



# Prenatal exposure to polychlorinated biphenyl and umbilical cord hormones and birth outcomes in an island population<sup>☆</sup>



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## ABSTRACT

Prenatal exposure to polychlorinated biphenyl (PCB) is suspected to interfere with fetal development including reproductive and thyroid function and birth outcomes, but published evidence are still sparse. We designed a cross-sectional study to analyze the associations between PCB levels in umbilical cord sera and hormones and birth outcomes of mothers and newborns who are residents from an island. Seven indicator-PCB (PCB-28, 52, 101, 118, 138, 153, 180), and five reproductive hormones including luteotropic hormones (LH), estradiol (E2), testosterone (T), follicle-stimulating hormones (FSH) and anti-Mullerian hormones (AMH), and three thyroid hormones including tri-iodothyronine (T3), tetra-iodothyronine (T4) and thyroid stimulating hormones (TSH) were measured in 106 cord sera specimens. Birth outcomes include birth weight, length, head circumference, and gestational age. Multiple linear regression and quartile regression were used to analyze the associations between PCB and each of the hormones and birth outcomes, adjusting for selected potential confounders. The median value of total PCB in umbilical cord sera was  $2.02 \mu\text{g L}^{-1}$  (IQR, 1.13–4.64). Several negative associations between PCB exposure and reproductive hormones were found. Among them, the  $\beta$  value of PCB-101 for FSH reached  $-0.38$  (95%CI,  $-0.69, -0.07$ ;  $p = 0.02$ ). Moreover, we also found some sex-specific associations i.e. PCB-28 was negatively correlated with LH and T and PCB-118 was negatively correlated with T in male newborns but not in female newborns. The associations between PCB and birth outcomes seem to differ by molecular weight of the PCB congeners i.e. the low-chlorinated PCB congeners were negatively associated with gestational age and head circumference while high-chlorinated PCB congeners were positively associated birth weight and gestational age. In this study, we found that PCB congeners with different molecular weight has different associations with hormones and birth outcomes, and future studies are recommended to investigate underlying mechanisms of these associations.

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## 1. Introduction

Fetal growth inhibition has been linked to exposures to environmental contamination and persistent organic pollutants (POPs) during fetal development. Many POPs are stable, lipophilic and have endocrine disruptive properties (Zhang et al., 2018). Fetal and infant endocrine systems may encounter POPs during intrauterine development and this may alter hormones (Berg et al., 2017) and

negatively affect birth outcomes (Casas et al., 2015). Birth weight, length, head circumference, and gestational age are indicators that are commonly used to study growth and development and adverse birth outcomes have been linked to later life health outcomes such as diabetes (Tang et al., 2014), overweight (Karlsen et al., 2017), and cancer (Vafeiadi et al., 2014).

Polychlorinated biphenyl (PCB) are industrial POPs used as heat transfer fluids in transformers and 209 chemical congeners may exist. Among them, seven indicator-PCB (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) which have greater bioaccumulation represent contamination according to the Global Environment Monitoring System (GEMS)/Food program of the World Health Organization (WHO). In China, PCB have been

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produced since 1965 but were not synthesized after 1974. During 1965 to 1974, overall 10 thousand tons of PCB were produced and along with imported PCB, usage reached 20 thousand tons (Xing et al., 2005). Due to nearby electronic waste (e-waste) recycling centers, pollutants on the studied island which is located in an estuary of two rivers were greater than in other places in China (Tang et al., 2016a, 2016b; Xu et al., 2016; Yin et al., 2017). Thus, mother and newborns have an exposure risk due to this PCB exposure (Song and Li, 2014).

PCB are related to adverse health effects including carcinogenesis (Rodgers et al., 2018), immunotoxicity (Heilmann et al., 2010), neurodevelopmental abnormalities (Fimm et al., 2017) and reportedly endocrine disruption (Eskenazi et al., 2017). Competitive binding to nuclear receptors or interfering with production, transport or regulation of hormones may explain how PCB can change the endocrine system (Koutros et al., 2015). The hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) axes are endocrine systems that regulate growth and development (Cao et al., 2008). Weak environmental estrogens as PCB (Bergeron et al., 1994) may interfere with reproductive hormones (Verner et al., 2015). Besides, due to structural similarities between PCB and thyroxine, competitively binding to transthyretin may be a mechanism of disrupted thyroid function due to PCB exposure (Brouwer et al., 1990).

Significant effects on reproductive (Warembourg et al., 2015), thyroid hormones (Hisada et al., 2014), and infant growth have been reported to be associated with PCB exposure in a few birth cohort studies. Pregnant women and newborns, may also be susceptible to health risks from PCB exposure. An American study that enrolled 160 children suggested that *in utero* PCB exposure is slightly associated with the increase of thyroid hormones at birth (Longnecker et al., 2000). Other associations were found between PCB and sex hormones in a French study that included 282 mother-child pairs (Warembourg et al., 2015). A study that used pool-data from 7 European birth cohorts including 2487 mother-child pairs reported that postnatal PCB-153 exposure was associated with decreased infant growth (Iszatt et al., 2015). Another study that combined 11 cohorts data also from Europe including 9377 mother-infant pairs, noted a 194 g decline in birth weight per  $1 \mu\text{g L}^{-1}$  increase in PCB-153 (Casas et al., 2015). Even so, isolated community from highly PCB polluted has not been well studied.

Previous study has suggested that noninvasive biomarkers, such as umbilical cord sera samples is safe for mothers and newborns and can represent PCB exposure *in utero* quite well (Jaraczewska et al., 2006). We used a cross-sectional study design to evaluate PCB exposure of Chinese fish consumers living on the easternmost island and assessed the associations with hormones and birth outcomes.

## 2. Materials and methods

### 2.1. Study population and area

We conducted a cross-sectional study based on samples from mother-newborn pairs living on Shengsi Island at the east end of China and between the Yangtze and Qiantang River mouths. The two rivers flow through the Yangtze River Delta which one of the largest e-waste recycling centers that release PCB on Shengsi Island. There were about 350 births from Shengsi Island annually. We reached out to 240 healthy pregnant women who planned to deliver in the only deliver hospital on the island from July 2011 to May 2012. We informed the procedure and significance of this study to all the women, and 113 (47%) eligible pregnant women with a singleton pregnancy filled out the questionnaires and signed the informed consent forms. In addition, umbilical cord blood

samples from 106 mother-newborn pairs were collected. All procedures were performed in compliance with relevant laws and institutional guidelines.

### 2.2. Sample collection and hormone levels detection

Umbilical cord blood was sampled just at birth in sterile centrifuge tubes and centrifuged to extract serum. Umbilical cord serums were frozen below  $-20^\circ\text{C}$  for laboratory detection. Considering hemolysis of the cord serum may affect the detection results of hormone levels negatively, we exclude these samples suspected to be affected by hemolysis and we finally analyzed the hormone levels in 76 cord serum samples. Enzyme linked immunosorbent assay (ELISA) kits (Cloud-Clone Corporation, the U.S.) were used to quantify the reproductive hormones levels including luteotropic hormone (LH), estradiol (E2), testosterone (T), follicle-stimulating hormone (FSH) and *Anti-Mullerian* hormones (AMH) and the thyroid hormone levels measured including triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone (TSH). The intra- and inter-assay coefficients of variation were less than 10% and 12% respectively which were the limits of the ELISA kit.

### 2.3. Outcome and covariate assessment

Birth outcome measurements included anthropometric variables, such as weight, height, head circumference, and gestational age. These were measured and recorded after birth by midwives and nurses according to standard procedures and recorded. Gestational age was calculated based on the date of the last menstrual period and delivery date and if menstrual date were unknown, a typical ultrasound measurement at 12 weeks was used.

Covariates including sociodemographic characteristics, reproductive histories (parity and pregnancy termination times), body mass index before pregnancy (preBMI), fish consumption (more than five days a week), drinking, smoking, and history of illness were collected from baseline questionnaires. Because almost all the tap water available to islanders were desalinated seawater, and the source of drinking water of some residents were well water (5.9%) and bottled water (24.5%), thus we added the covariate of water type in the multiple regression analyses.

### 2.4. PCB analyses in umbilical cord serum

In this study, we focused on the seven indicative PCB (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) recommended by WHO. All measurements were conducted in College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China. We modified the PCB analytical method in serum described in another study (Jaraczewska et al., 2006), and the detailed information of preparation, extraction, and Gas chromatograph-mass spectrometer (GC-MS) analyses were described in a recently published article (Tang et al., 2016a,b). Briefly, to determine PCB, 2 mL samples spiked with surrogate standard PCB-209 (J&K Chemical, Beijing, China) were extracted by 30 mL *n*-hexane and dichloromethane (2:1) three times. The combined extract was filtered by anhydrous sodium sulfate and purified by acidic silica gel to obtain the analyte. After rotary evaporation and gentle nitrogen blow-down to 50  $\mu\text{L}$ , GC-MS equipped with an HP-5 MS column was used to analyze the concentrated extracts. The helium flow rate was  $1.5 \text{ mL min}^{-1}$ . Initial temperature was  $90^\circ\text{C}$ , increased at  $10^\circ\text{C min}^{-1}$  to  $150^\circ\text{C}$ , held for 1 min, then increased at  $20^\circ\text{C min}^{-1}$  to 260, held for 5 min. The injector temperature was kept at  $250^\circ\text{C}$ . The limit of detection (LOD) of PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and

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