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Dichlorodiphenyltrichloroethane exposure and anogenital distance in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) birth cohort study, South Africa

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SUMMARY

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

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birth in either boys or girls. At 1 year, o,p′-DDE was negatively associated with anofourchette in girls (μ = −1.32 mm, 95% confidence interval (CI) = −2.27, −0.38) and positively associated with penile width in boys (μ = 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; dichlorodiphenyldichloroethylene

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 (AS) distance in boys, although some studies of males also consider the distance from the anus to the anterior base of the penis (anope-nile, AP) (Swan et al. 2005).

Anogenital distance has been examined in humans in relation to fetal exposures to various endocrine-disrupting chemicals such as phthalates (Swan et al., 2005, 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 antiandrogenic effect (Swan et al., 2005, 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., 2013) or phthalates (Swan et al., 2015).

Dichlorodiphenyltrichloroethane 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 antiandrogenic properties (Kelce et al., 1995; Danzo, 1997), while p,p′-DDT and o,p′-DDT are estrogenic (ASTDR, 2002). o,p′-DDT 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 17b-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 signifi-cant associations was observed 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 Anal Position Index (API) and 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 1-year old boys and girls were related to maternal blood concentrations of DDT and DDE at delivery.

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 min 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 1-year-old. A total of 1649 women were approached, 920 were eligible, 752 were enrolled, and 700 completed the 1-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 shortly 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 2 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 2 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. Sealed blanks, field and laboratory blanks, and spiked samples were use for quality control. Results were lipid adjusted based on a summation method using triglycerides and total cholesterol concentrations measured using standard enzymatic methods (Roche Chemicals, Indianapolis, IN, USA) (Phillips et al., 1989).

Maternal interview and measurements

Mothers were interviewed in TshiVenda (the local 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

Birth weight 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 1-year visit (mean age 372 ± 26 days), child weight (using Tanita Corporation BD-590) and recum-bent length (using a SECA Infantometer 416, Mortara Instrument UK Ltd) were measured. Weight-for-length z-scores at 1 year were calculated using growth standards provided by the WHO (World Health Organization 2006).

Anogenital distance measures were collected on the day of postnatal discharge and at the 1-year visit (see Fig. 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 using Swiss Precision Instruments (SPI) dial calipers (Enco Manufacturing, Fernley, Nevada), except penile length (PL), which was measured with a ruler. All measurements were performed in triplicate and averaged. The caliper measurements were all read to the nearest 0.1 mm and the ruler to 0.1 cm. For boys (Fig. 1a), AGD measurements included AS (from the center of the anus to the posterior base of the scrotum, AP (the center of the anus to the anterior base of the penis), anococcygeal (ACo – the anus to the coccyx), and PL (stretched) and penile 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 (CS) distance was calculated by adding the anococcygeal and anoscrotal measurements (Torres-Sanchez et al., 2008). The API was calculated as the ratio between the anoscrotal and coccyx-scrotal (AS + AC) distances.

Measurements for girls (Fig. 1b) included anococcygeal distance, as well as AF (distance between the anus and the four-chette) 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-four-chette (AF + AC) distances. Anococcygeal measurements were only performed for the delivery examination.

Statistical analysis

Anogenital distance 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 birth weight 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 (birth weight/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 1 year included child age, weight-for-length z-score, and examiner. We looked for evidence of non-linearity using generalized additive models with three degrees of freedom cubic splines. All the analyses were performed using STATA 13.1 (Stata Corp, College Station, TX,USA).

We also constructed models using generalized estimation equations (GEE), in which AGD measures from both delivery and 1 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 GEE, in which AGD measures from both delivery and 1 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 birth weight 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 [standard deviation (SD) ± 6.3]. More than half (54.9%) of the mothers had not com pleted 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 birth weight was 3125 g (SD ± 452), with 8.4% of children born of lowbirth weight (<2500 g) and 14.6% born preterm (<37 weeks). Weight-for-length at 1 year averaged close to the expected z-score for age based on the WHO standards.

p,p′-DDT and p,p′-DDE were detected in almost all samples with 98.1 and 100.0%, 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 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 1 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 Fig. 2a–d). In measurements taken at 1 year, o,p′-DDE was negatively associated with AF in girls (μ = – 1.32 mm, 95% confidence interval (CI) = – 2.27, – 0.38) and positively associated with PW in boys (μ = 0.30 mm, 95% CI = 0.003, 0.60) (see Fig. 3a). There were no associations of p, p′-DDT, p,p′–DDE, or o,p′-DDT in either boys or girls at 1 year (see Fig. 3b).

Longitudinal models showed no associations for DDT/E and AGD measurements or interactions between DDT/E and the visit (birth or at 1 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.

DISCUSSION

This study examined the associations between prenatal exposure to DDT and DDE and AGD measurements in newborns and 1-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 1 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; Sathyanarayana et al., 2010; Ozkan et al., 2011; Papadopoulou et al., 2013a; Alaee et al., 2014; Park et al., 2015). 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 weight new borns (Park et al., 2015), but shorter than reported from Nigeria 31.11 ± 0.64 mm (Avidime et al., 2011), and similar to that reported from Crete 27.1 ± 4.4 mm (Papadopoulou et al., 2013a) (see Fig. 4). The longer AS length persisted in the longitudinal comparison at 1 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 in AS from birth to 1 year was 14.4 mm (19.8–34.2 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 percentage increase in AS was 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 1 year, but the mean increase in AF of VHEMBE girls from birth to 1 year (5.2 mm) was almost the same as in the Cambridge study (5.4 mm) (Thankamony et al., 2009). However, the percentage increase in 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 et al., 2010).

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 with Torres-Sanchez et al., 2008 who reported associations in males with API.

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 1 year of age, but not at birth. In a previous report, o,p′-DDE, metabolite of o,p′-DDT, was present in breast milk samples from this study area (Bouwman et al. 2012); thus, infants could possibly be exposed through breast milk, but this is speculative as 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 because of 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 1 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 PW at 1 year. These results warrant replication, given the high proportion of samples below the limit of quantification for o,p′- DDE.

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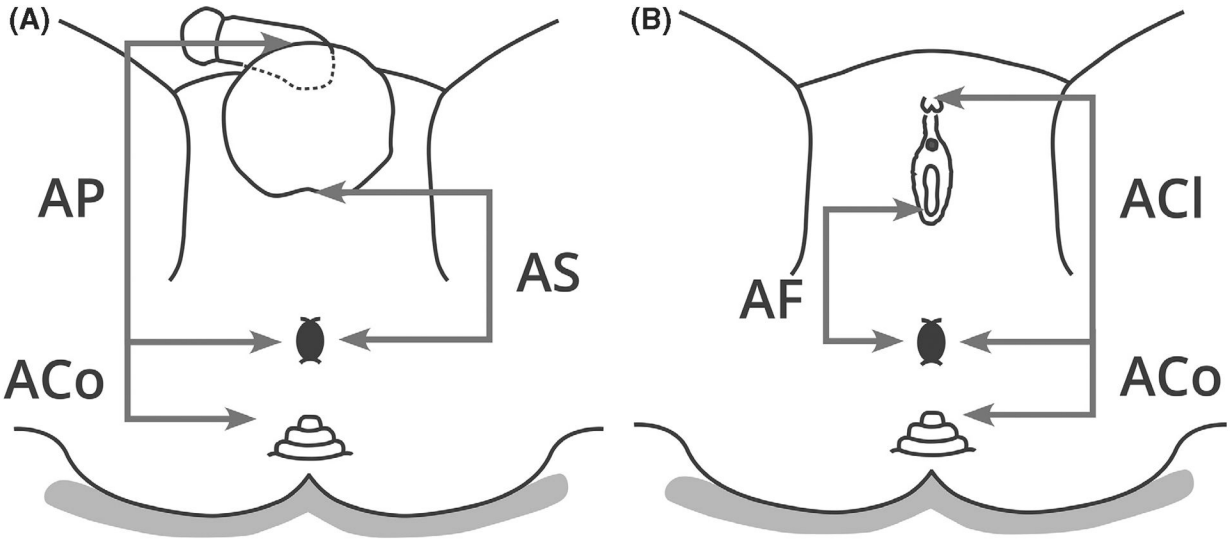

Figure 1.

(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).

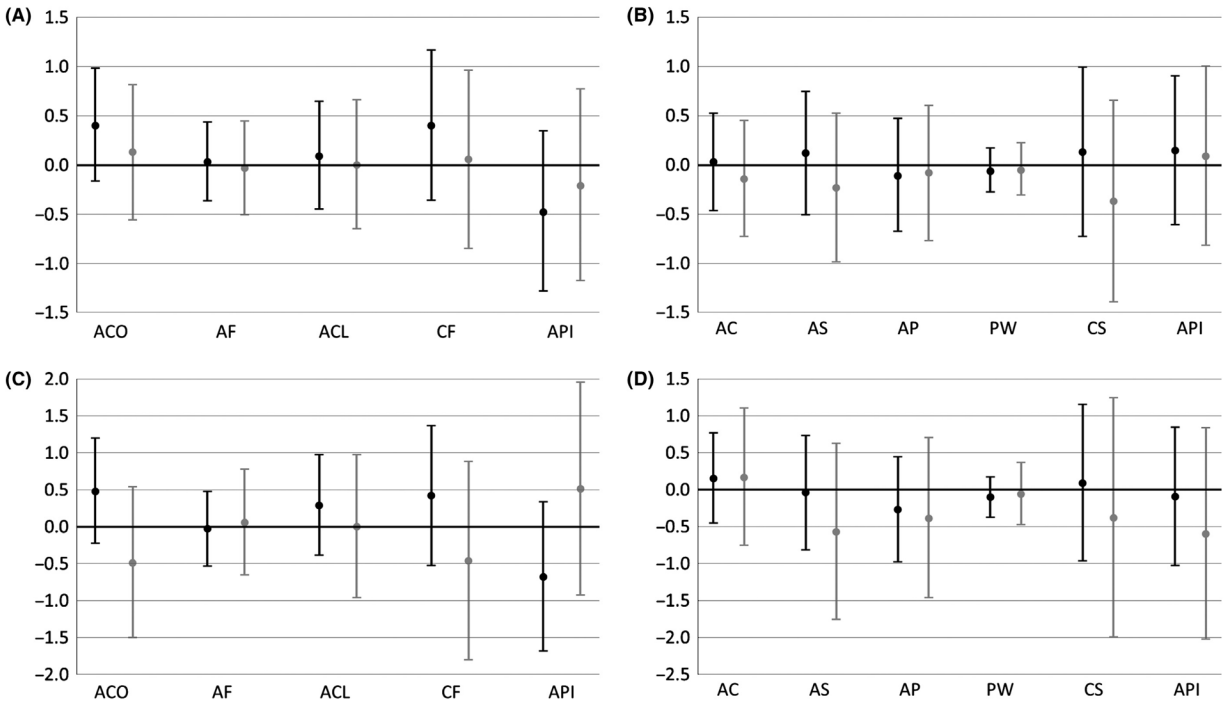

Figure 2.

(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.

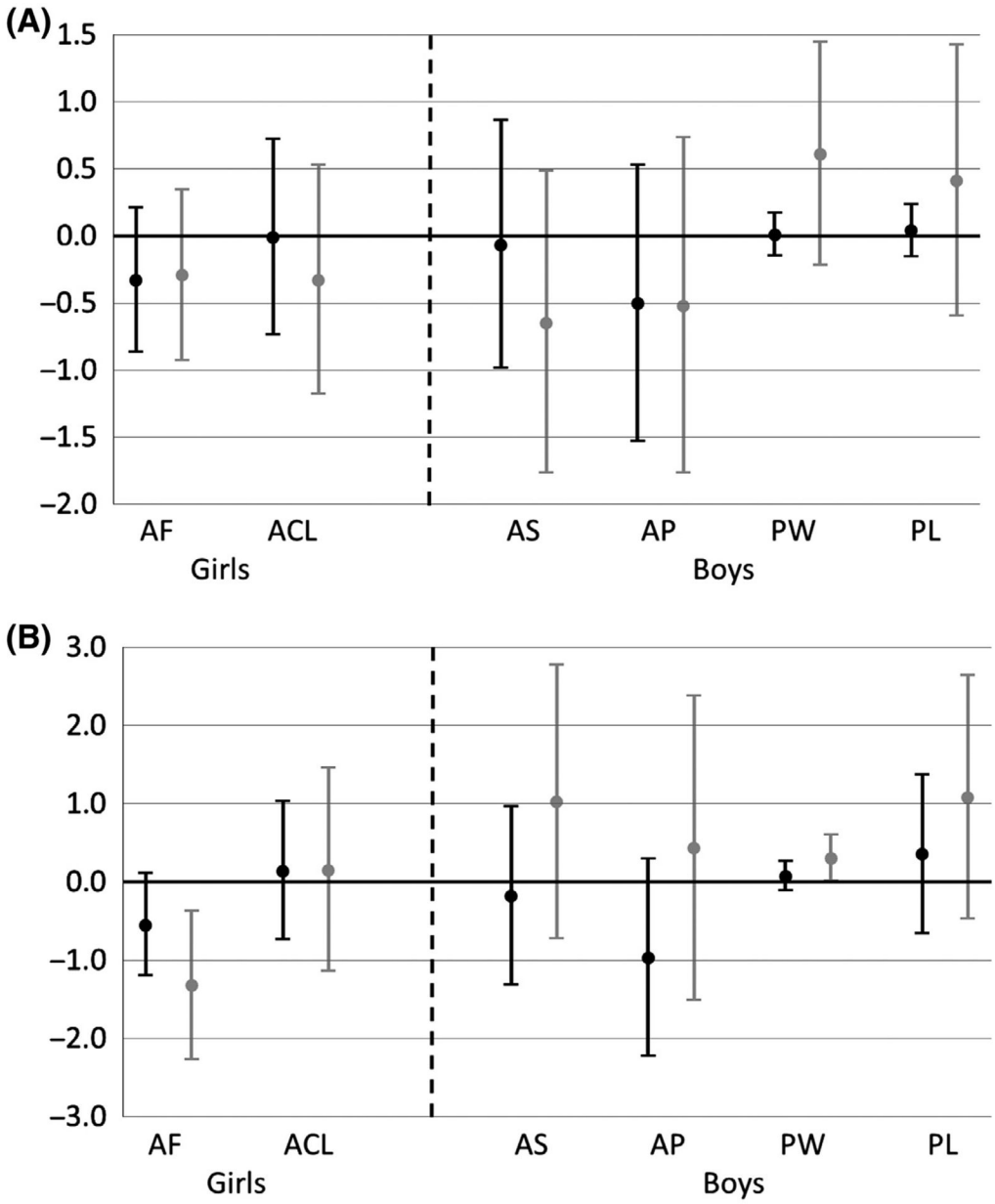

Figure 3.

(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.

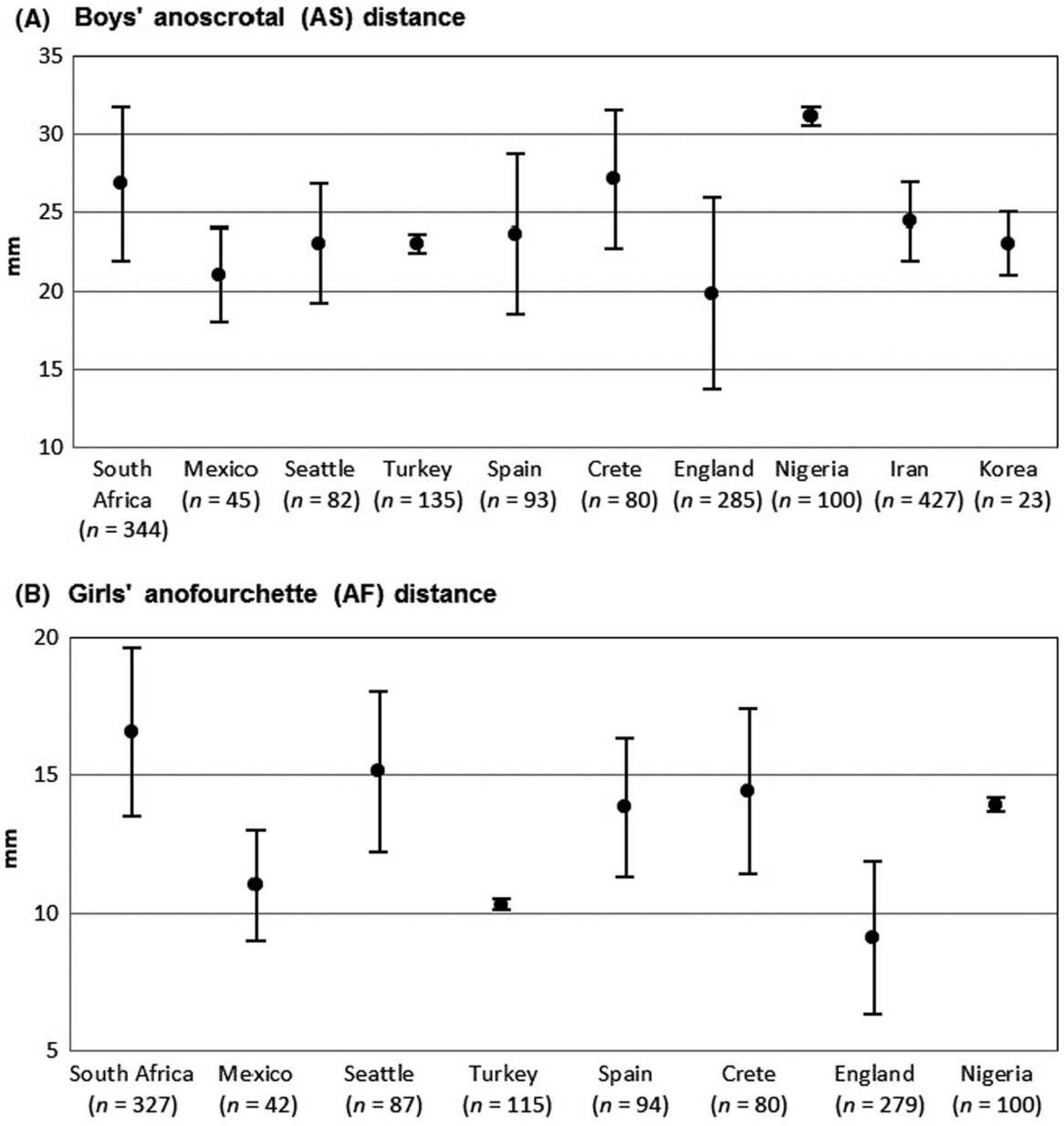

Figure 4.

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).

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)

	All children	
	Mean	**SD**
Girl	364	(48.4)
Birth weight (g), M ± SD	3125.2	(452.1)
Ponderal index (BWT/length^3)	26.7	(3.0)
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.

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.

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
		CS	336	46.0 ± 7.1
		API	336	58.3 ± 5.9
At 1 year	Girls	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, anope-nile; CS, coccyx-scrotal (AC + AS); API, anal position index (ratio of AS/CS); PW, penile width; PL, penile length.

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]						
Girls	Aco	322	0.40 (−0.1 7, 0.98)	0.1 3 (−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)
	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)
Boys	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)
	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	0.1 3 (−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]						
Girls	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)*
	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)
Boys	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)
	PW	340	0.01 (−0.15,0.17)	0.04 (−0.16, 0.23)	0.07 (−0.1 2, 0.26)	0.30(0.00,0.60)*
	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.

*$p < 0.05$.