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In-utero exposure to DDT and pyrethroids and child behavioral and emotional problems at 2 years of age in the VHEMBE cohort, South Africa

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Abstract

Background: Half the world's population is at risk for malaria. Indoor residual spraying (IRS) with insecticides has been effective in controlling malaria, yet the potential neurotoxicity of these insecticides is of concern, particularly for infants exposed *in utero*.

Objectives: To determine the association of prenatal exposure to DDT/DDE and pyrethroid insecticides and behavioral/emotional problems in two-year-old children.

Methods: The Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) birth cohort in South Africa, measured concentrations of *p,p'*-DDT and *p,p'*-DDE in maternal serum and pyrethroid metabolites (*cis*-DBCA, *cis*-DCCA, *trans*-DCCA, and 3-PBA) in maternal urine collected during pregnancy. At 2 years, 683 mothers were interviewed about their children's behavior and emotional development, using the Child Behavior Checklist (CBCL). We examined associations between behavioral or emotional problems and biomarkers of prenatal insecticide exposure.

Results: Maternal serum *p,p'*-DDT concentrations were associated with heightened withdrawn behavior in 2-year olds, with a 0.25 increase in raw scores (95%CI=0.00, 0.49) and a 12% increase (95%CI=1.01, 1.24) in risk of being at or above the borderline-clinical level, per 10-fold increase in concentrations. Ten-fold increases in *p,p'*-DDT and *p,p'*-DDE were related to 26% (RR=1.26; 95%CI= 0.99, 1.62) and 35% (RR=1.35; 95%CI=0.98, 1.85) higher risks, respectively, for increased oppositional-defiant behavior. *p,p'*-DDE concentrations were also related to increased risk of ADHD-related problems (RR=1.31; 95%CI=0.98, 1.73). Maternal urinary concentrations of *cis*-DBCA and 3-PBA were associated with increased risk of externalizing behaviors (RR

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= 1.29; 95%CI= 1.04, 1.61; RR = 1.34, 95%CI=1.02, 1.76 per 10-fold increase, respectively), with some evidence of an association between *cis*-DBCA and affective disorders (RR=1.24; 95%CI=0.99, 1.56). Some associations with maternal pyrethroid concentrations were stronger in girls than boys.

Conclusions: Prenatal exposure to DDT and pyrethroid insecticides may be associated with maternally-reported behavioral problems in two-year-old children. Given their long history and continued use, further investigation is warranted.

Keywords

Insecticides; DDT; pyrethroids; behavior; birth cohort; South Africa

Introduction

In 2020, there were an estimated 241 million cases of malaria, which were responsible for over 627,000 deaths, mostly among children under age 5 living in Sub-Saharan Africa (World Health Organization 2021). Indoor residual spraying (IRS), the application of insecticides on the walls of homes, is one of the core vector control methods for malaria prevention (World Health Organization 2016). In accordance with the Stockholm Convention on Persistent Organic Pollutants, eight malaria-endemic sub-Saharan African countries continue to use dichlorodiphenyltrichloroethane (DDT) for IRS (World Health Organization 2016). Pyrethroid insecticides, particularly deltamethrin and cypermethrin, are commonly used for IRS due to their rapid elimination in humans (half-lives on the order of hours or days instead of years for DDT) (Barlow et al. 2001).

DDT and pyrethroids are neurotoxic chemicals, interfering with voltage-gated sodium ion channels in insects, inducing excessive excitation of neurons and cell death. In animal models, these insecticides also act on potassium and chloride ion channels, which can lead to tremors, and even death when exposed at high doses (Eriksson et al. 1992; Malik et al. 2017). Both DDT and pyrethroids have endocrine disrupting properties. Pyrethroids insecticides disrupt androgen signaling and steroidogenesis (Brander et al. 2016; Chen et al. 2002; Sun et al. 2007; Tyler et al. 2000). *p, p'*-DDT acts as an antiandrogen while *o,p'*-DDT acts as a weak estrogen and p, p'-DDE is an androgen receptor antagonist (Diamanti-Kandarakis et al. 2009; Holm et al. 2006; Kelce et al. 1995; Soto et al. 1995).

Early-life exposure is of particular concern, since disruption by chemicals during development could lead to long-lasting consequences (Eriksson et al. 1990; Eriksson et al. 1992). Mice with early-life exposure to either DDT or pyrethroids showed increased density of muscarinic cholinergic (mAChR) receptors and increased motor activity, coupled with delayed learning (Eriksson 1992; Gupta and Gupta 2017; Shafer et al. 2005).

The relationship between developmental exposure to DDT and pyrethroids and behavioral problems in children has been investigated using maternal biomarkers to determine levels of exposure during pregnancy (Barkoski et al. 2021; Cheslack-Postava et al. 2013; Dalsager et al. 2019; Forns et al. 2016; Furlong et al. 2017; Kyriklaki et al. 2016; Rosenquist et al. 2017; Sagiv et al. 2010; Sagiv et al. 2012; Shelton et al. 2014; Sioen et al. 2013; Viel

et al. 2017). In a cohort from Massachusetts, *p,p'*-DDE concentrations in cord blood were linked with an increase in errors of omission among boys on the Continuous Performance Test (CPT) (Sagiv et al. 2012) and in attention-deficit hyperactivity disorders (ADHD)-associated behaviors on the Conner's Rating Scale (CRS-T) in both sexes combined at 8 years (Sagiv et al. 2010),. In a Belgian study, positive associations between cord blood concentrations of *p,p'*-DDE and hyperactivity were observed in school-aged girls but not boys (Sioen et al. 2013). In a Norwegian cohort of about 600 mother-child pairs, Forns et al. (2016) reported positive associations between *p,p'*-DDT concentrations in breast milk and regulatory behavioral problems measured by the Infant/Toddler Symptoms Checklist (ITSC) at 12 months of age but not at 24 months.

Prenatal exposure to pyrethroid insecticides has been linked with behavioral problems in children. In the PELAGIE cohort, maternal urinary concentrations of *cis*–(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid (*cis*-DCCA) during pregnancy were associated with maternal report of internalizing problems on the Strength and Difficulties Questionnaire (SDQ) in their children at age 6 years. They also found that prenatal 3-phenoxybenzoic acid (3-PBA) concentrations were weakly linked with decreased prosocial behavior. No associations were found with concentrations of other pyrethroid urinary metabolites *cis*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid (*cis*-DBCA) and *trans*-DCCA (Viel et al. 2017). In a Danish birth cohort, prenatal urine concentrations of 3-PBA were associated with increased odds of scoring above the 90th percentile on the Diagnostic and Statistical Manual (DSM) ADHD scale of the Child Behavior Checklist (CBCL) between 2 and 4 years of age (Dalsager et al. 2019). The MARBLES birth cohort, composed of children in California expected to have a higher risk of autism spectrum disorder (ASD) diagnosis, found that higher urinary concentrations of 3-PBA in samples taken from mothers in the second trimester were associated with an increased risk of being diagnosed with ASD (Barkoski et al. 2021). In addition, California children whose mothers lived closer to agricultural regions where pyrethroids were regularly used were found to have a higher odds of autism diagnosis (Shelton et al. 2014). In a previous analysis (Eskenazi et al. 2018), we reported an association between maternal urinary concentrations of *cis*-DCCA, *trans*-DCCA, and 3-PBA and decrements in Social-Emotional scores on the Bayley Scales of Infant Development in VHEMBE children at one-year; noadverse associations were found with maternal serum concentrations of *p,p'*-DDT or *p,p'*-DDE.

In the present study, we investigated the relationship of maternal prenatal exposure to DDT and pyrethroid insecticides and the behavior of two-year-old children. The maternal-child dyads were participants of the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) birth cohort study conducted in Limpopo, South Africa, where DDT and pyrethroids are used for malaria control.

Methods

Study population

The VHEMBE study aims to assess the environmental determinants of child health and development in a birth cohort. Detailed methods for the VHEMBE study have been

described elsewhere (Eskenazi et al. 2018; Gaspar et al. 2017). Briefly, between August 2012 and December 2013, pregnant women were screened and recruited at Tshilidzini Hospital in the Vhembe district of Limpopo Province, South Africa. Eligibility criteria included being 18 years or older, having contractions >5 minutes apart, speaking primarily TshiVenda at home, having a singleton pregnancy, living within 20 km of the hospital and planning to remain in the area for at least 2 years, and having no malaria diagnosis during the index pregnancy. Among the 752 mother/infant pairs enrolled at delivery, 24 children died before the 2-year visit, 43 were lost to follow-up, and 2 had mothers who did not complete the child behavioral evaluation at the 2-year follow-up, leaving a total of 683 (91%) children who completed the 2-year visit. Sociodemographic characteristics of those who completed the 2-year visit were similar to the 69 mother-child pairs who did not, except that 17% of the latter group were born of low birth weight (<2500g) compared to 7% among children who completed their 2-year visit (chi-square p-value = 0.01). Maternal concentrations of insecticide biomarkers did not differ between those followed to two years and those who were not.

Mothers provided informed consent before participating in the study. 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.

Procedures

Mothers were interviewed in TshiVenda by research staff shortly after delivery in the hospital or in our field office on the hospital grounds. The interviewers queried the mother on their socio-demographic characteristics, medical history, household composition, residential history, and habits. In addition, mothers completed the U.S. Household Food Security Survey Module (6-item short form) (USDA 2012), and the Stressful Life Events Scale (Yach et al. 1991). Questionnaires were translated into TshiVenda and back-translated to English, and then back-translations were reviewed by the publisher against the original scale in English to assure their accuracy (ASEBA). A study nurse abstracted the child's delivery information from medical records, including birth weight and gestational age at delivery.

At 1 and 2 years of age, children came back to the field office with either their mother or another primary caregiver. The mother/caregiver was interviewed regarding socio-demographic characteristics, household composition, residential history, breastfeeding history, and the child's health and learning environment at home (using a subset of the Home Observation for Measurement of Environment (HOME) inventory) (Kariger et al. 2012; Tu et al. 2016). We also administered the Self-Reporting Questionnaire (SRQ, 20-question version) (Arrebola et al. 2016) to screen for maternal depression. At the 1-year visit, psychometricians assessed the primary caregiver's nonverbal intelligence with the Raven's Coloured Progressive Matrices (Raven 1960). Household poverty was assessed at both visits using thresholds based on per capita monthly income (Statistics South Africa. 2017).

The Child Behavior Checklist (CBCL)

At the 2-year visit, psychometricians administered 64 out of the 99 items of the CBCL for children ages 1 ½ - 5 (Achenbach and Rescorla 2000) to mothers (n=656) or other primary caregivers (N=27) in order to assess the child's behavioral/emotional problems. We selected *a priori* four empirical scales (anxious/depressed, withdrawn, attention problems, and aggressive behavior), one composite scale for attention and aggressive behavior problems (externalizing), and five Diagnostic and Statistical Manual of Mental Disorders (DSM) scales (affective problems, anxiety problems, pervasive developmental problems, attention deficit/hyperactivity problems (ADHD), and oppositional defiant problems). For each item, the caregivers rated how well the problem described their child's behavior on a scale of 0 to 2, with 0 being "Not True", 1 being "Somewhat True," and 2 being "Very True." We summed the scores on appropriate items for each scale to calculate raw scores. Raw scores were standardized to T-scores using a normative U.S. sample and were categorized into normal, borderline clinical, and clinical groups using cut-points of T-scores of 65 (93^{rd} percentile) and 70 (98^{th} percentile) (Achenbach and Rescorla 2000).

With permission from the publishers (ASEBA, Burlington, VT), we created a TshiVenda version of the CBCL. To ensure that the translation was consistent with the meaning of the original English version, the CBCL was backtranslated to English and versions were compared; any differences in the translation were reconciled by the publisher.

DDT/DDE measurements

Blood samples were collected from mothers via venipuncture at the hospital before (n=538) or after delivery (but before leaving the hospital) (n=145). Samples were immediately processed and stored at −80°C prior to being shipped on dry ice to Emory University's Rollins School of Public Health for chemical analysis. Concentrations of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT, and *o,p'*-DDE were measured by high-resolution gas chromatography-isotope dilution mass spectrometry (GC-MS) (Barr et al. 2003). The limits of detection were 0.01 ng/mL wet-weight for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.03 ng/mL for *p,p'*-DDE. The limits of quantification were 0.05 ng/mL wet-weight for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.15 ng/mL 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 concentrations measured using standard enzymatic methods (Roche Chemicals, Indianapolis, IN) (Phillips et al. 1989).

Pyrethroid measurements

Maternal urine samples were collected before (N=418) or soon after (n=255) delivery. Samples were stored at −80°C at the field office until shipment to the Institut National de Santé Publique du Québec (INSPQ) for chemical analysis. We measured the major metabolites of the pyrethroid insecticides used for IRS in the region using GC-MS (Dewailly et al. 2014). *cis*-DBCA is a metabolite of deltamethrin and 4-F-3-PBA is a metabolite of cyfluthrin, while *cis*-DCCA, *trans*-DCCA, and 3-PBA are non-specific metabolites of several pyrethroid insecticides, including permethrin, cypermethrin, and/or cyfluthrin (Barr et al. 2010). The limits of detection were 0.0025 μg/L for *cis*-DBCA, 0.045 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

4-F-3-PBA. The 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-F-3-PBA. Quality control was conducted with reference materials produced at the laboratory as well as using ClinChek® non-certified reference material in urine (RECIPE; Munich, Germany). Specific gravity measurements were determined using an Atago PAL-10S refractometer (Atago Company Ltd, Tokyo, Japan).

Data analysis

We conducted descriptive analyses for demographic characteristics, maternal levels of DDT/E and pyrethroids, and maternally-reported behavioral outcomes measured with the CBCL. DDT and DDE concentrations were corrected for lipid levels and are given in units of ng/g-lipid; pyrethroid metabolites concentrations were corrected for urinary specific gravity and given in units of μg/L. We limited our analyses to those analytes for which at least 70% of samples were quantifiable; thus, we excluded *o,p'*-DDT, *o,p'*-DDE, and 4-F-3-PBA. We $\log_{10}$-transformed maternal serum DDT/E and urinary pyrethroid metabolite levels to reduce the influence of outliers. For all concentrations below the limit of detection (LOD), we randomly selected a value below theLOD based on a log-normal probability distribution whose parameters were estimated via maximum likelihood estimation (Lubin et al. 2004). Concentrations between the LOD and the limit of quantifications were assigned machine-read values.

To assess the relationship between metabolite levels and the behavioral problems assessed by CBCL, we constructed separate multiple regression models for each combination of analyte and CBCL measure. We examined associations between continuous measures of maternal concentrations of *p,p'*-DDT, *p,p'*-DDE, and the four pyrethroid metabolites (*cis*-DCCA, *trans*-DCCA, 3-PBA, and *cis*-DBCA) with CBCL scores for both continuous raw scores, as well as binary outcomes defined as T-scores above or equal to the 93rd percentile of the normative sample (the borderline-clinical and clinical range) vs. below the 93rd percentile. We examined the functional form of models using Generalized Additive Models (GAMs) with 3-degree of freedom cubic splines and plots of the GAM smoother. As we did not find evidence of non-linearity, we used multivariable linear regression to examine the associations between a 10-fold increase in biomarker of exposure to insecticide and the raw score for each scale. We used Poisson regression with a robust (Huber-White) variance estimator to estimate the relative risk of borderline-clinical and clinical behavior/emotional problems for each ten-fold increase in serum DDT, DDE and urinary pyrethroid metabolite levels.

We selected covariates using a directed acyclic graph (DAG). Covariates selected for the final models included maternal education category (less than 12th grade, 12th grade, more than 12th grade), age at delivery (continuous), Raven's z-score at 1 year (continuous), risk for depression at both 1 and 2 years (≥ 6 vs. <6 on the SRQ), HOME z-score (continuous) at 1-year, breastfeeding status at 1-year (yes vs. no), and food poverty status at 2 years (<R441 – about $35 U.S./month per capita – vs. ≥R441, for the year of 2015) (Statistics South Africa. 2017). Models with pyrethroid metabolites also included the time of urine sample collection (before delivery vs. after delivery). For Raven's score and HOME scores,

we used z-scores standardized within the study population. Missing values of child's birth weight, HOME score at 1-year, maternal Raven's score at 1-year and maternal risk for depression at 1 and 2 years (less than 3% for all variables), were randomly imputed based on observed probability distributions. Missing values of the 2-year poverty status (n=10) were replaced with values from the 1-year questionnaire, due to the high concordance between visits. Effect modification by child sex was assessed using cross-product interaction terms. Sensitivity analyses were performed excluding children whose mothers' urine samples were collected after delivery.

To examine associations with the joint exposure to DDT/E and pyrethroids, we used Bayesian Kernel Machine Regression (BKMR). BKMR models examine both individual and joint effects within a mixture of exposures by modeling the outcome as a flexible kernel function of the exposure variables. Here, we focus on BKMR's ability to identify associations with the overall mixture by comparing the estimated outcomes when holding all exposures at particular percentiles. If there are joint associations of insecticide exposures, we would expect to see a non-linear relationship between the overall mixture concentration and outcome estimates as the mixture concentration increases. For the sake of brevity, we confined BKMR analyses to continuous CBCL outcomes only.

Analyses were performed using Stata version 15 (StataCorp, College Station, TX) and R (version 3.3.1; R Development Core Team, Vienna, Austria).

Results

Demographic characteristics

Mothers averaged 26.4 years old at delivery, with more than half having less than a 12^{th} grade education (see Table 1). Less than half of the women were married, 43.3% were primiparous, and 11.6% and 6.6% were at risk for depression at 1- and 2-years post-partum, respectively. Eighty-six women (12.6%) were HIV-positive. The average birth weight of the children was 3143g (standard deviation (SD)=447 g) with 12.9% of the children born preterm (< 37 weeks gestation). The average duration of exclusive breastfeeding was 2.3 months (SD 1.9) with 77.9% of mothers still breastfeeding at one year. Nearly half of families lived below the food poverty level at 1 year (45.7%) (R417/month per capita) and at 2 years (42.4%) (R441/month per capita).

Maternal DDT/E and pyrethroid metabolite concentrations

The quantification frequencies for *p,p'*-DDT, *p,p'*- DDE, *cis*-DBCA, *cis*-DCCA, *trans*-DCCA, and 3-PBA were all greater than 90% (Supplemental Table 2). The geometric mean (GM) of maternal serum concentrations was 69.9 ng/g-lipid for *p,p'*-DDT and 292.2 ng/g-lipid for *p,p'*-DDE; the two analytes were highly correlated ($r = 0.85$, $p < 0.001$) (Supplemental Table 1). The GMs of the specific gravity-corrected maternal urinary pyrethroid metabolite concentrations were 0.35 μg/L for *cis*-DBCA, 0.48 μg/L for *cis*-DCCA, 0.56 μg/L for *trans*-DCCA, and 1.11 μg/L for 3-PBA. The pyrethroid metabolites were moderately to highly correlated ($r = 0.32$ to 0.88, $p<0.001$) (see Supplemental Table 1). The pyrethroid metabolites were not correlated with DDT or DDE ($r = -0.01$ to 0.05,

p=0.20 to 0.71). Uncorrected (wet-weight) pyrethroid metabolite concentrations are shown in Supplemental Table 3.

Caregiver-reported behavior/emotional problems

Supplemental Table 4 displays the average raw scores for each CBCL scale, as well as the percentage of two-year-old children whose caregivers reported child behaviors in the borderline-clinical and clinical (≥93rd percentile) range. Overall, we observed a higher proportion of children in the borderline-clinical and clinical categories than the 7% reported in the normative sample. The pervasive developmental (47%), anxiety (37%), and withdrawn (42%) measures were particularly high in our sample. Boys also scored higher than girls on the attention-related (ADHD and attention scales), externalizing, and anxiety measures.

Relationships between maternal serum DDT and DDE concentrations and behavior/ emotional problems

Overall, we observed consistently positive associations between maternal serum concentrations of *p,p'*-DDT and *p,p'*-DDE and caregiver report of behavioral problems in children (Table 2). Associations were generally weak and imprecise except for findings on the withdrawn and oppositional-defiant scales; for each 10-fold increase in maternal serum *p,p'*-DDT concentrations, there was 0.24 point increase (95% CI=0.00, 0.49) on the child's withdrawn scale raw score, with a 12% increased risk of scores falling at or above the borderline-clinical range (RR=1.12; 95% CI=1.01, 1.23) (Table 2). We observed a similar trend between withdrawn behavior and *p,p'*-DDE (β=0.24; 95% CI=−0.06, 0.53 per 10-fold increase). Maternal serum *p,p'*-DDT and *p,p'*-DDE concentrations were related respectively to a 30% (RR=1.30; 95% CI= 1.01, 1.67) and 39% (RR=1.39; 95% CI=1.01, 1.91) higher risk for oppositional-defiant behavior falling at or above the borderline-clinical range per 10-fold increase in concentrations. Maternal serum *p,p'*-DDE concentrations were also related to 30% higher risk of children having ADHD-related problems at or above the borderline-clinical range (RR=1.30; 95% CI=0.98, 1.72 per 10-fold increase). We found no evidence of effect modification by sex (Supplementary Tables 5.1 and 5.2).

Relationships between maternal urinary pyrethroid concentrations and behavior/emotional problems

We found that maternal urinary concentrations of *cis*-DBCA and 3-PBA were related to a higher risk of scores at or above the borderline-clinical range of externalizing behaviors (RR=1.30; 95% CI=1.05, 1.62, and RR=1.35; 95% CI=1.03, 1.78, per 10-fold increase, respectively) as well as some evidence of an association between *cis*-DBCA and maternal report of affective disorders (RR=1.25; 95% CI=0.99, 1.56 per 10-fold increase). Other models yielded weak and inconsistent associations.

We found very limited evidence of effect modification by sex (Supplementary Tables 6.1 and 6.2). Maternal urinary *cis*-DBCA was associated with somewhat higher anxiety scores for girls (β=0.70, 95% CI=−0.03, 1.43 per 10-fold increase) but not for boys (β=−0.19, 95% CI=−0.94, 0.55 per 10-fold increase; $p_{interact}$=0.08). Maternal urinary 3-PBA concentrations were associated with lower risks of scoring at or above the borderline-clinical range of the anxious/depressed scale among boys (RR=0.59, 95% CI=0.41, 0.84) but not in girls

(RR=1.11, 95% CI=0.65, 1.90 per 10-fold increase; $p_{interact}$=0.05). Similarly, we found an inverse association between 3-PBA and scoring at or above the borderline-clinical range of the affective scale among boys (RR=0.85; 95% CI=0.53, 1.35 per 10-fold increase) and a positive association among girls (RR=1.57; 95% CI=1.02, 2.44, per 10-fold increase; $p_{interact}$=0.08) but stratified estimates were imprecise.

Associations reported above were similar after restricting the analysis to children whose mothers provided a urine sample before giving birth (Supplemental Table 7).

When assessing the exposure response for the overall mixture using BKMR models (Supplemental Figure 1a–j), our data suggests there is no departure from linearity. This finding supports our conclusion of no apparent joint effects of the insecticide mixture that go beyond additive effects.

Discussion

Previously, we reported no association between the parent-reported Bayley social-emotional scale and prenatal serum *p,p'*-DDT or *p,p*'-DDE concentrations at 1 year (Eskenazi et al. 2018). In a follow-up at age 2 years, we now report an association between maternal serum *p,p'*-DDT concentrations and parent-reported withdrawn and oppositional-defiant behaviors. Higher maternal serum concentrations of *p,p'*-DDE were also associated with increased risks of oppositional-defiant and ADHD behaviors. In addition, similar to our previously reported association between lower performance on the Bayley social-emotional scale at one-year and maternal pyrethroid metabolite concentrations (Eskenazi et al. 2018), we found that maternal urinary concentrations of *cis*-DBCA and 3-PBA were associated with increasing risks of externalizing behaviors at or above the borderline-clinical range at age and of maternal report of affective disorder behavior. Some other associations were stronger in girls. We found no evidence of joint effects of DDT and pyrethroid insecticides on these measures.

Our findings suggest that prenatal exposure to *p,p'*-DDT may increase the risk of withdrawn behaviors in children at 2 years. The withdrawn scale measures lack of social engagement, and is considered to be a good tool for identifying preschoolers at risk for autism (Muratori et al. 2011). Although we are aware of no other study that specifically investigated withdrawn behavior with respect to insecticide exposure, our findings are consistent with the moderate trend of increasing odds of autism diagnosis related to higher maternal concentrations of *p,p'*-DDE reported by the Finnish Prenatal Study of Autism (Cheslack-Postava et al. 2013), but they run counter to the null finding from the Early Markers for Autism (EMA) study in California (Lyall et al. 2017).

Prior studies have reported mixed findings on the relationship between oppositional defiant behavior and DDT biomarkers. In the Inuit and European populations (INUENDO) cohort, *p,p'*-DDE increased measures of abnormal conduct behaviors on Strengths and Difficulties Questionnaire (which has a significant overlap with the CBCL oppositional-defiant scale) in children between 5 and 9 years of age (Rosenquist et al. 2017); while a non-significant

positive trend was observed in the mother-child cohorts in Belgium (Sioen et al. 2013) and Greece (Kyriklaki et al. 2016).

Only a few studies have examined the relationship of prenatal pyrethroid biomarkers and child behavior. Findings from birth cohorts in Denmark (Dalsager et al. 2019), the U.S. (Furlong et al. 2017), and France (Viel et al. 2017), were consistent with the positive associations that we observed between concentrations of 3-PBA and externalizing behavioral problems.

Emerging animal evidence supports our findings of behavioral changes associated with *in utero* pyrethroid exposure. Mice exposed to deltamethrin *in utero* showed hyperactivity, increased impulsivity, and altered attention, predominantly in males (Richardson et al. 2015); however, findings in rats showed decreased activity (Pitzer et al. 2019). Our finding that *cis*-DBCA, the metabolite associated with deltamethrin, on externalizing behavior is in line with rodent findings of impulsivity, although the association was not unique to males. Other studies have shown changes in the acoustic startle response following early life exposure to pyrethroids, with the direction of effect dependent on the pyrethroid, age, dose, and timing since dose (Pitzer et al. 2021). The mechanism of action has been hypothesized to be through perturbation in the dopamine system, including lower synaptic dopamine, elevated dopamine active transport, and increased dopamine D1 receptor levels (Richardson et al. 2015), although the glutamatergic and cholinergic pathways have also been implicated (Pitzer et al. 2021).

DDT and DDE also are neurotoxic. Administration of DDT and DDE *in utero* has been shown to result in behavioral and neurochemical changes in rodents, including impaired maze learning and memory functions, increased spontaneous motor activity, and increased urine marking behavior (ATSDR 2022). Both in insects and in mammals, DDT interferes with the sodium channels in the axonal membrane and neurochemical studies have also shown that DDT exposure alters the levels of some neurotransmitters such as acetylcholine, norepinephrine, and serotonin. Although it is possible that DDT and DDE's impact on the brain is through endocrine disruption, only one previous study in humans showed sex differences on behavior associated with *in utero* DDE exposure (Sagiv et al. 2012). We did not see any effect modification by sex in our study for DDT or DDE.

The strengths of the present study include its longitudinal design, which is ideally suited to measuring early life exposure and examining its longer-term effects. The VHEMBE population is highly exposed, making them a unique population for the study of the health effects of insecticides. The population is also unique in that co-exposure to DDT and pyrethroids has been observed in South Africa (Bouwman et al. 2006). To our knowledge, VHEMBE is the first prospective cohort to investigate possible associations between prenatal exposure to either DDT or pyrethroids with emotional and behavioral issues in children in a malaria-endemic region, where insecticides are heavily used for malaria vector control. Other cohort studies have investigated associations between prenatal exposure to DDE and child behavior in European or North American countries, where the use of DDT has been banned for several decades; hence, the exposure levels were low and few studies have been conducted on DDT (Cheslack-Postava et al. 2013; Kyriklaki

et al. 2016; Rosenquist et al. 2017; Sagiv et al. 2008; Sagiv et al. 2010; Sagiv et al. 2012; Sioen et al. 2013). The observed maternal concentrations of *p,p'*-DDE in our study population were two to ten times higher than the median values reported in the Flemish Environment and Health study (124 ng/g-lipid) and a cohort from New Bedford, MA (0.31 ng/g) (Oulhote and Bouchard 2013; Quiros-Alcala et al. 2014; Sagiv et al. 2010; Sioen et al. 2013). Observed concentrations of pyrethroids are also higher than in other studies of pregnant women (Dalsager et al. 2019; Furlong et al. 2017; Viel et al. 2017). Another strength of this study is that we accounted for a large array of socioeconomic factors that could impact neurobehavioral development, such as poverty, socio-demographics, home environment quality, maternal non-verbal intelligence, and health-related behaviors, making confounding by these factors less likely.

The present study also has some limitations. The children are quite young, and it is possible that their behavior patterns will change as they grow. Although we don't know precisely how these findings will track over time in this study population, the scales of interest have shown moderate to high correlations over time (r=0.4 to 0.7) when tracked from ages 2 to 9 years among US children (Achenbach and Rescorla 2000). Fortunately, the VHEMBE study will include additional follow-up visits. In addition, the psychometric properties of the CBCL have also held up well in a population in rural Kenya that is in many ways similar to the VHEMBE cohort (Kariuki et al. 2016). Another limitation is that only a single urine sample was obtained. Given the short half-life of pyrethroid metabolites, it would have been preferable for all samples to have been collected before delivery; however, this was logistically difficult given the study population. However, we found that both the urinary concentrations of 3-PBA and cis-DCCA were both somewhat elevated ($p \leq 0.10$) in women who had their home sprayed during their pregnancy (n=40). While it is possible that metabolite concentrations in samples taken after delivery reflect exposure in the hospital, the consistent results in the sensitivity analyses limiting analysis to prenatal specimens support our overall results. In addition, the prevalence of borderline-clinical and clinical behavioral measures was much higher in the VHEMBE population than in the U.S. normative sample. This may stem from the impoverished, rural population of the study, or from cultural differences reflected in the caregivers' assessment of child behaviors. Also, the CBCL was translated and back-translated but was not validated for this population and the high scores may reflect cross-cultural differences. Unfortunately, to our knowledge, no validated TshiVenda child behavior scale was available at the time of study. Within the population, correlations with pesticides would still hold.

In summary, the present findings suggest that prenatal exposure to DDT and pyrethroid insecticides may be associated with problematic behaviors among children living in a malaria-endemic rural region of South Africa. While IRS has been shown to be effective in controlling malaria, similar findings on neurodevelopment from prior studies suggest that educating families in the region about the potential adverse effects and the actions they can take to minimize exposure to insecticides may be beneficial, particularly for children and pregnant women (Eskenazi et al. 2019). Further studies are needed to confirm our results, to elucidate the effects of exposure on young children, and to inform early interventions to prevent life-long sequelae of social and emotional problems. As insecticides are continually used to prevent the spread of vector-related diseases such as malaria and Zika virus, further

studies are warranted among the populations exposed to a prolonged use of large quantities of these chemicals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic characteristics of VHEMBE cohort who completed the Child Behavior Checklist (CBCL) at 2 years (n=683)

Maternal characteristics		
Maternal age, M±SD	26.4	± 6.2
Education, n (%)		
< 12th grade	379	(55.5)
Grade 12	203	(29.7)
Further studies	101	(14.8)
Marital status, n (%)		
Married,	320	(46.9)
Not married	363	(53.1)
Parity, n (%)		
0	296	(43.3)
1	185	(27.1)
2+	202	(29.6)
HIV status		
Positive	86	(12.6)
Negative	595	(87.4)
Alcohol during pregnancy, n (%)		
Yes	38	(5.6)
No	645	(94.4)
Raven’s Coloured Progressive Matrices, M±SD		
Raw score	22.0	± 6.0
z-score within sample population	0.008	± 1.0
At risk for depression (SRQ-20) at 1 year, n (%)		
Yes	79	(11.6)
No	604	(88.4)
At risk for depression (SRQ-20) at 2 years, n (%)		
Yes	45	(6.6)
No	638	(93.4)
Child characteristics		
Sex, n (%)		
Boy	355	(52.0)
Girl	328	(48.0)
Preterm (<37 weeks), n (%)		
Yes	88	(12.9)
No	595	(87.1)
Low birth weight (<2500g), n (%)		
Yes	51	(7.5)
No	632	(92.5)
Birthweight (g), M±SD	3143	± 447

Breastfeeding at 1 year old, n (%)		
Yes	532	(77.9)
No	151	(22.1)
Duration of exclusive breastfeeding (months), M±SD	2.3	±1.9
Family Characteristics at 1 year Follow-up		
Below food poverty level (R417/month per capita), n (%)		
Yes	305	(45.7)
No	362	(54.3)
HOME score, M±SD		
Raw score (Maximum, 31)	22.2	± 3.5
Family Characteristics at 2 year Follow-up		
Below food poverty level (R441/month per capita), n (%)		
Yes	284	(42.4)
No	386	(57.6)
HOME score, M±SD		
Raw score (Maximum, 42)	31.7	± 4.2

Table 2.

Adjusted[a] beta coefficient (β) and 95% confidence interval (CI) for the total raw score of the behavioral/emotional scales of the CBCL with each log10 unit increase in serum DDT/DDE congener level (lipid-adjusted); Adjusted relative risk (RR) and 95% confidence interval (CI) for the borderline-clinical and clinical range to normal range of behavioral/emotional scales of the CBCL with a $\log_{10}$ unit increase in serum DDT/DDE congener levels (lipid-adjusted)(N=683)

	β (95% CI)		RR (95% CI) for Borderline-Clinical and Clinical Range	
Measures	***p,p'*-DDT**	***p,p'*-DDE**	***p,p'*-DDT**	***p,p'*-DDE**
DSM				
Pervasive developmental	0.22 (−0.13, 0.56)	0.26 (−0.16, 0.67)	1.07 (0.97, 1.18)	1.05 (0.94, 1.18)
Anxiety	0.07 (−0.24, 0.37)	−0.01 (−0.38, 0.35)	1.05 (0.94, 1.18)	1.01 (0.89, 1.16)
Affective	0.18 (−0.08, 0.43)	0.16 (−0.15, 0.47)	1.10 (0.97, 1.25)	1.09 (0.94, 1.27)
ADHD	−0.02 (−0.26, 0.22)	0.06 (−0.23, 0.35)	1.18 (0.94, 1.49)	1.30 (0.98, 1.72)
Oppositional-defiant	0.16 (−0.07, 0.40)	0.19 (−0.09, 0.47)	1.30 (1.01, 1.67)*	1.39 (1.01, 1.91)*
Empirical				
Withdrawn	0.24 (0.00, 0.49)*	0.24 (−0.06, 0.53)	1.12 (1.01, 1.23)*	1.07 (0.95, 1.22)
Externalizing	0.33 (−0.41, 1.07)	0.47 (−0.42, 1.37)	1.01 (0.89, 1.15)	1.02 (0.88, 1.18)
Anxious/Depressed	0.07 (−0.18, 0.32)	0.05 (−0.25, 0.36)	1.01 (0.89, 1.15)	1.02 (0.88, 1.19)
Aggressive behavior	0.32 (−0.33, 0.96)	0.47 (−0.30, 1.25)	1.10 (0.92, 1.31)	1.13 (0.91, 1.40)
Attention	0.01 (−0.14, 0.17)	0.00 (−0.19, 0.19)	1.15 (0.90, 1.46)	1.11 (0.81, 1.51)

Models adjusted for maternal education, and age at delivery; mother's Ravens score, and HOME score at 1-year; risk for depression at 1-year and 2-years; poverty at 2-years; breastfeeding through 1 year.

*$p<0.05$

Table 3

Adjusted beta coefficient and 95% confidence interval (CI) for the total raw score of the behavioral/emotional scales of the CBCL with a log10 unit increase in urinary pyrethroid metabolites (specific gravity-adjusted, log10); Adjusted relative risk and 95% confidence interval (CI) for the borderline and clinical range of behavioral/emotional scales of the CBCL with a log10 unit increase in urinary pyrethroid metabolites (specific gravity-adjusted, log10).

	β (95% CI)				RR (95% CI)			
Measures	***cis*-DBCA**	***cis*-DCCA**	***trans*-DCCA**	**3-PBA**	***cis*-DBCA**	***cis*-DCCA**	***trans*-DCCA**	**3-PBA**
DSM								
Pervasive developmental	0.08 (−0.50, 0.67)	−0.10 (−0.79, 0.60)	0.17 (−0.41, 0.75)	0.11 (−0.64, 0.85)	1.08 (0.92, 1.27)	0.97 (0.79, 1.18)	1.05 (0.90, 1.23)	1.05 (0.85, 1.29)
Anxiety	0.28 (−0.24, 0.79)	−0.03 (−0.65, 0.59)	−0.12 (−0.63, 0.40)	0.02 (−0.64, 0.69)	1.13 (0.93, 1.37)	0.92 (0.70, 1.21)	0.86 (0.69, 1.07)	0.92 (0.70, 1.22)
Affective	0.25 (−0.19, 0.69)	0.05 (−0.47, 0.57)	0.14 (−0.30, 0.58)	0.22 (−0.34, 0.79)	1.25 (0.99, 1.56)	1.04 (0.77, 1.41)	1.03 (0.80, 1.31)	1.14 (0.82, 1.58)
ADHD	0.15 (−0.26, 0.57)	0.08 (−0.42, 0.57)	0.06 (−0.36, 0.47)	0.14 (−0.40, 0.67)	0.77 (0.49, 1.21)	0.74 (0.41, 1.35)	0.78 (0.49, 1.24)	0.67 (0.35, 1.30)
Oppositional-defiant	0.25 (−0.15, 0.65)	0.00 (−0.47, 0.48)	0.09 (−0.30, 0.49)	0.13 (−0.38, 0.64)	0.90 (0.53, 1.53)	0.78 (0.33, 1.84)	0.91 (0.50, 1.64)	0.63 (0.28, 1.44)
Empirical								
Withdrawn	−0.07 (−0.48, 0.34)	0.01 (−0.48, 0.50)	0.06 (−0.35, 0.47)	−0.01 (−0.54, 0.52)	1.02 (0.85, 1.22)	1.05 (0.84, 1.31)	1.05 (0.87, 1.26)	1.03 (0.81, 1.32)
Externalizing	1.02 (−0.25, 2.28)	0.23 (−1.28, 1.74)	0.35 (−0.91, 1.61)	0.82 (−0.81, 2.45)	1.30 (1.05, 1.62) *	1.11 (0.84, 1.45)	1.13 (0.91, 1.42)	1.35 (1.03, 1.78) *
Anxious/ Depressed	0.02 (−0.41, 0.45)	0.09 (−0.43, 0.60)	0.01 (−0.41, 0.44)	0.07 (−0.48, 0.62)	0.91 (0.73, 1.14)	0.84 (0.63, 1.12)	0.83 (0.66, 1.05)	0.78 (0.58, 1.06)
Aggressive behavior	1.03 (−0.07, 2.13)	0.21 (−1.09, 1.52)	0.36 (−0.73, 1.45)	0.75 (−0.65, 2.16)	1.00 (0.72, 1.38)	0.88 (0.53, 1.46)	0.90 (0.60, 1.33)	0.92 (0.56, 1.53)
Attention	−0.01 (−0.28, 0.25)	0.02 (−0.30, 0.34)	−0.01 (−0.28, 0.25)	0.07 (−0.27, 0.41)	0.78 (0.47, 1.27)	0.59 (0.31, 1.14)	0.63 (0.38, 1.05)#	0.64 (0.32, 1.29)

Models adjusted for maternal education, and age at delivery; mother's Ravens score, and HOME score at 1-year; risk for depression at 1-year and 2-years; poverty at 2-years; breastfeeding through 1 year, and time of urine collection.

* p<0.05