# Determination of organochlorine pesticides in human umbilical cord and association with orofacial clefts in offspring

Wenlei Yanga#, Wenli Nia,b #, Lei Jina, Jufen Liua, Zhiwen Lia, Linlin Wanga\*, Aiguo Rena

**Affiliations:** a Institute of Reproductive and Child Health, National Health Commission Key Laboratory of Reproductive Health, Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, Beijing, China

b Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany

#These authors contributed equally to this work.

**Address correspondence to**: Dr. Linlin Wang, Institute of Reproductive and Child Health, Peking University, Beijing 100191, China. Telephone: +86(10)82802976. E-mail address: [linlinwang@bjmu.edu.cn](mailto:linlinwang@bjmu.edu.cn)

# **Abstract**

Studies based on questionnaires suggested that maternal exposure to pesticides increases the risk for orofacial clefts (OFCs). However, whether organochlorine pesticides (OCPs) exposure *in vivo* affects the occurrence of OFCs remains unclear.The aims of this study are to investigate the association of OCP exposure with the risk of OFCs by examining the concentrations of OCPs in human umbilical cords, and investigate the potential dietary sources of OCPs in umbilical cord tissues.A case-control study consisting of 89 OFC cases and 129 nonmalformed controls with available tissues of umbilical cord was conducted. Concentrations of twenty specific OCPs were determined in the umbilical cord by gas chromatograph-mass spectrometry, and seven OCPs with detection rate larger than 50% were included in analyses. The individual effect and joint effect of multiple OCPs in umbilical cords on the risk for OFCs were investigated using multivariate logistic models and Bayesian Kernel Machine Regression (BKMR). No difference was found in the median levels of ΣOCPs between cases (1.04 ng/g) and controls (1.03 ng/g). No significant associations were observed between levels of OCPs in umbilical cords and risk for OFCs in either multivariate logistic models or BKMR models. Maternal consumptions of beans or bean products were positively correlated with levels of β-hexachlorocyclohexane, heptachlor epoxide, p,p′-DDE, and ∑OCPs in umbilical cord, respectively. In conclusion,we didn’t find the association between *in utero* exposure to OCPs and the risk for OFCs. Maternal consumptions of beans or bean products may be a source of OCPs exposure.

# Key words

Umbilical cord tissue; orofacial clefts; organochlorine pesticides; joint effects

# Introduction

Orofacial clefts (OFCs) are among the most common and burdensome birth defects, which arise in about 1 in 700 livebirths, with ethnic and geographic variation (Mossey et al. 2009). Generally, children with OFCs need multidisciplinary care from birth to adulthood and have higher morbidity and mortality throughout life than do unaffected individuals (Mossey et al. 2009). Although there has been progress in identifying environmental and genetic factors for OFCs, the etiology of OFCs remains poorly elucidated (Dixon et al. 2011). Some environmental risk factors have been identified for OFCs, including maternal exposure to indoor air pollution, tobacco smoke, dioxins, and PM2.5 (Honein et al. 2007; Leskow et al. 2019; Liu et al. 2016; Zhou et al. 2017).

Organochlorine pesticides (OCPs) are a group of important organic pollutants, which have been widely used worldwide because of low cost and their good performance against plant disease and various pests (Li et al. 2014; Pirsaheb et al. 2015). Although the use of OCPs was banned worldwide since 1970s, the residues of different OCPs have been reported in human blood, fatty tissues, and breast milk (Konishi et al. 2001; Liu et al. 2004; Ma et al. 2013; Song et al. 2012; Yu et al. 2006). Additionally, dichlorodiphenyltrichloroethane (DDT) was identified as new main source of OCPs in recent years (Qiu et al. 2005), which resulted in OCPs becoming ubiquitously occurring environmental contaminants. The adverse effects of the OCPs on humans’ health are of high concern because of its worldwide use, its accumulation in the human food chain, and its long half-life in human tissues due to its lipophilicity (Konishi et al. 2001; Liu et al. 2004; Ma et al. 2013; Song et al. 2012; Yu et al. 2006). More importantly, OCPs have the ability to transfer from mother to fetus/baby through placental transport or breastfeeding (Mishra and Sharma 2011; Sala et al. 2001). Exposure to OCPs, such as Hexachlorocyclohexanes (HCHs), p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE) and hexachlorbenzene (HCB) are related to birth defects, such as neural tube defects (Ren et al. 2011), hypospadias (Rignell-Hydbom et al. 2012). Previous case-control studies based on questionnaires suggested that maternal exposure to pesticides increases the risk for OFCs (Garcia et al. 1999; Hao et al. 2015; Xu et al. 2015). However, these associations may be interfered due to the recall bias and reporting bias. To date, no study has explored the association between *in utero* exposures to OCPs, and the occurrence of OFCs.

Previous studies have found that human is generally exposed to multiple environmental pollutants, rather than just a single environmental pollutant at the same time (Crinnion 2010; Woodruff et al. 2011). Most pollutants are highly correlated to each other, so that an additive or synergic effect cannot be excluded (Billionnet et al. 2012). Recently, an increasing number of studies have used multi-pollutant analysis to assess the association between the environmental pollutants mixture exposure and health effects, instead of analyzing the effects of single pollutants (Lim et al. 2019; Valeri et al. 2017). However, traditional methods to analyze pollutants mixtures in association studies have methodological limitations (Billionnet et al. 2012). Bayesian Kernel Machine Regression (BKMR), a recently proposed method, is used to estimate the health effects of multi-pollutant mixtures (Bobb et al. 2015; Bobb et al. 2018). At present, no studies have investigated the combined effects of multiple OCPs on human health.

Humans could be exposed to pesticides through different pathways including air, dermal penetration and ingesting contaminated food. The two former pathways comprise only approximately less than 2% of the total absorption of pesticides, and the ingesting contaminated food is considered as the main potential for exposure to pesticides (Hu et al. 2004; Lu et al. 2004; Morgan et al. 2007; Pirsaheb et al. 2015).

Therefore, this study aimed to examine the association between concentrations of OCPs in umbilical cord tissues and risk for OFCs using multivariate logistic models and BKMR models in a case-control study. In addition, we investigated the potential dietary sources of OCPs in umbilical cord tissues.

# Materials and methods

## 2.1 Study Population and sample collection

We have carried out an on-going population-based case-control study since 2002, and details have been described elsewhere (Pi et al. 2018; Ren et al. 2011). Briefly, the subjects were recruited from five rural counties (Xiyang, Taigu, Pingding, Zezhou, and Shouyang) of Shanxi Province in northern China. For each fetus or newborn diagnosed with birth defect, a non-malformed newborn was selected as a control in the same hospital matching with last menstrual period (plus or minus 4 weeks), residence of the mother (the same county), and infant gender. Diagnoses were confirmed using physical examination and/or prenatal ultrasound examination by trained health workers. Umbilical cord tissues were obtained from the neonatal umbilicus after delivery, and immediately stored at −20°C. In the present study, 89 OFC cases (48 with cleft lip with cleft palate, 36 with cleft lip only, and 5 with cleft palate only) and 129 controls collected from 2003 to 2016, and with available umbilical cord samples (≥7g wet weight) were included.

Maternal face-to face interviews were conducted by trained local health workers using a structured questionnaire, within a week after delivery. We collected information regarding maternal age at pregnancy, body mass index (BMI), infant sex (male or female), education (“Primary school or lower”, “Junior high school” or “High school or above”), occupation (farmer or other), gravidity (1, 2 or >2 times), history of birth defects (yes or no), periconceptional use of a folic acid supplement (yes or no), and alcohol consumption (yes or no). Smoking exposure was defined as maternal active or passive smoking from any source (at home or in public places) measured through self-reported exposure (at least once per week, and at least one cigarette at a time) from one month before and two months after conception. Information on dietary intake habits was also collected, including frequency of consumption of fish or meat (<1, 1–3, 4–6, >6 times per week), frequency of consumption of milk or eggs (<1, 1–3, 4–6, >6 times per week), frequency of consumption of fresh green vegetables (<1, 1–3, 4–6, >6 times per week), frequency of consumption of fresh fruit (<1, 1–3, 4–6, >6 times per week), and frequency of consumption of beans or bean products (<1, 1–3, 4–6, >6 times per week). Our study was approved by the Institutional Review Board of Peking University. All participating mothers provided written informed consent.

## 2.2 Determination of umbilical cord OCPs

Gas chromatography-mass spectrometry (GC-MS, Agilent 7890B-5977A, USA) was used to quantify the concentrations of twenty specific OCPs. The detailed descriptions of the method are listed on the Supplementary materials. Firstly, about 0.700 g of lyophilized umbilical cord tissue sample was spiked with 20 ng of two recovery surrogates (polybrominated diphenyl ethers-77 and polybrominated diphenyl ethers-128, Dr. Ehrenstorfer, Germany) and then Soxhlet-extracted with 120 mL of dichloromethane for 24 hours. All the extracts were concentrated and their lipophilic material in extractions was filtrated using gel permeation chromatograph GPC. After that, a silica/alumina gel cleanup column was used to further [purification](https://www-sciencedirect-com.emedien.ub.uni-muenchen.de/topics/earth-and-planetary-sciences/purification). The sequential eluents were 20 mL n-hexane/dichloromethane (1:1, v/v). Pentachloronitrobenzene was then added as the injection internal standard. The fractions containing OCPs were further concentrated to about 100 μL prior to quantitative analysis. Finally, a total of twenty priority OCPs, including hexachlorobezene (HCB), α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), γ-hexachlorocyclohexane (γ-HCH), δ-hexachlorocyclohexane (δ-HCH), heptachlor (HEC), heptachlor epoxide (HCEAB), aldrin (ALD), endrin (END), isodrin (IDR), α-endosulfan (ENS I), β-endosulfan (ENS II), o,p’-DDE (op-DDE), o,p’-DDT (op-DDT), o,p’-DDD (op-DDD), p,p’-DDE (pp-DDE), p,p’-DDT (pp-DDT), p,p’-DDD (pp-DDD), mirex (MRI), and dieidrin (DIE), were determined with a gas chromatography coupled to GC-MS.

## 2.3 Quality control

Two analytical procedure blank samples were prepared with each batch of the fourteen umbilical cord samples. The operators were blinded to case/control status, and similar number of cases and controls were randomly ordered in each batch and GC-MS measurements. This system was calibrated using pentachloronitrobenzene (Dr. Ehrenstorfer, Germany) as the internal reference.

## 2.4 Statistical analysis

Subject characteristics in case and control groups were compared by Chi-squared test or Fisher’s exact test for categorical variables, and t-test for continuous variables. The concentrations of OCPs were described using the median with interquartile range because of the skewed distribution, and the difference between cases and controls were evaluated by the Wilcoxon rank sum test. OCP concentrations were categorized in dichotomy based on the median values among all subjects. Multivariate logistic regression models were used to estimate the associations between each of OCPs and risk for OFCs. Maternal age and BMI are the most important basic characteristics of subjects, and we empirically adjusted them in the model. Gestational age and maternal smoking showed significant statistical differences between the case and control groups, therefore we also adjusted them in the model. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the risk. Although the etiology of the two subtypes of orofacial clefts (cleft lip with or without cleft palate and cleft palate) is different, the sample size of the cleft palate (5) was so small that we did not perform a subgroup analysis of cleft palate. Furthermore, as a sensitivity analysis, we restricted association analyses to isolated OFCs.

To allow for potential synergistic and nonlinear effects among mixture components, in addition to the single-pollutant exposure model, we further applied Bayesian kernel machine regression (BKMR), which combines Bayesian and machine learning methods to regress an exposure-response function iteratively through the Gaussian kernel function. BKMR utilizes a non-parametric approach to flexibly model the joint effect of environmental mixtures (Bobb et al. 2015; Bobb et al. 2018). BKMR allows the visualization of the exposure-response association for each component of a mixture, while taking into account the correlation between the mixture components. The probit BKMR model used in this study is below: Yi=h (HCB, β-HCH, γ-HCH, IDR, HCEAB, ENS I, and pp-DDE) + βT Zi + ei. The function h (exposure) is an exposure-response function that accommodates nonlinearity and/or interaction among the mixture components and seven OCPs were included in this model with the detection rate >50%. The coefficients βare the effect estimates of the Zi covariate. The same covariates included in the multivariate logistic regression models were included in the BKMR model for confounding adjustment. OCPs concentrations were log-transformed in the BKMR model to improve model fit.

Spearman correlation tests were performed to examine the correlations between concentrations of each of OCPs, between gestational age and concentrations of OCPs, and between dietary intake habits and concentrations of OCPs.

Wilcoxon rank sum test and multivariate logistic regression controlled the type I errors on multiple comparisons by the Bonferroni correction, the significance level of the association analyses was calculated as 0.05/7 (the number of OCPs detected) = 0.007. Statistical tests were two-tailed and all *P* values < 0.05 were conventionally regarded as statistically significant.Analyses were performed with SPSS 23.0 software (SPSS, Chicago, IL, USA). The BKMR analysis was implemented with the R package bkmr (version 3.5.1; R Development Core Team).

# Results

## Study population Characteristics

The demographic characteristics of subjects summarized by case-control status are presented in Table S1. The majority of the mothers were identified as Han ethnicity (98.9%). Mothers of infant with OFC cases were more likely to have smoking exposure during early pregnancy and have shorter gestation age, as compared with the control group. We did not observe differences between the OFC cases and controls for other demographic characteristics.

## 3.2 Concentrations of OCPs in umbilical cord tissues

The levels of OCPs in umbilical cord tissues of OFC cases and controls were shown in Table 1. There was no significant difference between OFC cases and control groups. For α-HCH, δ-HCH, HEC, ALD, END, ENS II, op-DDE, op-DDT, op-DDD, pp-DDT, pp-DDD, MRI, and DIE, more than 50% of the subjects had levels lower than the limit of quantification (Table S2), therefore these chemicals were not analyzed in this study. There were low to moderate correlations among the seven OCPs in umbilical cord (r values ranging from 0.18 to 0.47, Figure S1).

A statistical difference in gestational age was observed between OFCs and controls. If the OCPs concentration changes with gestation age, gestation age will be an important confounding factor in this study. Hence, spearman correlation was used in this study to evaluate the correlation between OCPs concentrations in umbilical cord tissues and gestational age. We did not find correlation between OCP concentrations in umbilical cord tissues with gestational age (Table S3).

**Table 1 Concentrations of OCPs in umbilical cord samples from cases with OFCs and controls**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| OCPs (ng/g) *a* | Controls (n=129) |  | OFCs (n=89) | |  |
| Median  (P25–P75) |  | Median  (P25–P75) | *P b* |  |
| HCB | 0.04 (0.02-0.07) |  | 0.04 (0.03-0.08) | 0.259 |  |
| β-HCH | 0.66 (0.53-0.93) |  | 0.74 (0.44-1.05) | 0.487 |  |
| γ-HCH | 0.15 (0.01-0.02) |  | 0.01 (0.01-0.02) | 0.645 |  |
| IDR | 0.03 (0.02-0.05) |  | 0.04 (0.02-0.07) | 0.087 |  |
| HCEAB | 0.14 (0.01-0.02) |  | 0.01 (0.01-0.04) | 0.480 |  |
| ENS I | 0.14 (0.01-0.02) |  | 0.02 (0.01-0.02) | 0.354 |  |
| pp-DDE | 0.10 (0.04-0.30) |  | 0.11 (0.05-0.24) | 0.890 |  |
| ∑OCPs | 1.03 (0.72-1.45) |  | 1.04 (0.66-1.60) | 0.565 |  |

HCB, hexachlorobezene; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane; HCEAB, heptachlor epoxide; IDR, isodrin; ENS I , α-endosulfan; pp-DDE, p,p’-DDE; ΣOCPs is the sum of HCB, β-HCH,γ-HCH, HCEAB, IDR, ENS I, pp-DDE; OFCs, orofacial clefts

a The levels of OCPs in umbilical cord tissues of OFC cases and controls are based on a dry weight.

b Bonferroni corrections *P*-value threshold of 0.007 was considered statistically significant.

### 3.3.1 Multivariable logistic model to assess the association between OCPs concentration and OFCs

Table 2 showed the results from the multivariate logistic models for associations between the concentrations of the OCPs in umbilical cord and OFCs risk. No significant association was found between the concentration of the sum for OCPs (∑OCPs) in umbilical cord tissues and risk of OFCs in the models without adjusting confounders (crude OR [cOR] = 1.04, 95% CI: 0.61-1.78), or adjusting confounders (adjusted OR [aOR] = 0.81, 95% CI: 0.44-1.49). There was still no association between each single OCP (HCB, β-HCH, γ-HCH, IDR, HCEAB, ES1, and pp-DDE) in the umbilical cord tissue and risk for OFCs.

We also did the subgroup analysis of cleft lip with cleft palate and cleft lip only, and the results were similar to the primary analyses (Table S4).

**Table 2 Associations between concentrations of OCPs in umbilical cord and risks for OFCs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| OCPs (ng/g) | | Controls (n=129) | OFCs  (n=89) | | |
| n (%) | n (%) | cOR  (95% CI) | aOR a (95% CI) |
| HCB |  |  |  |  |  |
| Low | ≤0.043 | 66 (51.2 | 43 (48.3) | 1.12 (0.65-1.92) | 1.00 (0.56-1.83) |
| High | >0.043 | 63 (48.8) | 46 (51.7) |
| β-HCH |  |  |  |  |  |
| Low | ≤0.683 | 67 (51.9) | 42 (47.2) | 1.21 (0.70-2.08) | 0.890 (0.49-1.64) |
| High | >0.683 | 62 (48.1) | 47 (52.8) |
| γ-HCH |  |  |  |  |  |
| Low | ≤0.014 | 64 (49.6) | 45 (50.6) | 0.96 (0.56-1.65) | 0.98 (0.54-1.79) |
| High | >0.014 | 65 (50.4) | 44 (49.4) |
| IDR |  |  |  |  |  |
| Low | ≤0.036 | 69 (53.5) | 40 (44.9) | 1.41 (0.82-2.42) | 1.10 (0.61-2.00) |
| High | >0.036 | 60 (46.5) | 49 (55.1) |
| HCEAB |  |  |  |  |  |
| Low | ≤0.013 | 60 (46.5) | 49 (55.1) | 0.71 (0.41-1.22) | 0.56 (0.30-1.04) |
| High | >0.013 | 69 (53.5) | 40 (44.9) |
| ENS I |  |  |  |  |  |
| Low | ≤0.014 | 69 (53.5) | 40 (44.9) | 1.41 (0.82-2.42) | 1.22 (0.67-2.22) |
| High | >0.014 | 60 (46.5) | 49 (55.1) |
| pp-DDE |  |  |  |  |  |
| Low | ≤0.102 | 66 (51.2) | 43 (48.3) | 1.12 (0.65-1.92) | 1.00 (0.55-1.83) |
| High | >0.102 | 63 (48.8) | 46 (51.7) |
| ∑OCP |  |  |  |  |  |
| Low | ≤1.033 | 65 (50.4) | 44 (49.4) | 1.04 (0.61-1.78) | 0.81 (0.44-1.49) |
| High | >1.033 | 64 (49.6) | 45 (50.6) |

HCB, hexachlorobezene; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane; HCEAB, heptachlor epoxide; IDR, isodrin; ENS I, α-endosulfan; pp-DDE, p,p’-DDE; ΣOCPs is the sum of HCB, β-HCH,γ-HCH, HCEAB, IDR, ENS I, pp-DDE; aOR, adjusted odds ratio; cOR, crude odds ratio; CI, confidence interval; OFCs, orofacial clefts.

a Adjusted for maternal age at pregnancy, BMI, maternal smoking exposure, and gestational age.

### 3.3.2 BKMR model to assess the association between OCPs concentration and OFCs

Fig. 1 showed that there was no joint effect of seven OCP mixtures on the risk of OFCs, when all the OCPs were fixed to different percentiles (from 25th to 75th by 5th), as compared with when they were all fixed to the 50th percentile. Fig. 2 showed the variation in the association of each OCP levels when the single OCP increased from 25th to 75th percentile accompanied by the other OCPs set at different threshold (25th, 50th, or 75th percentile). We still have not observed an association between OCP individuals and risk for OFCs. We also used the BKMR to examine the potential non-linear exposure-response relationships when the levels of other OCPs were fixed at the median concentration (Fig. 3). β-HCH showed to have a U-shaped relationship with OFCs, and IDR appeared a little increasing relationship with OFCs, while other OCPs showed flat relationships with OFCs.

F:\R analysis-clp\log转换结果\总体.tiff

Figure 1 Overall association of the OCP mixture with OFCs risk. The black dots represent the estimates; solid line represent 95% credible intervals. The risk was estimated when all OCPs were at a particular percentile compared with the value when all of them were at their 50th percentile. Model adjusted for maternal age at pregnancy, BMI, maternal smoking exposure, and gestational age.

F:\R analysis-clp\log转换结果\dange.tiff

**Figure 2** **Individual pollutant association.** Point estimates show the difference in OFC risks when a single metal was at the 75th vs. 25th percentile, when all the other metals were fixed at either the 25th (red line), 50th (green line), or 75th percentile (blue line). "est" can be defined as the association between single OCP and OFC risk. Model adjusted for maternal age at pregnancy, BMI, maternal smoking exposure, and gestational age.

F:\R analysis-clp\log转换结果\fanyinghans.tiff

**Figure 3** **Dose-response function between individual OCP and OFCs while fixing other OCPs at median values.** The grey area represents 95% credible intervals. The results were estimated by Bayesian Kernel Machine Regression, adjusting for maternal age at pregnancy, BMI, maternal smoking exposure, and gestational age.

## Spearman correlations between dietary intake habits and concentrations of OCPs in umbilical cord tissues

The results of the correlation analysis are shown in Table 3. The frequencies of maternal ingestion of beans and bean products were positively correlated with β-HCH, HCEAB, pp-DDE, and ∑OCPs levels in umbilical cord tissues (*r* = 0.259, *r* = 0.153, *r* = 0.211, and *r* = 0.233, respectively), but the correlations were weak. There was no correlation between OCP levels in umbilical cord tissues and other dietary intake habits.

**Table 3 Spearman correlations between dietary intake habits and concentrations of OCPs in umbilical cord tissues**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Potential exposure sources | HCB | β-HCH | γ-HCH | IDR | HCEAB | ENS I | pp-DDE | ∑OCPs |
| Dietary intake habits |  |  |  |  |  |  |  |  |
| Fish or Meat | -0.003 | 0.015 | -0.056 | -0.026 | -0.022 | -0.098 | 0.011 | -0.003 |
| Milk or Eggs | 0.043 | 0.013 | -0.108 | -0.025 | -0.019 | -0.038 | 0.081 | 0.034 |
| Fresh green vegetables | -0.068 | -0.022 | -0.080 | -0.068 | -0.010 | -0.021 | 0.054 | -0.004 |
| Fresh fruit | -0.009 | 0.036 | -0.067 | -0.008 | -0.010 | 0.031 | 0.089 | 0.059 |
| Beans or bean products | 0.065 | 0.259\*\* | -0.116 | 0.052 | 0.153\* | 0.032 | 0.211\*\* | 0.233\*\* |

HCB, hexachlorobezene; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane; HCEAB, heptachlor epoxide; IDR, isodrin; ENS I, α-endosulfan; pp-DDE, p,p’-DDE; ΣOCPs is the sum of HCB, β-HCH,γ-HCH, HCEAB, IDR, ENS I, pp-DDE

\* *P* < 0.05, \*\* *P* < 0.001

## 3.4 Sensitivity Analysis

As a sensitivity analysis, we restricted our analyses to cases with isolated OFCs. When we excluded nine non-isolated OFC cases, the results did not change (Table S5). No associations between levels of OCPs and risk for OFCs were found.

# Discussions

This study for the first time determined the associations between OCPs levels in umbilical cord tissue and the risk for OFCs. Besides evaluating the individual effect of each OCPs exposure on the risk of OFCs, we also considered associations within the context of exposure mixture patterns. However, no statistically significant associations between levels of OCPs in umbilical cord tissue and the risk for OFCs in either multivariate logistic models (single-pollutant models) or BKMR models (multi-pollutant models) were found. In addition, maternal consumptions of beans and bean products may be sources of OCPs exposure in umbilical cord.

We compared the levels of OCPs in umbilical cord with results from previous studies. Our median DDE level among controls (0.10 ng/g dry weight) was lower than that reported in umbilical cord from the general population in Faroe Islands (1.19 ng/g dry weight) (Burse et al. 2000). Fukata et al. (2005) reported that based on the lipid weight, the median concentration of umbilical cord pp-DDT was 18 ng/g, while the HCB, op-DDT, pp-DDE, pp-DDD, ENS, HEC were not detected in Japan. In our study, the detection rate of pp-DDT in umbilical cord tissues was only 39%, and the median value of pp-DDT level based on the lipid weight in controls was 0.92 ng/g. The umbilical cords in these two studies were collected in 1986-1987 (Burse et al., 2000) and 2002-2003 (Fukata et al., 2005), respectively. In our study, most of umbilical cords (over 86.8%) were collected in 2010-2016. As the use of OCPs was banned worldwide since 1970s, the people exposed to OCP decreased over time. Although OCP is a persistent organic pollutant, the residual amount of OCP in the environment gradually decreases over time, which also results in the decrease of population's exposure to OCP. Therefore, this may explain why the OCP level in this study is lower than that in the previous two studies.

In our study, neither the applied multivariate logistic models nor BKMR models produced statistically significant associations between *in utero* exposure to single OCP or OCP mixtures and risk for OFCs. To our knowledge, this study is the first to report the association between *in utero* exposure to OCPs and risk for OFCs. A previous case-control study reported that no association of paternal/maternal occupational exposure to pesticides with OFCs risk (Shaw et al. 1999). A case-control studybased on questionnaires found that maternal pesticide exposure in early pregnancy was significantly associated with an increased risk of non-syndromic cleft of the lip and/or palate in China (OR = 8.90, 95% CI: 1.81-43.59) (Xu et al. 2015). A case-malformed control study found that oral clefts had significantly increased ORs of maternal occupational exposure to pesticides (aOR = 1.7, 95% CI: 1.0–3.1) when using nonchromosomal controls (Spinder et al. 2017). A meta-analysis suggested that maternal exposure to pesticides is associated with a modest but marginally significant risk of OFCs (Romitti et al. 2007). Another, more recent, meta-analysis which limited to studies using expert assessment of occupational exposure, could not calculate a pooled estimate for pesticide exposure and OFC risk, because of heterogeneity between published studies (Spinder et al. 2019). However, none of these previous studies did separate out which pesticide exposures (such as OCPs, organophosphorus pesticides, carbamate, pyrethroids, etc.) or the subtypes of OFCs. There may be significant differences in the toxicity and metabolism of different pesticides and in the etiology and pathogenesis between cleft lip with or without cleft palate and cleft palate. In addition, these previous studies were based on the external exposure, which may introduce recall bias and reporting bias on exposure to OCPs. Furthermore, because the fetus lives in the intrauterine and is not directly exposed to the external environment, the assessment of external environmental exposure to the pregnant woman cannot accurately reflect the fetal OCPs exposure. Inaccurate assessment of exposure level may seriously interfere with the true association between OCPs exposure and OFCs risk. In this study, an internal exposure assessment with detecting the OCPs level in umbilical cord tissues was performed, and no association between OCPs intrauterine exposure and the risk of OFCs was found. Our study may provide a new clue to illustrate the developmental toxicity of OCPs.

The evidence showed that the ingesting contaminated food is considered as the main source of human exposure to pesticides (Pirsaheb et al. 2015). In this study, our results indicated that maternal dietary intake beans and bean products may be a source of *in utero* exposure to OCPs. A previous study reported that the level of p,p’-DDT was statistically significant correlated with bean products (r =0.131) from 999 pregnant women in the Huaihe River Basin of China (Luo et al. 2016). Some studies have found that there were a variety of OCPs residues in beans (Latif et al. 2011; Oyeyiola et al. 2017). Although the use of OCPs has been banned in China, plants can still absorb OCPs from the soil due to their history of large-scale use and persistence in the environment, resulting in OCPs accumulation and potential harm to human health.

Our study had two strengths. Firstly, umbilical cord is a part of the fetal tissue derived from the yolk sac and the urine sac. The levels of OCPs in umbilical cord tissues can more accurately reflect fetal exposure. Secondly, in this study, in addition to analyzing the individual effects of each OCPs exposure on the risk of OFCs, BKMR model was used to evaluate the combined effects of OCPs mixtures on the risk of OFCs. The BKMR model can flexibly estimate the combined effects of multiple highly correlated OCPs exposures on OFCs. It is also possible to evaluate the exposure-response function and complex interactions by controlling other OCPs at specific levels. These are things that traditional regression models cannot analyze.

Our study is not without limitations. A study limitation is the window period of exposure: the umbilical cord tissues were collected from subjects after delivery, the key window of lip and palate development is in 4−12 weeks of gestation. However, for intrauterine exposure studies, this is a difficult problem to solve. Our study also found no correlation between OCPs level and gestational age, suggesting that OCPs level in late gestation may represent exposure in early gestation in some extent. Secondly, the relatively small sample sizes of this study, limited the statistical power and our ability to do further subgroup analysis. Thirdly, the intrinsic limitations of this case-control study are recall bias and report bias regarding dietary intakes. Finally, although the study was population-based, we could not completely exclude selection bias.

# 5. Conclusions

We found no evidence of associations between *in utero* exposure to OCPs and risk for OFCs, whether in estimating single effects of OCPs or joint effects of multiple OCPs. Maternal consumptions of bean and bean products may be sources of OCPs exposure in umbilical cord.

# CRediT authorship contribution statement

**LL Wang**: Conceptualization, Supervision, Writing - review & editing, Funding acquisition. **WL Yang**: Investigation, Writing - original draft, Formal analysis. **WL Ni**: Investigation, Writing - original draft, Formal analysis, Methodology. **JF Liu:** Data Curation, Writing - review & editing. **L Jin**: Writing - review & editing. **ZW Li**: Resources. **AG Ren**: Resources, Supervision, Funding acquisition.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Acknowledgments

This work was supported by the National Key Research and Development Program, Ministry of Science and Technology, People’s Republic of China (Grant No. 2016YFC1000501); and National Natural Science Foundation of China (Grant No. 81773441).

# References

Billionnet C, Sherrill D, Annesi-Maesano I, study G. 2012. Estimating the health effects of exposure to multi-pollutant mixture. Ann Epidemiol 22:126-141. https://doi.org/10.1016/j.annepidem.2011.11.004

Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, et al. 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16:493-508. https://doi.org/10.1093/biostatistics/kxu058

Bobb JF, Claus Henn B, Valeri L, Coull BA. 2018. Statistical software for analyzing the health effects of multiple concurrent exposures via bayesian kernel machine regression. Environ Health 17:67. https://doi.org/10.1186/s12940-018-0413-y

Burse VW, Najam AR, Williams CC, Korver MP, Smith BF, Jr., Sam PM, et al. 2000. Utilization of umbilical cords to assess in utero exposure to persistent pesticides and polychlorinated biphenyls. J Expo Anal Environ Epidemiol 10:776-788. https://doi.org/10.1038/sj.jea.7500125

Crinnion WJ. 2010. The cdc fourth national report on human exposure to environmental chemicals: What it tells us about our toxic burden and how it assist environmental medicine physicians. Altern Med Rev 15:101-109.

Dixon MJ, Marazita ML, Beaty TH, Murray JC. 2011. Cleft lip and palate: Understanding genetic and environmental influences. Nat Rev Genet 12:167-178. https://doi.org/10.1038/nrg2933

Fukata H, Omori M, Osada H, Todaka E, Mori C. 2005. Necessity to measure pcbs and organochlorine pesticide concentrations in human umbilical cords for fetal exposure assessment. Environ Health Perspect 113:297-303. https://doi.org/10.1289/ehp.7330

Garcia AM, Fletcher T, Benavides FG, Orts E. 1999. Parental agricultural work and selected congenital malformations. Am J Epidemiol 149:64-74. https://doi.org/10.1093/oxfordjournals.aje.a009729

Hao Y, Tian S, Jiao X, Mi N, Zhang B, Song T, et al. 2015. Association of parental environmental exposures and supplementation intake with risk of nonsyndromic orofacial clefts: A case-control study in heilongjiang province, china. Nutrients 7:7172-7184. https://doi.org/10.3390/nu7095328

Honein MA, Rasmussen SA, Reefhuis J, Romitti PA, Lammer EJ, Sun L, et al. 2007. Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. Epidemiology 18:226-233. https://doi.org/10.1097/01.ede.0000254430.61294.c0

Hu Y, Akland GG, Pellizzari ED, Berry MR, Melnyk LJ. 2004. Use of pharmacokinetic modeling to design studies for pathway-specific exposure model evaluation. Environ Health Perspect 112:1697-1703. https://doi.org/10.1289/ehp.6367

Konishi Y, Kuwabara K, Hori S. 2001. Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in osaka, japan. Arch Environ Contam Toxicol 40:571-578. https://doi.org/10.1007/s002440010212

Latif Y, Sherazi ST, Bhanger MI. 2011. Assessment of pesticide residues in commonly used vegetables in hyderabad, pakistan. Ecotoxicol Environ Saf 74:2299-2303. https://doi.org/10.1016/j.ecoenv.2011.07.030

Leskow A, Nawrocka M, Latkowska M, Tarnowska M, Galas N, Matejuk A, et al. 2019. Can contamination of the environment by dioxins cause craniofacial defects? Hum Exp Toxicol 38:1014-1023. https://doi.org/10.1177/0960327119855121

Li C, Cheng Y, Tang Q, Lin S, Li Y, Hu X, et al. 2014. The association between prenatal exposure to organochlorine pesticides and thyroid hormone levels in newborns in yancheng, china. Environ Res 129:47-51. https://doi.org/10.1016/j.envres.2013.12.009

Lim JT, Tan YQ, Valeri L, Lee J, Geok PP, Chia SE, et al. 2019. Association between serum heavy metals and prostate cancer risk - a multiple metal analysis. Environ Int 132:105109. https://doi.org/10.1016/j.envint.2019.105109

Liu SL, Qin QF, Li QQ. 2004. Analysis of the accumulative levels of organochlorine pesticides in human body fat and blood in xiaogan. J Environ Health 21:238-240.

Liu Y, Wang B, Li Z, Zhang L, Liu J, Ren A. 2016. Indoor air pollution and the risk of orofacial clefts in a rural population in shanxi province, china. Birth Defects Res A Clin Mol Teratol 106:708-715. https://doi.org/10.1002/bdra.23522

Lu C, Kedan G, Fisker-Andersen J, Kissel JC, Fenske RA. 2004. Multipathway organophosphorus pesticide exposures of preschool children living in agricultural and nonagricultural communities. Environ Res 96:283-289. https://doi.org/10.1016/j.envres.2004.01.009

Luo D, Pu Y, Tian H, Cheng J, Zhou T, Tao Y, et al. 2016. Concentrations of organochlorine pesticides in umbilical cord blood and related lifestyle and dietary intake factors among pregnant women of the huaihe river basin in china. Environ Int 92-93:276-283. https://doi.org/10.1016/j.envint.2016.04.017

Ma ZL, Mao XX, Ding ZY, Gao H, Huang T, Tian H, et al. 2013. Residual levels in air, soil and soil-air exchange of organochlorine pesticides in hami region of xinjiang and its potential ecological risk. Huan Jing Ke Xue 34:1120-1128.

Mishra K, Sharma RC. 2011. Assessment of organochlorine pesticides in human milk and risk exposure to infants from north-east india. Sci Total Environ 409:4939-4949. https://doi.org/10.1016/j.scitotenv.2011.07.038

Morgan MK, Sheldon LS, Croghan CW, Jones PA, Chuang JC, Wilson NK. 2007. An observational study of 127 preschool children at their homes and daycare centers in ohio: Environmental pathways to cis- and trans-permethrin exposure. Environ Res 104:266-274. https://doi.org/10.1016/j.envres.2006.11.011

Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. 2009. Cleft lip and palate. Lancet 374:1773-1785. https://doi.org/10.1016/s0140-6736(09)60695-4

Oyeyiola AO, Fatunsin OT, Akanbi LM, Fadahunsi DE, Moshood MO. 2017. Human health risk of organochlorine pesticides in foods grown in nigeria. J Health Pollut 7:63-70. https://doi.org/10.5696/2156-9614-7.15.63

Pi X, Li Z, Jin L, Liu J, Zhang Y, Zhang L, et al. 2018. Secondhand smoke during the periconceptional period increases the risk for orofacial clefts in offspring. Paediatr Perinat Epidemiol 32:423-427. https://doi.org/10.1111/ppe.12497

Pirsaheb M, Limoee M, Namdari F, Khamutian R. 2015. Organochlorine pesticides residue in breast milk: A systematic review. Med J Islam Repub Iran 29:228.

Qiu X, Zhu T, Yao B, Hu J, Hu S. 2005. Contribution of dicofol to the current ddt pollution in china. Environ Sci Technol 39:4385-4390. https://doi.org/10.1021/es050342a

Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, et al. 2011. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proc Natl Acad Sci U S A 108:12770-12775. https://doi.org/10.1073/pnas.1105209108

Rignell-Hydbom A, Lindh CH, Dillner J, Jonsson BA, Rylander L. 2012. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants and the risk of hypospadias. PLoS One 7:e44767. https://doi.org/10.1371/journal.pone.0044767

Romitti PA, Herring AM, Dennis LK, Wong-Gibbons DL. 2007. Meta-analysis: Pesticides and orofacial clefts. Cleft Palate Craniofac J 44:358-365. https://doi.org/10.1597/06-100.1

Sala M, Ribas-Fito N, Cardo E, de Muga ME, Marco E, Mazon C, et al. 2001. Levels of hexachlorobenzene and other organochlorine compounds in cord blood: Exposure across placenta. Chemosphere 43:895-901. https://doi.org/10.1016/s0045-6535(00)00450-1

Shaw GM, Wasserman CR, O'Malley CD, Nelson V, Jackson RJ. 1999. Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiology 10:60-66.

Song SY, Wang X, LI J, Feng XX. 2012. An investigation on the accumulative level of organochlorine pesticides in residents of tangshan area. Modern Preventive Medicine 39:2079-2081.

Spinder N, Bergman JEH, Boezen HM, Vermeulen RCH, Kromhout H, de Walle HEK. 2017. Maternal occupational exposure and oral clefts in offspring. Environ Health 16:83. https://doi.org/10.1186/s12940-017-0294-5

Spinder N, Prins JR, Bergman JEH, Smidt N, Kromhout H, Boezen HM, et al. 2019. Congenital anomalies in the offspring of occupationally exposed mothers: A systematic review and meta-analysis of studies using expert assessment for occupational exposures. Hum Reprod 34:903-919. https://doi.org/10.1093/humrep/dez033

Valeri L, Mazumdar MM, Bobb JF, Claus Henn B, Rodrigues E, Sharif OIA, et al. 2017. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20-40 months of age: Evidence from rural bangladesh. Environ Health Perspect 125:067015. https://doi.org/10.1289/ehp614

Woodruff TJ, Zota AR, Schwartz JM. 2011. Environmental chemicals in pregnant women in the united states: Nhanes 2003-2004. Environ Health Perspect 119:878-885. https://doi.org/10.1289/ehp.1002727

Xu LF, Zhou XL, Wang Q, Zhou JL, Liu YP, Ju Q, et al. 2015. A case-control study of environmental risk factors for nonsyndromic cleft of the lip and/or palate in xuzhou, china. Biomed Environ Sci 28:535-538. https://doi.org/10.3967/bes2015.076

Yu HF, Zhao XD, Zhao JH, Zhu ZQ, Zhao Z. 2006. Continuous surveillance of organochlorine pesticides in human milk from 1983 to 1998 in beijing, china. Int J Environ Health Res 16:21-26. https://doi.org/10.1080/09603120500397615

Zhou Y, Gilboa SM, Herdt ML, Lupo PJ, Flanders WD, Liu Y, et al. 2017. Maternal exposure to ozone and pm2.5 and the prevalence of orofacial clefts in four u.S. States. Environ Res 153:35-40. https://doi.org/10.1016/j.envres.2016.11.007