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Organochlorine pesticide residues in maternal blood, cord blood, placenta, and breastmilk and their relation to birth size

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HIGHLIGHTS

- There is no placental barrier against transfer of organochlorine pesticides across the maternal-fetal axis.
- Transplacentally transferred organochlorine pesticides are associated with a reduced birth size of the offspring.
- Amongst the organochlorine pesticides, HCH was most consistently associated with decreased birth size of the neonate.

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ABSTRACT

There is a growing concern that persistent organic pollutants like organochlorine pesticides (OCPs) can impair fetal growth and affect birth size. However, currently available epidemiological evidence is inconclusive. In this case-control study, we examined the association between exposure to hexachlorocyclohexane (HCH) and its isomers (α-HCH, β-HCH and γ-HCH), dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and birth size. We recruited 60 infant-mother pairs, comprising of 30 term, small for gestational age babies with their mothers (Case group), and another 30 term, appropriate for gestational age babies with their mothers (Control group). This study was conducted in a tertiary hospital in Delhi, India, between March, 2009 and February 2010. Organochlorine pesticides were estimated in maternal blood, cord blood, placenta and breastmilk samples, using gas–liquid chromatography. Transplacental and transmammary transfer of OCPs was assessed by correlating the maternal blood OCP levels with those in cord blood and breastmilk by simple linear regression. The birthweight, crown heel length, head circumference, mid-arm circumference and ponderal index of the neonates was correlated with OCP levels in the maternal blood, cord blood, placenta and breastmilk. The OCP estimates were compared between samples of the case and control group. There was a significant ($P < 0.001$) transplacental transfer of all OCPs, however the transmammary transfer was insignificant for most OCPs except α-HCH. The OCP levels in the case group were higher than the control group; these were significantly more for t-HCH in cord blood and breastmilk; β-HCH in maternal blood, cord blood and breastmilk; DDE in placenta and DDT in breastmilk. There was a significant negative correlation between birthweight and t-HCH levels in maternal blood ($P = 0.022$), cord blood ($P < 0.001$), placenta ($P = 0.008$) and breastmilk ($P = 0.005$); β-HCH in cord blood ($P < 0.001$) and placenta ($P = 0.020$); γ-HCH in placenta ($P = 0.045$); and DDT ($P = 0.009$). Length at birth had a significant negative correlation with t-HCH in cord blood ($P = 0.014$) and breastmilk ($P < 0.001$); β-HCH in cord blood ($P = 0.016$) and breastmilk ($P = 0.012$); DDE in placenta ($P = 0.016$); and DDT in breastmilk ($P = 0.006$). Similarly, OCP levels were also found to be negatively correlated with head circumference, ponderal index and chest circumference in neonates. We conclude that prenatal exposure to some OCPs could impair the anthropometric development of the fetus, reducing the birthweight, length, head circumference, chest circumference and ponderal index.

1. Introduction

Organochlorine pesticides (OCPs) are the most frequently used insecticides containing carbon, hydrogen and chlorine. Commonly used OCPs include DDT (dichlorodiphenyl trichloroethane), DDE (dichlorodiphenyldichloroethylene), hexachlorocyclohexane (HCH), dieldrin, aldrin, endrin, mirex, dicofol, methoxychlor, heptachlor, chlordane, pentachlorophenol, and endosulphan. Most of these OCPs are lipophilic, non-biodegradabable and possess residual activity resulting in biomagnification and bioaccumulation. Considerable concern has been focused on OCPs due to their broad

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spectrum of toxicity and adverse ecological effects. The Stockholm Convention on persistent organic pollutants (POPs) (2001) recommended elimination of several hazardous OCPs including aldrin and dieldrin, restricted the use of some OCPs like DDT, and permitted unintentional production of certain pesticides including HCH and its isomers. In 2009, in view of emerging scientific evidence on the detrimental effects of HCH on human health (Pierik et al., 2007; Alvarez-Pedrerol et al., 2008; Pathak et al., 2009; Richardson et al., 2009), the pesticides were reclassified and HCH and its isomers were recommended for elimination; albeit, lindane (γ-HCH) was permitted for use as second line drug for control of head lice and scabies. Production and use of DDT was permitted in developing countries for malarial vector control, in situations where no safe, effective and affordable alternative was locally available (United Nations Environmental Program, 2009). Inspite of the recommendations of the Stockholm Convention, α-HCH and β-HCH continue to be released in the atmosphere either during production of lindane or from the environmental stockpiles (Jit et al., 2011), while there is continued significant exposure of humans to DDT; more so in developing countries including India (Tanabe and Kunisue, 2007; Charan et al., 2010; Kaushik et al., 2012).

Recent epidemiological studies have stressed on the reproduction-related effects of OCPs, wherein they act as endocrine disruptors (Ropstad et al., 2006) resulting in detrimental effects on mothers and their offsprings (Eskenazi, 2004; Fenster et al., 2006). While several studies have shown a positive association of OCP exposure with spontaneous abortions (Axmon et al., 2000; Khanjani and Sim, 2006; Pathak et al., 2010), low birthweight (Siddiqui et al., 2003; Sagiv et al., 2007), decreased crown-heel length (Ribas-Fitó et al., 2002; Eggesbø et al., 2009), and prematurity (Pathak et al., 2009), some others have had inconclusive results (Longnecker et al., 2005; Khanjani and Sim, 2006). More than 20 million low birthweight babies are born every year, accounting for 17% of all births in the developing world. Unlike the developed countries where prematurity is the major cause for low birthweight, in developing countries most of the low birthweight babies are a consequence of fetal malnutrition and growth retardation. India alone accounts for 40% of all low birthweight (LBW) babies in the developing world (UNICEF, 2006). Three-fourths of the LBW babies in India are born at term gestation with intra-uterine growth retardation (IUGR), and are labelled as being small for gestational age (SGA). The cause of intra-uterine growth retardation cannot be ascertained in up to 40% of SGA infants; unknown environmental factors could have a possible implication (UNICEF, 2006). Therefore, we assessed the relationship between OCP residue levels (HCH and its isomers viz α-HCH, β-HCH and γ-HCH; DDT; and DDE) in maternal blood, cord blood, placenta, and breastmilk with the size of the neonate born at term gestation.

2. Methods and materials

2.1. Study population

The study was conducted between March 2009 to February 2010 following an informed written consent from all participants and an approval from the Institutional Ethics Committee. Thirty consecutive term (37–42 weeks gestation), small for gestational age babies [birthweight <10th centile for gestational age, (Lubchenco et al., 1963)] and their mothers comprised the case group. Another 30, term appropriate for gestational age babies (birthweight between 10th and 90th centile for gestation) and their mothers comprised the control group. Gestational age was estimated from the date of last menstrual period (LMP). New Ballard's Score was used where the LMP was unknown or not reliable (Ballard et al., 1991). Women with known risk factors for intra-uterine growth retardation i.e., age <18 or >35 year; malnutrition (hemoglobin < 8 g dL^{-1}, height < 145 cm or body mass index (BMI) < 19 kg m^{-2}); chronic illnesses (HIV, diabetes, renal disease, cardiovascular, hepatobiliary disorder, etc.); substance abuse (smoking or alcoholism); and third trimester complications (antepartum hemorrhage, pregnancy-induced hypertension, urinary tract infection, etc.) were excluded. Neonates with congenital malformations and multiple gestation were also excluded.

2.2. Data and sample collection

Mothers were enquired for details regarding age, socioeconomic status, drinking water source, alcohol consumption, dietary and smoking habits, which were recorded in a pre-designed proforma. The body mass index (BMI) of mothers was calculated using the 'pre-pregnancy weight' noted from the patient case records and height measured using a stadiometer. Placenta was weighed to the nearest 5 g using an electronic weighing scale (Gold MI Goldtech, Merino International) after clamping and cutting the umbilical cord nearest to the placenta. Birthweight (BW), crown heel length (CHL), mid-arm circumference (MAC) and chest circumference (CC) of neonates at birth were assessed at birth while head circumference (HC), was recorded between 48 and 72 h after birth. Baby was weighed in nude using electronic weighing scale (MI Goldtech, Merino International) to the nearest 5 g. CHL was measured to the nearest 1 mm using an infantometer (Seca) while HC, MAC and CC were measured using a non-stretchable cloth measuring tape to the nearest 1 mm by standard anthropometric technique. Ponderal index (PI) was calculated using the formula: PI = birthweight/length^3 (g cm^{-3}) × 100.

About 2 mL of maternal and cord blood were collected in EDTA vials and and 5 g of placental tissue was collected in a plain vacutainer at the time of delivery. On the third day of delivery, mothers were asked to manually express milk from both the breasts for about 15 min in a clean bowl and a 2 mL aliquot was taken in a plain vacutainer and stored at −20 °C until analysis.

2.3. Chemical analysis

Estimation of OCP residues in blood/tissue involved their extraction from samples with organic solvents, a clean-up step to remove lipids which could interfere with analysis, gas chromotography to separate a particular OCP from other compounds in the extract and its confirmation by an electron capture detector (ECD). One mL of blood/milk or 1 g of placental tissue was taken in a 50 mL flask. Hexane (6 mL) and acetone (3 mL) were added and the contents were shaken for 30 min in a mechanical shaker at room temperature. The extract was centrifuged for 10 min at 2000 rpm and the clear top layer of hexane was collected in a clean test tube. The remaining portion was again extracted twice using the same process and the hexane fractions were added to the previous solvent fractions. Clean up of the samples was done by column chromatography. A column packed with 10 cm of activated florisil and 1 cm anhydrous Na_2SO_4, was pre-wetted with 40–50 mL of *n*-hexane. Sample was allowed to pass through the column and 20 mL of hexane was added for complete elution of samples. This process was repeated three times for the remaining residues. Elute was collected in a 100 mL beaker and hexane was evaporated to concentrate the sample. The concentrated residue was dissolved in 2 mL of hexane for further analysis. Quantification of organochlorine residue levels was done by Perkin–Elmer Gas Chromatograph equipped with ^{63}Ni Electron Capture Detector. The carrier gas and the make-up gas was nitrogen with a 2.0 mL min^{-1} and 35 mL min^{-1} flow rate, respectively, employing the splitless mode. Final extract (μL) was injected at a temperature of 170 °C with a hold time of 1 min. The temperature was raised

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from 170 °C to 225 °C at a rate of 5 °C min^{-1} with a hold time of 5 min and finally from 225 °C to 275 °C at a rate of 6 °C min^{-1} with a hold time of 15 min. The total run length was 40 mm per sample. The samples were analyzed for the following organochlorine pesticide residues: DDT isomers, DDE, and HCH and its isomers. Quantitative analysis of OCP residues in each sample was done by comparing the peak area with those obtained from a chromatogram of a mixed organochlorines standard of known concentration. Further, a quality check sample was always run with each set of samples for pesticide analysis to maintain accuracy.

2.4. Data analysis

Demographic characteristics and anthropometry of all mother–infant pairs in both groups were compared to ascertain any confounding factors. Maternal age, weight, height, BMI, placental weight, weight gain in third trimester, hemoglobin and neonatal anthropometric parameters were compared by student's *t*-test while maternal socioeconomic status, residence, source of water, dietary habit, antenatal nutrient supplementation status and parity were compared by Chi-Square or Fischer's exact test. The correlation between OCP levels in the maternal blood and cord blood was ascertained to assess the transplacental transfer of OCPs using simple linear regression model with the maternal level of OCP as the independent variable and the cord blood level of the OCP as the dependent variable. Likewise, maternal OCP levels were correlated with OCP levels in breastmilk to ascertain the transmammary transfer of OCPs, and the maternal OCP levels were correlated with placental tissue OCP levels. Pesticide residue levels (mean ± SD) in maternal blood, cord blood, placental tissue and breastmilk were compared between the two groups by unpaired *t*-test. Relationship between the size of the neonate at birth (measured by BW, CHL, MAC, HC, CC and PI) and the OCP levels in the maternal blood, cord blood, placenta and breastmilk was determined by estimating the correlation coefficient. All data obtained were entered in an appropriate format and statistical analysis was done using SPSS version 13.0 software. $P < 0.05$ was taken to be statistically significant.

3. Results

3.1. Demographic characteristics of enrolled mothers

The baseline demographic characteristics of the 60 mothers included in the analysis are depicted in Table 1. Women delivering term, small for gestational age (SGA) babies and those delivering term, appropriate for gestational age (AGA) babies were not statistically different in terms of demographic characteristics such as age, weight, height, body mass index, dietary habits, drinking water supply, living style and socioeconomic status. However, the weight gain in third trimester and the placental weight were significantly lesser in women delivering small for gestational age babies.

3.2. Anthropometric comparison of infants

As expected the infants in the case group were significantly smaller than their counterparts in the control group at birth, as measured in terms of birthweight, length, head circumference, ponderal index, chest circumference and mid-arm circumference (Table 1).

3.3. Transplacental and transmammary transfer of OCP residues

There was a significant positive correlation between the maternal and cord blood levels of all OCPs signifying an increased transplacental transfer of OCPs. A significant positive correlation between maternal levels of t-HCH and β-HCH and the corresponding levels in placental tissue (t-HCH, $R = 0.349$, $P = 0.006$; β-HCH, $R = 0.437$, $P < 0.001$). No consistent correlation was observed between maternal blood OCP levels and those in the breastmilk (data not shown), except for α-HCH ($R = -0.272$, $P = 0.036$).

3.4. Case-control comparisons for OCP residues

OCP residues namely HCH and its isomers, DDT and its metabolite DDE, from SGA group were higher than the corresponding levels in the AGA group as shown in Table 2. SGA group had a statistically significant higher t-HCH in the cord blood and breastmilk; β-HCH in the maternal blood, cord blood and breastmilk; DDT in breastmilk and DDE in placenta compared to the AGA group.

3.5. Correlation between OCP levels in the maternal blood, cord blood, placenta and breastmilk and the size of the neonate

Table 3 depicts the correlation between OCP residues in maternal blood, cord blood, placental tissue and breastmilk with the size of the newborn at birth. There was a statistically significant negative correlation between t-HCH in maternal blood and BW, CC, and PI. Likewise, t-HCH in cord blood was significantly negatively correlated with BW, CHL, HC and PI, as also for t-HCH in placenta with BW and PI. Breastmilk t-HCH had a significant negative correlation with BW, CHL, HC and CC. The relationship between birthweight and t-HCH in maternal blood, cord blood, placenta and breastmilk is depicted in Fig. 1. α-HCH did not have a statistically significant correlation with the birth size of the neonate. β-HCH in maternal blood had a significant negative correlation with BW, HC and PI. Cord blood β-HCH was significantly negatively correlated with BW, CHL, HC, CC and PI. Placental β-HCH had a statistically significant negative correlation with BW, while breastmilk β-HCH had a statistically significant negative correlation with CHL, HC and CC. γ-HCH in placenta had a statistically significant negative correlation with BW and PI. DDT in breastmilk had an inverse relationship with BW and CHL. DDE levels in placenta affected the BW and CHL adversely. For all other OCPs, the relationship with anthropometric parameters of the newborn were not statistically significant.

4. Discussion

In our study, we documented a direct significant correlation between maternal and cord blood levels of OCPs indicating that there is a significant transplacental transfer of OCPs (HCH and its isomers, DDT and DDE). That organochlorine pesticides are transferred from the mother to the fetus through the placenta, has been shown previously also (Rogan et al., 1986; Skaare et al., 1988; Longnecker et al., 2005). In our study, we also estimated the OCPs in the placental tissue which did not have a significant correlation with the corresponding levels in maternal blood for most OCPs except t-HCH ($R = 0.349$, $P = 0.006$) and β-HCH ($R = 0.437$, $P < 0.001$). Our study has shown that the concentration of OCPs is high in maternal blood specimens and low in umbilical cord blood. The observed trend is: maternal blood > placenta > umbilical cord blood > breastmilk. This indicates that the mother has the highest burden of OCPs and there is a continued partial transfer of such compounds to the fetus.

We also found that the OCP levels in the maternal blood, cord blood, placenta and breastmilk of mothers delivering SGA babies were higher compared to mothers delivering AGA babies. These associations were independent of the prematurity as we recruited only term babies. In utero exposure to various OCPs has been

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Table 1
Characteristics of mothers and neonates in the two groups.

Parameters	Case group (n = 30)	Control group (n = 30)	P value
Maternal data (Proportions [n (%)])			
Lower socioeconomic status	7 (23.3%)	5 (16.7%)	0.29
Slum dwellers	7 (23.3%)	7 (23.3%)	1
Consuming non-potable water	7 (23.3%)	9 (30%)	0.91
Non-vegetarian	18 (60%)	17 (56.7%)	0.79
Not received iron, folic acid, calcium supplementation	11 (36.7%)	10 (33.3%)	0.12
Primipara	22 (73.3%)	14 (46.7%)	0.17
(Means ± SD)			
Age (y)	24.7 ± 3.5	25.1 ± 2.7	0.65
Pre-pregnancy weight (kg)	55.9 ± 4.12	55.5 ± 3.55	0.7
Height (cm)	155.1 ± 2.57	155.8 ± 2.72	0.26
Pre-pregnancy BMI (kg m^{-2})	23.3 ± 2.04	22.7 ± 1.67	0.23
Hemoglobin (g dL^{-1})	11.5 ± 0.90	11.2 ± 0.77	0.28
Weight gain in third trimester (kg)	7.9 ± 1.08	9.72 ± 1.12	**<0.001**
Placental weight (g)	356.0 ± 46.72	453.5 ± 54.31	**<0.001**
Neonatal data (means ± SD)			
Weight (kg)	1.9 ± 0.32	2.91 ± 0.22	**<0.001**
Length (cm)	47.7 ± 1.66	52.88 ± 1.06	**<0.001**
Head circumference (cm)	29.9 ± 1.54	35.42 ± 0.98	**<0.001**
Ponderal index	1.8 ± 0.30	1.96 ± 0.17	**<0.001**
Chest circumference (cm)	28.4 ± 1.49	33.20 ± 1.27	**<0.001**
Mid-arm circumference (cm)	9.4 ± 0.77	10.86 ± 0.84	**<0.001**

Bold values are statistically significant.

related with fetal outcomes including birth size. Some authors have related fetal growth retardation and reduced anthropometric measurements at birth to exposure to OCPs (Siddiqui et al., 2003; Weisskopf et al., 2005; Sagiv et al., 2007; Barr et al., 2010; Lopez-Espinosa et al., 2011). Some other investigators found no association between maternal exposure to OCPs and fetal growth (Farhang et al., 2005; Khanjani and Sim, 2006; Eggesbø et al., 2009). Amongst the OCPs, we found that t-HCH and β-HCH had a consistent significant adverse effect on birth size. This relationship corroborates our observation wherein β-HCH and t-HCH in maternal, cord blood and breastmilk, were significantly higher in the SGA group compared to the AGA group. Previously, fetal growth outcomes with HCH exposure have been assessed in several studies with variable results. Higher β-HCH in breastmilk has been associated with lower BW (Schade and Heinzow, 1998). In contrast, higher cord blood β-HCH was found to be associated with higher BW and HC (Tan et al., 2009); inconsistent results were reported by others (Ribas-Fitó et al., 2002; Siddiqui et al., 2003; Fenster et al., 2006; Khanjani and Sim, 2006]).

Amongst the isomeric forms of hexachlorocyclohexane (HCH), also known as benzene hexachloride (BHC), we found only β-HCH to have significant adverse effects on fetal growth. This may be explained by its recalcitrant chemical structure which makes it resistant to biodegradation. In addition, once ingested or inhaled, it leaves the body very slowly due to its high lipophilicity, thereby, and with chronic exposure, β-HCH is more toxic than any other isomer of HCH (ATSDR, 2005). Since the regulation imposed by the Stockholm Convention (2001), β-HCH by itself is no longer intentionally produced or available in the market, however it is produced as a part of technical grade HCH or as an intermediate chemical in the manufacturing of lindane (γ-HCH). Hence, the exact cause-effect relationship between continued HCH exposure and fetal growth retardation needs to be assessed in future also. We also assessed OCP levels in breastmilk, a possible route of continued transfer of pesticides to newborns. About 60% of lipids in breastmilk originate from the mother's adipose tissue which may explain how these persistent OCPs stored in the mother's adipose tissue may be transmitted to the infant through breastmilk. There

Table 2
Distribution and comparison of organochlorine pesticide levels (ng mL^{-1}) in maternal blood, cord blood, placenta and breastmilk of case group (SGA) and control group (AGA).

Pesticide	Maternal blood (mean ± SD)				Cord blood (mean ± SD)			
	Case group (SGA)	Control group (AGA)	P value	Odds ratio (95% CI)	Case group (SGA)	Control group (AGA)	P value	Odds ratio
HCH								
Total-HCH (t-HCH)	19.8 ± 8.15	16.8 ± 4.22	0.07	1.1 (0.92–1.17)	13.3 ± 5.37*	10.2 ± 3.59*	0.01	**1.2 (1.03–1.33)**
Alpha- HCH (α-HCH)	5.2 ± 2.74	4.9 ± 2.80	0.7	0.9 (0.86–1.25)	3.7 ± 1.81	3.3 ± 2.09	0.39	1.1 (0.86–1.46)
Beta-HCH (β-HCH)	8.1 ± 4.41*	5.9 ± 2.48*	**0.02**	**1.2 (1.01–1.47)**	6.0 ± 3.44*	4.0 ± 1.85*	**<0.01**	**1.4 (1.20–1.74)**
Gamma-HCH (γ-HCH)	6.6 ± 3.65	5.9 ± 2.32	0.41	1.08 (0.91–1.19)	3.5 ± 2.55	2.9 ± 1.53	0.25	1.2 (0.90–1.50)
DDT	1.5 ± 1.88	1.4 ± 2.02	0.73	1.1 (0.8–1.4)	1.1 ± 1.45	0.9 ± 1.26	0.56	1.1 (0.76–1.66)
DDE	2.3 ± 2.61	2.1 ± 2.12	0.67	1.1 (0.84–1.31)	1.9 ± 2.28	1.7 ± 2.03	0.78	1 (0.82–1.32)
	Placenta (mean ± SD)				Breastmilk (mean ± SD)			
HCH								
Total-HCH (t-HCH)	18.9 ± 10.93	15.4 ± 7.85	0.15	1 (0.99–1.10)	15.8 ± 8.48*	8.5 ± 5.81*	**<0.001**	**1.1 (1.06–1.26)**
Alpha-HCH (α-HCH)	3.9 ± 3.43	3.4 ± 4.20	0.59	1 (0.88–1.24)	3.9 ± 3.62	2.4 ± 3.29	0.07	1.1 (0.98–1.35)
Beta-HCH (β-HCH)	8.5 ± 4.73	6.6 ± 5.69	0.16	1.2 (1.03–1.43)	5.9 ± 6.77*	2.2 ± 2.96*	**<0.01**	**1.2 (1.03–1.38)**
Gamma-HCH (γ-HCH)	6.5 ± 5.06	5.4 ± 3.44	0.33	1.1 (1.01–1.20)	5.8 ± 5.47	4.0 ± 3.64	0.15	1.1 (0.97–1.22)
DDT	1.9 ± 1.60	1.7 ± 1.24	0.56	1.117 (0.780–1.610)	1.8 ± 1.12*	0.8 ± 0.71*	**<0.001**	**2.9 (1.54–5.50)**
DDE	3.9 ± 2.76*	2.5 ± 1.87*	**0.02**	**1.304 (1.030–1.650)**	2.9 ± 2.54	2.2 ± 1.70	0.19	1.2 (0.92–1.50)

Bold values are statistically significant.
All pesticide levels are mentioned as mean ± standard deviation.
* Significant at $P < 0.05$.

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Table 3
Correlation between OCP levels in maternal blood and cord blood and size of the newborn at birth ($n = 60$).

Pesticide	Birthweight	Length	Mid-arm circumference	Head circumference	Chest circumference	Ponderal index
	Pearson's correlation coefficient, R (P value)	Pearson's correlation coefficient, R (P value)	Pearson's correlation coefficient, R (P value)	Pearson's correlation coefficient, R (P value)	Pearson's correlation coefficient, R (P value)	Pearson's correlation coefficient, R (P value)
Maternal blood						
HCH						
t-HCH	**−0.29 (0.02)***	−0.20 (0.11)	−0.16 (0.64)	**−0.33 (<0.01)**	**−0.33 (<0.01)***	**−0.27 (0.04)***
α-HCH	−0.15 (0.26)	−0.06 (0.62)	−0.13 (0.30)	−0.13 (0.34)	−0.15 (0.24)	−0.16 (0.21)
β-HCH	**−0.34 (<0.01)***	−0.22 (0.09)	−0.03 (0.81)	**−0.34 (<0.01)***	**−0.32 (0.01)**	**−0.32 (0.01)***
γ-HCH	−0.09 (0.51)	−0.12 (0.35)	−0.06 (0.64)	−0.19 (0.13)	−0.13 (0.16)	−0.05 (0.70)
DDT	−0.06 (0.65)	−0.14 (0.29)	−0.10 (0.44)	−0.02 (0.87)	−0.03 (0.81)	−0.05 (0.72)
DDE	0.07 (0.62)	−0.04 (0.75)	−0.10 (0.45)	−0.02 (0.87)	−0.02 (0.84)	−0.15 (0.24)
Cord blood						
HCH						
t-HCH	**−0.40 (<0.001)***	**−0.32 (<0.01)***	−0.122 (0.35)	**−0.39 (<0.01)***	−0.01 (0.91)	**−0.34 (<0.01)***
α-HCH	−0.21 (0.10)	−0.14 (0.28)	−0.010 (0.94)	−0.16 (0.22)	−0.16 (0.21)	−0.19 (0.14)
β-HCH	**−0.41 (<0.001)***	**−0.31 (<0.01)***	−0.136 (0.30)	**−0.39 (<0.01)***	**−0.39 (<0.01)***	**−0.34 (<0.01)***
γ-HCH	−0.14 (0.27)	−0.16 (0.23)	−0.079 (0.55)	−0.20 (0.12)	−0.15 (0.23)	−0.11 (0.39)
DDT	−0.09 (0.48)	−0.13 (0.31)	−0.123 (0.35)	−0.03 (0.81)	−0.07 (0.57)	−0.00 (0.98)
DDE	−0.07 (0.58)	−0.19 (0.89)	−0.074 (0.57)	−0.04 (0.76)	−0.01 (0.95)	−0.14 (0.29)
Placental tissue						
HCH						
t-HCH	**−0.34 (<0.01)***	−0.12 (0.37)	−0.12 (0.37)	−0.31 (0.01)	−0.21 (0.10)	**−0.48 (<0.001)***
α-HCH	−0.15 (0.26)	−0.04 (0.73)	−0.10 (0.43)	−0.10 (0.44)	−0.07 (0.57)	−0.20 (0.12)
β-HCH	**−0.30 (0.02)***	−0.13 (0.39)	−0.04 (0.74)	−0.29 (0.02)	−0.24 (0.07)	**−0.38 (<0.01)***
γ-HCH	**−0.26 (0.04)***	−0.117 (0.37)	−0.12 (0.36)	−0.25 (0.05)	−0.12 (0.36)	**−0.42 (<0.001)***
DDT	0.80 (0.07)	−0.116 (0.38)	−0.04 (0.76)	−0.03 (0.83)	−0.06 (0.65)	−0.07 (0.58)
DDE	**0.09 (0.02)***	**−0.309 (0.01)***	−0.01 (0.97)	−0.09 (0.47)	−0.11 (0.41)	−0.02 (0.86)
Breastmilk						
HCH						
t-HCH	**−0.35 (<0.01)***	**−0.44 (<0.001)***	−0.25 (0.05)	**−0.44 (<0.001)***	**−0.36 (<0.01)***	−0.09 (0.47)
α-HCH	−0.17 (0.19)	−0.17 (0.192)	−0.14 (0.30)	−0.23 (0.08)	−0.16 (0.21)	−0.11 (0.38)
β-HCH	−0.24 (0.06)	**−0.32 (0.01)***	−0.24 (0.07)	**−0.31 (0.01)***	**−0.33 (0.01)***	−0.01 (0.91)
γ-HCH	−0.18 (0.16)	−0.22 (0.09)	−0.01 (0.91)	−0.21 (0.10)	−0.10 (0.43)	−0.06 (0.63)
DDT	**−0.33 (<0.01)***	**0.35 (<0.01)***	−0.23 (0.08)	**−0.36 (<0.01)***	**−0.39 (<0.01)***	−0.12 (0.35)
DDE	−0.18 (0.16)	−0.14 (0.30)	0.17 (0.20)	−0.14 (0.29)	−0.04 (0.74)	−0.12 (0.37)

Bold values are statistically significant.
* Significant at $P < 0.05$.

are very few studies ascertaining the relationship between maternal levels of OCPs with those in breastmilk. While a few authors (Khanjani and Sim, 2006; Kumar et al., 2006) have reported a direct correlation of OCP levels in maternal blood with those in breastmilk; some others (Nair et al., 1996) did not observe any relationship between the two. We did not observe any significant correlation between maternal levels of OCPs with those in breastmilk for any pesticide other than α-HCH which had a negative correlation ($R = -0.272$, $P = 0.036$). This may be explained if we understand that breastfeeding is a major excretory route for OCPs and the longer a woman breastfeeds the more she reduces her body burden of OCPs (Rogan et al., 1986). Thereby, implicating that breastmilk is a preferred mode of excretion for maternal α-HCH. While this holds true for α-HCH, no such relationship was seen for other OCPs. This may be because some maternal OCPs may selectively partition into breastmilk, while others may have a transplacental transmission, related to the varying lipophilicity of different OCPs. In addition, lack of consistent relationship with other OCPs may have been due to a faulty technique of collecting breastmilk samples. Some mothers may have expressed only the foremilk which has a lesser fat content compared to hind milk; Nair et al., (1996) found that hind milk had 80% more DDT and HCH compared to maternal serum. Hence, the measured OCPs in breastmilk may not be truly representative of actual transfer of OCPs to the infant through continued breastfeeding. There were very few studies showing relationship of pesticides in breastmilk in relation to IUGR. Few authors have reported an association of low birthweight with DDE in breastmilk (Siddiqui et al., 2003; Khanjani and Sim, 2006). In Norway, an association was reported between increased hexachlorobenzene in breastmilk with decreased BW, CHL and HC, although this association was restricted to smokers (Eggesbø et al., 2009). Siddiqui et al., (2003) did not find any association between β-HCH in breastmilk with birthweight. In contrast, Schade and Heinzow, (1998) reported an association between higher breastmilk β-HCH and lower BW in females. We found breastmilk DDT to significantly affect the fetal growth (BW, CHL, HC and CC).

Ponderal index was found to be negatively associated with HCH levels implicating that the fetal insult due to OCPs continues during pregnancy resulting in asymmetrical growth retardation, which may have grave implications for the newborn (Henriksen, 1999). OCP exposure did not affect the mid-arm circumference significantly, unlike the other anthropometric estimates which were affected in some ways. Different mechanisms have been postulated for growth retardation due to OCPs such as genotoxic, immunotoxic, endocrine disruption (anti-progesterone effects), cytotoxic (lipid peroxidation) and fetotoxic effects (placental dysfunction) (Pathak et al., 2010). The chemicals used in the present study are known endocrine disruptors and have a capacity to alter the estrogen and thyroid hormones (Janosek et al., 2006; Lopez-Espinosa et al.,

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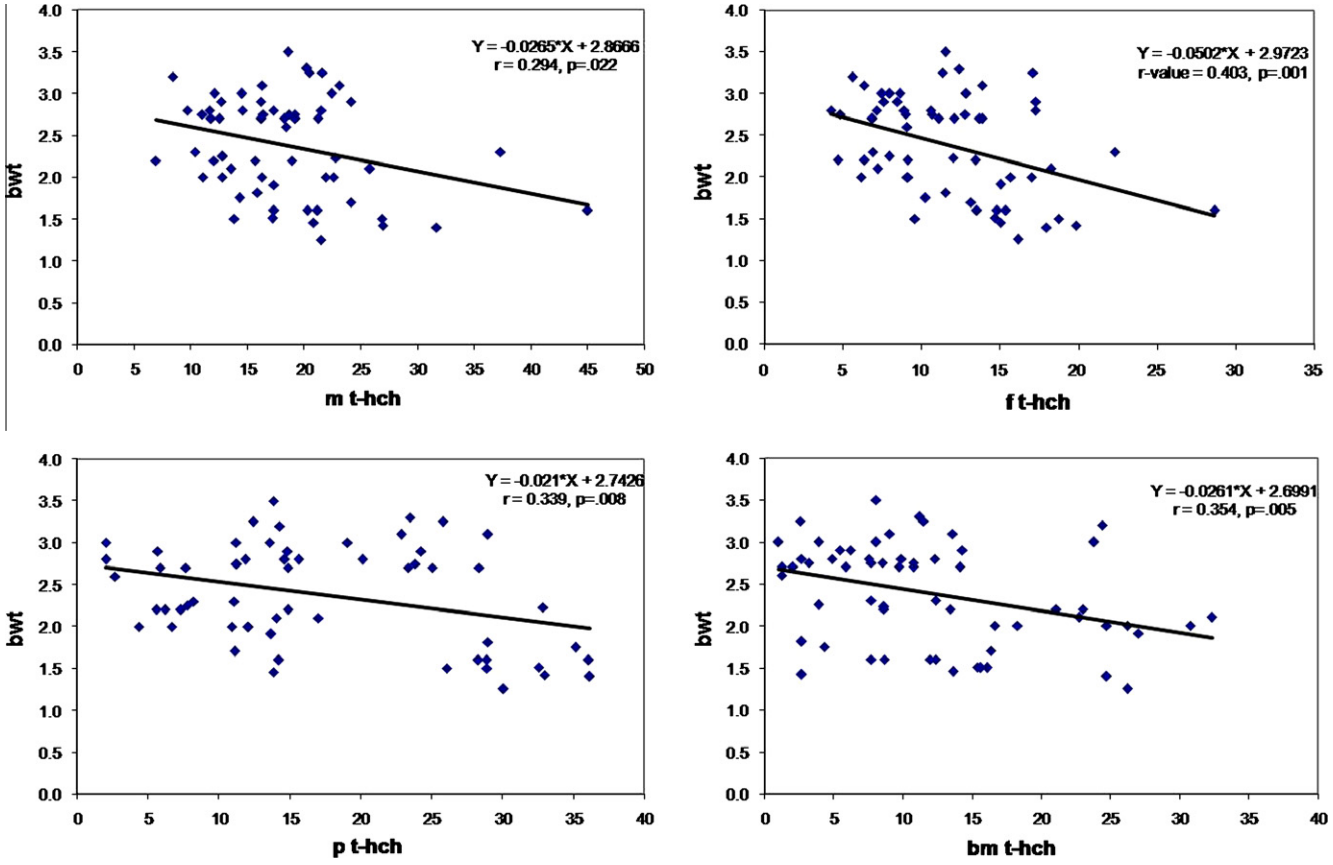

Fig. 1. Correlation between total HCH (t-HCH) in maternal blood (m t-HCH), cord blood (f t-HCH), placenta (p t-HCH) and breastmilk (bm t-HCH) and birthweight.

2009) which may have an effect on the somatic growth. We, however, did not evaluate the exact mechanism of toxic injury due to OCPs in our study. For a study with ramifications on the population we agree that our sample was small. However, we have obtained significant results probably due to the strength of the associations. By recruiting only term infants, the effect of gestational age was eliminated. The simultaneous evaluation of OCP levels in maternal blood, cord blood, placenta and breastmilk enabled us to ascertain the biotransmission process. These findings need to be confirmed in a larger and wider sample.

We conclude that exposure to organochlorine pesticides in pregnant women may be an important etiology in fetal growth restriction. Given the persistence of DDT and γ-HCH in the environment, studies using more robust data should continue to assess this relation. Till then, indeterminate use of the pesticides should be checked and appropriate measures to decrease human exposure to these persistent organic pollutants by allowing their minimal need-based use needs to be implemented.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.09.083.

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