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Maternal Exposure to DDT, DDE, and Pyrethroid Insecticides for Malaria Vector Control and Hypospadias in the VHEMBE Birth Cohort Study, Limpopo, South Africa

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Abstract

Hypospadias is the ectopic opening of the urethra on the penis or scrotum. Exposure to estrogenic and/or anti-androgenic chemicals *in utero* may play an etiologic role. DDT and the pyrethroids cypermethrin and deltamethrin, are used to control malaria. DDT is estrogenic and its breakdown product DDE is anti-androgenic; cypermethrin and deltamethrin can also disrupt androgen pathways. We examined the relationship between maternal exposure to these insecticides during pregnancy and hypospadias among boys participating in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) in Limpopo Province, South Africa. We measured peripartum levels of *p,p'*-DDT and *p,p'*-DDE in maternal serum and urinary pyrethroid metabolites. We conducted urogenital examination on 359 one-year-old boys. A total of 291 (81.0%) had phimosis, which prevented full urogenital examination, leaving a final sample of 68 boys for determination of the presence of hypospadias. Diagnosis was based on concordance of two independent physicians. We identified hypospadias in 23 of the 68 boys (34%). Maternal urinary concentrations of *cis*-DCCA and *trans*-DCCA metabolites of cypermethrin and other pyrethroids, were associated with an increased risk for hypospadias, but the other metabolite 3-PBA was not (adjusted relative risk per 10-fold increase=1.58, 95% CI 1.07–2.34; 1.61, 95% CI 1.09–2.36; and 1.48, 95% CI 0.78– 2.78, respectively). No associations were found between *p,p'*-DDT, *p,p'*-DDE, 3-PBA or *cis*-DBCA and hypospadias. We observed a high prevalence of hypospadias among boys without phymosis. Boys with higher prenatal exposure to pyrethroid insecticides were at higher risk of hypospadias. Our findings may have global implications given that pyrethroid insecticides are widely used for malaria control, in agriculture and for home use.

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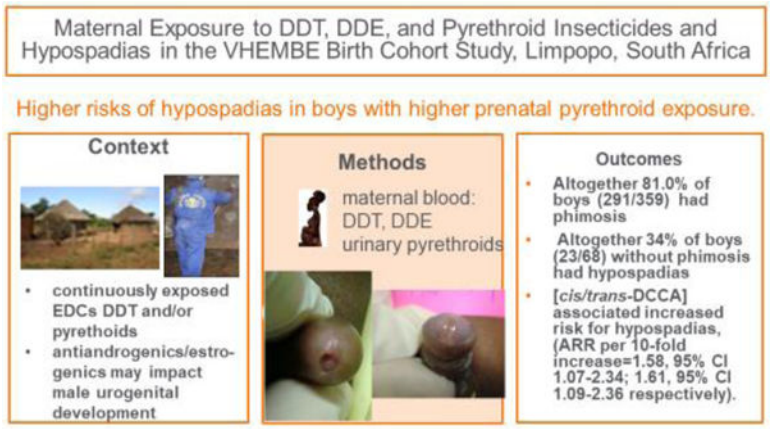
GRAPHICAL ABSTRACT



Keywords

hypospadias; urogenital; insecticides; DDT; pyrethroid; South Africa

1. INTRODUCTION

Indoor residual spraying (IRS) with insecticides is used by 84 countries for malaria vector control (1). South Africa introduced dichlorodiphenyltrichloroethane (DDT) in 1946 (2) and continues using DDT in malaria endemic regions (3). Pyrethroid insecticides are currently used worldwide for malaria control as well as for household and agricultural pest control (4, 5). In South Africa, pyrethroids are gradually replacing DDT for IRS (6), where cypermethrin and deltamethrin are primarily used (5).

Technical-grade DDT formulated for spraying, contains 65%–80% of the active insecticidal ingredient *p,p′*-DDT (7) and also includes *p,p′*-dichlorodiphenyl dichloroethylene (*p,p′*-DDE), a breakdown product (8, 9). Both DDT and DDE are persistent organic pollutants with a long half-life of about 4 and 10 years, respectively (10). They are known to interfere with endocrine function in *in vitro* and animal studies (9). *In vitro*, *p,p′*-DDT is estrogenic (11–13), whereas DDE competitively binds with the androgen receptor, blocks androgen-induced transcription (14, 15), and inhibits androgen action in developing, pubertal, and adult male rats *in vivo* (14).

Human exposure to pyrethroids is thought to occur mainly via residues in the diet or exposure via inhalation, but ingestion of contaminated household dust as well as dermal exposure may occur after indoor application (16). Urinary pyrethroid metabolites are commonly used as biomarkers of exposure (17). As pyrethroids degrade rapidly in the body, these biomarkers reflect exposure over the preceding hours or days (16). Cypermethrin and other pyrethroids are metabolized to *cis*-3-and *trans*-3- (2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*- and *trans*-DCCA) and 3-phenoxybenzoic acid (3-PBA) (17). Deltamethrin breaks down to the specific metabolite *cis*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DBCA).

Pyrethroids may affect estrogen receptors in Sertoli cells (18), interfere with androgen pathways (19, 20, 21) and affect seminal vesicle weight (22). *In vivo* exposure to cypermethrin adversely impacts fertility, spermatogenesis including sperm count, and sex hormones in the adult male rat (23), while rats exposed to deltamethrin showed adverse effects on sex hormones and arrest of spermatogenesis and apoptosis (24).

Human exposure to endocrine disrupting chemicals (EDCs) has been hypothesized to be related to decreased semen quality and increases in testicular cancer in adult males and undescended testis and hypospadias in newborns—collectively termed the testicular dysgenesis syndrome (25). Animals exposed *in utero* to exogenous estrogenic chemicals, such as diethylstilbestrol or to anti-androgens, can develop reproductive system birth defects, including hypospadias in males (26). Based on these findings in animals, several authors argued that male sexual differentiation in humans may likewise be disrupted by EDCs and result in hypospadias (26–30). Hypospadias develops *in utero* when an incomplete midline fusion of the genital fold causes the urethra to be located in an ectopic position on the ventral side of the penis or on the scrotum (29, 31, 32).

Few epidemiologic studies have examined male reproductive health in the context of IRS. Nonoccupational exposure to DDT has been associated with impaired seminal parameters in men living in the Vhembe District of Limpopo, South Africa — a malaria-endemic region sprayed with DDT (33). Bornman et al. (3) found that mothers who lived in DDT-sprayed villages in this area had a 33% increased odds of having a boy with a urogenital birth defect compared to mothers whose villages were not sprayed (OR 1.33, 95% CI 1.04–1.72). Although this study (3) did not measure biomarkers of exposure, recent studies from the same area have reported a ten-fold difference in serum DDT and DDE concentrations between males living in sprayed and unsprayed villages (34), and a 5 to 7-fold increase in women whose homes had been sprayed compared to those whose homes had not (35). Pyrethroids are also used in large quantites for malaria control in this region (36).

People living in malaria risk areas are exposed to IRS insecticides while inside their houses and seems be a “captive population” for continuous exposure to DDT and/or pyrethoids; insecticides with both toxicity and endocrine disrupting properties, but no study has addressed the health effects. Considering *in vitro* and *in vivo* animal studies and the limited scientific evidence from human studies, the Vhembe District, in Limpopo Province, South Africa offers a unique study opportunity to provide some information of exposure to IRS insecticides and the impact on foetal development. Living in IRS sprayed structures may carry a higher risk for pregnant mothers and their developing boys, particular for urogenital development, a hormone-dependent process.

In the present study, we examined whether exposure to DDT, DDE, and pyrethroid insecticides during pregnancy was associated with higher risk of hypospadias among participants in the Venda Health Examination of Mothers, Babies and the Environment (VHEMBE) study.

2. MATERIALS AND METHODS

2.1 Study population

VHEMBE, a longitudinal birth cohort study, aims to investigate the environmental determinants of child health and development. Study methods have been presented elsewhere in greater detail (37); briefly, we recruited pregnant women between August 2012 and December 2013 at Tshilidzini Hospital in the Vhembe District Municipality of Limpopo Province, South Africa. Mothers were eligible if they were 18 years or older, spoke TshiVenda as their main language at home, lived within 20 km of the hospital, planned to remain in the area for at least 2 years, did not contract malaria during pregnancy, had contractions at least 5 minutes apart when approached by study staff, and gave birth to a live singleton infant. Of 920 eligible women, 752 (81.7%) completed a baseline questionnaire and provided maternal samples at delivery, of whom 388 (51.6%) delivered a male infant. By age 1 year, 11 infants died and 18 missed the 1-year visit, leaving 359 boys (92.5%) in the current investigation.

All mothers or guardians gave written informed consent. 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 approved the study.

2.2 Procedures

Trained bilingual, English-TshiVenda speaking interviewers queried mothers in TshiVenda shortly after delivery (mean 1.1 ± 1.3 days). The questionnaire gathered information on socio-demographic characteristics, reproductive and medical history, personal habits, occupational and residential history, and use of pesticides around their home or for malaria control. Maternal height and weight were measured in triplicate after delivery using a Beurer PS06 scale (Ulm, Germany) and Charder HM200P stadiometer (Taichung, Taiwan), respectively. Infant birth weight was measured by hospital staff using a Tanita BD-815U neonatal scale (Tanita Corporation of America, Inc., Arlington Heights, Illinois) and abstracted from medical records and the hospital birth registry. Medical records from pregnancy and delivery were abstracted by registered nurses. Approximately 1 week after delivery, staff visited participants' homes and collected information on the home environment, including pesticide use in the home and grounds. When the boys were one year of age (12.2 ± 0.6 months), they received a urogenital examination (described below).

2.3 DDT/DDE measurements

Maternal blood samples were collected from the mother before (n=278) or after (n=81) delivery (and before leaving the hospital) and were immediately processed, aliquoted and frozen at −80°C. Samples were shipped on dry ice to Emory University's Rollins School of Public Health for the measurement of *p,p'* and *o,p'* isomers of DDT and DDE using high resolution gas chromatography-isotope dilution mass spectrometry (GC-MS) (38). The detection limits were 0.01 ng/g wet weight for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; 0.03 ng/g for *p,p'*-DDE. The quantification limit was 0.05 ng/g for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.15 ng/g for *p,p'*-DDE. Quality control samples included sealed blanks,

field blanks, and spiked samples. Total lipid concentrations were estimated based on triglycerides and total cholesterol (39), measured using standard enzymatic methods (Roche Chemicals, Indianapolis, IN).

2.4 Pyrethroid metabolite measurements

Spot urine samples were collected from the mother prior to (n=215) or as soon as possible after (n=142) delivery (and before leaving the hospital).Specific gravity was determined using an Atago PAL-10S refractometer (Tokyo, Japan). Urine samples were stored at −80°C in the field office, shipped on dry ice to the UC Berkeley School of Public Health Biorepository before being shipped to the Institut National de Santé Publique du Québec to measure pyrethroid metabolites using GC-MS (17). (17). Five main urinary pyrethroid metabolites were measured: *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DBCA); *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DCCA); *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*trans*-DCCA); 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). The analysis was performed by gas chromatography-mass spectrometry (GC-MS) on an Agilent 6890 Network gas chromatograph equipped with an Agilent 7683B series automatic injector and an Agilent 5975 mass spectrometer (Agilent Technologies; Mississauga, Ontario, Canada) operated in the single ion monitoring mode following negative ion chemical ionization (NCI) with methane (99.97 %) as the reagent gas. In house reference materials at three levels of concentrations and ClinChek control 8928 lot: 041 non-certified reference material in urine (RECIPE; Munich, Germany) were used for internal quality controls (QCs). The overall quality and accuracy of the analyses was monitored by the participation twice a year to the The detection limits were 0.0025 μg/L for *cis*-DBCA, 0.0045 μg/L for *cis*-DCCA, 0.0038 μg/L for *trans*-DCCA, 0.0047 μg/L for 3-PBA, and 0.005 μg/L for 4F3-PBA. Limits of quantification were 0.0082 μg/L for *cis*-DBCA, 0.015 μg/L for *cis*-DCCA, 0.013 μg/L for *trans*-DCCA, 0.016 μg/L for 3-PBA, and 0.011 μg/L for 4-fluoro-3-phenoxybenzoic acid (4F3-PBA).

Analyte concentrations between the detection and quantification limits were assigned machine-read values and those below the limit of detection were imputed at random based on a log-normal probability distribution whose parameters were estimated via a maximum likelihood method (40).

2.5 Urogenital examination and case definition

Two professional registered nurses, trained in clinical urogenital examination by an andrologist (RB), performed the urogenital examination. With the boys in a frog leg position, a nurse visually examined the penis, scrotum, and testes. The foreskin was gently retracted to expose the glans and the nurse determined the presence and severity of phimosis based on the ability of the foreskin to be retracted (41). If the foreskin could be retracted (i.e., no phimosis), the nurses evaluated the presence and position of hypospadias. Digital pictures were taken of the distal penis and pictures were stored in an encrypted and password-protected database until abnormalities could be classified. Two physicians, one trained in male reproductive health (RB) and the other in pediatrics (CLA), blinded to prenatal insecticide exposure, assessed the pictures independently for hypospadias and noted

the meatal position. Hypospadias was recorded when the opening of the urethra was not located on the tip of the penis, but lower on the glans, at the corona, or on the ventral penis or scrotum. For these analyses, hypospadias was considered to be present if both experts agreed, irrespective of the exact position of the urethral opening.

A total of 291 (81.0%) of these males had phimosis, which prevented examination for hypospadias, leaving a final sample of 68 boys. Of those 68 without phimosis, 38 (55.9%) were diagnosed with hypospadias by at least one physician and 23 (33.8%) were diagnosed by both. We included only those as having hypospadias if both clinicians agreed; infants for whom only one of the two experts identified hypospadias were not considered to have hypospadias.

2.6 Data analysis

We limited our statistical analyses to those analytes quantified in at least 70% of samples, thus excluding *o,p'*-DDT, *o,p'*-DDE, and 4-F-3-PBA from further analysis. DDT and DDE (DDT/E) as well as pyrethroid metabolite levels were $\log_{10}$-transformed to reduce the influence of outliers. DDT and DDE concentrations were lipid-corrected and expressed in ng/g lipid, and pyrethroid concentrations were standardized using specific gravity measurements and expressed in μg/L urine. We constructed separate models for the relationship between hypospadias and the six analytes/metabolites (*p,p'*-DDT, *p,p'*-DDE, *cis*-DBCA, *cis*-DCCA, *trans*-DCCA, and 3-PBA). Relative risks associated with a 10-fold increase in analyte concentrations were estimated using Poisson regression with a robust (Huber-White) variance estimator.

Several demographic and maternal characteristics have been associated with hypospadias in the literature, including family history of hypospadias; maternal socioeconomic status, ethnicity, older age, nulliparity, higher body mass index (BMI), younger age at menarche, gestational hypertension or preeclampsia in index pregnancy, and pre-existing diabetes or other endocrine disorders; and infants from a multiple birth, low birth weight, preterm, or small for gestational age (42–45). We determined the final covariates based on a directed acyclic graph (DAG) (46, 47). Maternal age (continuous), BMI category (<25 kg/m^2, 25–29.9 kg/m^2, or ≥30 kg/m^2), and previous parity (yes vs no); and household poverty (household income above or below the food poverty threshold of R386 monthly per capita) (48) were retained in the final adjusted models.

We conducted sensitivity analyses. Of the 68 boys without phimosis, 43 urine samples were collected before delivery and 25 were collected after delivery. There were no differences in distributions and geometric means for metabolite concentrations collected pre- vs. post-delivery (data not shown); however, because the half-life of pyrethroids is short and urine collected post-delivery might partly reflect in-hospital exposures, we conducted sensitivity analyses using only pyrethroid metabolite measurements from maternal urinary samples collected pre-delivery (n=43) (N.B. no sensitivity analyses were conducted for DDT/E because of their long half-lives). In another sensitivity analysis, we included the 291 boys whose corona was not fully visible due to phimosis and conservatively assumed that all those with phimosis did not have hypospadias. Although in the main models we considered

those considered to have hypospadias by only one clinician as controls, in sensitivity analyses we entirely excluded those boys from analysis (n=15 in the main sample).

We conducted analyses using STATA version 15 (StataCorp, College Station, TX).

3. RESULTS

The characteristics of the mothers and boys are summarized in Table 1. All VHEMBE mothers were black African women. More than half the 68 mothers with sons in this study were younger than 25 years (54.4%; mean ± SD = 26.5 ± 6.8 years), 54.4% had less than a 12^{th} grade education, 52.9% were nulliparous, and 57.4% lived in households below the food poverty threshold. Of the 68 male infants, 7.4% weighed below 2500 g at birth and 10.3% were born preterm (<37 weeks); the mean ± SD birth weight was 3172 ± 435g. Pesticide containers were observed in 41.8% of homesteads during the home walkthrough. Proportions were similar in the larger group of 359 boys followed to one year, except that they were slightly less likely to be first born.

Of the 359 boys, 291 had phimosis and the urethral opening and glans could therefore not be fully examined. In boys without phimosis (n=68) and we identified hypospadias in 23 boys (34%) - altogther 23 of 359 singleton male livebirths (6.4% (23 of 359)). The demography for children with and without a phimosis diagnosis are summarized in Supplemental Table 1 and no characteristic was significantly different. Since we could not fully examine the boys with phimosis, the sample size was effectively 68. In this case, we get a prevalence of 23 out of 68: 33.8% (22.6 – 45.0). The hypospadias cases included megameatus intact preputium (MIP), glanular and coronal hypospadias and no proximal cases. No information on family history, in particular the father was available.

Table 2 summarizes the maternal serum concentrations of DDT/E and the urinary concentrations of pyrethroid metabolites for the 68 boys. Both *p,p'*- DDT and *p,p'*- DDE were detected in 100% of serum samples. The median serum concentrations of *p,p'*- DDT and *p,p'*- DDE were 76.7 and 191.9 ng/g lipid, respectively, with a strong correlation between the two analytes (r=0.91). There was no correlation between DDT/E and pyrethroid metabolite levels (r=−0.12 to 0.01). All urine samples had detectable *cis*-DCCA, *trans*-DCCA, and 3-PBA residues, but *cis*-DBCA was detected in 97.1% samples The median urinary concentrations were 0.505 μg/L for *cis*-DBCA, 0.548 μg/L for *cis*-DCCA, 0.497 μg/L for *trans*-DCCA, and 1.192 μg/L for 3-PBA. Three pyrethroid metabolites, *cis*- DCCA, *trans*-DCCA, and 3-PBA, were strongly correlated (r=0.80 to 0.89), but correlations between *cis*-DBCA and other pyrethroid metabolites were weaker (r=0.29–0.53).

Average levels of *cis*- and *trans*-DCCA were nearly double among boys with hypospadias compared to those who did not have hypospadias (Supp Table 3).

We also present the crude (RR) and adjusted (aRR) relative risks for associations between a 10-fold increase in maternal peripartum serum DDT/E and urinary pyrethroid metabolite concentrations and the presence of hypospadias within the group of 68 one year-old boys without phimosis in Table 3. We observed no association between maternal *p,p'*-DDT and *p,p'*-DDE serum concentrations and *cis*-DBCA urinary concentrations and risk of

hypospadias, after controlling for potential confounders. However, maternal *cis*-DCCA and *trans*-DCCA urinary concentrations were associated with an increased risk of hypospadias (aRR=1.58; 95% CI: 1.07, 2.34 and aRR=1.61; 95% CI: 1.09, 2.36, respectively). Although the association of hypospadias and 3-PBA was not statistically significant in the adjusted analyses (aRR=1.48; 95% CI: 0.78, 2.78), the magnitude was close to that for *cis*-DCCA and *trans*-DCCA.

In the sensitivity analyses restricted to those with maternal samples collected pre-delivery (n=43; 16 cases and 27 controls), the magnitude of the associations was similar (Supplemental Table 3). In the sensitivity analyses, in which we assumed that all those with phimosis did not have hypospadias, the magnitude of the associations increased slightly but the overall estimates did not differ (Table 3; Supplemental Table 1). When excluding the boys for whom the two raters disagreed on the presence of hypospadias, associations were slightly attenuated in the sample of boys without phimosis; for example, the relative risk for *trans*-DCCA decreased from 1.61 (1.09, 2.36) to 1.46 (0.97, 2.18) (Supplemental Table 2). However, in the larger sample considering all boys with phimosis as non-cases, excluding the boys with conflicting diagnoses did not change the results.

4. DISCUSSION

The purpose of this study was to examine the relationship between maternal exposure to DDT, DDE, and pyrethroids currently used for malaria control in South Africa and the presence hypospadias in boys at age one year. This novel study is the first to address hypospadias in a population where IRS has annually been used for over 60 years. We found that 10-fold increases in maternal *cis*- and *trans*-DCCA urinary concentrations were associated with an approximately 60% increased risk of hypospadias. Both *cis*-DCCA and *trans*-DCCA metabolites are produced in the breakdown of cypermethrin as well as other common pyrethroids and have been shown in *in vitro* and *in vivo* studies to disrupt estrogen and androgen function (19, 20, 21).

Of all boys (n=359) examined, phimosis in 291 (81.1%) excluded complete examination of the urethral opening and glans - this was only possible in 68 (18.9%) boys, therefore the sample size investigated was n=68. The prevalence of hypospadias was 33.8% (22.6 – 45.0) and in singleton live-birth boys it was 6.4% (23 of 359) and is comparable to 5.6% previously reported in newborn boys of the same area in Vhembe District Municipality of Limpopo Province (3). These figures are likely underestimations of the true prevalences since 81% of the boys could not be adequately examined for hypospadias. The observed prevalence in this study is orders of magnitude higher than globally reported (1/1000 to 1/100 (28, 29, 49). Although the prevalence of hypospadias in African countries is not well documented (50), two South African studies on different ethnic groups living outside malaria endemic areas reported much lower prevalences of 0.29/1000 (51) and 0.79/1000 live births (52), respectively, which were within the global estimates.

We observed no association between maternal *p,p'*-DDT and *p,p'*-DDE serum concentrations or *cis*-DBCA urinary concentrations and risk of hypospadias. However, maternal *cis*- and *trans*-DCCA urinary concentrations were associated with an increased

risk of hypospadias (aRR=1.58; 95% CI: 1.07, 2.34 and aRR=1.61; 95% CI: 1.09, 2.36, respectively), but not 3-PBA (aRR=1.48; 95% CI: 0.78, 2.78). Average levels of *cis*- and *trans*-DCCA were nearly double among boys with hypospadias compared to those who did not have hypospadias (Supplemental Table 3). Our study was the first to study women continuously exposed to IRS for more than 60 years, but several previous studies from other countries have evaluated the association between maternal DDT/DDE exposure in general (based on matrix measurements) and hypospadias. Although the serum values of contaminants were higher in the case mothers compared with controls, the increases in the risk of hypospadias were not statistically significant (53–55) However, Rignell-Hydbom et al., (56) reported that fetal exposure to DDE might be a risk factor for hypospadias. In a systematic review and meta-analysis of the epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders, Bonde et al., (57) concluded that p,p′-DDE was related to an elevated risk: OR 1.35 (95% CI 1.04–1.74) for the development of TDS disorders, but not any specific disorder. Our results also add to the growing body of evidence that some environmental chemicals with endocrine-disrupting properties may adversely affect male reproductive health and contribute to the ‘testicular dysgenesis syndrome’ (25, 32, 58, 59). Male sex differentiation is androgen-dependent (59, 60), and any defect in androgen biosynthesis, metabolism or action (60), or exposure to anti-androgenic and/or estrogenic compounds, could disturb the androgen:estrogen balance (A:E), resulting in demasculinizing and feminizing effects in the developing male fetus (58, 59). However, the exact cause of hypospadias is unknown (32) and findings from *in vivo* and animal studies may not be generalizable to human (61). It is likely that a combination of causes and shared pathways may be involved (42) and that endocrine disruption may be one of many critical factors (58, 59, 62). Environmental factors on polymorphic genes or induction of epigenetic changes may contribute to hypospadias development (61).

To our knowledge, this is the first study reporting associations between maternal pyrethroid exposure during pregnancy and hypospadias in boys. However, we found no significant associations between hypospadias and the other well-known endocrine disruptors DDT and DDE. *In vitro* studies with mixtures of either estrogenic or anti-androgenic chemicals indicated that endocrine disrupting substances can act synergistically/additively to yield a greater response than elicited by a single substance (63, 64). Although the boys in this study were exposed to a cocktail of chemicals demonstrated by various DDT and pyrethroid metabolite residues, a small sample size limited statistical power and prevented us from doing mixture models of the kind presented in Coker et al. (65).

This study had a number of limitations, including the sharp reduction in sample size due to phimosis. Phimosis is common in uncircumcised children and over time usually loosens. Thus, a follow-up study when the children are older and less likely to have phimosis is warranted to determine the true incidence of hypospadias. Given the short half-life of pyrethroids, a further limitation of this study is the single measure of pyrethroid metabolites around the peripartum period to estimate exposure during pregnancy. However, we previously reported associations between pyrethroid metabolite concentrations and long-term markers of pesticide use among VHEMBE women (such as IRS spraying, pesticide storage in homes and report of pesticide use around the home during pregnancy), suggesting

that a single sample may reliably reflect exposure in this population (36). Strengths of this study include the independent and blind confirmation of hypospadias by two independent clinicians.

5. CONCLUSIONS

In summary, we found a high incidence of hypospadias in the VHEMBE population in Limpopo, South Africa, which was associated with maternal exposure to pyrethroid insecticides as estimated via maternal urinary metabolite levels around delivery. Although DDT and DDE are known EDCs, we did not find an association between maternal exposure to these pesticides and hypospadias. Corrective surgery is the only treatment option for hypospadias and adds to increased cost of pediatric health care. Untreated hypospadias can lead to urinary problems, infertility, delayed sexual development, and decreased sexual satisfaction later in life (66) and should be prevented. Future studies should confirm the high incidence of hypospadias in other African populations and the potential role of *in utero* exposure to pyrethroid insecticides and other endocrine disrupting chemicals in the aetiology of hypospadias.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic characteristics of mothers and their sons who completed the urogenital examination at one year in the VHEMBE cohort without a diagnosis of phimosis, Limpopo, South Africa (n=68).

Characteristics	N (%)
Maternal age (years)	
<25	37 (54.4)
25–35	21 (30.9)
>35	10 (14.7)
Maternal BMI (post-delivery)	
Normal (<25)	26 (38.2)
Overweight (25–29.9)	19 (27.9)
Obese (≥30)	23 (33.8)
Maternal education	
<12th grade	37 (54.4)
Grade 12	21 (30.9)
Further studies started	4 (5.9)
Diploma/further	6 (8.8)
Marital status	
Married or living as married	28 (41.2)
Not married or living as married	40 (58.8)
Maternal hypertension	
No	62 (91.2)
Yes	6 (8.8)
Maternal HIV status	
Negative	56 (82.4)
Positive	12 (17.6)
Previous parity	
0	36 (52.9)
1	12 (17.7)
2 or more	20 (29.4)
Low birthweight (<2500g)	
No	63 (92.7)
Yes	5 (7.4)
Preterm delivery (<37 weeks gestation)	
No	61 (89.7)
Yes	7 (10.3)
Mother worked during pregnancy	
No	47 (69.1)
Yes	21 (30.9)

Characteristics	N (%)
Pesticides observed on homestead[a]	
No	39 (58.2)
Yes	28 (41.8)

[a]Numbers do not add to total due to 1 missing value

Table 2.

Maternal serum concentrations of *p,p'*- and *o,p'*-DDT and DDE (ng/g, lipid-adjusted), and maternal urinary concentrations of pyrethroid metabolites (ug/L, specific-gravity adjusted); VHEMBE study, Limpopo South Africa

							Percentile					
Exposure	**n**	**% Detected[a]**	**% Quantifiable[b]**	**GM**	**± GSD**	**Min**	**10**	**25**	**50**	**75**	**90**	**Max**
p,p'-DDT	68	100%	97.1%	93.7	± 5.63	4.1	11.3	27.6	76.4	307.6	1521.1	5829.5
p,p'-DDE	68	100%	98.5%	311.2	± 4.68	5.8	63.5	99.1	191.8	1086.7	2673.6	7440.3
o,p'-DDT	68	92.7%	57.4%	11.1	± 4.77	<LOD	1.5	3.8	10.4	32.0	91.3	691.6
o,p'-DDE	68	86.8%	19.1%	4.4	± 2.75	<LOD	<LOD	2.4	4.1	6.9	19.5	82.3
cis-DBCA	68	100%	97.1%	0.469	± 3.81	0.017	0.079	0.157	0.505	1.132	2.889	7.948
cis-DCCA	68	100%	100%	0.535	± 2.76	0.092	0.171	0.311	0.548	0.896	1.196	209.488
trans-DCCA	68	100%	100%	0.582	± 3.17	0.095	0.162	0.284	0.497	1.046	1.878	268.945
3-PBA	68	100%	100%	1.275	± 2.57	0.175	0.382	0.781	1.192	2.214	3.725	88.217
4-F-3-PBA	65	18.5%	10.7%	N/A		<LOD	<LOD	<LOD	<LOD	<LOD	0.021	0.047

GM: geometric mean; GSD: geometric standard deviation.

[a]Detection limits are 0.01 ng/g wet weight for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; 0.03 ng/g for *p,p'*-DDE; and 0.0025 ug/L for *cis*-DBCA, 0.0045 ug/L for *cis*-DCCA, 0.0038 ug/L for *trans*-DCCA, 0.0047 ug/L for 3-PBA, and 0.005 ug/L for 4-F-3 PBA.

[b]Quantification limits are 0.05 ng/g wet weight for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.15 ng/g for *p,p'*-DDE; 0.0082 ug/L for *cis*-DBCA, 0.015 ug/L for *cis*-DCCA, 0.013 ug/L for *trans*-DCCA, 0.016 ug/L for 3-PBA, and 0.011ug/L for 4-F-3 PBA.

Table 3.

Adjusted Relative Risks and 95% confidence interval (CI) for the association between maternal prenatal serum DDT/E (lipid-adjusted) and urinary pyrethroid metabolite concentrations (specific gravity-adjusted) with the risk of hypospadias in 1 year-old boys, VHEMBE study, Limpopo, South Africa.

Exposure	RR (95% CI)
Boys without a diagnosis of phimosis (n=68)	
p,p'-DDT	1.01 (0.61, 1.67)
p,p'-DDE	0.89 (0.51, 1.57)
cis-DBCA	0.86 (0.49, 1.48)
cis-DCCA	1.71 (1.20, 2.45) *
trans-DCCA	1.64 (1.14, 2.35) *
3-PBA	1.61 (0.87, 2.98)
All boys (n=359)[a]	
p,p'-DDT	1.25 (0.84, 1.86)
p,p'-DDE	1.11 (0.65, 1.92)
cis-DBCA	1.23 (0.56, 2.69)
cis-DCCA	1.97 (1.31, 2.97) *
trans-DCCA	1.71 (1.10, 2.66) *
3-PBA	1.99 (1.10, 3.59) *

[a]Models with all boys consider those with a phimosis diagnosis (n=291) to not have hypospadias.

Relative risks show the change in the risk of hypospadias associated with a 10-fold increase in maternal serum DDT/E or urinary pyrethroid metabolite concentrations. Models adjusted for maternal BMI category. Models with pyrethroids also adjusted for time of urine collection (before or after delivery).

*p<0.05