Dichlorodiphenyltrichloroethane (DDT) Exposure and Anogenital Distance in the VHEMBE Birth Cohort Study, South Africa

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is used for malaria control by 10 countries, nine of which are in Africa. Technical DDT contains various isomers with 65–80% insecticidal *p,p'*-DDT and 15–21% *o,p'*-DDT, an estrogenic chemical, while the persistent metabolite of *p,p'*-DDT, dichlorodiphenyldichloroethylene (*p,p'*-DDE), is an antiandrogen. *In utero* antiandrogenic exposure reduces anogenital distance in animal models and the anal position index in a single study. This study examined the associations between mother's serum DDT and DDE levels at delivery and anogenital distance in their children at birth and age 1 year. Data were collected as part of the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a

birth cohort study located in rural South Africa. DDT and DDE concentrations were measured in blood samples collected from 752 mothers at delivery. Anogenital distance measurements, taken at birth (n = 671) and age 1 year (n = 674), included anofourchette and anoclitoral distances in girls, and anoscrotal and anopenile lengths in boys. We also measured anococcygeal and coccyx-fourchette distances in girls, while in boys, we measured anococcygeal and coccyx-scrotal distances as well as penile length and penile width. The anal position index is calculated for both sexes as anoscrotal/coccyx-scrotal in boys and anofourchette/coccyx-fourchette in girls. We found no associations between *p,p′*-DDT/-DDE or *o,p′*-DDT and anogenital distance measurements at birth in either boys or girls. At 1 year, *o,p′*-DDE was negatively associated with anofourchette in girls (β =−1.32 mm, 95% confidence interval (CI) = −2.27, −0.38) and positively associated with penile width in boys (β = 0.30 mm, 95% CI = 0.00, 0.60). The results do not suggest an overt antiandrogenic or estrogenic effect on anogenital distance after long-term DDT exposure. These weak associations may be due to chance.

KEYWORDS:

Anogenital distance, boys, girls, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyl-dichloroethylene (DDE)

INTRODUCTION

The anogenital distance (AGD), measured from the center of the anus to the genitals, is influenced by androgens. The development of the perineum and external genitalia is determined by dihydrotestosterone, resulting in a greater AGD in males than females and the AGD seems to

persist in mammals throughout life (Salazar-Martinez *et al.* (2004)Romano-Riquer *et al.*, 2007). In humans, AGD is measured as the anofourchette (AF) distance in girls (Salazar-Martinez *et al.* (2004) and the anoscrotal distance (AS) distance in boys, although some studies of males also consider the distance from the anus to the anterior base of the penis (anopenile, AP) (Swan *et al.* (2005).

AGD has been examined in humans in relation to fetal exposures to various endocrine disrupting chemicals (EDC) such as phthalates (Swan *et al.*, 2005, Swan *et al.*, 2015, Suzuki *et al.*, 2012, Bustamante-Montes *et al.*, 2013, Bornehag *et al.*, 2015); dioxin (Papadopoulou *et al.*, 2013b, Vafeiadi *et al.*, 2013), and bisphenol A (Miao et al., 2011) and the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolite, dichlorodiphenyldichloroethylene (DDE) (Longnecker *et al.*, 2007, Torres-Sanchez *et al.*, 2008). In boys, elevated prenatal phthalate exposure was associated with shorter AGD, suggesting an anti-androgenic effect (Swan *et al.*, 2005, Swan *et al.*, 2015, Suzuki *et al.*, 2012, Bustamante-Montes *et al.*, 2013, Bornehag *et al.*, 2015). Similarly, AGD was shortened in male newborns after *in utero* exposure to bisphenol A (Miao *et al*., 2011), dioxin and dioxin-like compounds (Vafeiadi *et al.*, 2013). Most studies have been conducted only in male newborns, but some have included females (Salazar-Martinez *et al*. 2004; Sathyanarayana *et al.* (2010); Swan *et al.* 2015; Thankamony *et al.* (2009). No associations were found between AGD in newborn females and exposure to dioxin and dioxin-like compounds (Vafeiadi et al., 213) or phthalates (Swan *et al.*, 2015).

DDT was a commonly-used insecticide until it was banned in 1972 in the United States and in 1986 in Europe. DDT use is regulated under the Stockholm Convention on Persistent Organic Pollutants (Anon 2004) but countries such as South Africa are still allowed to use DDT

for malaria vector control. For example, DDT was introduced for malaria control in the Limpopo Province, South Africa, in 1943 and has since been sprayed annually for vector control (Bornman *et al.*, 2010). During indoor residual spraying (IRS), DDT is applied in a mixture of 65–80% of the insecticidal 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (*p,p'*-DDT) and 15–21% of the less insecticidal 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane (*o,p'*-DDT) (Metcalf, 1995) to the inside walls and eaves of homes. DDT and DDE isomers have different endocrine effects: *p,p'*-DDE has anti-androgenic properties (Kelce *et al.*, 1995) (Danzo, 1997) while *p,p'*-DDT and *o,p'*-DDT are estrogenic (ASTDR, 2002). *o,p'*-DDT is less insecticidal than *p-p'*-DDT (Metcalf, 1995), but is the most estrogenic DDT isomer and initiates both estrogen receptor (ER) and ER-independent gene expression (ASTDR, 2002, Bratton *et al.*, 2012). *o,p'*-DDE, the breakdown product of *o,p'*-DDT, is weakly estrogenic in recombinant receptor-reporter gene assays (Balaguer et al. 1999) and competes with 17β-estradiol for binding to the estrogen receptor in uterine extracts of rabbits (Danzo 1997) and immature rats (Kelce et al. 1995).

In one population from Mexico where DDT had been used, but banned before the commencement of the study, no significant associations between maternal DDT or DDE concentrations and infant AGD (Longnecker *et al.*, 2007). However, in another Mexican population, *p-p'*-DDE levels measured during the first trimester of pregnancy was reported to be associated with a reduced in the Anal Positionl Index (API) or the ratio between the anoscrotal and coccyx-scrotal distances in boys (Torres-Sanchez et al., 2008). No study has explored the relationship between prenatal exposure to DDT isomers and AGD in infant boys and girls in a population with current DDT use. In this birth cohort study conducted in Limpopo, South Africa,

we examined whether AGD in newborn and one-year old boys and girls were related to maternal blood concentrations of DDT and DDE during pregnancy.

METHODS

Study population

Between August 2012 and December 2013 we initiated the Venda Health Examination of Mothers, Babies and their Environment (The VHEMBE Study). Women presenting in the early stages of labor at Tshilizidini hospital in the Vhembe district of South Africa's Limpopo Province were approached for participation in this longitudinal birth cohort study. Eligible women were ≥18 years old, spoke Tshivenda at home, lived within 20 km of the hospital and planned to remain in the area, had not been diagnosed with malaria during pregnancy, had contractions >5 minutes apart, and gave birth to a live singleton infant. Written consent was obtained from mothers before study participation. All human subject protocols were approved by the Institutional Review Boards at the University of California, Berkeley, McGill University, the University of Pretoria, the Limpopo Department of Health and Social Development, and the Ethics Committee of Tshilidzini Hospital. Follow-up assessments were conducted when the infants were one year old. A total of 1649 women were approached, 920 were eligible, 752 were enrolled, and 700 completed the one-year visit.

Exposure measurement

Maternal blood samples were collected into vacutainer tubes by two study nurses prior to delivery if possible (n=595), or otherwise after delivery (n=157). All but three samples collected after delivery were collected on the day of delivery or the day after (the other three were

collected two days after). Samples were immediately separated into serum and clot and frozen at -80°C in our field office located on the grounds of the hospital. Aliquots of two mL of maternal serum were sent on dry ice to Emory University for measurement of *p,p'* and *o,p'* isomers of DDT/E using high resolution gas chromatography-isotope dilution mass spectrometry (GC-MS) (Barr *et al.*, 2003). The limits of quantification ranged between 0.03 and 0.06 ng/mL for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and between 0.09 and 0.18 ng/mL for *p,p'*-DDE. Quality control samples including sealed blanks, field and laboratory blanks, and spiked samples. Results were lipid-adjusted based on a summation method using triglycerides and total cholesterol concentrations measured using standard enzymatic methods (Roche Chemicals, Indianapolis, IN) (Phillips *et al.*, 1989).

Maternal interview and measurements

Mothers were interviewed in TshiVenda (the local tribal language) by trained bilingual (TshiVenda and English) staff originating from the study area. The questionnaire gathered information on demographic and family characteristics, medical history, lifestyle and diet during pregnancy as well as exposure information. After delivery, maternal weight and height were measured, from which the mother's post-delivery body mass index (BMI - kg/m^2) was calculated.

Child measurements

Birthweight measurements were performed by hospital nurses immediately after birth using a Tanita Newborn Scale (BD-MA III 815U scale) provided and routinely calibrated by the study team. At the one-year visit (mean age 372 ± 26 days), child weight (using Tanita Corporation BD-590) and recumbent length (using a SECA Infantometer 416) were measured.

Weight-for-length z-scores at one year were calculated using growth standards provided by the WHO (World Health Organization, 2006).

AGD measures were collected on the day of postnatal discharge and at the one-year visit (see Figure 1) by two study nurses who were blinded to maternal DDT/E levels. Measures of AGD followed the protocol developed by Sathyanarayana *et al.* (2015) and study nurses were trained with a videotape and consultation with the pediatrician trainer involved in that study. Of the 752 infants initially enrolled in the study, 737 (98.0%) had at least one AGD measurement at either delivery or 1 year. All measurements were performed with Swiss Precision Instruments (SPI) dial calipers (Enco Manufacturing, Fernley, Nevada), except penile length, which was done with a ruler. All measurements were performed in triplicate and averaged. The caliper measurements were all read to the nearest 0.1mm. For boys (Figure 1a), AGD measurements included anoscrotal distance (AS - from the center of the anus to the posterior base of the scrotum, anopenile (AP - the center of the anus to the anterior base of the penis), anococcygeal (ACo - the anus to the coccyx), and penile length (stretched) (PL) and width (PW). PL of the flaccid penis was measured by holding a ruler perpendicular at the penopubic junction against the bone and gently stretching the penis to read the distance to the nearest 1 mm. PW was measured as the diameter at the base of the flaccid penis held perpendicular to the baby's body and in contact with the left base of the penis, but not compressing the skin of the penile shaft on either side. The coccyx-scrotal distance (CS) was calculated by adding the anococcygeal and anoscrotal measurements (Torres-Sanchez et al., 2008). The anal position index (API) was calculated as the ratio between the anoscrotal and coccyx-scrotal (AS+AC) distances.

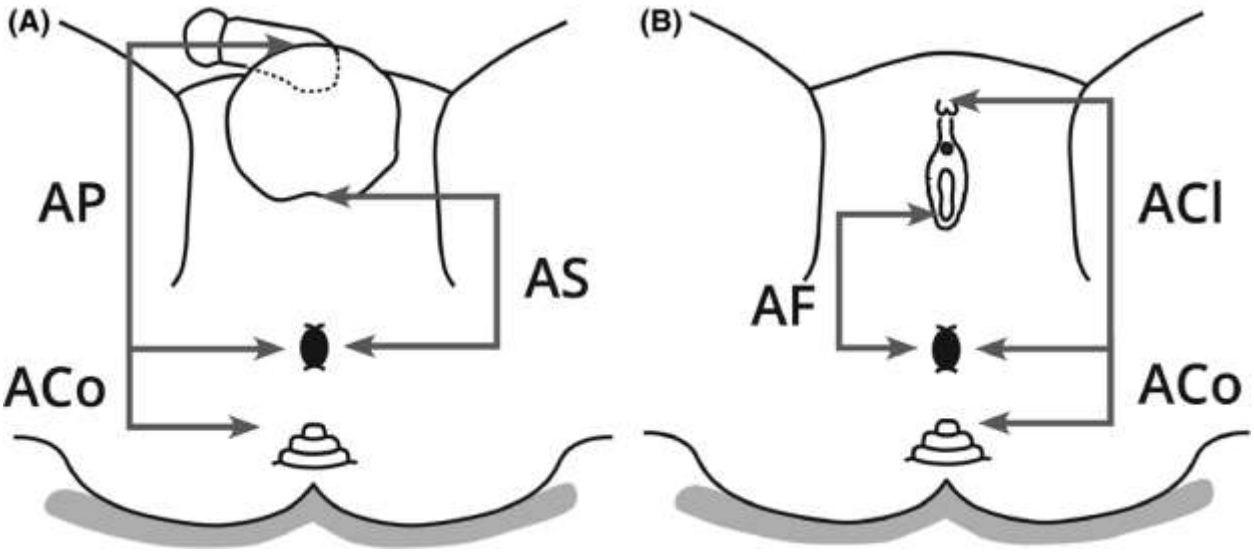

Figure 1. (a) Boys' anopenile (AP), anoscrotal (AS), and anococcygal (ACo) measurements. (Adapted from Sathyanarayana *et al*., 2010). (b) Girls' anococcygeal (ACO), anofourchette (AF), and anoclitoral (ACL) measurements. (Source: Torres-Sanchez *et al*., 2008).

Measurements for girls (Figure 1b) included anococcygeal distance, as well as anofourchette (AF - distance between the anus and the fourchette) and anoclitoral (ACl – between the anus and the clitoris). Coccyx-fourchette distance (CF) was calculated by adding the anofourchette and anococcygeal distances, and the API was calculated as the ratio between the anofourchette and coccyx-fourchette (AF+AC) distances. Anococcygeal measurements were only performed for the delivery examination.

Statistical analysis

AGD measurements were approximately normally distributed, and were treated as continuous variables using linear regression. Distributions for all DDT and DDE congeners were right-skewed, and were $\log_{10}$-transformed to reduce the influence of outliers. Values below the

LOD were imputed based on a log-normal probability distribution whose parameters were determined by maximum likelihood estimation (Lubin et al., 2004).

Potential covariates for linear regression models were identified based on Directed Acyclic Graphs (DAGs). Child variables included birthweight and length, gestational age at birth (calculated from the mother's self-reported date of her last menstrual period and verified using maternal medical records), ponderal index (birthweight/length^3). Maternal variables considered for inclusion were age, education, post-delivery BMI, energy intake during pregnancy, poverty status, smoking and alcohol use during pregnancy (see Table 1 for categories). Final models for measurements at delivery included gestational age, ponderal index, and examiner; models for measurements at one year included child age, weight-for-length z-score, and examiner. We looked for evidence of non-linearity using generalized additive models (GAM) with three degrees of freedom cubic splines. All analyses were performed using Stata 13.1 (Stata Corp, College Station, TX).

We also constructed models using generalized estimation equations (GEE), in which AGD measures from both delivery and one year were included in the same models. Covariates included gestational age at birth, and child's weight and examiner at each time point. We assessed the GEE models for interactions by time point.

We also constructed longitudinal models using generalized estimation equations (GEE), in which AGD measures from both delivery and one year were included in the same models. Covariates included gestational age at birth, and child's weight and examiner at each time point. We assessed the GEE models for interactions by time point.

In sensitivity analyses, we restricted the sample to only full-term infants. We also used child weight-adjusted AGD measures instead of controlling for weight in the model. Because

Table 1. Demographic characteristics, VHEMBE cohort, 2012–2014

	All children	
	Mean	**SD**
Maternal characteristics at pregnancy		
Maternal age	26.4	(6.3)
Education, *n* (%)		
<12th grade	412	(54.9)
Grade 12	229	(30.5)
Further studies started	50	(6.7)
Diploma or further degree	60	(8.0)
Parity, *n* (%)		
0	326	(43.4)
1	201	(26.7)
2+	225	(29.9)
Maternal height (cm)	158.1	(6.8)
Post-delivery BMI	27.6	(5.4)
Energy consumption over 18,000 kJ, *n* (%)		
No	714	(95.1)
Yes	37	(4.9)
Mother diagnosed with high blood pressure/pre-eclampsia, *n* (%)		
No	651	(86.7)
Yes	100	(13.3)
Mother worked during pregnancy, *n* (%)		
No	567	(75.6)
Yes	183	(24.4)
Below the food poverty level (R370/mother per capita), *n* (%)		
No	310	(41.3)
Yes	438	(58.3)
Don't know	3	(0.4)
Alcohol during pregnancy		
No	711	(94.5)
Yes	41	(5.5)
Ever smoked		
No	745	(99.2)
Yes	6	(0.8)
Smoked in past year		
No	748	(99.6)
Yes	3	(0.4)
Child characteristics		
Sex		
Boy	388	(51.6)
Girl	364	(48.4)
Birth weight (g), M ± SD	3125.2	(452.1)
Ponderal index (BWT/length^3)	26.7	(3.0)

	All children	
	Mean	**SD**
Low birth weight (<2500 g), *n* (%)		
No	688	(91.6)
Yes	63	(8.4)
Preterm birth (<37 weeks)		
No	641	(85.4)
Yes	110	(14.6)
Weight-for-length *z*-score (1-year)	−0.001	(1.2)

BMI, body mass index; SD, standard deviation; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.

birthweight may be on the causal pathway, we also ran the models of delivery measures without ponderal index included.

RESULTS

The average age of mothers was 26.4 years (SD ± 6.3). More than half (54.9%) of the mothers had not completed high school and 43.4% of mothers were nulliparous (Table 1). Many of the women were economically impoverished, with 58.3% from households below the food poverty level, and almost all reporting insufficient energy consumption during pregnancy. Very few women reported ever smoking (<1%) or consuming alcohol during pregnancy (5.5%). The average birthweight was 3125g (SD ± 452), with 8.4% of children born of low birthweight (<2500g) and 14.6% born preterm (<37 weeks). Weight-for-length at 1-year averaged close to the expected z score for age based on WHO standards.

p,p'-DDT and *p,p'*-DDE were detected in almost all samples with 98.1% and 100.0% detected, respectively. The geometric mean for the maternal *p,p'*-DDT and -DDE serum concentrations were 69.2 and 287.0 ng/g lipid and the median levels were 54.1 and 242.2 ng/g lipid, respectively. Detection frequencies of *o,p'*-DDT (90.5%) and *o,p'*-DDE (82.8%) were

Table 2. *p,p′* – and *o,p′*-dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) lipid-adjusted concentrations (ng/g lipids) geometric means, detection and quantification frequencies, and distributions for girls and boys, Venda Health Examination of Mothers, Babies and their Environment (VHEMBE)

Variable	*n*	% Detected[a]	% Quantifiable[b]	GM	Min	10th%	25th%	50th%	75th%	90th%	Max
p,p'-DDT	752	98.0	94.4	69.4	<LOD	8.1	18.8	55.2	259.3	946.2	15027.6
p,p'-DDE	752	100	98.4	256.5	6.9	44.3	91.7	241.3	878.7	2577.7	26301.3
o,p'-DDT	752	90.6	66.6	8.9	<LOD	1.5	3.4	7.1	22.6	72.0	2029.3
o,p'-DDE	752	82.7	48.3	4.1	<LOD	<LOD	2.3	4.2	6.9	13.0	117.5

[a] Detection limit is 0.01 ng/g wet weight for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.03 ng/g for *p,p'*-DDE.

[b] Quantification limit is 0.03 ng/g wet weigh for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.09 ng/g for *p,p'*-DDE.

lower. Table 2 shows the distribution of concentrations. Table 3 presents the means and standard deviations for the various AGD measures for both boys and girls after delivery and at one-year.

In the adjusted analyses (Table 4), we found no associations between DDT or DDE congeners and AGD measures at delivery in either boys or girls (see Figure 2a,b,c,d). In measurements taken at one year, *o,p'*-DDE was negatively associated with AF in girls (β =-1.32 mm, 95% CI = -2.27, -0.38) and positively associated with PW in boys (β = 0.30 mm, 95% CI = 0.003, 0.60) (see Figure 3a). There were no associations of *p,p'*-DDT, *p,p'*–DDE, or *o,p'*-DDT in either boys or girls at one year (see Figure 3b).

Table 3. Mean and standard deviation (SD) of anogenital distance (AGD) measurements (mm) at delivery and 1 year for girls and boys, Venda Health Examination of Mothers, Babies and their Environment (VHEMBE)

	Sex	Measure	*n*	Mean ± SD
At birth	Girls	Aco	323	19.1 ± 4.7
		AF	327	16.5 ± 3.1
		Acl	326	35.7 ± 4.1
		CF	323	35.7 ± 6.3
		API	323	46.8 ± 6.1
	Boys	AC	336	19.2 ± 4.2
		AS	344	26.8 ± 4.9
		AP	341	47.3 ± 4.4
		PW	340	9.6 ± 1.7
At 1 year	Girls	CS	336	46.0 ± 7.1
		API	336	58.3 ± 5.9
		AF	324	21.7 ± 4.1
		Acl	315	44.5 ± 5.9
	Boys	AS	344	36.6 ± 7.4
		AP	335	72.5 ± 9.1
		PW	340	12.1 ± 1.2
		PL	345	27.7 ± 6.3

All measurements are in mm, except API.

Girls: ACo, anococcygeal; AF, anofourchette; ACl, anoclitoral; CF, coccyx-fourchette (AC + AF); API, anal position index (ratio of AF/CF).

Boys: AC, anococcygeal; AS, anoscrotal (mm); AP, anopenile; CS, coccyx-scrotal (AC + AS); API, analposition index (ratio of AS/CS); PW, penile width; PL, penile length.

Longitudinal models showed no associations for DDT/E and AGD measurements or interactions between DDT/E and the visit (birth or at one year); thus, longitudinal models were redundant with cross-sectional results (data not shown). In sensitivity analyses, results did not differ when analyses were restricted to full-term infants, when we used weight-adjusted AGD measures, or when we removed ponderal index from models; therefore these analyses are not shown.

Table 4. Adjusted linear regression β coefficient and 95% confidence interval (CI) associations between DDT/DDE exposure and infants' AGD measurements at delivery for girls and boys, Venda Health Examination of Mothers, Babies and their Environment (VHEMBE)

	Measure	*n*	*p,p'*-DDT β (95% CI)	*p,p'*-DDE β (95% CI)	*o,p'*-DDT β (95% CI)	*o,p'*-DDE β (95% CI)
At birth[a]						
	Aco	322	0.40 (−0.17, 0.98)	0.13 (−0.56, 0.81)	0.48 (−0.23, 1.19)	−0.49 (−1.51, 0.53)
	AF	326	0.03 (−0.37, 0.43)	−0.03 (−0.51, 0.44)	−0.03 (−0.54, 0.47)	0.06 (−0.66, 0.77)
Girls	Acl	325	0.09 (−0.45, 0.64)	0.00 (−0.65, 0.66)	0.29 (−0.39, 0.97)	0.00 (−0.97, 0.97)
	CF	322	0.40 (−0.36, 1.16)	0.06 (−0.85, 0.96)	0.42 (−0.53, 1.36)	−0.46 (−1.81, 0.88)
	API	322	−0.48 (−1.29, 0.34)	−0.21 (−1.18, 0.77)	−0.68 (−1.69, 0.33)	0.51 (−0.93, 1.95)
	AC	335	0.03 (−0.47, 0.52)	−0.14 (−0.73, 0.45)	0.15 (−0.46, 0.76)	0.17 (−0.76, 1.10)
	AS	343	0.12 (−0.51, 0.74)	−0.23 (−0.99, 0.52)	−0.04 (−0.82, 0.73)	−0.57 (−1.76, 0.62)
	AP	340	−0.11 (−0.68, 0.47)	−0.08 (−0.77, 0.60)	−0.27 (−0.98, 0.44)	−0.39 (−1.47, 0.70)
Boys						
	PW	339	−0.06 (−0.28, 0.17)	−0.05 (−0.31, 0.22)	−0.10 (−0.38, 0.17)	−0.06 (−0.48, 0.36)
	CS	335	0.13 (−0.73, 0.99)	−0.37 (−1.40, 0.65)	0.09 (−0.97, 1.15)	−0.38 (−2.00, 1.24)
	API	335	0.15 (−0.61, 0.90)	0.09 (−0.82, 1.00)	−0.09 (−1.03, 0.84)	−0.60 (−2.03, 0.83)
At 1 year[b]						
	AF	324	−0.33 (−0.87, 0.21)	−0.29 (−0.93, 0.34)	−0.55 (−1.20, 0.10)	−1.32 (−2.27, −0.38)[c]
Girls						
	AC	315	−0.01 (−0.74, 0.72)	−0.33 (−1.18, 0.53)	0.14 (−0.74, 1.02)	0.15 (−1.15, 1.45)
	AS	344	−0.07 (−0.99, 0.86)	−0.65 (−1.77, 0.48)	−0.18 (−1.32, 0.96)	1.02 (−0.73, 2.77)
	AP	335	−0.50 (−1.53, 0.53)	−0.52 (−1.77, 0.73)	−0.97 (−2.23, 0.29)	0.43 (−1.52, 2.37)
Boys						
	PW	340	0.01 (−0.15, 0.17)	0.04 (−0.16, 0.23)	0.07 (−0.12, 0.26)	0.30 (0.00, 0.60)[c]
	PL	345	0.61 (−0.22, 1.44)	0.41 (−0.60, 1.42)	0.36 (−0.66, 1.37)	1.08 (−0.48, 2.64)

AC, anococcygeal; AF, anofourchette; AGD, anogenital distance; CF, coccyx-fourchette; API, anal position index; AC, anococcygeal; AS, anoscrotal; AP, anopenile; CS, coccyx-scrotal; PW, penile width; PL, penile length; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane.

Coefficients show the change in AGD measurements associated with a 10-fold increase in exposure.

[a] Models for birth outcomes adjusted for gestational age at birth, ponderal index (birth weight/length^3), and examiner.

[b] Models for 1-year outcomes adjusted for child age, weight-for-length z-score, and examiner.

[c] $p < 0.05$.

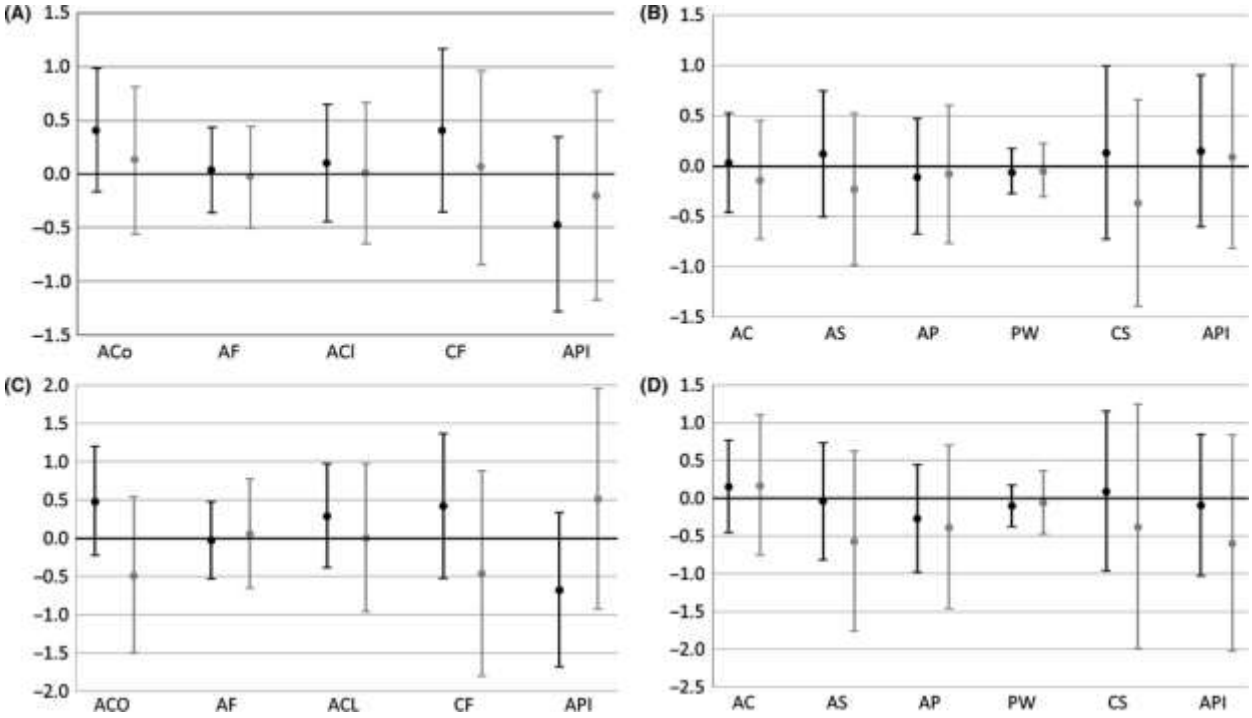

Figure 2. (a) Change in girls' delivery anogenital distance (AGD) associated with a 10-fold increase in *p,p*'-dichlorodiphenyltrichloroethane (DDT) (black) and *p,p*'-dichlorodiphenyldichloroethylene (DDE) (gray). All measurements are in millimeters, except anal position index (API), which is a number. Error bars indicate 95% confidence intervals. (b): Change in boys' delivery AGD associated with a 10-fold increase in *p,p′*-DDT (black) and *p,p′*-DDE (gray). All measurements are in millimeters, except API, which is a number. Error bars indicate 95% confidence intervals. (c): Change in girls' delivery AGD associated with a 10-fold increase in *o,p′*-DDT (black) and *o,p′*-DDE (gray). All measurements are in millimeters, except API, which is a number. Error bars indicate 95% confidence intervals. (d): Change in boys' delivery AGD associated with a 10-fold increase in *o,p′*-DDT (black) and *o,p*'-DDE (gray). All measurements are in millimeters, except API, which is a number. Error bars indicate 95% confidence intervals.

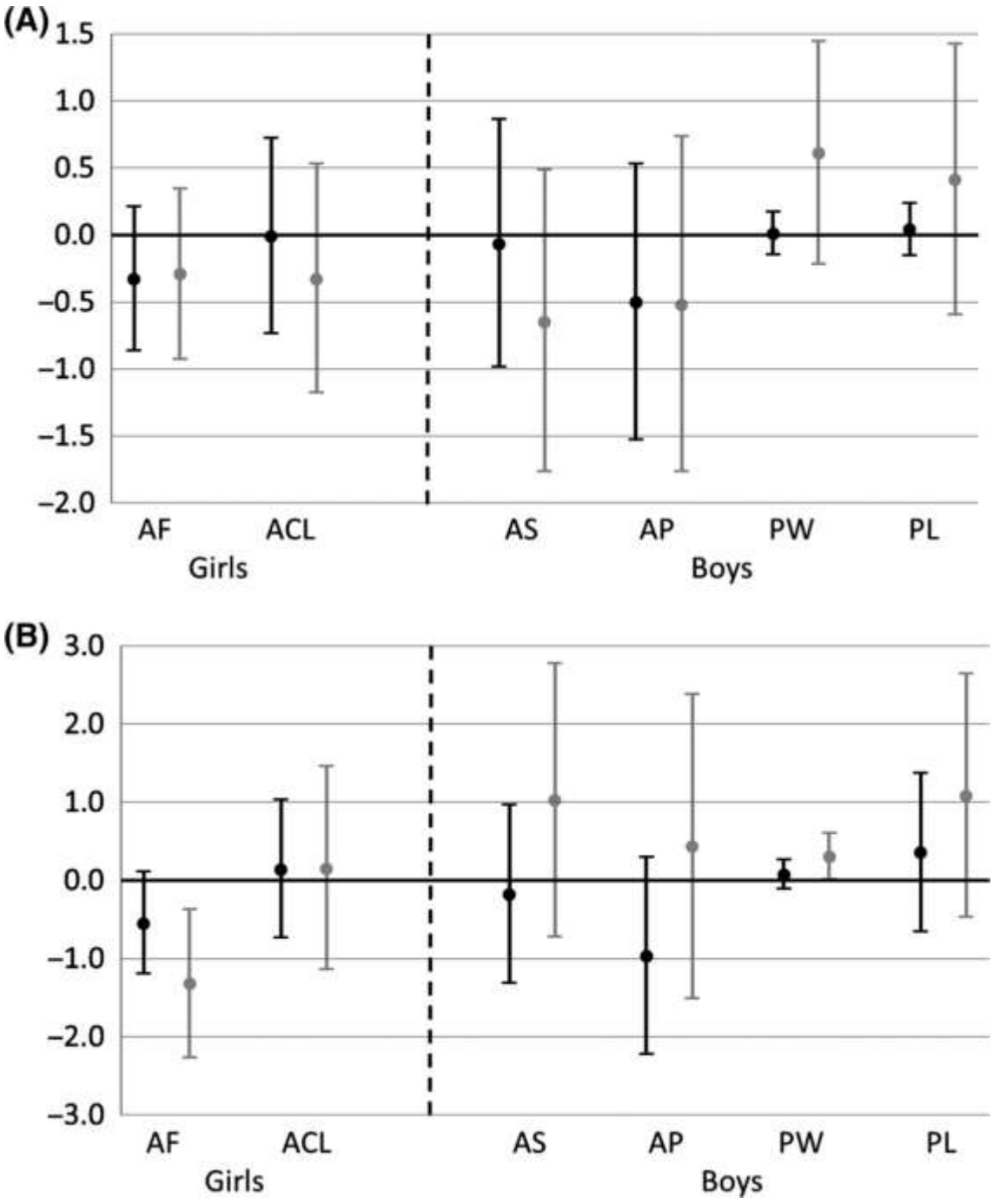

Figure 3. (a): Change in boys' and girls' 1-year anogenital distance (AGD) associated with a 10-fold increase in *p*,*p*'-dichlorodiphenyltrichloroethane (DDT) (black) and *p*,*p*'-dichlorodiphenyldichloroethylene (DDE) (gray). All measurements are in millimeters. Error bars indicate 95% confidence intervals. (b): Change in boys' and girls' 1-year AGD associated with a 10-fold increase in *o*,*p*'-DDT (black) and *o*,*p*'-DDE (gray). All measurements are in millimeters. Error bars indicate 95% confidence intervals.

DISCUSSION

This study examined the associations between prenatal exposure to DDT and DDE and AGD measurements in newborns and one-year old infants from an area where DDT is used for IRS. No associations were found between any DDT or DDE isomers and AGD measurements in boys and girls at birth. However, at age one year, maternal serum *o,p'*-DDE concentrations at delivery were associated with shorter AF measurements in girls and larger PW measurements in boys.

Most studies of AGD in boys measured AS, but in six studies AP measurements were also included (Romano-Riquer *et al.*, 2007, Ozkan *et al.*, 2011, Sathyanarayana *et al.*, 2010, Papadopoulou *et al.*, 2013a, Park *et al.*, 2015, Alaee *et al.*, 2014). The mean (± SD) of 26.8 ± 3.1 mm in VHEMBE boys at birth was somewhat longer than that reported from Cambridge, England 19.6 ± 6.1 mm (Thankamony *et al.* (2009)); Morelos, Mexico 21.0 ± 3.0 (Salazar-Martinez *et al.* (2004)); Tapachula, Mexico median=19.1 mm (Romano-Riquer *et al.* (2007)); Seattle, USA 23.0 ± 3.8. mm (Sathyanarayana *et al.* (2010)); Turkey 23.0 ± 0.6 mm (Ozkan *et al.* (2011)); Spain 24.7 ± 5.1 mm (Papadopoulou *et al.* (2013a)); Iran 24.5 ± 2.5 mm (Alaee *et al.* (2014)); and Korea 23.0 ± 0.2 mm in normal birth weights newborns (Park *et al.* (2015)), but shorter than reported from Nigeria 31.11 ± 0.64mm (Avidime *et al.*, 2011), and similar to that reported from Crete 27.1±4.4 mm (Papadopoulou et al., 2013a) (see Figure 4). The longer AS length persisted in the longitudinal comparison at one year when VHEMBE boys (36.6 ± 7.4 mm) were compared to the Cambridge cohort (mean 29.0 ± 7.5 mm) (Thankamony *et al.*, 2009). The average increase of AS from birth to one year was 14.4 mm (19.8 to 34.2 mm; 72.7% increase) in the Cambridge cohort, whereas in our study, boys' AS increased an average of 9.8 mm (from 26.8 to 36.6 mm, 36.6% increase). However, the percentages increase of AS was.

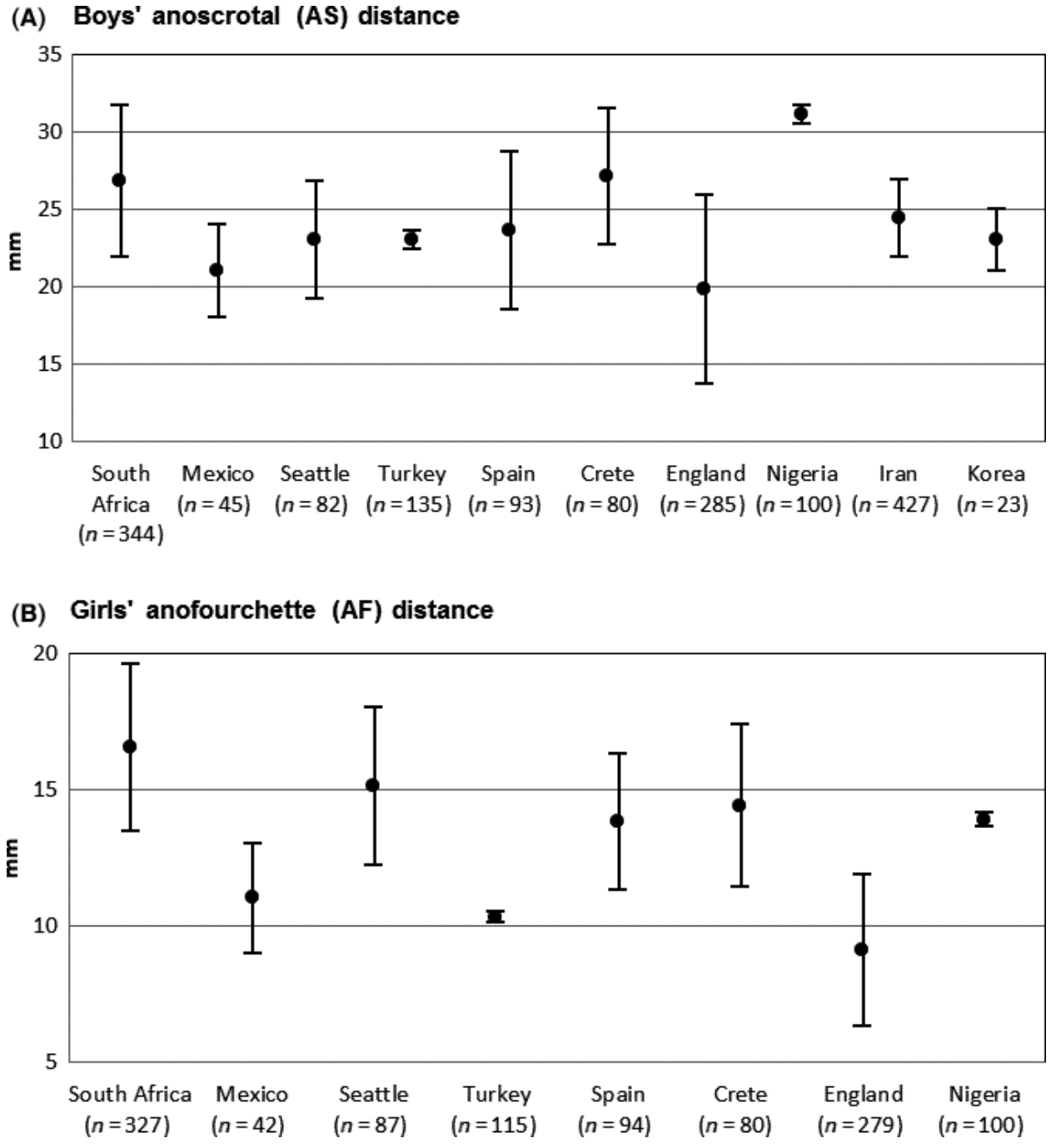

Figure 4. Comparison of mean boys' anoscrotal (Box A) and girls' anofourchette (Box B) distances across studies. All measurements are in millimeters. Error bars represent the standard deviation. Data obtained from Salazar-Martinez *et al*., 2004; (Mexico), Sathyanarayana *et al*., 2010; (Seattle), Ozkan *et al*., 2011; (Turkey), Papadopoulou *et al*., 2013a; (Spain and Crete), Thankamony *et al*., 2009; (England), Avidime *et al*., 2011; (Nigeria), Alaee *et al*., 2014; (Iran), and Park *et al*., 2015; (Korea).

36.6% in the VHEMBE boys, compared to 72.7% in the UK study (Thankamony *et al.*, 2009). Thus, the rate of growth may differ among populations

Figure 4 also shows the comparison of AGD measurements from different studies reported in girls. The mean AF in newborn girls in VHEMBE was longer than measurements reported from Mexico (Salazar-Martinez *et al.* (2004)); Seattle (Sathyanarayana *et al.* (2010)); Turkey (Ozkan *et al.* (2011)); Spain and Crete (Papadopoulou *et al.* (2013a)); Cambridge England (Thankamony *et al.* (2009)) and from Nigeria (Avidime *et al.* (2011)) . The longer AF persisted at one year, but the mean increase in AF of VHEMBE girls from birth to one year (5.2mm) was almost the same as in the Cambridge study (5.4mm) (Thankamony *et al.*, 2009). However, the percentage increase of AF was 31.5% in the VHEMBE girls, compared to 59.3% in the UK study (Thankamony *et al.*, 2009). These comparisons indicate that there is considerable variation among different study populations and factors like ethnicity/race, differences in observers, and measuring techniques may explain some of the variation. There may also be regional/geographical variation because of differing environmental factors that could be contributing to genital development (Sathyanarayana al., 2105).

Our findings that AGD measurements were not associated with either *p,p'*-DDT or *p,p'*-DDE levels agree with those of (Longnecker *et al.*, 2007) from Mexico, but not Torres-Sanchez *et al.*, 2008 who reported associations in males with API. The maternal exposure levels in Chiapas, Mexico were 2700 ng/g lipid for *p,p'*-DDE and 250 ng/g lipid for *p,p'*-DDT (Longnecker *et al.*, 2007), while mothers from Morelos, Mexico had a median of 1274 ng/g lipid for *p,p'*-DDE and 12.3 ng/g lipid for *p,p'*-DDT in measurements taken in mothers of male infants in the third trimester of pregnancy (Torres-Sanchez *et al.*, 2008). The respective levels in VHEMBE mothers of 242.2 ng/g lipid of *p,p'*-DDE were lower than in the Mexican studies.

However, our levels of 54.1 ng/g lipid for *p,p′*-DDT were higher than in Morelos, but lower than in Chiapas; however, it should be noted that that not all VHEMBE mothers were living in DDT sprayed houses.

The only significant finding of this study was that maternal *o,p'*-DDE levels were associated with shorter AF measurement in girls and wider PW in boys at one year of age, but not at birth. In a previous report, *o,p'*-DDE, metabolite of *o,p'*-DDT, was present in breastmilk samples from this study area (Bouwman *et al.* 2012); thus, infants could possibly be exposed through breastmilk, but this is speculative since we have not measured residues in human milk. In addition, as infant's mobility increases, with close proximity to the ground and greater hand to mouth behavior (Beamer et al., 2009), they are likely to have increased exposure to contaminated soil and dust. Dust samples collected in buildings previously sprayed for malaria control had significantly higher detection frequencies of *p,p′*-DDT (34%) and *p,p′*-DDE (58%), while *o,p′*-DDE and *o,p′*-DDD (14% and 16%, respectively) were the least commonly detected analytes. However, VHEMBE mothers women living in a home with dust levels of *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, and *o,p′*-DDE above the LOQ had significantly higher serum concentrations of those chemicals (Gaspar et al. 2015). Although it remains possible that the shorter AF associated with higher exposures could be related to a feminizing effect, the mechanism is not clear and health effects of *o,p′*-DDE have yet to be identified. Although most of the samples (82.8%) were detected, less than half (48.3%) were above the limit of quantification for *o,p′*-DDE (0.01 and 0.03 ng/g wet weight, respectively), making these findings less reliable and possibly due to chance. Clearly, these results need to be replicated

This study has several strengths. AGD measurements were performed by the same two trained professional nurses at birth and one year. We conducted AGD measurements on both

boys and girls. Also, all four DDT isomers were included in the statistical analysis, which made it possible to find associations with *o,p'*-DDE.

In conclusion this study investigated whether AGD in newborn boys and girls were affected by maternal exposure to DDT during pregnancy. No associations were found for *p,p'*-DDT, *p,p'*-DDE, or *o,p'*-DDT, but *o,p'*-DDE levels were negatively associated with anofourchette measurements and positively with penile width at one year. These results warrant replication, given the high proportion of samples below the limit of quantification for *o,p'*-DDE.

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