfamily A member 1 (CYP19A1), better known as aromatase. The androstenedione-DHEA ratio represents the hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (HSD3B1) index; the testosterone-androstenedione ratio represents the hydroxysteroid 17-beta dehydrogenase 1 (HSD17B1) index; and the cortisone-cortisol ratio represents the HSD3B1 index. Increasing and decreasing ratios suggest the up- and downregulation of enzyme activity, respectively. Additionally, the adrenal androgen (the sum of DHEA and androstenedione)-glucocorticoid (sum of cortisol and cortisone) ratio was examined to determine the balance shift of adrenal androgen (C19-steroids) and glucocorticoid (C21-steroids) (Goudarzi et al. 2016). Similarly, testosterone-LH and FSH-inhibin B ratios were examined as indices of gonadal function. The inclusion of covariates was examined based on biological considerations, and included maternal age (continuous), parity (primipara or multipara), and gestational age (continuous). All statistical analyses were performed using the Japanese version of IBM SPSS Statistics 19 (IBM Analytics, NC, USA) and the Japanese version of JMP Pro 12 (SAS Institute Inc., NC, USA).

2.6 Ethical approval

This study was approved by the Institutional Ethical Board for Epidemiological Studies at Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences, in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

3. Results

Table 1 shows the characteristics of the participants included in this study as well as those of the original cohort. Compared to the original cohort, the mean birth weight and gestational age were slightly larger among the participants; the vaginal delivery rate among participants was 99.1%. One infant with cryptorchidism was included in the study.

The concentrations of OCP in maternal blood samples are shown in Table 2. There were 15 OCPs (3 chlordanes [*ci*s-nonachlor, *trans*-nonachlor, and oxychlordane], 5 DDTs [*o,p'*-DDT, *p,p'*-DDT, *o,p'*- DDE, *p,p'*-DDE, p,p*'*-DDD], dieldrin, *cis*-HCE, HCB, β-HCH, Mirex, and 2 toxaphenes [Parlar-26 and Parlar-50]) that were above 80% of their detection limits; these OCPs were subjected to further analysis. The median concentration of *p,p'*-DDE was the highest at 619.26 pg/g-wet, followed by β-HCH at 154.31 pg/g-wet, and HCH at 103.99 pg/g-wet. The distributions of OCPs in the original cohort are shown in Supplemental Table S1; *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor levels of participants in this study were slightly higher compared with the levels in those who were excluded (Mann-Whitney U test, P=0.06, 0.039, and 0.040, respectively; data not shown). Associations between OCP levels and maternal and child characteristics are shown in Supplemental Table S1. The levels of most OCPs increased with the ages of the mothers. OCP concentrations were not significantly associated with infant characteristics such as sex, birth weight, or gestational age.

The steroid and reproductive hormone levels in infants are shown in Table 3. Testosterone and DHEA levels were significantly higher and lower in boys than in girls, respectively. The detection rate of LH, FSH, and inhibin B was below 30% in girls, among whom only 16 samples were analyzed for INSL3. Therefore, no further tests of LH, FSH, inhibin B, and INSL3 were conducted for girls. The distribution of all measured hormone levels are shown in Supplemental Table S3; there were no differences between subjects included and excluded from the study.

The associations between OCPs and steroid and reproductive hormone levels for both sexes combined were examined (Supplemental Table S4). Sex-specific OCP interactions (P<0.05) between one or more hormones and cis-nonachlor, trans-nonachlor, *p,p'*-DDE, *o,p'*-DDT, dieldrin, and Mirex were observed, suggesting that the effects of OCPs differ according to sex.

Adjusted regression coefficients (β) and 95% confidence intervals (CIs) for the association between a 10-fold increase of OCP and $\log_{10}$-transformed hormone levels as determined by a linear regression model in boys and girls are shown in Tables 4 and 5, respectively. Among infant boys, Mirex was inversely associated with testosterone, and *p,p'*-DDE showed a positive association with the estradiol-testosterone ratio. Oxychlordane, *cis*-nonachlor, *trans*-nonachlor, dieldrin, *cis*-HCE, HCB, Mirex, Parlar-26, and Parlar-50 were positively associated with DHEA. Oxychlordane, *trans*-nonachlor, *cis*-HCE, and Mirex were inversely associated with the androstenedione-DHEA ratio. Oxychlordane, *cis*-nonachlor, *trans*-nonachlor, dieldrin, *cis*-HCE, HCB, Mirex, and Parlar-50 showed inverse associations with the testosterone-androstenedione ratio. Both *trans*-nonachlor and Mirex were inversely associated with cortisol and cortisone. Oxychlordane, *cis*-nonachlor, *trans*-nonachlor, *cis*-HCE, Mirex, and Parlar-50 were positively associated with the adrenal androgen-glucocorticoid ratio. β-HCH was inversely associated with SHBG, and β-HCH, Mirex, Parlar-26, and Parlar-50 were positively associated with FSH. *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDT, *p,p*'-DDT, dieldrin, β-HCH, Mirex, and Parlar-50 were all inversely associated with prolactin. Finally, *cis*-nonachlor, *p,p*'-DDE, *p,p*'-DDT, *cis*-HCE, HCB, β-HCH, Mirex, Parlar-26, and Parlar-50 were inversely associated with the FSH-inhibin B ratio. There was no statistically significant association between OCPs and progesterone, estradiol, androstenedione, FSH, inhibin B, or INSL3. Among girls, *p,p*'-DDD was inversely associated with DHEA and the adrenal androgen-glucocorticoid ratio, and was positively associated with cortisone. There was no association between OCPs and progesterone, estradiol, testosterone, androstenedione, cortisol, SHBG, or prolactin among girls.

Figure 1 shows the relationships between hormones and OCPs in quartile models for boys (only in cases where $P<0.05$ was observed). Testosterone showed a decreasing trend in relation to quartiles of Mirex, while LSM analysis of the estradiol-testosterone ratio showed an increasing trend of *p,p'*-DDE in relation to quartiles. DHEA showed increasing trends in relation to the quartiles of *cis*-Nonachlor, Dieldrin, Parlar-26, and Parlar-50. Moreover, the LSM method showed that the 4th quartile of *cis*-nonachlor was significantly increased compared to the 1st quartile *cis*-nonachlor. LSM analysis of the testosterone-androstenedione ratio showed decreasing trends in relation to *cis*-nonachlor, *trans*-nonachlor, Dieldrin, Mirex, and Parlar-50. Moreover, LSM analysis of the adrenal androgen-corticoid ratio showed an increasing trend of *cis*-nonachlor, Parlar-26, and Parlar-50, while SHBG showed a decreasing trend of β-HCH. Prolactin showed decreased trends of *p,p*'-DDE and *o,p*'-DDT. Statistically significant relationships between hormones and OCPs in the quartile models were not found among girls.

4. Discussion

In linear models, we found that relatively low levels of OCPs were inversely associated with testosterone, cortisol, cortisone, SHBG, and prolactin, but positively associated with DHEA in newborn boys after stratification by sex. Positive associations between OCPs and each of estradiol-testosterone and adrenal androgen-glucocorticoid, as well as an inverse association between OCPs and each of androstenedione-DHEA, testosterone-androstenedione, corticoid-cortisone, and the FSH-inhibin B ratio, were also observed among boys. In quartile models, testosterone was inversely associated with OCPs, whereas DHEA was positively associated with them. The estradiol-testosterone and adrenal androgen-glucocorticoid ratios tended to increase when OCP concentrations were the higher quartile, while the testosterone-androstenedione ratio tended to decrease. SHBG and prolactin showed inverse associations with OCPs. Among girls, *p,p'*-DDD was inversely associated with DHEA levels and positively associated with levels of cortisol; it was also inversely associated with the glucocorticoid-adrenal androgen ratio. However, these associations were not observed in the quartile models, which would have provided more credence to the findings of the linear models. Overall, our data suggested that the natures of the associations between OCP and hormones differ according to sex, and that clear associations were observed only among infant boys.

The levels of OCPs in maternal blood have been measured in several studies performed in various countries. In this study, the median *p,p*'-DDE value in maternal whole blood was 619.26 pg/g-wet. In a study in Chiapas, Mexico, the median values of DDT and DDE in maternal serum collected in 2002–2003 were 1.9 and 19.5 µg/L, respectively (Longnecker et al. 2007). A study of Mexican-Americans in the US state of California found that the maternal geometric mean values of *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDE were 22.0, 1.8, and 1436.9 ng/g-lipid, respectively (Eskenazi et al. 2006). A study in China showed geometric means of *p,p*'-DDE, HCB, and β-HCH in maternal serum, at 203.54 ng/g, 70.62 ng/g, and 67.67 ng/g, respectively (Guo et al. 2014). In another recent study in South Africa, the median maternal *p,p*'-DDE level was 241.3 ng/g lipids (Bornman et al. 2016). A meta-analysis of 12 European cohorts found that the median cord serum *p,p'*-DDE concentration was 527.9 ng/L (ranging from 49.8

ng/L to 1208 ng/L in various studies) (Govarts et al. 2012). By using Govarts et al.'s conversion factor to calculate cord serum levels from maternal whole blood levels in our study (cord serum level = 0.36 × maternal whole blood level), the estimated median level of *p,p*'-DDE in the cord serum of our study was 229.1 ng/L, which is approximately half the median value of European cohorts. Taken together, the OCP exposure levels in our cohort were relatively low in comparison.

Only 1 study, performed in France, examined prenatal OCP exposure and hormone levels at birth (Warembourg et al. 2016). The investigators found that higher levels of HCB and HCE were associated with reduced levels of testosterone and elevated levels of SHBG among boys. HCE was also associated with higher levels of estradiol and a lower testosterone-estradiol ratio among girls. There was no association between *p,p*'-DDE and any hormone levels. Their results were partly consistent with ours. In our study, OCPs including HCB and HCE showed inverse associations with testosterone; these were not significant except for Mirex among boys. Although we did not find any association between estradiol and OCPs, their levels tracked together in a manner also observed by Warembourg et al. (2016). Moreover, the positive association between the estradiol-testosterone ratio and OCPs, which was statistically significant for *p,p'*-DDE in this study, are consistent with the findings of Warembourg et al. (2016), as is the significant decrease in the aromatase index (testosterone/estradiol) with increasing HCE. On the other hand, SHBG had a significant inverse correlation with Mirex in our study; which was in direct contrast to Warembourg et al.'s findings; we are unable to explain this discrepancy, as the sampling period (2002–2006) and sample sizes (n=282 for their study and n=232 for ours) were comparable. Additionally, the exposure levels of DDE, HCE, and HCB in our study and in that of Warembourg et al. were comparable. However, the detection rates of HCB and HCE were higher in our study, and Warembourg et al. did not measure chlordanes, Mirex, or toxaphenes. Because of the low detection percentage, Warembourg et al. divided OCP levels into two or three categories to examine the association of each with hormones; this could explain why their results do not match ours more closely. We found no other studies that investigated associations between OCPs and DHEA, androstenedione, cortisol, or cortisone. More studies are therefore warranted to ascertain the association between OCPs and steroidogenesis at birth.

In this study, DDT, DDE, and DDD were not associated with steroid hormones except for *p,p*'-DDE, which showed a positive association with the estradiol-testosterone ratio in boys. The increased estradiol-testosterone ratio suggested increased CYP19A1 enzyme activity. A previous animal study showed that *p,p'*-DDE induces CYP19A1 in hepatic microsomal samples of adult male rats (You et al. 2001), which is consistent with our results. In girls, *p,p*'-DDD was inversely associated with DHEA and the adrenal androgen-glucocorticoid ratio, but was positively associated with cortisone levels. However, these associations were not observed in the quartile models; therefore, false positive associations are likely. Longnecker et al. (2007) conducted a study in Mexico where maternal DDT levels are relatively high, and found no evidence that in utero exposure to DDE was related to anogenital distance or penile dimensions at birth among male infants. Additionally, Bornman et al. (2016) conducted a similar study in South Africa and reported no associations between *p,p′*-DDT/-DDE or *o,p′*-DDT and anogenital distance measurements at birth in either boys or girls. Yet another study in Denmark found no association between prenatal *p,p*-DDE levels and reproductive hormones at approximately 20 years of age (Vested et al. 2014). Taken together, these data indicate that DDTs may not alter infant steroid hormones at the levels detected in these studies.

Among boys, *p,p*'-DDE and *o,p*'-DDT were inversely associated with prolactin in quartile models. Although there have been no specific studies of the mechanisms of OCP influence on prolactin, other investigations suggest that newborns with lower levels of prolactin in their cord blood are more likely to have an increased risk of respiratory distress syndrome than those with higher levels of prolactin (Parker et al. 1989; Padvi et al. 2017). Combined with data from the Canadian Health Measures Survey that DDT exposure is inversely associated with lung function parameters in adults (Ye et al. 2015), long term follow-up of children who were exposed to these chemicals is needed to clarify the health-related consequences of such exposure.

Studies of OCPs other than DDTs are scarce. We found that *cis/trans*-nonachlor, Dieldrin, Mirex, and toxaphenes (Parlar-26 and 50) were positively associated with DHEA as well as with the adrenal androgen-glucocorticoid ratio, but were inversely associated with testosterone, the testosterone-androstenedione ratio, and prolactin in boys. Decreasing testosterone-androstenedione ratio trends suggests the downregulation of HSB17B1; previous animal studies have shown that aldrin inhibits HSB17B1 (Chatterjee et al. 1988), which is consistent with our findings. DHEA is the main precursor of sex hormones and cortisol antagonists (Mastorakos and Ilias 2003). The increasing adrenal androgen-glucocorticoid ratio suggests that chlordanes, Dieldrin, Mirex, and toxaphenes may shift steroidogenesis towards androgenic hormones (C19-steroids), although we observed no evidence of this. However, circulating hormones are regulated by several cascade reactions. Translocation of cholesterol from the outer mitochondrial membrane to the inner membranes is a critical step in steroidogenesis;

this process is enhanced by steroidogenic acute regulatory protein (StAR), which is encoded by *STARD1*. Other key human steroidogenic genes are *CYP11A1*, *CYP17A1*, *CYP11B1*/*CYP11B2*, *CYP21A2*, *SRD5A1*, and *SRD5A2*. Moreover, *SULT2A1* and *SULT2B1* encode enzymes that convert DHEA to DHEA-sulfate and vice versa. Although we have not measured methoxychlor, another synthetic organochlorine insecticide and a derivative of DDT showed decreased expression of CYP19A1, HSD17B1, *CYP17A1*-encoded cytochrome P450 family 17 subfamily A member 1, HSD3B1, *CYP11A1*-encoded cytochrome P450 family 11 subfamily A member 1, and StAR, but increased expression of *CYP1B1*-encoded cytochrome P450 family 1 subfamily B member 1 enzyme levels, *in vitro* (Basavarajappa et al. 2011; Vaithinathan et al. 2008). Therefore, our overall findings do not rule out that many of the OCPs measured in this study may alter steroid hormones by inhibiting such steroidogenesis enzymes. Moreover, a previous study notably examined 15 organochlorines (OCs) mixture similar to those found in the bladders of Arctic ringed seals. Exposing male rats *in utero* to this OC mixture caused disruptions in the development of androgen-dependent organs such as the testis, epididymis, seminal vesicle, and prostate (Anas et al. 2005). They also investigated that OC mixture's direct inhibition of Leydig cell steroidogenesis by disrupting cholesterol transport into the mitochondria via decreasing StAR protein levels, and by converting cholesterol into pregnenolone by modulating adrenodoxin reductase and CYP11A1 proteins (Enangue Njembele et al. 2014). We speculate that different OCPs influence steroidogenesis similarly. Additional studies on altered steroidogenic and metabolic enzymes with different OCPs are required to test these hypotheses.

In our linear regression model, HCH, Mirex, and toxaphenes were positively associated with FSH, as was the FSH-inhibin B ratio. Sertoli cells secrete inhibin B, which was first identified by its ability to negatively regulate FSH (Carlson et al. 2009). Increasing levels of FSH together with the FSH-inhibin B ratio point to a negative feedback mechanism as well as the influence of OCPs on Sertoli cells. However, these associations were not statistically significant in our quartile model; hence, the effects of OCPs on Sertoli cells remain unclear.

Our study found a significant association between OCPs and hormones only among boys. Although the reason for this remains unclear, one possible explanation for this sex preference is that most OCPs have an affinity for hormone receptors including androgen receptor, estrogen receptor, and/or aryl hydrocarbon receptor (Mnif et al. 2011; Kojima et al., 2004). Thus, OCPs may mimic or block the natural hormone's action (as an agonist or antagonist, respectively), and can interfere with the synthesis, transport, metabolism, and elimination of hormones (Mnif et al. 2011). Estrogen and androgen receptors are primarily involved in sexual differentiation and reproduction (Busillo et al. 2009). While estrogens and progestins are essential for normal female development, androgens are involved in various aspects of male reproductive physiology. One animal study showed that *p,p*'-DDE inhibits androgen binding to the AR, androgen-induced transcriptional activity, and androgen action in developing (Kelce W.R., et al., 1995). Androgen receptors are expressed in variety of tissues, but their levels differed in human fetal tissues (Wilson and McPhaul, 1996). During fetal genital development, the female phenotype is considered to be the baseline (or default) condition, and the development of maleness requires additional secretions produced by the testis (Carlson 2009). Thus, we speculate that the effect of anti-androgenic activities of OCPs on males is more severe than the effect of their estrogenic activities on females. The physiological differences in the roles of these hormones may explain the observation of sex differences in OCP-hormone associations. One epidemiological study in Mexico found that higher exposure to *p,p'*-DDE *in utero* shortened the anal position index among boys but not among girls (Torres-Sanchez et al., 2008). The authors explained that this was due to the putative androgen deficiency occurring due to the reduction of transcriptional activity that occurs when AR is blocked by *p'p*-DDE *in utero*. Although the exposure levels in Torres-Sanchez et al.'s study were 10 times higher than that in ours, our results were in line with those of their study. In vitro reporter gene assays showed that not only *p,p'*-DDE, but also other DDTs, dieldrin, and heptachlors showed AR antagonist effects (Kojima et al., 2004). Hence, we assume that those OCPs studied herein with AR antagonist properties share similar modes of action in terms of male-specific responses.

The strength of this study is that we examined a wide range of OCPs. Investigating seven steroids enabled us to construct a clearer picture of the association between steroidogenesis and OCPs levels. Measuring steroid hormones by LC-MSMS is considered more accurate than other methods such as radioimmunoassays, which is another strength of this study. However, there are several limitations as well. First, the 15 OCPs are moderately-to-highly correlated with each other (Spearman's rho: 0.230–0.918, P<0.001). Thus, we were unable to clarify the effect of individual OCPs on hormones. Moreover, there may be other residual confounding factors. Previous studies suggested that reproductive hormones in cord blood may be affected by factors such as diurnal variation, duration of labor, placental weight, and the presence of pre-eclampsia (Hollier et al. 2014; Keelan et al. 2012). A recent cross-sectional study among adults suggested an association between OCP concentrations and total cholesterol levels (Arrebola et al. 2014), which may also modify steroid hormones levels. Second, multiple tests of 15 OCPs and 13 hormones may have found statistically significant associations by chance. However, the associations between OCPs and hormones exhibited consistent trends, which suggest that the results are robust and that OCP exposure is likely to alter infants' hormones in utero. Finally, there is a possibility of selection bias in this study, as only participants with available cord blood samples were included in the analysis. Because the life and the safety of the mother and child were of utmost priority, cord blood samples were seldom acquired during Caesarian sections. Thus, two infants were delivered Caesarian sections and others included in this study were delivered vaginally; they had longer gestational ages and heavier birth weights than the infants who were excluded, which indicated that the analysis was biased towards healthier infants. Therefore, the effects of OCPs may have been underestimated in this study.

5. Conclusion

We found that exposure to relatively low levels of OCPs such as *cis/trans*-nonachlor, *p,p'*-DDE, *o,p'*-DDT, Dieldrin, β-HCH, Mirex, and toxaphenes *in utero* was significantly associated with levels of hormones and their ratios in male fetuses. These results suggest that OCPs that include but are not limited to DDTs ought to be examined, as the existing data are limited. Disrupting the balance of steroid hormones may cause adverse effects on reproductive growth, development, and other health outcomes in later life. The clinical significance of these findings is unclear at present, as it remains unknown whether these small hormonal alterations are of any future health consequences in these individuals. Therefore, further studies investigating the long-term effects of OCP exposure are required.

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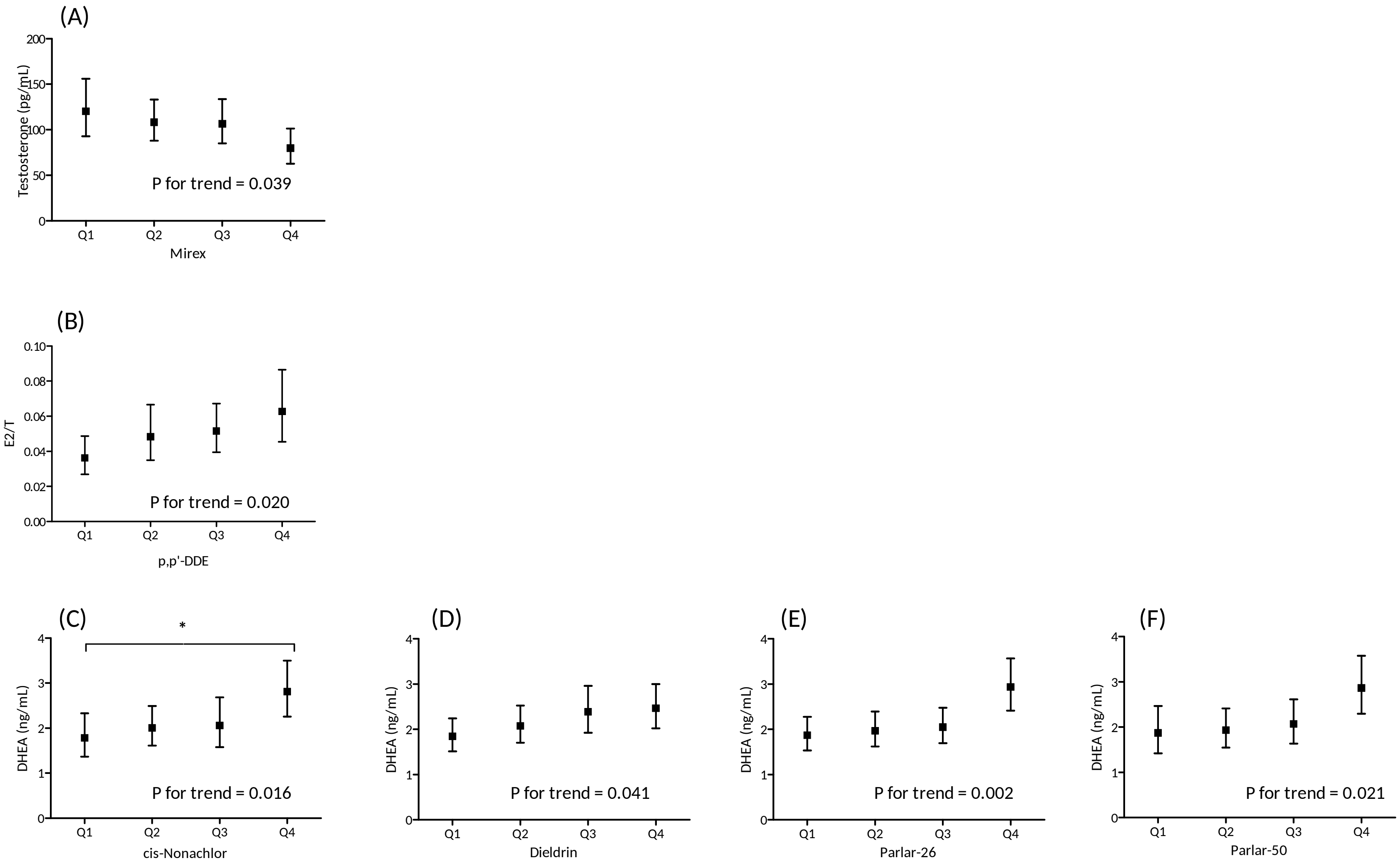
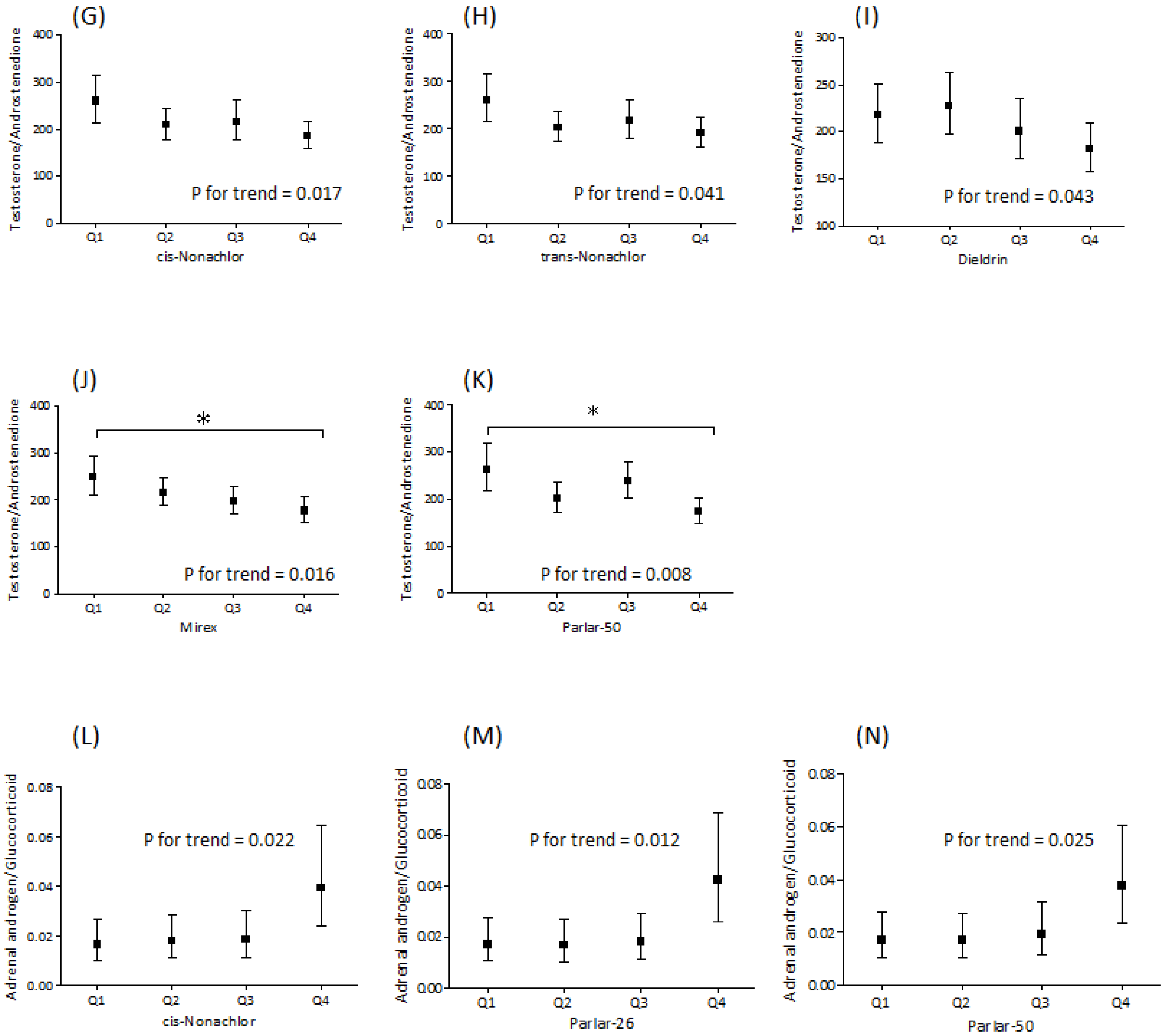
Figure legends

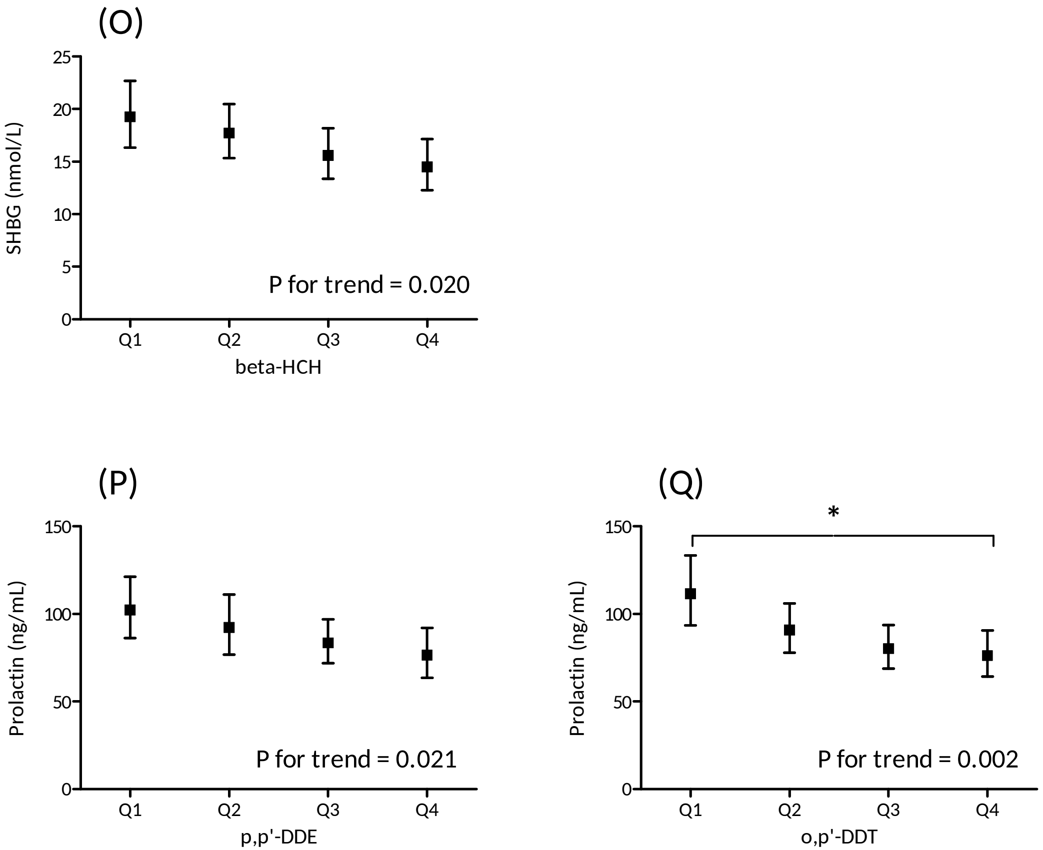
Fig. 1. Least square means (LSMs) of hormone levels according to maternal organochlorine pesticide (OCP) concentration quartiles in boys. The X-axes show the OCP quartiles, while the Y-axes show each hormone level calculated using the LSM in the boxes; the error bars are the 95% confidence intervals. The four OCP categories were, *cis*-nonachlor: Quartile 1 (Q1) (≤7.07 pg/g-wet), Q2 (7.08–10.37 pg/g-wet), Q3 (10.38–15.06 pg/g-wet), and Q4 (≥15.07 pg/g-wet); *trans*-nonachlor: Q1 (≤52.25 pg/g-wet), Q2 (52.26–75.60 pg/g-wet), Q3 (52.26–75.60 pg/g-wet), and Q4 (≥110.29 pg/g-wet); *p,p'*-DDE: Q1 (≤0.99 pg/g-wet), Q2 (1.00–1.65 pg/g-wet), Q3 (1.66–2.54 pg/g-wet), and Q4 (≥2.55 pg/g-wet); *o,p'*-DDT: Q1 (≤2.28 pg/g-wet), Q2 (2.29–3.36 pg/g-wet), Q3 (3.37–4.66 pg/g-wet), and Q4 (≥4.67 pg/g-wet); Dieldrin: Q1 (≤12.17 pg/g-wet), Q2 (12.17–16.68 pg/g-wet), Q3 (16.69–22.05 pg/g-wet), and Q4 (≥22.06 pg/g-wet); β-hexachlorocyclohexane (β-HCH): Q1 (≤104.33 pg/g-wet), Q2 (104.34–154.31 pg/g-wet), Q3 (154.32–238.06 pg/g-wet), and Q4 (≥238.07 pg/g-wet); Mirex: Q1 (≤4.12 pg/g-wet), Q2 (4.13–6.04 pg/g-wet), Q3 (6.05–8.52 pg/g-wet), and Q4 (≥8.53 pg/g-wet); Parlar-26: Q1 (≤2.84 pg/g-wet), Q2 (2.85–4.46 pg/g-wet), Q3 (4.47–7.11 pg/g-wet), and Q4 (≥7.12 pg/g-wet); and Parlar-50: Q1 (≤4.31 pg/g-wet), Q2 (4.31–6.56 pg/g-wet), Q3 (6.56–9.83 pg/g-wet), and Q4 (≥9.83 pg/g-wet). (A) Testosterone according to Mirex, (B) Estradiol-testosterone ratio (E2/T) according to *p,p*'-dichlorodiphenyldichloroethylene (DDE), (C) dehydroepiandrosterone (DHEA) according to cis-nonachlor, (D) DHEA according to Dieldrin, (E) DHEA according to Parlar-26, (F) DHEA according to Parlar-50, (G) Testosterone-androstenedione ratio (T/Adione) according to *cis*-Nonachlor, (H) T/Adione according to *trans*-Nonachlor, (I)

T/Adione according to Dieldrin, (J) T/Adione according to Mirex, (K) T/Adione according to Parlar-50, (L) Adrenal androgen-glucocorticoid ratio according to *cis*-Nonachlor, (M) Adrenal androgen-glucocorticoid ratio according to Parlar-26, (N) Adrenal androgen-glucocorticoid-ratio according to Parlar-50, (O) sex hormone-binding globulin (SHBG) according to β-HCH, (P) Prolactin according to *p,p*'-DDE, (Q) Prolactin according to *o,p'*-DDT. The first quartile is compared to the second, third, and fourth quartile OCPs as calculated using the Dunnett-Hsu method; the statistical significance of the *P* value was *$P<0.017$ based on Bonferroni's correction. LSMs were adjusted for maternal age, parity, and gestational age.


(A)
Testosterone (pg/mL)
0
50
100
150
200
Q1
Q2
Q3
Q4
Mirex
P for trend = 0.039
(B)
E2/T
0.00
0.02
0.04
0.06
0.08
0.10
Q1
Q2
Q3
Q4
p,p'-DDE
P for trend = 0.020
(C)
*
DHEA (ng/mL)
0
1
2
3
4
Q1
Q2
Q3
Q4
cis-Nonachlor
P for trend = 0.016
(D)
DHEA (ng/mL)
Q1
Q2
Q3
Q4
Dieldrin
P for trend = 0.041
(E)
DHEA (ng/mL)
Q1
Q2
Q3
Q4
Parlar-26
P for trend = 0.002
(F)
DHEA (ng/mL)
Q1
Q2
Q3
Q4
Parlar-50
P for trend = 0.021



(G)
Testosterone/Androstenedione
P for trend = 0.017
Q1
Q2
Q3
Q4
cis-Nonachlor
(H)
Testosterone/Androstenedione
P for trend = 0.041
Q1
Q2
Q3
Q4
trans-Nonachlor
(I)
Testosterone/Androstenedione
P for trend = 0.043
Q1
Q2
Q3
Q4
Dieldrin
(J)
Testosterone/Androstenedione
*
P for trend = 0.016
Q1
Q2
Q3
Q4
Mirex
(K)
Testosterone/Androstenedione
*
P for trend = 0.008
Q1
Q2
Q3
Q4
Parlar-50
(L)
Adrenal androgen/Glucocorticoid
P for trend = 0.022
Q1
Q2
Q3
Q4
cis-Nonachlor
(M)
Adrenal androgen/Glucocorticoid
P for trend = 0.012
Q1
Q2
Q3
Q4
Parlar-26
(N)
Adrenal androgen/Glucocorticoid
P for trend = 0.025
Q1
Q2
Q3
Q4
Parlar-50



(O)
SHBG (nmol/L)
25
20
15
10
5
0
P for trend = 0.020
Q1
Q2
Q3
Q4
beta-HCH
(P)
Prolactin (ng/mL)
150
100
50
0
P for trend = 0.021
Q1
Q2
Q3
Q4
p,p'-DDE
(Q)
*
Prolactin (ng/mL)
150
100
50
0
P for trend = 0.002
Q1
Q2
Q3
Q4
o,p'-DDT

Fig. 1.

Table 1 Maternal and infant characteristics

		This study (n=232)			Original cohort (n=514)		
		No.	%	Mean ± SD	No.	%	Mean ± SD
Mother							
Age at delivery (years)		232		30.45 ± 4.81	510		30.4 ± 4.9
Pre-pregnancy BMI (kg/m^2)		232		21.03 ± 2.92	506		21.2 ± 3.2
Educational level (years)	≤12	100	43.1		225	44.3	
	>12	132	56.9		283	55.7	
Annual Household income	<5	166	71.6		345	68.3	
(million yen per year)	≥5	66	28.4		160	31.7	
Smoking during pregnancy	No	190	81.9		404	79.7	
	Yes	42	18.1		103	20.3	
Alcohol consumption during pregnancy	No	154	66.4		351	69.1	
	Yes	78	33.6		157	30.9	
Parity	Primiparous	120	51.7		240	47.7	
	Multiparous	112	48.3		263	52.2	
Type of delivery	Vaginal	230	99.1		397	78.8	
	Caesarian section	2	0.9		107	21.2	
Blood sampling timing	During pregnancy	159	68.5		296	69.5	
	After delivery	73	31.5		130	30.5	
Infant							
Sex	Boys	106	45.7		246	48.1	
	Girls	126	54.3		265	51.9	
Birth weight (grams)		232		3130.5 ± 332.5	511		3025.6 ± 420.7
Gestational Age (weeks)		232		39.3 ± 1.1	511		38.9 ± 1.5
cryptorchidism		1	0.9		1	0.4	
hypospadias		0	0.0		0	0.0	

Table 2 Concentrations of organochlorine pesticides in maternal blood

Persistent organochlorine pesticides	Detection limit (pg/g-wet)	>DL (%)	Minimum	Percentile			Maximum
				25th	50th	75th	
Aldrin	1.00	0.4				<DL	12.83
Chlordanes							
oxychlordane	0.90	100.0	7.93	28.87	40.04	57.32	250.94
cis-Chlordane	0.70	62.1		<DL	1.15	2.29	17.53
trans-Chlordane	0.50	49.6			<DL	0.84	3.79
cis-Nonachlor	0.40	100.0	1.63	7.07	10.37	15.07	37.58
trans-Nonachlor	0.50	100.0	13.45	52.09	75.60	110.54	513.52
DDTs							
o,p'-DDD	0.50	14.2				<DL	1.16
p,p'-DDD	0.40	88.8	<DL	0.98	1.65	2.54	9.04
o,p'-DDE	0.40	86.6	<DL	0.72	1.25	1.78	4.60
p,p'-DDE	0.60	100.0	99.52	409.79	619.26	968.05	2686.23
o,p'-DDT	0.60	96.6	<DL	2.28	3.36	4.67	17.15
p,p'-DDT	0.40	100.0	2.38	16.22	23.17	33.94	104.76
Dieldrin	0.80	100.0	4.11	12.16	16.68	22.21	71.52
Endrin	1.00	0.0					<DL
Heptachlors							
Heptachlor	0.80	0.9				<DL	1.14
trans-HCE	1.00	0.0					<DL
cis-HCE	0.40	100.0	6.17	18.81	26.25	37.45	200.53
HCB	0.90	100.0	34.94	83.04	103.99	131.61	245.48
HCHs							
α-HCH	0.70	68.5		<DL	0.91	1.31	3.10
β-HCH	0.60	100.0	19.95	104.25	154.31	238.45	717.67
γ-HCH	0.90	57.3		<DL	1.09	1.73	100.92
δ-HCH	0.70	1.3				<DL	1.11
Mirex	0.50	100.0	0.88	4.11	6.04	8.53	30.11
Toxaphenes							
Parlar-26	1.00	97.0	<DL	2.84	4.46	7.13	20.82
Parlar-41	0.70	28.4			<DL	0.73	1.96
Parlar-40	2.00	0.9				<DL	2.43
Parlar-44	2.00	2.2				<DL	2.77
Parlar-50	2.00	96.1	<DL	4.30	6.56	9.86	29.29
Parlar-62	6.00	0.0					<DL

DL, detection limit; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HCE, heptachlor epoxide; HCH, hexachlorocyclohexane.

Table 3 Distribution of steroids and reproductive hormones

	DL	n	>DL (%)	Total (n=232) 25%	Total Med	Total 75%	Boys (n=106) n	Boys >DL (%)	Boys 25%	Boys Med	Boys 75%	Girls (n=126) n	Girls >DL (%)	Girls 25%	Girls Med	Girls 75%	P-value
Steroid hormones																	
Progesterone (ng/mL)	0.01	232	100.0	177.381	219.036	278.019	106	100.0	184.087	224.121	280.633	126	100.0	169.619	216.065	276.129	0.460
Testosterone (pg/mL)	0.01	232	100.0	59.750	82.050	114.025	106	100.0	76.788	101.875	132.050	126	100.0	50.975	68.675	92.400	<0.001
Estradiol (ng/mL)	0.01	232	99.6	3.220	4.546	7.053	106	99.1	3.248	4.458	7.311	126	100.0	3.140	4.645	6.672	0.393
DHEA (ng/mL)	0.01	232	100.0	1.763	2.160	2.970	106	100.0	1.590	2.090	2.830	126	100.0	1.898	2.270	3.140	0.040
Androstenedione (ng/mL)	0.01	232	99.6	0.360	0.460	0.588	106	99.1	0.380	0.470	0.613	126	100.0	0.350	0.450	0.580	0.414
Cortisol (ng/mL)	0.25	232	98.7	22.808	41.485	65.258	106	99.6	22.415	41.065	66.213	126	98.4	24.725	42.455	63.953	0.773
Cortisone (ng/mL)	0.10	232	96.1	76.393	95.145	124.655	106	98.1	72.825	94.175	122.963	126	94.4	77.150	95.650	125.123	0.807
Steroid Hormone Binding Globulin (nmol/L)	1.10	232	99.6	13.500	15.700	18.875	106	100.0	13.575	16.200	19.300	126	99.2	13.300	15.450	18.425	0.239
Lutheling Hormone (mIU/mL)	0.50	226	17.3			<DL	103	36.9		<DL	0.870	123	0.8			<DL	<0.001
Follicle Stimulating Hormone (mIU/mL)	0.50	225	22.2			<DL	103	48.5		<DL	0.660	122	0.0			<DL	<0.001
Inhibin B (pg/mL)	11	232	61.6	<DL	23.500	45.325	106	98.1	34.200	44.350	61.300	126	31.0		<DL	13.900	<0.001
Insulin-like factor 3 (ng/mL)	0.01	119	100.0	0.230	0.280	0.330	103	100.0	0.250	0.290	0.340	16	100.0	0.180	0.185	0.235	<0.001
Prolactin (ng/mL)	1.0	226	99.6	64.600	87.600	118.250	103	100.0	63.400	87.200	119.000	123	99.2	64.600	87.800	116.000	0.921

P values were calculated by Mann-Whitney U test;

DHEA, dehidroepiandrostenedione; DL, detection limit

Table 4 Associations between OCPs exposure and steroid and reproductive hormone levels among boys

	Progesterone				Estradiol				Testosterone				Estradiol/Testosterone				DEHA				Androstenedione				Androstenedione/DHEA			
	β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI		
Oxychlordane	-0.090	-0.416	0.236		0.007	-0.359	0.372		-0.176	-0.421	0.068		0.183	-0.140	0.505		0.243	0.028	0.459	*	0.006	-0.214	0.226		-0.238	-0.470	-0.005	*
cis-Nonachlor	-0.149	-0.424	0.125		-0.005	-0.314	0.304		-0.095	-0.303	0.113		0.090	-0.184	0.363		0.247	0.066	0.427	**	0.070	-0.116	0.255		-0.177	-0.375	0.021	†
trans-Nonachlor	-0.162	-0.436	0.112		-0.027	-0.335	0.282		-0.161	-0.367	0.045		0.134	-0.138	0.407		0.255	0.076	0.435	**	0.038	-0.147	0.224		-0.217	-0.413	-0.021	*
p,p'-DDD	-0.097	-0.279	0.085		-0.151	-0.354	0.051		-0.065	-0.203	0.073		-0.086	-0.267	0.095		-0.023	-0.147	0.101		-0.016	-0.139	0.108		0.007	-0.126	0.141	
o,p'-DDE	-0.046	-0.234	0.143		0.050	-0.161	0.260		-0.029	-0.171	0.114		0.078	-0.108	0.265		0.080	-0.046	0.207		0.013	-0.114	0.140		-0.067	-0.204	0.069	
p,p'-DDE	0.034	-0.218	0.286		0.192	-0.088	0.472		-0.105	-0.295	0.084		0.297	0.054	0.541	*	0.108	-0.061	0.278		-0.041	-0.211	0.128		-0.150	-0.331	0.032	
o,p'-DDT	-0.056	-0.248	0.136		0.048	-0.167	0.263		-0.035	-0.180	0.110		0.083	-0.107	0.273		0.118	-0.010	0.246	†	0.010	-0.119	0.140		-0.108	-0.246	0.031	
p,p'-DDT	-0.077	-0.339	0.184		0.031	-0.262	0.324		-0.065	-0.263	0.132		0.096	-0.163	0.356		0.151	-0.024	0.325	†	0.016	-0.161	0.192		-0.135	-0.324	0.054	
Dieldrin	-0.178	-0.482	0.125		0.069	-0.273	0.410		-0.023	-0.254	0.208		0.092	-0.211	0.394		0.267	0.067	0.467	**	0.155	-0.048	0.359		-0.112	-0.333	0.109	
cis-HCE	-0.090	-0.416	0.236		0.007	-0.359	0.372		-0.176	-0.421	0.068		0.183	-0.140	0.505		0.243	0.028	0.459	*	0.006	-0.214	0.226		-0.238	-0.470	-0.005	*
HCB	0.025	-0.438	0.489		0.114	-0.404	0.633		-0.139	-0.489	0.210		0.254	-0.203	0.711		0.311	0.004	0.619	*	0.058	-0.254	0.370		-0.253	-0.586	0.081	
β-HCH	0.118	-0.157	0.392		0.168	-0.139	0.474		-0.091	-0.299	0.117		0.259	-0.010	0.528	†	0.136	-0.049	0.320		-0.004	-0.190	0.181		-0.140	-0.339	0.059	
Mirex	-0.108	-0.418	0.203		-0.007	-0.355	0.342		-0.262	-0.492	-0.032	*	0.256	-0.050	0.561		0.213	0.007	0.420	*	-0.061	-0.270	0.149		-0.274	-0.494	-0.054	*
Parlar-26	-0.124	-0.338	0.091		0.124	-0.116	0.364		0.002	-0.161	0.165		0.122	-0.090	0.335		0.235	0.097	0.373	**	0.100	-0.044	0.244		-0.135	-0.290	0.020	†
Parlar-50	-0.099	-0.315	0.116		0.072	-0.170	0.313		-0.020	-0.183	0.144		0.091	-0.123	0.305		0.236	0.097	0.374	**	0.103	-0.041	0.248		-0.132	-0.288	0.023	†

	Testosterone/Androstenedione				Cortisol				Cortisone				Cortisone/Cortisol				Adrenal androgen/Glucocorticoid				SHBG				Prolactin			
	β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI		
Oxychlordane	-0.182	-0.339	-0.024	*	-0.363	-0.763	0.038		-0.398	-0.858	0.062	†	-0.035	-0.316	0.246		0.571	0.042	1.100	*	-0.082	-0.245	0.081		-0.077	-0.261	0.107	
cis-Nonachlor	-0.165	-0.297	-0.032	*	-0.218	-0.559	0.124		-0.310	-0.700	0.079		-0.092	-0.330	0.145		0.487	0.040	0.935	*	-0.040	-0.178	0.098		-0.150	-0.303	0.003	†
trans-Nonachlor	-0.199	-0.330	-0.069	**	-0.372	-0.708	-0.036	*	-0.426	-0.810	-0.041	*	-0.054	-0.291	0.184		0.609	0.168	1.050	**	-0.027	-0.165	0.111		-0.075	-0.230	0.080	
p,p'-DDD	-0.049	-0.140	0.041		0.061	-0.166	0.289		0.014	-0.248	0.275		-0.048	-0.205	0.110		-0.049	-0.352	0.255		0.055	-0.036	0.146		0.021	-0.082	0.124	
o,p'-DDE	-0.042	-0.134	0.051		-0.108	-0.342	0.126		-0.148	-0.415	0.119		-0.040	-0.202	0.122		0.200	-0.109	0.510		-0.030	-0.124	0.064		-0.120	-0.224	-0.015	*
p,p'-DDE	-0.064	-0.188	0.060		-0.290	-0.600	0.019	†	-0.249	-0.606	0.108		0.041	-0.176	0.258		0.354	-0.059	0.766	†	-0.080	-0.206	0.045		-0.226	-0.361	-0.092	**
o,p'-DDT	-0.045	-0.140	0.049		-0.172	-0.410	0.065		-0.153	-0.426	0.120		0.019	-0.146	0.185		0.260	-0.055	0.574		-0.017	-0.114	0.079		-0.172	-0.275	-0.069	**
p,p'-DDT	-0.081	-0.210	0.048		-0.073	-0.400	0.253		-0.192	-0.565	0.180		-0.119	-0.344	0.105		0.276	-0.155	0.707		-0.019	-0.150	0.112		-0.155	-0.299	-0.011	*
Dieldrin	-0.178	-0.325	-0.032	*	0.028	-0.352	0.409		-0.207	-0.641	0.227		-0.235	-0.494	0.023	†	0.367	-0.134	0.867		-0.049	-0.202	0.103		-0.209	-0.377	-0.040	*
cis-HCE	-0.182	-0.339	-0.024	*	-0.363	-0.763	0.038	†	-0.398	-0.858	0.062	†	-0.035	-0.316	0.246		0.571	0.042	1.100	*	-0.082	-0.245	0.081		-0.077	-0.261	0.107	
HCB	-0.198	-0.424	0.028	†	-0.255	-0.830	0.321		-0.185	-0.845	0.476		0.070	-0.329	0.469		0.474	-0.289	1.236		-0.167	-0.397	0.062		-0.239	-0.495	0.017	†
β-HCH	-0.087	-0.222	0.048		-0.153	-0.495	0.189		-0.076	-0.469	0.317		0.077	-0.160	0.314		0.221	-0.234	0.676		-0.147	-0.282	-0.012	*	-0.182	-0.332	-0.031	*
Mirex	-0.202	-0.350	-0.053	**	-0.588	-0.959	-0.218	**	-0.572	-1.002	-0.142	*	0.016	-0.252	0.285		0.744	0.249	1.239	**	-0.083	-0.238	0.073		-0.180	-0.352	-0.008	*
Parlar-26	-0.098	-0.203	0.007	†	-0.174	-0.441	0.092		-0.289	-0.592	0.013	†	-0.115	-0.299	0.069		0.457	0.111	0.803	*	-0.017	-0.125	0.091		-0.107	-0.229	0.015	†
Parlar-50	-0.123	-0.227	-0.019	*	-0.156	-0.424	0.111		-0.231	-0.537	0.075		-0.075	-0.260	0.111		0.418	0.069	0.767	*	-0.048	-0.156	0.060		-0.131	-0.252	-0.010	*

	LH				FSH				Inhibin B				INSL3				T/LH				FSH/InhibinB			
	β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI			β	95%CI		
Oxychlordane	0.037	-0.305	0.380		0.097	-0.143	0.337		-0.093	-0.301	0.115		-0.064	-0.190	0.063		-0.224	-0.684	0.236		0.190	-0.119	0.499	
cis-Nonachlor	0.087	-0.202	0.376		0.199	0.000	0.399	†	-0.140	-0.315	0.034		-0.015	-0.123	0.092		-0.185	-0.574	0.203		0.341	0.086	0.595	**
trans-Nonachlor	0.077	-0.211	0.366		0.121	-0.080	0.323		-0.115	-0.290	0.060		-0.061	-0.167	0.045		-0.245	-0.632	0.141		0.236	-0.023	0.494	†
p,p'-DDD	-0.045	-0.236	0.146		0.057	-0.077	0.191		-0.050	-0.167	0.067		-0.044	-0.114	0.027		-0.023	-0.281	0.235		0.105	-0.068	0.277	
o,p'-DDE	-0.042	-0.240	0.156		-0.012	-0.151	0.127		-0.090	-0.209	0.029		0.003	-0.070	0.077		0.019	-0.248	0.287		0.086	-0.093	0.265	
p,p'-DDE	0.084	-0.179	0.347		0.145	-0.038	0.328		-0.127	-0.286	0.033		-0.069	-0.165	0.028		-0.184	-0.538	0.169		0.284	0.052	0.517	*
o,p'-DDT	0.047	-0.154	0.248		-0.005	-0.146	0.137		-0.077	-0.199	0.045		-0.003	-0.078	0.071		-0.075	-0.346	0.196		0.080	-0.102	0.262	
p,p'-DDT	-0.014	-0.288	0.259		0.159	-0.031	0.349		-0.156	-0.320	0.009	†	-0.014	-0.116	0.087		-0.061	-0.430	0.308		0.307	0.066	0.548	*
Dieldrin	-0.040	-0.362	0.282		0.071	-0.155	0.297		-0.183	-0.375	0.009	†	-0.048	-0.167	0.071		0.011	-0.424	0.446		0.246	-0.043	0.535	†
cis-HCE	0.037	-0.305	0.380		0.097	-0.143	0.337		-0.093	-0.301	0.115		-0.064	-0.190	0.063		-0.224	-0.684	0.236		0.190	-0.119	0.499	
HCB	-0.116	-0.598	0.367		0.213	-0.124	0.549		-0.290	-0.581	0.000	†	-0.041	-0.220	0.138		-0.032	-0.683	0.620		0.496	0.069	0.924	*
β-HCH	0.030	-0.257	0.317		0.203	0.006	0.401	*	-0.130	-0.304	0.045		-0.028	-0.134	0.078		-0.130	-0.516	0.256		0.324	0.072	0.577	*
Mirex	0.137	-0.188	0.461		0.229	0.004	0.453	*	-0.069	-0.267	0.130		-0.061	-0.181	0.060		-0.401	-0.833	0.031		0.299	0.009	0.589	*
Parlar-26	0.075	-0.154	0.304		0.164	0.006	0.323	*	-0.099	-0.235	0.038		-0.058	-0.142	0.027		-0.073	-0.383	0.237		0.267	0.065	0.469	*
Parlar-50	0.033	-0.197	0.262		0.173	0.015	0.331	*	-0.124	-0.260	0.012	†	-0.028	-0.113	0.057		-0.056	-0.365	0.254		0.297	0.097	0.497	**

Adjusted regression coefficients (β) and 95% confidence intervals (CIs) for the association between a 10-fold increase of OCP and log10-transformed hormone levels as determined by a linear regression model

Each OCP was introduced into the model separately and adjusted for maternal age, parity, and gestational age.

*P < 0.05, **P < 0.01, †P <0.1

Table 5 Associations between OCPs exposure and steroid and reproductive hormone levels among girls

	Progesterone			Estradiol			Testosterone			Estradiol/Testosterone			DEHA			Androstenedione		
	β	95%CI		β	95%CI		β	95%CI		β	95%CI		β	95%CI		β	95%CI	
Oxychlordane	0.136	-0.149	0.420	0.047	-0.175	0.270	0.050	-0.219	0.320	-0.003	-0.222	0.216	-0.087	-0.377	0.203	0.032	-0.189	0.254
cis-Nonachlor	0.194	-0.069	0.456	0.019	-0.188	0.225	0.035	-0.215	0.284	-0.016	-0.220	0.187	-0.136	-0.404	0.132	0.006	-0.199	0.211
trans-Nonachlor	0.192	-0.065	0.449	0.035	-0.167	0.237	0.065	-0.179	0.310	-0.030	-0.229	0.169	-0.163	-0.425	0.099	0.038	-0.163	0.239
p,p'-DDD	0.099	-0.046	0.244	-0.059	-0.172	0.054	-0.041	-0.178	0.096	-0.018	-0.130	0.094	-0.160	-0.305	-0.014 *	-0.069	-0.181	0.043
o,p'-DDE	-0.042	-0.212	0.127	-0.082	-0.214	0.049	-0.038	-0.198	0.122	-0.044	-0.174	0.086	0.000	-0.173	0.173	-0.066	-0.197	0.065
p,p'-DDE	0.018	-0.201	0.237	-0.116	-0.285	0.054	0.035	-0.172	0.241	-0.151	-0.317	0.015 †	-0.156	-0.377	0.065	-0.077	-0.246	0.092
o,p'-DDT	-0.071	-0.282	0.140	-0.109	-0.272	0.055	-0.024	-0.222	0.175	-0.085	-0.246	0.076	0.013	-0.202	0.227	-0.072	-0.235	0.091
p,p'-DDT	0.042	-0.205	0.289	-0.063	-0.256	0.129	0.075	-0.158	0.308	-0.138	-0.327	0.050	-0.102	-0.353	0.149	-0.041	-0.233	0.150
Dieldrin	-0.012	-0.310	0.287	-0.028	-0.261	0.204	-0.092	-0.373	0.188	0.064	-0.165	0.293	0.068	-0.235	0.371	-0.204	-0.432	0.024 †
cis-HCE	0.136	-0.149	0.420	0.047	-0.175	0.270	0.050	-0.219	0.320	-0.003	-0.222	0.216	-0.087	-0.377	0.203	0.032	-0.189	0.254
HCB	0.216	-0.174	0.606	-0.052	-0.357	0.253	0.026	-0.344	0.395	-0.077	-0.378	0.223	-0.052	-0.450	0.346	0.051	-0.252	0.354
β-HCH	-0.032	-0.286	0.222	-0.050	-0.248	0.147	0.057	-0.182	0.296	-0.107	-0.301	0.087	-0.016	-0.274	0.242	-0.006	-0.203	0.190
Mirex	0.218	-0.068	0.503	0.043	-0.182	0.267	-0.030	-0.302	0.241	0.073	-0.148	0.294	-0.166	-0.457	0.126	0.025	-0.198	0.248
Parlar-26	0.031	-0.167	0.229	-0.006	-0.160	0.149	-0.012	-0.198	0.175	0.006	-0.146	0.158	0.022	-0.180	0.223	-0.029	-0.182	0.124
Parlar-50	0.046	-0.177	0.270	-0.012	-0.186	0.162	-0.002	-0.213	0.209	-0.010	-0.182	0.162	0.021	-0.207	0.248	-0.015	-0.189	0.158

	Androstenedione/DHEA			Testosterone/Androstenedione			Cortisol			Cortisone			Cortisone/Cortisol			Adrenal androgen/Glucocorticoid		
	β	95%CI		β	95%CI		β	95%CI		β	95%CI		β	95%CI		β	95%CI	
Oxychlordane	0.119	-0.217	0.455	0.018	-0.140	0.175	0.214	-0.265	0.692	0.374	-0.302	1.050	0.160	-0.145	0.465	-0.361	-1.139	0.416
cis-Nonachlor	0.142	-0.169	0.453	0.029	-0.117	0.175	0.229	-0.215	0.672	0.463	-0.161	1.087	0.234	-0.046	0.515	-0.463	-1.181	0.255
trans-Nonachlor	0.201	-0.103	0.505	0.027	-0.116	0.170	0.276	-0.157	0.709	0.517	-0.092	1.126 †	0.241	-0.033	0.516 †	-0.533	-1.234	0.168
p,p'-DDD	0.091	-0.080	0.262	0.028	-0.052	0.108	0.208	-0.034	0.450 †	0.358	0.017	0.699 *	0.150	-0.004	0.304 †	-0.412	-0.803	-0.021 *
o,p'-DDE	-0.066	-0.266	0.134	0.027	-0.066	0.121	-0.176	-0.460	0.108	-0.030	-0.434	0.374	0.146	-0.034	0.327	0.088	-0.376	0.551
p,p'-DDE	0.079	-0.179	0.337	0.112	-0.007	0.231 †	0.106	-0.261	0.474	0.285	-0.233	0.803	0.179	-0.054	0.411	-0.333	-0.928	0.261
o,p'-DDT	-0.084	-0.333	0.164	0.048	-0.068	0.164	-0.269	-0.620	0.083	-0.058	-0.560	0.443	0.210	-0.013	0.433 †	0.143	-0.432	0.718
p,p'-DDT	0.061	-0.231	0.353	0.116	-0.019	0.251 †	-0.045	-0.461	0.371	0.207	-0.380	0.795	0.253	-0.009	0.514 †	-0.167	-0.842	0.508
Dieldrin	-0.272	-0.621	0.076	0.112	-0.051	0.275	-0.203	-0.704	0.297	-0.274	-0.982	0.434	-0.071	-0.391	0.249	0.256	-0.557	1.069
cis-HCE	0.119	-0.217	0.455	0.018	-0.140	0.175	0.214	-0.265	0.692	0.374	-0.302	1.050	0.160	-0.145	0.465	-0.361	-1.139	0.416
HCB	0.102	-0.359	0.564	-0.025	-0.241	0.191	0.317	-0.339	0.973	0.499	-0.428	1.425	0.182	-0.237	0.600	-0.420	-1.486	0.647
β-HCH	0.010	-0.290	0.309	0.063	-0.077	0.202	0.004	-0.422	0.431	0.012	-0.592	0.615	0.007	-0.265	0.280	-0.014	-0.707	0.679
Mirex	0.191	-0.148	0.529	-0.055	-0.214	0.103	0.229	-0.254	0.712	0.510	-0.170	1.189	0.280	-0.025	0.585 †	-0.525	-1.306	0.257
Parlar-26	-0.051	-0.285	0.183	0.017	-0.092	0.127	0.044	-0.289	0.377	0.091	-0.380	0.562	0.047	-0.165	0.260	-0.040	-0.581	0.501
Parlar-50	-0.036	-0.300	0.228	0.014	-0.110	0.137	0.028	-0.348	0.404	0.123	-0.408	0.655	0.095	-0.144	0.334	-0.050	-0.661	0.560

	SHBG			Prolactin		
	β	95%CI		β	95%CI	
Oxychlordane	0.060	-0.118	0.238	0.092	-0.149	0.332
cis-Nonachlor	0.057	-0.108	0.221	0.076	-0.147	0.298
trans-Nonachlor	0.059	-0.102	0.220	0.085	-0.134	0.303
p,p'-DDD	0.064	-0.026	0.155	0.120	0.000	0.241 †
o,p'-DDE	-0.004	-0.109	0.102	-0.048	-0.191	0.094
p,p'-DDE	0.074	-0.061	0.210	0.092	-0.091	0.275
o,p'-DDT	-0.002	-0.133	0.130	-0.009	-0.186	0.169
p,p'-DDT	0.034	-0.120	0.188	0.029	-0.178	0.237
Dieldrin	0.014	-0.172	0.200	-0.199	-0.453	0.055
cis-HCE	0.060	-0.118	0.238	0.092	-0.149	0.332
HCB	0.116	-0.127	0.359	0.128	-0.202	0.458
β-HCH	0.125	-0.031	0.282	0.009	-0.207	0.225
Mirex	0.021	-0.159	0.200	0.120	-0.121	0.362
Parlar-26	0.037	-0.086	0.160	-0.022	-0.188	0.145
Parlar-50	0.030	-0.110	0.169	-0.002	-0.190	0.186

Adjusted regression coefficients (β) and 95% confidence intervals (CIs) for the association between a 10-fold increase of OCP and log10-transformed hormone levels as determined by a linear regression model
Each OCP was introduced into the model separately and adjusted for maternal age, parity, and gestational age.

*P < 0.05, **P < 0.01, † P <0.1

Supplemental Table S1 Concentrations of organochlorine pesticides in maternal blood all measured of original cohort (n=379)

Persistent organochlorine pesticides	Detection limit (pg/g-wet)	>DL (%)	Minimum	Percentile			Maximum
				25th	50th	75th	
Aldrin	1.00	0.3				<DL	12.83
Chlordanes							
oxychlordane	0.90	100.0	7.93	27.05	39.67	56.02	250.94
cis-Chlordane	0.70	58.8		<DL	1.15	2.29	17.53
trans-Chlordane	0.50	45.4			<DL	0.71	3.79
cis-Nonachlor	0.40	100.0	1.63	6.76	9.97	14.36	38.07
trans-Nonachlor	0.50	100.0	13.14	49.70	71.52	107.59	513.52
DDTs							
o,p'-DDD	0.50	12.4				<DL	1.16
p,p'-DDD	0.40	89.7	<DL	0.94	1.48	2.29	9.04
o,p'-DDE	0.40	85.0	<DL	0.75	1.27	1.82	6.20
p,p'-DDE	0.60	100.0	99.52	401.53	650.99	1011.48	4575.67
o,p'-DDT	0.60	7.6	<DL	2.27	3.48	4.86	17.15
p,p'-DDT	0.40	100.0	2.38	16.63	23.16	33.99	121.52
Dieldrin	0.80	100.0	4.11	12.08	16.42	22.62	71.52
Endrin	1.00	0.0					<DL
Heptachlors							
Heptachlor	0.80	0.5				<DL	1.14
trans-HCE	1.00	0.0					<DL
cis-HCE	0.40	100.0	6.17	18.78	26.44	37.28	200.53
HCB	0.90	100.0	34.94	80.24	101.65	130.06	245.48
HCHs							
α-HCH	0.70	69.1		<DL	0.90	1.32	3.89
β-HCH	0.60	100.0	19.95	105.05	154.45	244.76	1667.12
γ-HCH	0.90	59.1		<DL	1.05	1.63	100.92
δ-HCH	0.70	0.8				<DL	1.11
Mirex	0.50	100.0	0.88	4.07	5.95	8.26	34.97
Toxaphenes							
Parlar-26	1.00	97.1	<DL	2.87	4.39	6.65	20.82
Parlar-41	0.70	26.9			<DL	0.72	1.96
Parlar-40	2.00	0.5				<DL	2.43
Parlar-44	2.00	1.6				<DL	2.84
Parlar-50	2.00	96.0	<DL	4.36	6.52	9.68	29.29
Parlar-62	6.00	0.0					<DL

DL, detection limit; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HCE, heptachlor epoxide; HCH, hexachlorocyclohexane.

Supplemental table S2 Maternal and infant characteristics and concentrations of OCPs

characteristics		n	o,p'-DDT	p,p'-DDT	o,p'-DDE	p,p'-DDE	p,p'-DDD	cis-Nonachlor	trans-Nonachlor	oxychlordane
Mother			ρ	ρ	ρ	ρ	ρ	ρ	ρ	ρ
Age at delivery (years)			0.009	0.102	0.030	0.270**	0.137*	0.267**	0.311**	0.350**
Pre-pregnancy BMI (kg/m2)			0.062	0.118	0.119	0.003	0.052	0.089	-0.011	-0.093
			Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)
Educational level	≤12 years	100	3.01 (2.09, 4.47)	21.59 (15.41, 32.84)	1.16 80.70, 1.71)	565.05 (376.81, 942.71)	1.62 (0.88, 2.44)	9.88 (7.22, 14.64)	72.73 (52.69, 108.21)	39.83 (27.1, 56.02)
	>12 years	132	3.54 (2.42, 4.99)	25.32 (16.37, 34.77)	1.33 (0.96, 1.86)	634.71 (422.04, 976.14)	1.69 (1.03, 2.58)	10.78 (6.85, 15.13)	78.85 (51.80, 118.08)	40.22 (28.87, 57.78)
Annual Household income	<5 million yen per year	166	3.27 (2.22, 4.66)	22.35 (16.38, 33.65)	1.26 (0.71, 1.74)	619.26 (411.26, 942.60)	1.62 (0.99, .53)	9.93 (6.71, 14.35) *	70.06 (50.04, 111.09) *	39.07 (27.61, 56.02) *
	≧5 million yen per year	66	3.64 (2.41, 5.06)	24.18 (15.54, 38.90)	1.23 (0.77, 2.05)	633.52 (392.24, 1302.85)	1.68 (0.98, 2.58)	11.91 (8.21, 15.98)	86.66 (62.34, 110.04)	45.83 (30.02, 66.07)
Smoking during pregnancy	No	190	3.46 (2.37, 4.66)	23.74 (16.38, 33.93)	1.26 (0.73, 1.77)	617.59 8409.71, 971.83)	1.61 (0.98, 2.57)	10.70 (7.17, 15.02)	76.63 (54.06, 112.12)	40.31 (29.25, 57.75)
	Yes	42	3.03 (1.79, 5.03)	21.09 (14.17, 34.22)	1.17 (0.70, 1.84)	625.95 (409.34, 944.10)	1.79 (1.07, 2.39)	8.94 (6.01, 15.10)	69.19 (48.19, 109.46)	38.64 (23.48, 51.97)
Alcohol consumption during pregnancy	No	154	3.10 (2.21, 4.67) *	22.62 (15.54, 33.44)	1.22 (0.69, 1.72)	60.5.48 (412.66, 971.83)	1.62 (0.99, 2.55)	10.14 (6.90, 14.76)	74.14 (51.75, 112.12)	40.13 (28.81, 58.13)
	Yes	78	3.86 (2.56, 4.98)	24.14 (17.27, 33.98)	1.27 (0.90, 1.87)	648.50 (406.30, 976.56)	1.67 (0.87, 2.41)	11.04 (7.29, 15.25)	78.85 (52.92, 108.95)	40.04 (28.87, 56.02)
Parity	0	120	3.39 (2.38, 4.67)	24.38 (17.17, 33.92)	1.35 (0.81, 1.83)	652.69 (453.78, 1002.45)	1.64 (1.00, 2.52)	11.52 (7.50, 15.13) *	87.74 (56.35, 117.19) *	42.99 (32.00, 60.65) **
	≥1	112	3.24 (2.22, 4.78)	21.98 (14.92, 34.69)	1.13 (0.69, 1.71)	564.40 (336.51, 938.47)	1.65 (0.91, 2.62)	9.5 (6.37, 14.06)	70.05 (45.60, 97.48)	38.97 (26.35, 54.44)
Blood sampling period	During pregnancy	159	3.42 (2.25, 4.72)	22.49 (15.57, 33.95)	1.22 (0.73, 1.83)	626.50 (412.96, 941.47)	1.51 (0.99, 2.28)	10.01 (7.06, 14.87)	70.38 (21.93, 105.61)	39.19 (27.65, 55.64)
	After delivery	73	3.36 (2.28, 4.48)	23.77 (19.33, 34.17)	1.35 (0.71, 1.73)	614.51 (339.28, 1008.37)	1.76 (0.87, 2.84)	11.15 (6.86, 16.79)	87.82 (51.95, 124.70)	44.66 (29.31, 60.98)
Type of delivery	Vaginal	230	3.36 (2.27, 4.68)	22.84 816.14, 33.93)	1.25 (0.72, 1.77)	619.26 8410.14, 964.80)	1.62 (0.98, 2.53)	10.33 (7.03, 15.04)	75.08 (51.89, 110.01)	39.87 (28.87, 57.31)
	Caesarian section	2	3.70 (3.03, 4.36)	36.58 (23.77, 49.38)	1.40 (0.91, 1.88)	688.72 (304.51, 1072.92)	2.87 (2.23, 3.5)	18.84 (12.12, 25.55)	119.01 (91.04, 146.97)	55.41 (40.2, 70.62)
Infant			ρ	ρ	ρ	ρ	ρ	ρ	ρ	ρ
Birth weight			0.022	-0.025	0.005	0.002	-0.087	-0.057	-0.057	-0.061
Gestational Age			0.066	0.037	0.039	0.117	-0.013	0.025	0.035	0.045
			Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)
Sex	Male	106	3.39 (2.42, 4.42)	23.90 (16.56, 33.88)	1.18 (0.72, 1.66)	641.93 (405.32, 934.78)	1.70 (1.04, 2.35)	10.29 (7.11, 15.11)	75.82 (53.44, 118.34)	40.22 (29.26, 57.54)
	Female	126	3.36 (2.20, 4.97)	21.96 (15.78, 34.45)	1.30 (0.72, 1.87)	611.15 (4101.00, 998.41)	1.60 (0.93, 2.66)	10.42 (6.80, 15.04)	74.66 851.75, 107.06)	39.87 (28.34, 57.15)

Supplemental table S1 Maternal and infant characteristics and concentrations of OCPs (continued)

Characteristics		n	Dieldrin	cis-HCE	HCB	β-HCH	Mirex	Parlar-26	Parlar-50
Mother			ρ	ρ	ρ	ρ	ρ	ρ	ρ
Age at delivery (years)			0.108	0.254**	0.119*	0.464**	0.513**	0.176**	0.181**
Pre-pregnancy BMI (kg/m2)			0.221**	0.177**	0.101	0.147*	-0.074	0.240**	0.240**
			Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)
Educational level	≤12 years	100	16.55 (12.52, 22.84)	26.07 (18.56, 40.28)	101.71 (80.26, 130.96)	154.79 (96.08, 262.30)	5.99 (4.08, 8.34)	4.85 (3.17, 7.55)	7.02 (4.61, 1028)
	>12 years	132	16.80 (12.11, 21.59)	26.91 (18.96, 37.13)	106.79 (85.26, 167.25)	154.31 (109.21, 220.69)	6.11 (4.31, 8.59)	4.05 (2.56, 6.68)	6.50 (3.88, 9.48)
Annual Household income	<5 million yen per year	166	15.93 (12.02, 20.99)	26.07 (18.44, 35.00)	102.17 (80.36, 129.52)	153.64 (100.87, 209.23)	5.76 (3.88, 7.79) **	4.32 (2.69, 6.82)	6.48 (4.25, 9.48)
	≧5 million yen per year	66	17.89 (12.60, 24.41)	26.55 (19.17, 43.07)	111.51 (85.53, 145.40)	165.66 (113.97, 279.53)	7.44 (4.82, 11.64)	5.22 (3.14, 7.61)	7.69 (4.33, 11.64)
Smoking during pregnancy	No	190	16.86 (12.35, 22.47)	26.79 (19.14, 37.90)	103.82 (83.69, 131.84)	158.46 (103.79, 241.30)	6.16 (4.51, 8.61)	4.50 (2.84, 6.60)	6.82 (4.32, 9.74)
	Yes	42	15.51 (11.41, 21.31)	24.39 (16.80, 35.42)	104.48 (74.82, 132.31)	150.87 (103.84, 184.91)	5.69 (3.73, 7.82)	4.23 (2.73, 8.24)	5.88 (3.75, 11.43)
Alcohol consumption during pregnancy	No	154	16.80 (12.14, 22.82)	27.18 (18.91, 39.08)	105.46 (81.20, 133.02)	162.80 (104.40, 244.79)	6.04 (4.11, 8.38)	4.19 (2.82, 6.82)	6.40 (4.28, 9.58)
	Yes	78	16.35 (12.42, 21.66)	24.82 (18.42, 35.69)	99.34 (86.16, 130.72)	146.89 (102.56, 202.87)	6.09 (4.11, 8.61)	4.54 (2.91, 7.58)	7.32 (4.44, 10.56)
Parity	0	120	17.16 (12.52, 21.71)	26.79 (19.16, 36.85)	109.93 (91.03, 131.94) **	165.19 (114.52, 271.53) *	5.98 (4.14, 8.81)	5.23 (2.96, 7.20)	7.54 (4.54, 9.99)
	≥1	112	16.35 (12.05, 22.66)	25.17 (18.19, 40.63)	95.36 (72.71, 129.15)	143.66 (90.00, 211.57)	6.19 (4.11, 8.47)	4.02 (2.72, 6.68)	5.94 (3.85, 9.66)
Blood sampling period	During pregnancy	159	17.59 (12.39, 22.87)	26.44 (18.91, 38.12)	104.06 (85.45, 130.56)	154.13 (108.08, 218.60)	5.88 (4.07, 7.86)	4.50 (2.93, 7.08)	7.16 (4.25, 9.72)
	After delivery	73	15.51 (12.06, 21.32)	25.10 (18.50, 36.78)	103.73 (75.77, 138.73)	158.62 (94.33, 254.44)	6.76 (4.54, 10.19)	4.33 (2.56, 7.49)	6.42 (4.45, 10.72)
Type of delivery	Vaginal	230	16.61 (12.16, 22.44)	26.15 (18.76, 37.42)	103.99 (82.85, 131.26)	154.31 (104.40, 237.67)	6.04 (4.11, 8.51)	4.41 (2.84, 7.09)	6.55 (4.28, 9.79)
	Caesarian section	2	18.66 (16.82, 20.50)	36.20 (28.26, 44.14)	134.17 (85.55, 182.79)	154.45 (68.50, 240.39)	13.99 (4.84, 23.14)	7.67 (5.34, 10.00)	10.15 (7.63, 12.67)
Infant			ρ	ρ	ρ	ρ	ρ	ρ	ρ
Birth weight			-0.049	-0.101	-0.066	-0.128	-0.030	-0.024	-0.022
Gestational Age			-0.035	-0.082	0.052	0.061	0.027	0.024	-0.018
			Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)	Med (min-max)
Sex	Male	106	15.92 (12.07, 22.55)	26.39 (19.22, 40.80)	101.76 (85.53, 130.85)	153.65 (107.63, 237.67)	6.04 (4.53, 8.94)	4.50 (2.91, 6.86)	6.64 (4.33, 10.27)
	Female	126	17.07 (12.51, 21.96)	26.15 (18.49, 35.42)	104.60 (78.58, 133.01)	155.04 (102.56, 240.78)	6.04 (4.03, 8.41)	4.24 (2.73, 7.36)	6.50 (4.25, 9.74)

Supplemental Table S3 Distribution of steroids and reproductive hormones among all measured of original cohort (n=295)

	DL	n	>DL (%)	Total (n=295)			Boys (n=106)					Girls (n=126)					P-value
				25%	Med	75%	n	>DL (%)	25%	Med	75%	n	>DL (%)	25%	Med	75%	
Steroid hormones																	
Progesterone (ng/mL)	0.01	295	100	175.5	218.8	278.8	135	100	183.8	225.9	285.6	160	100	167.6	209.0	275.6	0.184
Testosterone (pg/mL)	0.01	295	100	59.8	83.9	114.1	135	100	76.5	98.9	126.3	160	100	52.1	70.1	96.8	<0.001
Estradiol (ng/mL)	0.01	295	99.7	3.29	4.70	7.10	135	99.3	3.33	4.86	7.42	160	100	3.16	4.68	6.61	0.227
DHEA (ng/mL)	0.01	295	100	1.77	2.19	2.99	135	100	1.59	2.08	2.76	160	100	1.91	2.34	3.22	
Androstenedione (ng/mL)	0.01	295	99.3	0.36	0.46	0.58	135	98.5	0.38	0.47	0.61	160	99.4	0.35	0.45	0.58	
Cortisol (ng/mL)	0.25	295	97.6	22.7	38.9	63.6	135	98.5	22.5	38.3	65.3	160	96.9	22.8	39.2	62.8	
Cortisone (ng/mL)	0.10	295	94.9	69.9	93.9	123.0	135	97.0	70.5	95.3	123.4	160	93.1	69.7	93.0	123.0	
Steroid Hormone Binding Globulin (nmol/L)	1.10	295	100	13.3	15.8	19.0	135	100	13.9	16.5	19.3	160	100	13.0	15.5	18.7	0.079
Lutheling Hormone (mIU/mL)	0.50	288	16.3			<DL	132	34.8		<DL	0.84	156	0.6			<DL	<0.001
Follicle Stimulating Hormone (mIU/mL)	0.50	287	20.9			<DL	132	45.5		<DL	0.66	155	0			<DL	<0.001
Inhibin B (pg/mL)	11	295	59.7	5.5	23.2	44.6	135	98.5	33.9	44.0	58.3	160	26.9		<DL	12.4	<0.001
Insulin-like factor 3 (ng/mL)	0.01	157	100	0.23	0.27	0.32	132	100	0.25	0.29	0.34	25	100	0.17	0.18	0.23	<0.001
Prolactin (ng/mL)	1.0	289	99.7	63.1	85.8	116.0	132	100	65.4	85.2	115.0	157	99.6	61.4	86.0	118.0	0.986

P values were calculated by Mann-Whitney U test;

DHEA, dehidroepiandrostenedione; DL, detection limit

Supplemental Table S4 Associations between OCPs exposure and steroid and reproductive hormone levels and sex interaction

	Progesterone					Estradiol					Testosterone					Estradiol/Testosterone					DEHA					Androstenedione				
	β	95%CI		$p^{a)}$	$p^{b)}$	β	95%CI		$p^{a)}$	$p^{b)}$	β	95%CI		$p^{a)}$	$p^{b)}$	β	95%CI		$p^{a)}$	$p^{b)}$	β	95%CI		$p^{a)}$	$p^{b)}$	β	95%CI		$p^{a)}$	$p^{b)}$
Oxychlordane	0.024	-0.189	0.238			0.025	-0.178	0.229			-0.065	-0.249	0.118			0.091	-0.099	0.281			0.067	-0.119	0.253			0.019	-0.137	0.175		
cis-Nonachlor	0.022	-0.166	0.210		*	0.010	-0.170	0.190			-0.030	-0.192	0.132			0.040	-0.128	0.208			0.055	-0.108	0.218		*	0.039	-0.098	0.176		
trans-Nonachlor	0.014	-0.172	0.200		*	0.005	-0.173	0.184			-0.049	-0.209	0.112			0.054	-0.112	0.221			0.046	-0.116	0.208		*	0.038	-0.098	0.175		
p,p'-DDD	0.004	-0.111	0.119		†	-0.102	-0.211	0.008	†		-0.054	-0.153	0.045			-0.047	-0.150	0.055			-0.091	-0.191	0.009	†		-0.043	-0.126	0.041		
o,p'-DDE	-0.044	-0.169	0.082			-0.017	-0.136	0.102			-0.034	-0.142	0.074			0.017	-0.094	0.128			0.039	-0.070	0.149			-0.027	-0.118	0.064		
p,p'-DDE	0.017	-0.149	0.182			0.027	-0.130	0.183			-0.031	-0.172	0.110			0.058	-0.087	0.202		*	-0.029	-0.172	0.114			-0.060	-0.179	0.060		
o,p'-DDT	-0.069	-0.210	0.073			-0.035	-0.169	0.099			-0.024	-0.146	0.097			-0.011	-0.136	0.114			0.067	-0.056	0.190			-0.031	-0.134	0.071		
p,p'-DDT	-0.020	-0.198	0.158			-0.016	-0.186	0.154			0.007	-0.146	0.160			-0.023	-0.181	0.136			0.024	-0.130	0.179			-0.012	-0.141	0.118		
Dieldrin	-0.093	-0.304	0.118			0.022	-0.179	0.224			-0.059	-0.240	0.122			0.081	-0.106	0.269			0.164	-0.019	0.347	†		-0.022	-0.174	0.130		*
cis-HCE	-0.040	-0.238	0.158		†	-0.009	-0.198	0.181			-0.053	-0.224	0.118			0.044	-0.133	0.221			0.098	-0.074	0.271			-0.028	-0.173	0.117		
HCB	0.122	-0.178	0.422			0.033	-0.253	0.319			-0.060	-0.317	0.198			0.093	-0.173	0.359			0.126	-0.135	0.387			0.053	-0.165	0.271		
β-HCH	0.035	-0.151	0.220			0.050	-0.126	0.227			-0.014	-0.173	0.145			0.065	-0.099	0.229			0.056	-0.106	0.217			-0.004	-0.139	0.130		
Mirex	0.061	-0.146	0.268		†	0.027	-0.171	0.226			-0.154	-0.332	0.024	†		0.182	-0.003	0.366	†		0.018	-0.163	0.200			-0.019	-0.171	0.133		
Parlar-26	-0.046	-0.190	0.099			0.061	-0.077	0.198			-0.004	-0.128	0.121			0.065	-0.064	0.193			0.127	0.003	0.252	*		0.036	-0.069	0.140		
Parlar-50	-0.027	-0.181	0.127			0.032	-0.114	0.179			-0.010	-0.142	0.123			0.042	-0.095	0.179			0.127	-0.006	0.260	†		0.044	-0.067	0.156		

	Androstenedione/DHEA					Testosterone/Androstenedione					Cortisol					Cortisone					Cortisone/Cortisol					Adrenal androgen/ Glucocorticoid				
Oxychlordane	-0.051	-0.263	0.161			-0.083	-0.195	0.029			-0.084	-0.402	0.234			-0.014	-0.436	0.408		†	0.071	-0.138	0.281			0.100	-0.386	0.585		†
cis-Nonachlor	-0.018	-0.205	0.168			-0.067	-0.166	0.032		†	-0.009	-0.290	0.272			0.066	-0.306	0.438		*	0.076	-0.108	0.260		†	0.022	-0.405	0.450		*
trans-Nonachlor	-0.009	-0.194	0.175		*	-0.086	-0.184	0.011	†	*	-0.057	-0.334	0.220		*	0.040	-0.328	0.407		*	0.097	-0.085	0.280		†	0.044	-0.378	0.466		*
p,p'-DDD	0.050	-0.065	0.164			-0.013	-0.074	0.048			0.133	-0.038	0.305			0.186	-0.040	0.413			0.053	-0.059	0.165		†	-0.230	-0.490	0.031	†	
o,p'-DDE	-0.066	-0.190	0.058			-0.008	-0.074	0.058			-0.139	-0.325	0.048			-0.088	-0.336	0.161			0.052	-0.071	0.174			0.142	-0.143	0.428		
p,p'-DDE	-0.032	-0.195	0.131			0.029	-0.057	0.115		†	-0.092	-0.337	0.153			0.017	-0.308	0.343			0.107	-0.054	0.269			0.006	-0.368	0.380		†
o,p'-DDT	-0.097	-0.237	0.042			0.006	-0.068	0.081			-0.215	-0.424	-0.006	*		-0.104	-0.384	0.176			0.110	-0.028	0.248			0.199	-0.123	0.520		
p,p'-DDT	-0.037	-0.213	0.140			0.019	-0.075	0.112		*	-0.064	-0.331	0.202			0.003	-0.350	0.356			0.067	-0.106	0.240		*	0.058	-0.348	0.465		
Dieldrin	-0.189	-0.397	0.019	†		-0.035	-0.145	0.075		*	-0.094	-0.409	0.221			-0.243	-0.661	0.176			-0.149	-0.356	0.057			0.312	-0.168	0.793		
cis-HCE	-0.189	-0.326	0.068			-0.024	-0.127	0.080		*	0.001	-0.297	0.298			-0.092	-0.486	0.302			-0.094	-0.289	0.101			0.139	-0.314	0.592		
HCB	-0.076	-0.373	0.221			-0.112	-0.269	0.046			0.014	-0.433	0.461			0.146	-0.448	0.740			0.132	-0.162	0.426			0.036	-0.647	0.719		
β-HCH	-0.063	-0.246	0.121			-0.009	-0.106	0.089			-0.070	-0.347	0.206			-0.035	-0.402	0.333			0.035	-0.147	0.217			0.101	-0.321	0.524		
Mirex	-0.037	-0.243	0.169			-0.134	-0.243	-0.026	*		-0.207	-0.515	0.100		*	-0.047	-0.456	0.362		*	0.161	-0.042	0.364			0.122	-0.349	0.593		*
Parlar-26	-0.092	-0.235	0.051			-0.039	-0.115	0.037			-0.070	-0.286	0.145			-0.100	-0.386	0.186			-0.031	-0.172	0.111			0.209	-0.119	0.536		
Parlar-50	-0.084	-0.237	0.068			-0.053	-0.134	0.028			-0.070	-0.300	0.159			-0.058	-0.362	0.247			0.012	-0.139	0.163			0.186	-0.163	0.536		

	SHBG					Prolactin				
Oxychlordane	-0.014	-0.136	0.108			0.011	-0.144	0.167		
cis-Nonachlor	0.004	-0.104	0.112			-0.041	-0.177	0.096		
trans-Nonachlor	0.012	-0.095	0.119			0.003	-0.133	0.139		
p,p'-DDD	0.058	-0.008	0.123			0.070	-0.013	0.153	†	
o,p'-DDE	-0.016	-0.087	0.056			-0.082	-0.172	0.009	†	
p,p'-DDE	0.001	-0.092	0.095			-0.060	-0.178	0.058		*
o,p'-DDT	-0.006	-0.087	0.075			-0.090	-0.191	0.011	†	
p,p'-DDT	0.007	-0.095	0.109			-0.063	-0.191	0.065		
Dieldrin	-0.020	-0.140	0.101			-0.201	-0.354	-0.048	*	
cis-HCE	0.040	-0.074	0.153			-0.070	-0.215	0.075		
HCB	-0.029	-0.200	0.141			-0.057	-0.273	0.159		
β-HCH	-0.002	-0.107	0.103			-0.078	-0.212	0.056		
Mirex	-0.041	-0.160	0.078			-0.029	-0.180	0.121		
Parlar-26	0.008	-0.075	0.090			-0.066	-0.171	0.040		
Parlar-50	-0.011	-0.099	0.077			-0.069	-0.181	0.043		

βs were changes of hormone levels (log 10 transformed) of 10 fold increase of each OCP, calculated by linear regression model.

a) P for OCP adjusted for Maternal age, Parity, Gestational age, and (sex) x (each OCP); b) P for interaction of sex and OCP.

*P < 0.05, **P < 0.01, †P <0.1