Journal of Developmental Origins of Health and Disease

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Original Article

Cite this article: Torres-Sánchez L, Escamilla-Nuñez MC, Cebrian ME, Ancira-Moreno M, Rivera-Pasquel M, Lope V, Ramos-Treviño J, and Blanco-Muñoz J. (2023) Exposure to *p,p´*-DDE during early pregnancy, anthropometry, and gestational age at birth, in a flower-growing region of Mexico. *Journal of Developmental Origins of Health and Disease* **14**: 15–23. doi: 10.1017/S2040174422000277

Received: 28 September 2021
Revised: 17 March 2022
Accepted: 24 April 2022
First published online: 20 May 2022

Keywords:
p,p´-DDE; anthropometry at birth; small for gestational age

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Exposure to *p,p´*-DDE during early pregnancy, anthropometry, and gestational age at birth, in a flower-growing region of Mexico

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Abstract

Prenatal exposure to dichlorodiphenyldichloroethylene (*p,p´*-DDE) may interfere with fetal development; however, studies evaluating anthropometry and gestational age at birth show inconsistent results. Typically, *p,p´*-DDE exposure has been measured during the third trimester and missed the key early pregnancy period. We evaluated the association between *p,p´*-DDE exposure before week 18 of pregnancy and anthropometry at birth, as well as gestational length, in 170 mother–child pairs from a cohort study in a flower-growing mexican region. Maternal serum *p,p´*-DDE concentrations were determined by gas chromatography. The associations between *p,p´*-DDE and z-scores of birth weight, birth length, and gestational age were evaluated by linear multiple regression models. Logistic regression models were used for low birth weight and small size for gestational age. Effect modification by child's sex was explored. The average gestational age at the blood sample extraction was 10.6 weeks. *p,p´*-DDE was detected in 64.7% of mothers, at a geometric mean of 0.24 ng/mL. Prenatal *p,p´*-DDE exposure was not associated with the birth outcomes in the whole sample. However, a high *p,p´*-DDE exposure was marginally associated with greater small for gestational age risk in male newborns ($OR_{\geq 0.076\ ng/mL\ vs\ <0.076\ ng/mL} = 3.09$, 95% CI: 0.61; 15.58), but not in female (*p* for interaction = 0.08).

Even though, we found no reductions in anthropometric measurements or gestational length associated with early prenatal *p,p´*-DDE exposure, the potential effect modification by infant's sex in terms of small for the gestational age risk deserves future studies.

Introduction

Anthropometry and gestational age at birth are important neonatal health indicators that reflect the fetus' development progress from conception time to birth; these factors have been associated with adverse health effects in early childhood, as well as with chronic diseases in adulthood.[1,2] During fetal development, complex and sequential changes happen and they are characterized by fetal alterations at the molecular and cellular levels. Any adverse influence on this process may have consequences whose magnitude will depend on the nature, moment, duration, and severity of the disturbance.[3] Therefore, "fetal programming" could permanently affect fetal's physiology as a result of "*in utero*" environmental conditions.[4]

Dichlorodiphenyltrichloroethane (DDT), and its main metabolite, dichlorodiphenyldichloroethylene (*p,p´*-DDE) are endocrine disruptors with estrogenic, anti-androgenic[5] and anti-thyroid properties.[6] Both sexual steroid and thyroid hormones play key roles in fetal growth and development[7]; thus, many authors have hypothesized that maternal or neonatal thyroid disruption[8,9] may mediate potential negative effects of exposure to these compounds on the fetus's development.

Nevertheless, studies of the effects of prenatal *p,p´*-DDE exposure on the weight, length, and gestational age at birth have yielded discordant results. Some studies find no association with low birthweight,[10,11] small for gestational age (SGA),[12,13] or preterm birth[13]; on the other hand others studies associated it with low[8,14,15] or high weight at birth,[9] increased risk of SGA,[16] intrauterine fetal growth restriction,[17] or preterm birth.[16,18] Recently, Chevrier *et al.*[19] reported an association of prenatal exposure to *o,p´*-DDT, *p,p´*-DDT, and *p,p´*-DDE with increased weight, length, and head circumference at birth in girls but not among boys. None association with gestational age at birth was observed. Differences in exposure levels, biological collection

temporality, and matrices for exposure assessment could partially explain the observed inconsistencies. Additionally, most of the studies do not consider standardized anthropometric measurements, such as z-scores for weight and length according to sex and gestational age, which would allow a better comparison between them.[11,20] Only two studies examined the potential mediating effect of maternal or fetal thyroid function[8,9] on anthropometric measurements or gestation length; however, their results did not confirm this hypothesis.

Despite the persistent nature and the prolongated half-life of *p,p′*-DDE, the concentrations of persistent organic pollutants may vary across critical windows of development.[21] Most studies have focused on third-trimester exposure using maternal serum concentrations at delivery or umbilical cord serum concentrations, despite increasing evidence that the early pregnancy period may be a critical window for fetal growth and development.[22]

In Mexico, DDT was used massively in agriculture, antimalarial campaigns, as well as for controlling other vector-transmitted diseases. Its use in agricultural pest and malaria control was restricted in 1991 and 1999, respectively.[23] However, due to its high persistence in the environment and its ability to biomagnify through the trophic chain, *p,p′*-DDE can still be detected in biological serum samples,[24] fatty tissue,[25] maternal and umbilical cord blood.[26] Two prior studies in Mexico found no association between prenatal exposure to DDT and growth during the first years of life[20,27]; however, we have not found Mexican epidemiological studies evaluating specifically its relationship with fetal growth and gestational age at birth.

We aimed to evaluate the association between early prenatal exposure to *p,p′*-DDE and different fetal growth indicators (birth weight, length at birth, low birth weight [LBW], and SGA), gestational age at birth, and preterm birth on children whose mothers live in a flower-growing region of the State of Mexico, where DDT was used in the past to control pests that affect flower crops. Because there are sex-specific differences in fetal growth and sensitivity to endocrine disruptors,[28] we evaluated whether the child's sex modifies the effect of *p,p′*-DDE exposure on the evaluated outcomes. Furthermore, we explored the potential role of maternal thyroid status as a mediator.

Method

Study population

Details on the study methodology have been previously published.[6] Between June 2013, and December 2015, a cohort study was carried out in the flower-growing region of the State of Mexico. Its objective was to evaluate the association between pesticide exposure during pregnancy and adverse reproductive outcomes as well as maternal thyroid function before 18 week of pregnancy, a critical time window of human prenatal development.

Pregnant women were recruited at the local primary health care facilities during the first prenatal visit or at the laboratory, where pregnancy tests were performed. Eligible women were pregnant between 15 and 40 years old, residing in the cohort's location region for a period frame equal to 1 year or more than, and gestational age <18 weeks. Women with a history of chronic diseases (cancer, cardiovascular diseases, nephropathy, diabetes, and endocrinopathies) were excluded from the study. At recruitment, each woman responded to a structured interview and provided a blood sample. After the delivery, the women were contacted for follow-up.

The study was approved by the Ethics and Research Committees of the National Institute of Public Health of Mexico. All eligible women were informed about the study's aim, and those who accepted to participate read and signed informed consent.

Data collection

At enrollment, each pregnant woman responded to a structured questionnaire providing information on last menstruation date, sociodemographic characteristics (age, education, occupation, marital status), reproductive history (number and outcomes of previous pregnancies), alcohol, tobacco, and coffee consumption before and during the index pregnancy. Only a few women reported having consumed tobacco (two women) or alcohol (seven women) during pregnancy; therefore, these variables were categorized as consumption over a lifetime (yes or no). Maternal height and weight were measured following standardized procedures (standing up, barefooted, and with light clothing) using a portable Seca stadiometer and a Tanita electronic digital scale.

After delivery, the mothers were interviewed again to collect data on the date of birth, sex, weight, and newborn's length, health conditions at birth (born alive or dead, malformations), type of delivery (vaginal or C-section), and complications during the pregnancy and birth (preeclampsia, premature membrane rupture, dystocia).

Out of 635 eligible pregnant women, 480 (75.6%) agreed to participate in the study; the main reason for nonparticipation was lack of time. We were able to obtain information on 192 births from 189 pregnancies. Twenty-two newborns were excluded for the following reasons: Twin pregnancies, stillbirth, congenital malformations or whose mother reported preeclampsia during pregnancy, or missing data on *p,p′*-DDE, or thyroid function. The results presented then correspond to a subsample of 170 mother–child pairs, with information related to *p,p′*-DDE exposure, thyroid function, and birth outcomes (weight, length, and gestational age at birth).

Collection of biological samples and chemical determinations

At the first interview, we collected a blood sample (10 mL) in a Vacutainer tube without anticoagulant; the samples were centrifuged at 2500 rpm. Two serum aliquots from each participant were kept at −80°C until analyzed; one was stored in Eppendorf cryovials for thyroid hormones determination, and the other was kept in glass cryovials prewashed with hexane and covered with a Teflon top, for *p,p′*-DDE determination.

Determination of p,p′-DDE

p,p′-DDE serum concentrations were determined by gas chromatography with an Agilent 7890 micro electron capture detector (CG-μECD) and the procedure followed the protocol recommended by the US Environmental Protection Agency (EPA).[29] Lipid concentration (mg/dL) was estimated with the SPINREACT colorimetric kit. Concentrations of *p,p′*-DDE were reported as ng/mL in wet weight basis and as ng/g in lipid basis. For every 10 study samples, one sample of bovine serum with known quantities of *p,p′*-DDE, was analyzed, with a recovery of 99%. In addition, one randomly selected sample was analyzed in duplicate in each bach with a coefficient of variation <10%. The recovery percentage of the samples enriched with 40 μL of aldrin (1×10^{-7} mg/mL) was maintained between 92.99–99.36%. Detection limits (DL) and quantification limits (QL) for the wet

https://doi.org/10.1017/S2040174422000277 Published online by Cambridge University Press

weight basis samples were 0.076 and 0.232 ng/mL, respectively, while for the lipid basis samples they were 1.75 and 5.30 ng/g. For levels below DL or QL, an imputed value equal to one-half the LD (0.038 ng/mL) or LQ (0.116 ng/mL)was used as suggested by EPA.[30]

p,p′-DDE determinations were performed at the Toxicology Laboratory of the Center for Advanced Research of the National Polytechnic Institute (CINVESTAV), Mexico.

Determination of TSH and Thyroid hormones

Serum concentrations of TSH and free T4 were determined by Enzyme-Linked ImmunoSorbent Assay (ELISA), using an automated immunoassay system (DRG International, Inc., USA). Reference values for the concentrations of thyroid hormones in the pregnant women were assessed according to the information provided in the commercial kits, as follows: a) TSH, 0.5–5.0 mlU/L; and b) free T4, 0.76–2.24 ng/dL. The intra-assay coefficients of variation for serum TSH and free T4 were 3.36–3.88%, and 3.2–10.9%, respectively. The interassay coefficients of variation for each biomarker were 3.3–9.1%, and 7.9–10.8%. The analytical sensitivity for TSH and free T4 were 0.06 mIU/mL, and 0.05 ng/dl, respectively.

Thyroid determinations were performed at the Reproduction Biology Laboratory of the Autonomous University of Coahuila (Universidad Autónoma of Coahuila), Mexico.

Gestational age and anthropometry at birth

Gestational age at birth was calculated based on the last menstruation date. Newborns were classified as preterm when gestational age at birth was <37 complete weeks.

Z-scores and percentiles for weight and length, according to the sex and gestational age at birth were calculated and standardized according to the standards of the International Fetal and Newborn Growth Consortium for the 21st Century.[31] A newborn was considered to be SGA when his/her weight was below the percentile 10 corresponding to his/her sex and gestational age at birth. We considered LBW when weight at birth was <2500 g.

Statistical analysis

Maternal and newborn selected characteristics were compared across the birth outcomes using the Chi-squared test or Fisher's exact test for categorical variables, and the *t*-test, ANOVA, or Kruskal–Wallis test for continuous variables. Likewise, to identify a potential selection bias, we compared the maternal characteristics' distribution between pregnancies included and not included in the analysis.

The geometric mean (GM) and median concentration of *p,p′*-DDE, was calculated using the laboratory imputed values for levels below the DL or QL. Because 50% of mothers had *p,p′*-DDE concentrations below QL, we decided to categorize this variable as follows: <DL; ≥DL and <QL; ≥QL. The association between *p,p′*-DDE exposure (taking as a reference <DL category) and anthropometry at birth (weight, length, *z*-scores), and gestational age was estimated using independent linear regression models. Additionally, we used independent logistic regression models to evaluate the association between prenatal *p,p'*-DDE exposure and SGA (yes or no), LBW (yes or no), and whether the birth was preterm (yes or no). Infants delivered by C- section were excluded from the models that evaluated the association between *p,p'*-DDE and gestational age or preterm birth.

We considered as potential confounders: maternal age (continuous [years] and categorized as <18, 18–34, and 35 and more); gestational age at the time when the blood samples were collected (weeks); body mass index (BMI - [kg/m^2]) at the study enrollment (continuous and categorized as <18.5 [underweight], 18.5–24.9 [normal weight], 25–29.9 [overweight], ≥30 [obesity]); education (≤elementary, junior high school, >junior high); marital status (with or without partner); alcohol or tobacco consumption over mother's life course (yes or no); consumption of coffee during pregnancy (≥one cup/day or no), and previous pregnancies (continuous variable and categorized as none, 1, 2, and ≥3). All the final models included maternal age, gestational age at the biological samples were collected, and also, those co-variables that modified the estimators by 10% or more. The newborns' sex was only included in models where the association with non-standardized weight and length at birth was estimated, as well as with LBW and gestational age at birth.

Since prior studies suggest that *p,p'*-DDE anti-androgenic effects[5] may impact fetal development depending on the fetus' sex,[16] potential interactions between *p,p'*-DDE, and sex were explored by adding interaction terms.

A potential mediator role of maternal thyroid function was evaluated by including TSH and thyroid hormones separately in the regression models, as applied by Kezios *et al.*[8] Mediation by maternal thyroid function was only evaluated if *p,p'*-DDE exposure was associated with the birth outcome. Maternal thyroid function would be a mediator if *p,p'*-DDE exposure is associated with TSH and/or free T4 levels, and they in turn are associated with the birth outcome. In this case, the inclusion of TSH or free T4 in the model would reduce the estimators (beta-coefficient and OR) for the *p,p'*-DDE exposure and birth outcomes.

All analyses were done using the Stata 14 software (Stata Corp., TX, USA).

Results

The average gestational age at enrollment in the study and blood sample extraction was 10.6 weeks (range: 4 to 17 weeks); 72.4% of biological samples were taken during the first 12 gestational weeks. Most of the women lived with partners and had an education level of junior high school; 38.2% were primiparous. Most of the women had never smoked, only 2 reported active smoking during pregnancy. 70% of the women had a history of alcohol consumption, but only 7 consumed it during pregnancy. At enrollment, the mean BMI was 24.5 kg/m^2, and approximately 36% were overweight or obese. The medians of TSH and free T4 were 0.83 mIU/L and 0.71 ng/dL, respectively. Half of the pregnant women (50.6%) had *p,p′*-DDE concentrations ≥QL. The GMs of the p,*p′*-DDE concentration in wet weight and lipid bases were 0.24 ng/mL and 8.0 ng/gr, respectively, and medians were 0.13 ng/mL and 7.60 ng/g. The mothers of infants included in the analysis drank coffee more frequently and had higher free T4 levels than those not included. No other significant differences were observed (Table 1).

Regarding newborns, the proportion of boys and girls was similar. Almost 60% of newborns were born through vaginal delivery. The average gestational age at birth was 38.3 weeks, and approximately 13% of the births were preterm. The weight and length's mean at birth were 3039 g and 50 cm, respectively; 10.6% of newborns weighed <2500 g, and 18.2% were SGA. The majority of *z*-scores for weight and length were within one standard deviation (Table 2).

https://doi.org/10.1017/S2040174422000277 Published online by Cambridge University Press

Table 1. Maternal selected characteristics of children included and not included in the analysis

Maternal characteristics	Included ($n = 170$)	Not included ($n = 310$)	*P* value*
Gestational age[a] (weeks)			
Mean ± SD	10.6 ± 2.8	10.1 ± 2.8	0.07
≤12 semanas	123 (72.4%)	244 (78.7%)	0.19
Maternal age[a] (years)			
Median (25^{th}, 75^{th})	23 (20–30)	23 (20–27)	0.11
<18	12 (7.1%)	26 (8.4%)	0.11
18–34	141 (82.9%)	268 (86.5%)	
≥35	17 (10.0%)	16 (5.2%)	
Marital status[a]			
With partner	150 (88.2%)	277 (89.6%)	0.64
Education[a]			
≤Elementary school	37 (21.8%)	57 (18.4%)	0.31
Junior high school	87 (51.2%)	152 (49.0%)	
>Junior high school	46 (27.0%)	101 (32.6%)	
Previous pregnancies			
0	65 (38.2%)	129 (41.6%)	0.34
1	40 (23.5%)	86 (27.7%)	
2	40 (23.5%)	58 (18.7%)	
≥3	25 (14.7%)	37 (11.9%)	
Tobacco consumption history[b]			
Yes	52 (30.6%)	105 (33.9%)	0.46
No	118 (69.4%)	205(66.1%)	
Alcohol consumption history[b]			
Yes	119 (70.0%)	166 (62.6%)	0.09
No	51 (30.0%)	116 (37.4%)	
Coffee intake during pregnancy[c]			
Yes	121 (71.2%)	251 (81.0%)	0.01
No	49 (28.8%)	59 (19.0%)	
Body mass index[a] (kg/m^2)			
<25	109 (64.1%)	147 (62.6%)	0.9
25 to 29.9	42 (24.7%)	59 (25.1%)	
≥30	19 (11.2%)	29 (12.3%)	
p,p'-DDE concentration[a,d] (ng/mL)			
Median[d] (25^{th}, 75^{th})	0.13 (0.038–1.02)	0.19 (0.038–2.4)	0.11
<0.076	60 (35.3%)	104 (39.1%)	0.56
0.076 to <0.232	24 (14.1%)	29 (10.9%)	
≥0.232	86 (50.6%)	133 (50.0%)	
Thyroid hormones			
Median[a](25^{th}, 75^{th})			
TSH (mUI/L)	0.83 (0.43–1.71)	0.87 (0.48–1.61)	0.90
Free T4 (ng/dL)	0.71 (0.62–0.81)	0.76 (0.65–0.86)	0.01

Standard deviation (SD); 25^{th} and 75^{th} centiles.
**t*-Test; Chi-squared test; U-Mann–Whitney test.
[a]At enrollment.
[b]Consumption over a lifetime.
[c]≥1 coup per day.
[d]Values below the QL or DL were replaced by half the QL or DL.

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Table 2. Newborn selected characteristics ($n = 170$)

Characteristics	*n* (%)	Mean	SD[a]	Median	25 th	75th
Sex						
Female	85 (50.0)					
Type of birth						
Vaginal delivery	101 (59.4)					
Gestational age at birth (weeks)		38.3	2.6	39.0	38.0	40.0
Preterm birth (<37 weeks)	22 (12.9)					
Anthropometry at birth						
Birthweight (g)		3039.2	558.7	3035.0	2740.0	3350.0
Weight z-scores		−0.22	1.15	−0.28	−0.95	0.55
Low birthweight (<2500 g)	18(10.6)					
Small for gestational age(<10th centile)	31 (18.2)					
Lenght at birth (cm)		50.0	6.4	49.0	48.0	51.0
Length z-scores		0.27	1.6	0.43	−0.69	1.28

[a]Standard deviation.

Table 3. Adjusted associations[a] between maternal *p,p'*-DDE concentrations and birth outcomes

	Prenatal p,p'-DDE exposure (ng/mL)		
Birth outcomes	<0.076	0.076 to <0.232	≥0.232
Birth weight (g)			
β[b] (95% CI)	Ref	94.27 (−129.86, 318.48)	−32.11 (−193.86, 129.63)
Birth weight z-scores			
β[c] (95% CI)	Ref	0.23 (−0.32, 0.78)	0.01 (−0.38, 0.41)
Birth length (cm)			
β[d] (95% CI)	Ref	0.32 (−1.36, 1.99)	−0.62 (−1.84, 0.59)
Birth length z-scores			
β[e] (95% CI)	Ref	−0.05 (−0.84, 0.73)	−0.30 (−0.85, 0.25)
Low birth weight			
OR[b] (95% CI)	Ref	0.46 (0.05, 4.44)	0.98 (0.28, 3.43)
Gestational age at birth*			
β[f] (95% CI)	Ref	1.34 (−0.47, 3.15)	0.89 (−0.32, 2.10)

*Newborns delivered by C-section were excluded.
[a]All models were adjusted for maternal age and gestational age at enrollment (when blood samples were collected) both as continuous variables, taking the category of *p,p'*-DDE < 0.076 ng/ml as comparison group.
[b]Adjusted for newborn sex, gestational age at birth (weeks), number of previous pregnancies (as a continuous variable), tobacco consumption history (yes or no) and body mass index (as a continuous variable).
[c]Adjusted for number of previous pregnancies (as a continuous variable), tobacco consumption history (yes or no) and body mass index (as a continuous variable).
[d]Adjusted for newborn sex, gestational age at birth (weeks), marital status and maternal education.
[e]Adjusted for marital status and maternal education
[f]Adjusted for newborn sex, body mass index (included as a continuous variable) and maternal education.

The birth outcomes distribution by newborn's sex and selected maternal characteristics (Supplementary Table S1) showed that girls had a significantly lower weight at birth than boys (2904.5 g vs 3173.8 g; $p < 0.01$) and the LBW frequency was marginally higher among girls (15.3 vs 5.9%, $p = 0.05$) than the boys; nevertheless, there were no significant sex-related differences in the SGA and preterm birth frequency. Among the primiparous women or those with only one previous pregnancy, the proportion of children born SGA was marginally higher ($p = 0.10$) than the one observed in women who had two or more previous pregnancies. Maternal alcohol consumption was marginally associated with higher SGA (21.9 vs. 10.0%; $p = 0.06$) and lower preterm birth frequency (10.1 vs. 19.6%; $p = 0.09$).

No significant associations were found between *p,p'*-DDE exposure and the evaluated birth outcomes either in crude (data not shown) or adjusted models (Table 3). In the adjusted models, girls had significantly less weight and length at birth than boys. Birth weight and length increased as gestational age at birth increased; in contrast, the risk of LBW decreased. Maternal age was associated

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Table 4. Sex-stratified adjusted models[a] for the association between maternal *p,p'*-DDE concentrations and the risk of small for gestational age (SGA)

	All sample ($n = 170$)		Male ($n = 85$)		Female ($n = 85$)	
	Small for gestational age (SGA)					
p,p'-DDE (ng/mL)	Yes/No (31/139)	OR (95% CI)	Yes/No (13/72)	OR (95% CI)	Yes/ No (18/67)	OR (95% CI)
<0.076	11/49	1.00	2/23	1.00	9/26	1
≥0.076	20/90	1.00 (0.43, 2.32)	11/49	**4.40* (0.82, 23.58)**	9/41	0.44 (0.14, 1.42)

*$p = 0.08$.
[a]Adjusted for maternal age, maternal body mass index, and gestational age at enrollment, all as continuous variables.

Table 5. Adjusted models[a] for interaction between maternal *p,p'*-DDE concentrations and newborn's sex on the risk of small for gestational age (SGA)

	Male ($n = 85$)		Female ($n = 85$)		
	Small for gestational age (SGA)				
p,p'-DDE (ng/mL)	Yes/No (13/72)	OR (95% CI)	Yes/No (18/67)	OR (95% CI)	*p* for interaction
< 0.076	2/23	1.00	9/26	4.68 (0.89, 24.61)	**0.08**[b]
≥ 0.076	11/49	3.09 (0.61, 15.58)	9/41	2.50 (0.49, 12.83)	

[a]Adjusted for maternal age, maternal body mass index, and gestational age at enrollment, all as continuous variables.
[b]OR for interaction = 0.17 (CI 95 % 0.02, 1.22, $p = 0.08$).

with lower gestational age at birth. Previous pregnancies and higher BMI at the beginning of pregnancy were associated with higher birth weight and weight z-scores. Married women had children with a higher length z-score. Also, maternal education level above junior high school was associated with a greater *z*-score for length and a lower gestational age at birth (Supplementary Table S2). As the number of infants born preterm was low when excluding those delivered by C-section, we were unable to assess the effect of *p,p'*-DDE on preterm birth.

Prenatal exposure to *p,p'*-DDE ≥ DL was not associated with SGA risk. Nevertheless, a newborn's sex-stratified analysis suggested a potential interaction with *p,p'*-DDE (Table 4). *p,p'*-DDE concentrations above the DL were associated with higher odds of SGA among males (OR = 4.40; CI 95%: 0.82; 23.58) but not among females (OR = 0.44; CI 95%: 0.14; 1.42). The interaction analysis showed that only among male newborns, SGA risk seems to increase (OR = 3.09 CI 95%: 0.61–15.58; p for interaction = 0.08) with *p,p'*-DDE concentrations above DL (Table 5). No other interaction was observed between *p,p'*-DDE exposure and the newborn's sex in any of the other evaluated outcomes (data not shown in tables).

We observed no evidence regarding any mediator effect of maternal thyroid function between *p,p'*-DDE exposure and birth outcomes. Likewise, the inclusion of TSH or free T4 did not change the results in relation to the potential effect of the interaction between *p,p'*-DDE exposure and newborn's sex on SGA, (Supplementary Table S3 A–B).

Discussion

Overall, the results of this study do not support an association of early prenatal low exposure to *p,p'*-DDE with the anthropometric parameters at birth, or with gestational age at birth. Nevertheless, our results suggest a differentiating effect on SGA risk according to the newborn's sex. Contrary to female newborns, the *p,p'*-DDE concentrations above the DL were associated with a higher SGA risk among the males. We did not find evidence to support mediation by maternal thyroid status.

Our results are partially consistent with those from two recent meta-analyses that included European cohorts where *p,p'*-DDE concentrations in the umbilical cord (50–1323 ng/L) were not associated with birth weight or gestational age at birth,[10] or with SGA risk.[12] Although *p,p'*-DDE concentrations of the mentioned studies may be considered to be low, studies such as the cohort established during the 1960s in San Francisco (USA) (median maternal serum concentrations of *p,p'*-DDE = 6.9 µg/g of lipids), also exhibited no association with anthropometry at birth, gestational age,[11] prematurity, or SGA.[13] Two recent studies conducted in China, neither found significant associations.[15,32]

Longnecker *et al.*[16] found that children of pregnant women with *p,p'*-DDE concentrations ≥60 µg/L had higher probabilities of preterm birth and SGA. Also, other authors have reported a negative association between maternal *p,p'*-DDE concentrations and newborn's weight at birth,[14] and a positive association with preterm birth.[18] Kezios *et al.*[8] found that, when controlling for *p,p'*-DDT concentrations, maternal *p,p'*-DDE levels were associated with a reduction in birth weight and in gestational age. On the other hand, Arrebola *et al.*,[9] in Bolivia, found a positive association between *p,p'*-DDE concentrations (median 1.01 ng/mL) and birth weight ($\beta = 0.012$, $p = 0.006$), and a negative association with gestational age ($\beta = -0.004$, $p = 0.012$).

It seems unlikely that the differences in the magnitude of prenatal exposure to *p,p'*-DDE can explain the observed inconsistent results because both relatively low and high exposures have been positively and negatively associated with the evaluated birth outcomes. Differences in the pregnancy stage when the blood samples were taken for *p,p'*-DDE determination, or whether they were obtained from maternal blood or the umbilical cord, could partially explain the discrepancies in the results, because both exposure opportunities and vulnerability to physiological/biological effects of exposure may vary from preconception to delivery.[33] On the other hand, not all the studies used standardized weight and length for the gestational age and the newborn's sex, limiting the study's comparison. (Supplementary Table S4)

Some studies have evaluated the interaction between the newborn' sex and *p,p'*-DDE exposure and its effect on fetal growth,

https://doi.org/10.1017/S2040174422000277 Published online by Cambridge University Press

with contradictory results. While most of them did not yield results that support this interaction,[8,9,11] Chevrier *et al.*[19] found a greater birthweight among girls prenatally exposed to *p,p′*-DDE (*p* for interaction = 0.074), but not among boys. As in the present study, Longnecker *et al.*[16] observed that prenatal *p,p′*-DDE exposure was associated with higher SGA risk in boys than in girls, but the interaction was not significant. Other authors have reported a decrease in the boys' ponderal index at birth[34] or in the birthweight,[35] associated with an increase in *p,p′*-DDE exposure, without association among girls; however, they did not analyze the interaction between the newborn's sex and the exposure.

Evidence exists that intrauterine growth is greater in boys than in girls, and some authors suggest that this could be due to sex-specific differences in sensitivity to androgens that are mediated by the differentiating expression of the androgen receptor (AR) at the placental level. It appears that androgens and AR are critical for appropriate male growth and development in utero, and any alteration to this signaling pathway can result in adverse outcomes.[28] Maccoby *et al.*[36] have reported that levels of tissue-specific androgens were measurable in first-trimester human male placentae. Because *p,p′*-DDE blocks the union between androgens and AR, it could be hypothesized that exposure during the early pregnancy window could have a higher impact on the growth of male fetuses than that of female ones. Nevertheless, in view of our small sample size, additional studies with a larger sample size are needed to evaluate the interaction between sex and *p,p′*-DDE exposure with respect to SGA.

Regarding a potential mediation effect of maternal thyroid status on the association between *p,p′*-DDE exposure and SGA in male newborns, our results are consistent with those reported by Kezios *et al.*[8] and Arrebola *et al.*[9] We did not find evidence to support this hypothesis, which suggests that *p,p′*-DDE exposure may have an impact on male fetal growth via a different mechanism, as we previously suggested.

Although our study population is a subsample of the original cohort, we consider that the likelihood of a selection bias is low because the maternal characteristics of the newborns included in the analysis were similar to those who were not. Maternal free T4 levels in the included infants were slightly lower than those not included; however, we think this is unlikely to have skewed our results because *p,p′*-DDE was not associated with maternal free T4 neither in the original sample nor in this subsample.

On the other hand, the frequency of LBW was higher to that recently reported for Mexico by UNICEF[37] but similar to that found in studies carried out in rural areas of Mexico.[38] The percentage of women who reported not ever having smoked was similar to that found by the 2011 National Survey of Addictions,[39] and the frequency of C-sections was consistent with reports in other studies carried out in Mexico.[40] Moreover, the main known risk factors which have been associated with the anthropometry at birth (female sex, maternal BMI, gestation duration) were confirmed in this study, thus supporting the validity of our data.

Our study has limitations that are important to highlight. Firstly, the *p,p′*-DDE concentration in our study population was low, and we only had a measurement corresponding to the first half of the pregnancy. Maternal and paternal preconception exposure to persistent organic pollutants, including DDT metabolites,[22] as well as exposure during the third trimester of pregnancy, have been associated with offspring size at birth; however, we may assume that we evaluated a key stage in placental development[41] which, if it is inadequate, leads to impaired fetal growth.[42]

Secondly, length and weight at birth were reported by mothers and we assume that there could be some degree of measurement error. However, in a previous study in the same region, we found a high agreement between the information reported by the mothers and that recorded in the birth information card provided from healthcare centers discharge where they gave birth to their children.[43] On the other hand, potential misclassification regarding these variables would be non-differential, since the pregnant women did not know their *p,p′*-DDE concentrations, and the staff member who determined this metabolite did not know the newborn's characteristics.

Although we do not dismiss the presence of residual confusion, we believe it is unlikely that tobacco and alcohol consumption during pregnancy has confounded our results because there were practically no women who were exposed. Nevertheless, in the statistical analysis, we decided to categorize this consumption as sometimes or never over life course, assuming that some women may have underreported consumption of these substances during pregnancy, as suggested by some studies.[44,45]

Even though the BMI at enrollment was included in the models, we do not have information on weight gain during pregnancy. We considered this lack of information as a potential source of residual confounding because other authors[12] have found maternal weight gain during pregnancy to be an important confounder in the relationship between environmental contaminants and birth weight. However, it has also been reported that its inclusion might produce an over adjustment.[46]

Among the strengths of our study, this is one of the few prospective studies that have evaluated the association between early pregnancy *p,p′*-DDE exposure, and standardized anthropometric parameters, as well as fetal growth indicators with clinical repercussions, such as LBW and SGA. Previous research usually have focused on third-trimester exposure despite increasing evidence that the early pregnancy period may be a critical window for fetal growth and development. Besides, in the analyses, we controlled for important covariables that potentially could confound the results. A particular strength of this study is that it allowed us to evaluate the interaction with newborns' sex, as well as whether maternal thyroid function mediates the effect of *p,p′*-DDE exposure on fetal growth.

In conclusion, although the overall results of this study show no association between low prenatal early exposure to *p,p′*-DDE and anthropometric parameters at birth or duration of gestation, they suggest a potential differentiating effect according to the newborn's sex, respecting the SGA risk. These results need to be replicated and, perhaps, pooled with those of similar studies to provide a larger sample size.

Acknowledgements. We express our gratitude towards the pregnant women who participated in this project and to the Health Services of the State of Mexico. We also acknowledge the work of biologist Rosa María García-Hernández for her support in the laboratory analysis, and of Evelyn Aron for the manuscript translation.

The authors are entirely responsible for the scientific content of the paper.

Financial support. This study has been funded by the Consejo Nacional de Ciencia y Tecnología of Mexico (Conacyt; grant n° 180956). The funding source had not involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation (Mexico) and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees (Ethics Committee of the Instituto Nacional de Salud Pública of México/National Institute of Public Health).

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