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# Prenatal exposure to persistent organochlorine pollutants and female reproductive function in young adulthood

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ABSTRACT

*Background:* The biopersistent organochlorine pollutants dichlorodiphenyldichloroethylene (p,p′-DDE), hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs) can be detected in humans worldwide. The chemicals can cross the placenta and may interfere with endogenous hormonal homeostasis.
*Objectives:* To investigate effects on female reproduction following intrauterine exposure to selected biopersistent organochlorines.
*Methods:* We used data from a Danish pregnancy cohort with follow-up on 436 eligible daughters at approximately 20 years of age. Information on age of menarche ($n = 335$), menstrual cycle length ($n = 230$) and serum concentrations of reproductive hormones ($n = 243$) was obtained. Number of antral follicles was counted by vaginal ultrasound ($n = 147$). Of 244 daughters who attended clinical examination, 170 used hormonal contraceptives and 74 were non-users. Concentrations of p,p′-DDE, HCB and six PCB congeners were analysed in maternal serum samples obtained in pregnancy week 30.
*Results:* Age of menarche and menstrual cycle length were found not to be statistically significant associated with prenatal organochlorine exposure. Among non-users of hormonal contraceptives with information on antral follicle number ($n = 43$), daughters exposed to the highest tertile of p,p′-DDE had 28% (95% confidence interval (95% CI): 5; 46%) lower follicle number compared to the low-level exposed reference group. Those exposed to medium and higher levels of HCB had 30% (95% CI: 5; 48%) and 28% (95% CI: 7; 44%) lower follicle number compared to the reference group. Furthermore, maternal serum HCB concentrations were inversely associated with free androgen index among non-users of hormonal contraceptives ($n = 73$). These associations were not found in users of hormonal contraceptives.
*Conclusions:* Among non-users of hormonal contraceptives, we found indications of adverse long-term effects on female reproduction following prenatal exposure to biopersistent organochlorines. These findings may have wide implications for public health as intrauterine exposure occurs worldwide.


## 1. Introduction

The persistent organochlorine pollutants dichlorodiphenyldichloroethylene (p,p′-DDE), hexachlorobenzene (HCB) and the polychlorinated biphenyls (PCBs) are suspected to interfere with endogenous hormonal homeostasis (Crain et al., 2008; Peña et al., 2012). Since these chemicals can pass the placental barrier (Bergonzi et al., 2009), concern exists about the impact of prenatal exposure (Crain et al., 2008). Many important steps in formation of the female reproductive system take place during intrauterine life and disruptions in development may affect the function permanently (Newbold, 2004).

The p,p′-DDE precursor dichlorodipenyltrichloroethane (DDT), HCB and PCBs have been extensively used in a wide range of agricultural and

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industrial applications. The primary exposure route is through ingestion of contaminated food of animal origin but exposure can also occur through inhalation of contaminated air (Frederiksen et al., 2012; Reed et al., 2007; Roosens et al., 2010). The compounds accumulate in adipose tissue and are slowly metabolised or excreted (Bergonzi et al., 2009). Although production and use of the organochlorine pollutants now have been banned or restricted, the compounds are widely present in the environment (Bergonzi et al., 2009). Hence, pregnant women and thereby their offspring are still exposed as reflected in detection of the chemicals in maternal serum and cord blood (Bergonzi et al., 2009; Lopes et al., 2014).

Human data of the possible impact on female reproductive function following early life exposure to p,p′-DDE, HCB and PCBs have been investigated through evaluations of hormone levels, ultrasonographic measures, timing of puberty and menstrual cycle characteristics in childhood and adolescence (Axmon, 2006; Blanck et al., 2000; Cao et al., 2008; Gladen et al., 2000; Su et al., 2012; Vasiliu et al., 2004; Yang et al., 2005). Associations between prenatal exposure and reproductive function in adulthood have so far only been studied in one cohort, where maternal levels of p,p′-DDE and some PCB congeners were found to affect time-to-pregnancy (Cohn et al., 2003, 2011). Hence, there is a paucity of human studies with sufficient follow-up time to investigate the possible long-term effects of intrauterine exposure to persistent organochlorine pollutants. To our knowledge, no previous studies have evaluated the implications of in utero exposure to HCB. Furthermore, the number of antral follicles and serum levels of anti-Müllerian hormone (AMH), which are both markers of ovarian reserve (Gleicher et al., 2013), have not been studied as parameters of reproductive function in adulthood.

In this study, we aimed to investigate whether maternal levels of persistent organochlorine pollutants during pregnancy were associated with reproductive hormone levels, menstrual cycle characteristics and ultrasonographic measures of adult daughters using data from a population-based follow-up study.

## 2. Methods

### 2.1. Study population

The participants were female offspring from a Danish pregnancy cohort established in 1988–89 (Olsen et al., 1995). The mothers were recruited at a midwifery clinic in the city of Aarhus at a routine visit in pregnancy week 30. Serum samples were obtained from 965 of 1212 eligible women with uncomplicated pregnancies. Furthermore, the mothers were interviewed and completed a questionnaire. Information on age, parity, height, pre-pregnancy weight, socioeconomic status and smoking and alcohol habits during pregnancy was collected.

Follow-up of the offspring was initiated in 2008 when the daughters were approximately 20 years of age. Questionnaires on reproductive health and lifestyle habits were sent to 436 eligible daughters. Furthermore, daughters were invited to participate in a clinical examination which was conducted from August 2008 to August 2009.

Maternal serum samples were missing for 21 participants. The final study population comprised 341 women (78% of eligible daughters) of which 252 had participated in the examination and 89 had answered the questionnaire only. Among the 252 daughters who had participated in the examinations, eight of them were excluded from analyses on outcome measures besides age of menarche because of breastfeeding at time of examination ($n = 1$), signs of premature ovarian failure ($n = 1$), and lack of several key information including hormonal contraceptive use ($n = 6$).

The study was approved by the local ethical committee of Central Denmark Region (registration number M-20070157) and informed written consent was obtained from all participants.

### 2.2. Exposure

Maternal serum samples from pregnancy week 30 were cryopreserved at −20 °C until analyses in 2011. Concentrations of p,p′-DDE, HCB and six PCB congeners (118, 138, 153, 156, 170 and 180) were measured by liquid-liquid extraction, silica column cleanup and gas chromatography high-resolution mass spectrometry analyses. Details on sample pre-treatment for persistent organochlorine analyses have been described previously (Koponen et al., 2013). In each batch of 25 samples, two blanks were included to control for laboratory contamination. Additionally, two control samples were added. Average recoveries of measured POPs in control samples were 97–106% of the certified values. The between-assay coefficient of variation was 2.1% (at 11.5 ng/ml) for p,p′-DDE, 4.0% (at 0.08 ng/ml) for HCB, 4.0% (at 0.17 ng/ml) for PCB 118, 4.2% (at 0.54 ng/ml) for PCB 138, 2.7% (at 0.94 ng/ml) for PCB153, 6.5% (at 0.07 ng/ml) for PCB 156, 6.7% (at 0.21 ng/ml) for PCB 170, and 2.6% (at 0.52 ng/ml) for PCB 180. The limit of quantification (LOQ) for HCB was 25 pg/ml. For p,p′-DDE and the six PCBs, LOQs were between 2 and 5 pg/ml. The analyses were performed at the National Institute of Health and Welfare, Chemical Exposure Unit, Kuopio, Finland. Chemical Exposure Unit is testing laboratory number T077 accredited according to ISO/IEC 17025 by Finnish Accreditation Services (FINAS).

### 2.3. Outcome measures

The reproductive function of the daughters was evaluated as age of menarche, menstrual cycle length, number of ovarian follicles and serum levels of total testosterone, sex hormone-binding globulin (SHBG), free androgen index (FAI) (calculated as 100 × total testosterone / SHBG), FSH, luteinizing hormone (LH), estradiol and AMH.

Data on age at menarche was obtained from the questionnaire and provided by 335 daughters. Information on current menstrual cycle length was obtained at the examination ($n = 230$). For users of hormonal contraceptives, recalled cycle length from prior to commencement of these medications was used in statistical analyses.

In order to measure levels of reproductive hormones, a blood sample was obtained between 8:00 and 12:30 h ($n = 243$). After centrifugation, serum samples were stored at −80 °C until assessment. Immunoassay analyses were performed to quantify levels of total testosterone, FSH, LH and estradiol (cobas 6000 e 601, Roche Diagnostics, Mannheim, Germany) with CVs of 2.2–4.5%, 1.9–2.1%, 1.1–2.4% and 1.5–4.5%, respectively, and SHBG (IMMULITE 2000, Siemens Healthcare, Gwynedd, UK) with CV of 4.5–4.7%. Analyses were performed at Department of Clinical Biochemistry, Aarhus University Hospital, Denmark. Concentrations of AMH were measured using specific ELISA kits (DSL-10-14400; Diagnostic System Laboratories Inc., Webster, TX, USA) with inter- and intra-assay variations <10% at the Laboratory of Reproductive Biology, Copenhagen University Hospital, Rigshospitalet, Denmark. For data included in statistical analyses, two measurements of testosterone were below the detection limit (LOD) and were set at LOD/2 (0.20/2 nmol/l).

Ovarian antral follicles measuring 2–9 mm were counted in 147 participants by transvaginal ultrasonography using VOLUSON e (GE Healthcare, Zipf, Austria) devices as previously reported (Kristensen et al., 2010). All examinations were performed by the same physician. The number of follicles per ovary was counted by scanning from one margin of the ovary to the other. The follicle number used in statistical analyses was calculated as the mean follicle number of right and left ovary. For 39 women (30 users and 9 nonusers of hormonal contraceptives) only one ovary could be visualised sufficiently to count the number of antral follicles. If it was not possible to count follicles in both ovaries, the number from only one ovary was used in analyses.

### 2.4. Statistics

PCB exposure was evaluated as a summed measure of all six PCB congeners ($\sum$ PCBs). Before summation, the PCB wet weight concentrations