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Prenatal Exposure to DDT and Pyrethroids for Malaria Control and Child Neurodevelopment: The VHEMBE Cohort, South Africa

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Background: Although indoor residual spraying (IRS) with dichlorodiphenyltrichloroethane (DDT) and pyrethroids effectively controls malaria, it potentially increases human exposure to these insecticides. Previous studies suggest that prenatal exposure to these insecticides may impact human neurodevelopment.

Objectives: We aimed to estimate the effects of maternal insecticide exposure and neurodevelopment of toddlers living in a malaria-endemic region currently using IRS.

Methods: The Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) is a birth cohort of 752 mother–child pairs in Limpopo, South Africa. We measured maternal exposure to DDT and its breakdown product, dichlorodiphenyldichloroethylene (DDE), in maternal serum, and measured pyrethroid metabolites in maternal urine. We assessed children's neurodevelopment at 1 and 2 y of age using the Bayley Scales of Infant Development, third edition (BSID-III), and examined associations with maternal exposure.

Results: DDT and DDE were not associated with significantly lower scores for any BSID-III scale. In contrast, each 10-fold increase in *cis*-DCCA, *trans*-DCCA, and 3-phenoxybenzoic acid were associated, respectively, with a −0.63 (95% CI: −1.14, −0.12), −0.48 (95% CI: −0.92, −0.05), and −0.58 (−1.11, −0.06) decrement in Social-Emotional scores at 1 y of age. In addition, each 10-fold increase in maternal *cis*-DBCA levels was associated with significant decrements at 2 y of age in Language Composite scores and Expressive Communication scores [$\beta = -1.74$ (95% CI: −3.34, −0.13) and $\beta = -0.40$ (95% CI: −0.77, −0.04), respectively, for a 10-fold increase]. Significant differences by sex were estimated for pyrethroid metabolites and motor function scores at 2 y of age, with higher scores for boys and lower scores for girls.

Conclusions: Prenatal exposure to pyrethroids may be associated at 1 y of age with poorer social-emotional development. At 2 y of age, poorer language development was observed with higher prenatal pyrethroid levels. Considering the widespread use of pyrethroids, these findings deserve further investigation. https://doi.org/10.1289/EHP2129

Introduction

The World Health Organization (WHO) reported that there were 214 million cases of malaria in 2015, resulting in about 438,000 deaths, with most deaths occurring in children under 5 y of age (WHO 2015). Increased prevention and control measures have led to a 60% reduction in global malaria mortality rate since 2000 (WHO 2015). Indoor residual spraying (IRS), or systematic insecticide application to the interior walls of homes to kill malaria-infected mosquitoes as they rest, is one of the main techniques for controlling the *Anopheles* mosquito vector. In malarial-endemic regions of South Africa, IRS with dichlorodiphenyltrichloroethane (DDT) has been ongoing since 1946 (Bouwman et al. 2006), even as DDT use was effectively banned in most Western countries due to its toxicity. DDT and its main breakdown product, dichlorodiphenyldichloroethylene (DDE), have long half-lives in the human body (6 y and up to 10 y, respectively) and in the environment (ATSDR 2002; Longnecker 2005; Wolff et al. 2000). In recent years, pyrethroids, such as alpha-cypermethrin or deltamethrin, have become South Africa's IRS insecticides of choice (Hlongwana et al. 2013). Unlike DDT, pyrethroids are rapidly metabolized and have short half-lives in humans (hours to days) (Barlow et al. 2001), and are still widely used in global agriculture (van Balen et al. 2012). Both DDT and pyrethroids are neurotoxic in developing animals through a number of mechanisms, including effects on cholinergic muscarinic receptors and on the sodium channels of axons (Eriksson et al. 1992; Malik et al. 2017). In addition, both DDT (Patisaul and Adewale 2009) and some pyrethroids (Ben Slima et al. 2017) are endocrine disruptors. Without question, effective prevention of malaria is of critical importance to human health; nonetheless, there is growing concern about the potential health effects of IRS insecticides on developing children given their demonstrated neurotoxicity in animals (Mandhane and Chopde 1997; Nasuti et al. 2003) and suggested evidence of developmental neurotoxicity in humans.

The majority of studies investigating DDT exposure and children's neurobehavioral development have focused on its main breakdown product, DDE, and have found null (Eskenazi et al. 2006; Gaspar et al. 2015; Gladen et al. 1988; Jusko et al. 2012; Lyall et al. 2017; Pan et al. 2009; Ribas-Fitó et al. 2006; Rogan and Gladen 1991; Torres-Sánchez et al. 2007) or transient inverse associations (Eskenazi et al. 2006; Ribas-Fitó et al. 2003; Torres-Sánchez et al. 2009), although some have found clear adverse associations (Torres-Sánchez et al. 2013). Fewer studies have measured prenatal DDT exposure directly and/or had sufficient detection frequencies to meaningfully assess impact. In our study of California children whose immigrant mothers were likely exposed to DDT in Mexico, maternal prenatal DDT levels were related to poorer scores on the Bayley Scales of Infants Development-II (BSID-II) between 6 and 24 months of age (Eskenazi et al. 2006), and slower processing speed as assessed on the Wechsler Intelligence Scale for Children (WISC-IV) at 7 y of age, particularly in girls (Eskenazi et al. 2006; Gaspar et al. 2015); however, by 10.5 y of age, this result had attenuated. A study of Spanish children reported associations of cord serum DDT levels

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with poorer performance of 4-y-olds on the McCarthy Scales of Children's Abilities (Ribas-Fitó et al. 2006). In contrast, a large study of U.S. children circa 1960, when DDT exposure was quite high, reported no association of maternal serum DDT levels with either BSID scores at 8 months of age or WISC scores at 7 y of age (Jusko et al. 2012; Ribas-Fitó et al. 2006).

Few studies have examined pyrethroid exposure and child neurodevelopment. In a New York City (NYC) study, maternal prenatal permethrin exposure was not associated with infants' BSID-II scores (Horton et al. 2011), and a French study likewise found no association between prenatal urinary pyrethroid metabolites and 6-y-olds' performance on WISC Verbal Comprehension or Working Memory (Viel et al. 2015). On the other hand, a Chinese study reported the sum of prenatal urinary levels of *cis*–(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid (DCCA), *trans*-DCCA, and 3-phenoxybenzoic acid (3PBA) to be associated with lower cognitive scores in 1-y-olds (Xue et al. 2013), and a Mexican study found higher 3PBA levels to be related with lower cognitive scores (BSID) at 2 and 3 y of age (Watkins et al. 2016). The French study reported evidence of adverse effects on behavior at 6 y of age. Specifically, maternal prenatal *cis*-DCCA was associated with higher scores for internalizing difficulties and 3PBA with higher externalizing difficulties scores and higher odds of abnormal or borderline social behavior based on maternal responses to the Strengths and Difficulties Questionnaire (Viel et al. 2017). This finding is in line with a recent NYC study that reported evidence of adverse behavioral outcomes associated with detectable levels of 3PBA and *cis*-DCCA in maternal prenatal samples (Furlong et al. 2017).

In the current study, we examine exposure to DDT, pyrethroids, and the pesticide mixture among pregnant women in Limpopo, South Africa, in association with the neurobehavioral development of their young children. To our knowledge, this is the first study to investigate the effects of DDT and pyrethroids in a malaria-endemic region currently using IRS.

Methods

Study Population

The Venda Health Examination of Mothers, Babies and the Environment (VHEMBE) is a longitudinal birth cohort study on the effects of *in utero* exposure to IRS insecticides on the health of children. Pregnant women at the early stage of labor (contractions >5 min apart) were screened and recruited between August 2012 and December 2013 at Tshilizidini Hospital in the Vhembe district of Limpopo Province, South Africa. Eligible women were ≥18 y of age, spoke TshiVenda as the main language at home, lived within 20 km (12.4 mi) of the hospital and planned to remain in the area (to aid in study retention), were not diagnosed with malaria during pregnancy, and gave birth to a live singleton infant. Almost all women in the area deliver at the hospital. Among 920 eligible women, 752 completed a baseline questionnaire and provided a blood sample (81.7%). Among the enrolled children, 24 children died (4 within the first week of life, 13 between delivery and 1 y of age, and 7 between 1 and 2 y of age). A total of 665 children (88.4% of enrollees) completed both 1- and 2-y visits, and 40 (5.3% of enrollees) children completed only one of the two visits: 24 at 1 y and 16 at 2 y of age. We include in the present analysis the 705 children (93.8% of 752 enrolled mothers) who completed a neurodevelopment assessment at either 1 or 2 y of age.

Written informed consent was obtained from mothers or guardians. The study was 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.

Procedures

Shortly after delivery and before leaving the hospital, mothers were queried in TshiVenda by trained bilingual (English-TshiVenda) interviewers. The interview gathered information on sociodemographic characteristics, reproductive and medical history, personal habits (smoking and use of alcohol or drugs), occupational and residential history, household composition, and use of pesticides around the home, at work, in their fields, on their livestock, or for malaria control. The interview included the U.S. Department of Agriculture (USDA) Food Security Questionnaire (six-item short form) (Blumberg et al. 1999; USDA 2012), parts of the South Africa National Income Dynamics Study (NIDS) (Southern Africa Labour and Development Research Unit, 2010), an adapted version of the Quantitative Food Frequency Questionnaire developed for the regional diet (QFFQ) (MacIntyre et al. 2001a, 2001b, 2001c), and the Stressful Life Events Scale (Yach et al. 1991). Medical records for the pregnancy and delivery were abstracted by a registered nurse.

A home visit was conducted at approximately 1 wk postpartum. During this visit, we performed a home walkthrough (recorded information about sanitation facilities, materials of the walls and floors, food and water storage, condition of housekeeping, DDT residues on the walls and floors, and location and contents of pesticide containers and nearby agricultural fields). We also took Global Positioning System (GPS) coordinates and interviewed the mother about water source and use, housekeeping, and cooking practices including fuel use, livestock, breastfeeding, social support using the Duke University of North Carolina Social Support Scale (Broadhead et al. 1988), and depressive postpartum symptoms using the Edinburgh Postpartum Depression Scale (Cox et al. 1987).

At 1 y and 2 y of age, children came with their mothers or another primary caregiver ($n = 34$) to our field office on the hospital grounds. At these visits, the primary caregiver was interviewed on conditions and events in the previous year, including household composition, proximity of home to agricultural fields, malarial control interventions, the child's health and development (including history of malaria), child care arrangements, breastfeeding, child dietary intake using a food frequency questionnaire developed for this region, use of pesticides at work or at home, the USDA Food Insecurity Survey (Blumberg et al. 1999; USDA 2012), the Family Care Indicators (Kariger et al. 2012), a version of the Home Observation for Measurement of the Environment (HOME) modified for field office use (MAL-ED Network Investigators 2014), the Stressful Life Events scale (Yach et al. 1991), and the 20-question Self-Reporting Questionnaire (SRQ-20) for risk of maternal depression (Beusenberg and Orley 1994). At the 1-y visit, the primary caregiver also completed the Raven's Coloured Progressive Matrices (1998 edition), a nonverbal test of abstract reasoning (Raven 1960). At each visit, we photocopied the *Road to Health*, a booklet that mothers brought to all children's clinic visits for completion by the nurse about their child's health status, including human immunodeficiency virus (HIV) information, immunization status, and growth measurements. We also requested medical records for any hospital admissions. All medical information was abstracted by a registered nurse.

Blood was collected from children via venipuncture at the 1- and 2-y visits. Blood lead levels were quantified from the child's blood sample collected at the 1-y visit, using an inductively coupled plasma–mass spectrometer (ICP-MS) at the Lancet laboratory (South Africa). The limit of detection for lead in whole blood was 0.1 μg/dL. Children's hemoglobin levels were measured from the

1-y-olds' blood sample using an EKF Diagnostic Hemo Control meter (EKF-diagnostic GmbH).

Bayley Scales of Infant Development

At both the 1- and 2-y visits, one of two bilingual English-TshiVenda psychologists, trained by a TshiVenda developmental neuropsychologist, administered the Bayley Scales of Infant Development, third edition (BSID-III) (Bayley 2006) in TshiVenda to the children. At both visits, we administered the Cognitive, Language (Receptive and Expressive), and Motor (Fine and Gross) subtests, and at the 1-y assessment only, the Social-Emotional subtest. The Social-Emotional scores are based on maternal report, whereas the other domains are based on direct assessment; hence in a few cases where the child was unable to complete the assessment, the mother was still able to complete this subtest ($n = 7$). Scaled scores for a particular domain are age-standardized to a mean ± SD of 10 ± 3. Composite scores of language or motor domains are age-standardized with a mean ± SD of 100 ± 15. A portion of the assessments were videotaped for quality control (26.9% at the 1-y and 32.5% at 2-y visits). Each week, one video was randomly selected for review by all three psychologists to assure consistency in administration and scoring. The duration of the test averaged 60 and 69 min at the 1- and 2-y visits, respectively. In cases of children born preterm, the ages were prematurity-adjusted using the estimated date of conception as outlined in the BSID test manual. The mean prematurity-adjusted ages at assessment were 12.1 ± 0.8 months at the 1-y visit and 24.0 ± 0.6 at the 2-y visit.

DDT/DDE Measurements

Blood samples were collected from the mother by venipuncture before ($n = 555$) or after ($n = 150$) delivery (but before leaving the hospital). Samples were frozen at −80°C (−112°F), and 2-mL serum aliquots were sent on dry ice to Emory University's Rollins School of Public Health for analysis. DDT/DDE isomers (*p,p′* and *o,p′*) were measured using high-resolution gas chromatography–isotope dilution mass spectrometry (GC-MS) (Barr et al. 2003). The limit of quantification was 0.05 ng/mL 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 (Phillips et al. 1989), measured using standard enzymatic methods (Roche Chemicals). We limited our statistical analyses to those analytes with at least 70% of sample results over the limits of quantification (Lubin et al. 2004), thus excluding *o,p′*-DDT and *o,p′*-DDE from further analysis.

Pyrethroid Metabolite Measurements

We collected a spot urine sample for 432 women prior to delivery and for 263 women postdelivery (but before leaving the hospital); 10 women did not provide a urine sample. Urine samples were stored at −80°C (−112°F) at the field office and then at the Biorepository at the University of California, Berkeley, before shipment to the Institut National de Santé Publique du Québec. At the time of collection, specific gravity was determined using an Atago PAL-10S refractometer. Pyrethroid metabolites were measured using GC-MS (Barr et al. 2010). Limits of quantification were 0.0082 μg/L for *cis*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid (*cis*-DBCA), 0.015 μg/L for *cis*-DCCA, 0.013 μg/L for *trans*-DCCA, 0.016 μg/L for 3PBA, and 0.011 μg/L for 4-fluoro-3-phenoxybenzoic acid (4F3PBA). *cis*-DBCA is a metabolite of deltamethrin, whereas *cis*-DCCA, *trans*-DCCA, and 3PBA are nonspecific metabolites of several pyrethroid pesticides, including permethrin, cypermethrin, and cyfluthrin (Barr et al. 2010). In-house quality control materials and ClinChek noncertified reference material in urine (RECIPE) were used for internal quality control. The overall quality and accuracy of the analyses at the laboratory was monitored by twice annual participation in the German External Quality Assessment Scheme (G-EQUAS). We limited our statistical analyses to those metabolites with at least 70% of sample results over the limits of quantification (Lubin et al. 2004), thus excluding 4F3PBA from analysis.

Data Analysis

Prenatal DDT and pyrethroid metabolite levels were $\log_{10}$-transformed to reduce the influence of outliers. DDT and DDE concentrations were lipid-adjusted; pyrethroid concentrations were standardized using specific gravity measurements. We analyzed the demographic characteristics for mother–child pairs who completed the BSID at the 1-y and/or 2-y visits. We constructed separate multivariable regression models for the six analytes/metabolites (*p,p′*-DDT, *p,p′*-DDE, *cis*-DBCA, *cis*-DCCA, *trans*-DCCA, 3PBA) with each measure of the BSID at 1 and 2 y of age. Covariates for the multivariable models were identified using a directed acyclic graph (DAG) (see Figure S1) (Textor et al. 2011). Generalized additive models (GAMs) with a 3-degree of freedom cubic spline and residual plots were used to confirm the linearity assumption for the models and did not show evidence of nonlinearity; hence we used linear models. To assess relationships across the 1- and 2-y visits, longitudinal models were constructed using generalized estimating equations (GEEs).

Final covariates were coded as shown in Table 1 and included maternal age, education, poverty status, and marital status at delivery; breastfeeding status and Raven's Coloured Progressive Matrices (RCPM) (Raven 1960) *z*-score for the mother and/or primary caregiver at the 1-y visit; and child's preterm birth and birthweight *z*-scores. Household poverty status was determined by household per capita monthly income using thresholds provided by Statistics South Africa (Ruch 2016). Maternal scores on the RCPM were standardized within the study sample. Birthweight *z*-scores were calculated using age- and sex-standardized growth curves from a meta-analysis using WHO data and other population-based studies (Fenton and Kim 2013). Models also included several covariates derived from interview data collected at the time of specific visit (at 1 or 2 y): risk for maternal depression (SRQ-20 ≥6) (Beusenberg and Orley 1994), USDA Food Insecurity Score (0–6, continuous) (Blumberg et al. 1999; USDA 2012), modified HOME *z*-score (MAL-ED Network Investigators 2014), and the psychometrician administering the BSID-III. The HOME instrument differed between the 1- and 2-y visits, so scores for each time point were standardized to the study sample at each visit.

In preliminary analysis, we found evidence of differences in distributions and geometric means for three pyrethroid metabolites collected pre- versus postdelivery [geometric means (GMs) were higher predelivery for *cis*-DCCA (GM = 0.32 predelivery vs. 0.28 postdelivery, $p = 0.08$), *trans*-DCCA (GM = 0.39 predelivery vs. 0.30 postdelivery, $p = 0.01$) and 3PBA (GM = 0.76 predelivery vs. 0.64 postdelivery, $p = 0.04$)]; there was no difference in the pre- and postdelivery distributions for *cis*-DBCA. Therefore, in the main analyses using pyrethroid metabolites as the exposure, we controlled for whether the maternal urine was collected pre- or postdelivery.

We tested for effect modification by child sex using stratification and cross-product terms. We also conducted a number of sensitivity analyses. Because the half-life of pyrethroids is short and a measure made on urine collected postdelivery might reflect exposures in the hospital, we repeated models of associations with pyrethroid metabolites restricted to participants whose maternal urinary samples were collected predelivery. We reran models considering child blood lead concentrations, child hemoglobin levels, and maternal HIV status as covariates. Because child lead levels were not available for 205

Table 1. Demographic characteristics of the VHEMBE cohort who completed the Bayley Scales of Infant Development at either the 1 and/or 2-y visit in Limpopo, South Africa, 2012–2013 ($n = 705$).

Characteristics	Mean ± SD or *n* (%)
Maternal characteristics	
Maternal age	26.3 ± 6.2
Education	
<Grade 12	390 (55.3)
Grade 12	212 (30.1)
Further studies started	46 (6.5)
Diploma or further degree	57 (8.1)
Marital status	
Married or living as married	333 (47.2)
Not married	372 (52.8)
Parity	
0	308 (43.7)
1	190 (26.9)
≥2	207 (29.4)
Alcohol use during pregnancy	
Yes	41 (5.8)
No	664 (94.2)
Smoking cigarettes during pregnancy	
Yes	3 (0.4)
No	702 (99.6)
HIV during pregnancy	
Yes	91 (12.9)
No	611 (86.7)
Missing	3 (0.4)
Breastfeeding at 1-y visit	
Yes	547 (77.6)
No	158 (22.4)
Exclusive breastfeeding (months)	2.3 ± 1.9
At risk for depression (SRQ-20 ≥0.6) at 1 y visit	
Yes	82 (11.6)
No	623 (88.4)
At risk for depression (SRQ-20 ≥6) at 2 y visit	
Yes	48 (6.8)
No	657 (93.2)
Maternal Raven's score	
Raw score (maximum, 36)	22.0 (6.0)
Child characteristics	
Birthweight (g)[a]	3,137 (446)
Sex	
Boy	365 (51.8)
Girl	340 (48.2)
Preterm (<37 wk)	
Yes	91 (12.9)
No	614 (87.1)
Low birth weight (<2,500 g)	
Yes	55 (7.8)
No	649 (92.2)
Family characteristics at pregnancy	
Below poverty level (R386/month per capita)	
Yes	429 (60.9)
No	276 (39.1)
Food security (USDA Food Security Survey)[b]	
High	405 (57.4)
Low	220 (31.2)
Very low	80 (11.3)
Family characteristics at 1-y visit	
Food security (USDA Food Security Survey)[b]	
High	426 (60.4)
Low	182 (25.8)
Very low	97 (13.8)
HOME score	
Raw score (maximum, 31)	22.2 ± 3.5
Family characteristics at 2-y visit	
Food security (USDA Food Security Survey)[b]	
High	426 (60.4)
Low	203 (28.8)
Very low	76 (10.8)
HOME score	
Raw score (maximum, 42)	31.7 ± 4.2

Note: HIV, human immunodeficiency virus; HOME, Home Observation Measurement of the Environment; R386, 386 South African Rands; SRQ-20, Self-Reporting Questionnaire 20-Item; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.
[a]Birthweight not available for one infant.
[b]Food security was based on a scale of 0–6, and is here categorized into high food security (0–1), low food security (2–4), and very low food security (5–6) (Blumberg et al. 1999; USDA 2012). For the multivariate model, food security was considered as a continuous variable.

children and child hemoglobin levels were not available for 130 children, we reran models comparing results with and without controlling for these variables in the restricted samples.

A single-pollutant modeling framework does not reflect the reality of the exposure scenario to populations where IRS is conducted. Therefore, we also implemented Bayesian kernel machine regression (BKMR), which allowed for the consideration of health effects from insecticide mixtures while simultaneously penalizing credible intervals of individual insecticide effect estimates for multiple comparisons (Scott and Berger 2010; Valeri et al. 2017). Using a Bayesian framework for variable selection has been shown to be particularly helpful within the context of a large number of correlated covariates because it appropriately handles model uncertainty (Greenland 1993; MacLehose et al. 2007; Thomas et al. 2007). With BKMR, we can consider individual insecticide exposure responses (and potential nonlinearity) with co-occurring insecticides, and evaluate each exposure's relative importance with respect to the outcome via Bayesian variable selection, the exposure response of the overall mixture, interaction between any two insecticide measures, and each chemical's exposure response while holding the other insecticide measure at various percentiles of exposure.

Although the output from BKMR is rich with information for inference, we focus our presentation on posterior inclusion probabilities (PIPs) to highlight the relative ranking of variable importance for each pesticide class as well as each pesticide within a particular class of insecticides. We present the PIPs that result from the Bayesian Markov chain Monte Carlo (MCMC) iterations (25,000). A PIP for an exposure (or group PIP) is calculated as the posterior mean of the indicator variable across the MCMC iterations, whereby the indicator variable equals 1 if the variable (or group) is selected into the model at a given iteration or 0 otherwise (O'Hara and Sillanpää 2009). For the BKMR models, we fit each outcome-specific model using the same covariates as in the single-pollutant models. To mitigate against the problems of multicollinearity, we use hierarchical variable selection with default "slab-and-spike" priors (Bobb et al. 2015), and we group p,p'-DDT and p,p'-DDE measures together and the pyrethroid metabolite measures together. The relative ranking of the PIPs computed with hierarchical variable selection in the BKMR analysis is an indication of the relative importance of each exposure relative to other exposures of interest within an exposure group. When interpreting PIPs, particularly the conditional (within group) PIPs for specific compounds, emphasis should be placed on relative ranks, rather than absolute values. In addition, we interpreted a group PIP ≥0.50 as an indication that an exposure group (p,p'-DDT and p,p'-DDE, or the pyrethroid metabolites) was important to an outcome-specific multipollutant model, consistent with the median probability model proposed by Barbieri and Berger (2004). To assess potential binary between-chemical interactions, we also examined bivariate plots of exposure to evaluate whether individual chemical exposure responses change substantively as the level of another chemical increases from low quantiles to higher quantiles. Although BKMR is capable of examination of higher-order interactions or the overall combined

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 (μg/L, specific-gravity adjusted), VHEMBE study, Limpopo, South Africa.

Exposure	*n*	Percent detected[a]	Percent quantifiable[b]	GM	± GSD	Percentile						
						Min	10	25	50	75	90	Max
p,p′-DDT	705	98.2	90.8	69.0	± 6.64	<LOD	8.1	18.6	55.3	254.0	947.9	15027.6
p,p′-DDE	705	100.0	97.3	286.2	± 4.82	4.0	45.3	92.2	240.4	832.5	2585.4	26301.3
o,p′-DDT	705	90.2	43.6	8.9	± 4.67	<LOD	1.5	3.4	7.2	7.2	73.4	2029.3
o,p′-DDE	705	82.7	15.9	4.1	± 2.76	<LOD	<LOD	2.3	4.2	6.9	13.9	117.5
cis-DBCA	695	100	99.6	0.223	± 3.42	0.005	0.050	0.097	0.223	0.475	1.115	17.827
cis-DCCA	695	100	99.9	0.306	± 2.95	0.015	0.084	0.151	0.301	0.601	1.025	103.502
trans-DCCA	695	100	99.6	0.357	± 3.43	0.008	0.078	0.159	0.340	0.785	1.481	132.878
3PBA	694	100	100	0.712	± 2.80	0.022	0.214	0.374	0.700	1.372	2.381	58.899
4F3PBA	672	12.5	7.7	N/A		<LOD	<LOD	<LOD	<LOD	<LOD	0.008	0.423

Note: 3PBA, 3-phenoxybenzoic acid; 4F3PBA, 4-fluoro-3-phenoxybenzoic acid; DBCA, (2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; DCCA, (2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; Max, maximum; Min, minimum; N/A, not available; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.
[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 μg/L for *cis*-DBCA, 0.0045 μg/L for *cis*-DCCA, 0.0038 μg/L for *trans*-DCCA, 0.0047 μg/L for 3PBA, and 0.005 μg/L for 4F3PBA.
[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 μg/L for *cis*-DBCA, 0.015 μg/L for *cis*-DCCA, 0.013 μg/L for *trans*-DCCA, 0.016 μg/L for 3PBA, and 0.011 μg/L for 4F3PBA.

exposure levels, we limit our review of the BKMR output to bipollutant interactions to hedge against extrapolating beyond the observed co-exposures patterns in our population.

The Bayesian analysis was performed using the statistical program R (version 3.3.1; R Development Core Team), and all other analyses were performed with STATA version 13 (StataCorp). *p*-Values of 0.05 were considered statistically significant, except in interaction models, where $p = 0.10$ was considered significant.

Results

Almost all mothers were from the Vhavenda people and all were Black African. The mean age of mothers at delivery was 26.3 y old. More than half of the mothers had less than 12 y of education and were multiparous (Table 1). About half of the women were married or living as married. At delivery, 60.9% lived below the poverty level [in South African Rand, R (R386/month per capita)] and 42.5% had low or very low food security (Blumberg et al. 1999; USDA 2012). Few women reported smoking cigarettes during the pregnancy (0.4%) or drinking any alcohol (5.8%). About 12.9% of the women reported being HIV-positive. Of the 705 children who completed either the 1-y or the 2-y assessment, 365 (51.8%) were boys, 12.9% were born preterm (<37 wk gestation) and 7.8% were born of low birth weight. The average duration of exclusive breastfeeding was 2.3 ± 1.9 months of age, but 77.6% were still breastfeeding when the child was 1 y of age. By the 1-y follow-up, 11.6% of the mothers were at risk for depression and 42.5% were food insecure; at the 2-y follow-up, 6.8% of the mothers were at risk for depression and 39.6% were food insecure.

Table 2 presents the maternal serum concentrations of DDT/DDE and the urinary concentrations of pyrethroid metabolites. The median serum concentration for *p,p′*-DDT was 55.3 ng/g lipid and for *p,p′*-DDE was 240.4 ng/g lipid. The median urinary concentrations were 0.223 μg/L for *cis*-DBCA, 0.301 μg/L for *cis*-DCCA, 0.340 μg/L for *trans*-DCCA, and 0.700 μg/L for 3PBA (see Table S1 for wet-weight concentrations and creatinine-adjusted pyrethroid levels). *p,p′*-DDT and *p,p′*-DDE concentrations were strongly correlated ($r = 0.85$, $p < 0.001$) with each other but were not correlated with the pyrethroid metabolite levels ($r = -0.03$ to 0.04) (see Table S2). However, three pyrethroid metabolite concentrations were highly correlated with each other, with an approximate *r* of 0.9 between *cis*-DCCA, *trans*-DCCA, and 3PBA. *cis*-DBCA concentrations were moderately correlated with the other pyrethoid metabolites ($r = 0.46$–0.62).

Average BSID scaled and composite scores are shown in Table 3. The Cognitive scaled scores at the 2-y follow-up were particularly low, with a mean ± SD of 5.7 ± 1.6 and a Cognitive Composite of 78.7 ± 8.1.

As shown in Table 4, neither *p,p′*-DDT nor *p,p′*-DDE was associated with any of the child's BSID scores at the 1- or 2-y visit. Maternal *p,p′*-DDT and *p,p′*-DDE levels were associated with higher average Cognitive scores at the 1-y but not at the 2-y visit. In general, there was no clear evidence or consistent pattern of differences in associations by sex at 1 y of age (Table 5). At 2 y of age, associations for almost all outcomes were inverse in girls and positive for boys, with interaction *p*-values <0.1 for *p,p′*-DDT and the composite motor function score and for *p,p′*-DDE and the receptive communication score, the expressive communication score, and the composite language score (Table 5).

Table 6 shows the relationship of maternal urinary levels of the four pyrethroid metabolites and children's BSID scores at the 1- and 2-y visits. At the 1-y visit, we found only Social-Emotional scores to be inversely associated with pyrethroid metabolites *cis*-DCCA [$\beta = -0.63$ (95% CI = −1.14, −1.12)], *trans*-DCCA [$\beta = -0.48$ (95% CI = −0.92 to −0.05)], and 3PBA [$\beta = -0.58$ (95% CI = −1.11, −0.06)]. These associations were similar for

Table 3. Children's performance (mean ± SD) at the 1- and 2-y visits on the Bayley Scales of Infant Development (3rd edition), VHEMBE study, Limpopo, South Africa.

BSID Measures	Mean ± SD
At 1-y visit (*n* = 689)	
Cognitive[a]	8.7 ± 2.0
Receptive Communication	7.0 ± 1.8
Expressive Communication	11.2 ± 2.0
Fine Motor	9.2 ± 2.3
Gross Motor	9.0 ± 2.8
Language Composite	94.9 ± 8.8
Motor Composite	94.7 ± 12.2
Social-Emotional	8.3 ± 3.1
At 2-y visit (*n* = 681)	
Cognitive[a]	5.7 ± 1.6
Receptive Communication	7.7 ± 1.8
Expressive Communication	9.1 ± 2.5
Fine Motor	10.3 ± 2.0
Gross Motor	7.2 ± 1.7
Language Composite	90.8 ± 11.3
Motor Composite	92.6 ± 9.0

Note: BSID, Bayley Scales of Infant Development (3rd edition); VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.
[a]Cognitive scaled scores can be converted to Cognitive Composite scores standardized to a mean of 100 and SD of 15. The Cognitive Composite score at the 1-y visit had a mean ± SD of 93.6 ± 9.9) and at the 2-y visit, 78.7 ± 8.1.

Table 4. Adjusted linear regression β coefficient and 95% confidence interval for the association between maternal prenatal *p,p′*-DDT and *p,p′*-DDE serum concentrations (log_{10} transformed; lipid-adjusted) and children's performance on the Bayley Scales of Infant Development (3rd edition) at the 1- and 2-y visits, VHEMBE study, Limpopo, South Africa.

BSID Measure	*p,p′*-DDT β (95% CI)	*p,p′*-DDE β (95% CI)
At 1-y visit ($n = 689$)		
Cognitive	0.15 (−0.02, 0.33)	0.24 (0.05, 0.43)*
Receptive Communication	−0.02 (−0.17, 0.14)	0.00 (−0.18, 0.19)
Expressive Communication	−0.09 (−0.27, 0.09)	−0.04 (−0.26, 0.18)
Fine Motor	−0.02 (−0.24, 0.20)	0.04 (−0.19, 0.28)
Gross Motor	0.11 (−0.13, 0.34)	0.08 (−0.20, 0.36)
Language Composite	−0.32 (−1.10, 0.46)	−0.11 (−1.08, 0.86)
Motor Composite	0.25 (−0.83, 1.32)	0.36 (−0.88, 1.60)
Social-Emotional[a]	0.08 (−0.21, 0.36)	0.26 (−0.08, 0.60)
At 2-y visit ($n = 681$)		
Cognitive	−0.04 (−0.19, 0.10)	0.05 (−0.13, 0.24)
Receptive Communication	0.00 (−0.14, 0.15)	0.03 (−0.15, 0.22)
Expressive Communication	−0.06 (−0.28, 0.16)	0.05 (−0.23, 0.34)
Fine Motor	−0.07 (−0.24, 0.10)	0.02 (−0.19, 0.23)
Gross Motor	0.02 (−0.11, 0.16)	0.06 (−0.11, 0.23)
Language Composite	−0.15 (−1.10, 0.80)	0.28 (−0.94, 1.50)
Motor Composite	−0.15 (−0.89, 0.59)	0.24 (−0.67, 1.15)

Note: Coefficients show the change in scaled BSID score associated with a 10-fold increase in maternal DDT/DDE serum concentrations. Models adjusted for maternal education, age, marital status, poverty status at delivery, risk for depression (CES-D), and Raven's Coloured Progressive Matrices score (at 1-y visit); food insecurity (USDA Food Security Survey); HOME score; preterm delivery; and psychometrician. BSID, Bayley Scales of Infant Development (3rd edition); CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HOME, Home Observation Measurement of the Environment; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.
[a]Models include 696 participants. *$p < 0.05$.

girls and boys (p-Int ≥0.43; Table 7). There was a significant interaction of *cis*-DBCA and sex on Receptive Communication and Language Composite with a negative coefficient in girls and a positive coefficient in boys, although neither sex stratum was significant. At 2 y of age, we observed that a 10-fold increase in maternal *cis*-DBCA concentrations was associated with a 0.40-unit decrease in Expressive Communication scores [95% confidence interval (CI): −0.77, −0.04] and a 1.74-unit decrease in Language Composite scores (95% CI : −3.34, −0.13) (Table 6). These associations were similar by sex (p-Int = 0.93 and 0.62, respectively; Table 7). However, there were significant differences in associations between Motor Composite scores and each of the four metabolites (p-Int 0.01–0.02), which were negative for girls and positive for boys [e.g., for *trans*-DCCA, β = −1.76 (95% CI: −3.27, −0.25) for girls and β = 1.93 (95% CI: −0.23), 4.09 for boys, p-Int = 0.01] as well as significant differences in fine and/or gross motor scores between girls and boys (P-Int ≤0.09) for all four metabolites (Table 7). Several exposure–time interaction terms in GEE models did not indicate a consistent longitudinal association across the two assessment ages for either DDT/DDE or pyrethroids, and therefore we deemed the results at each time point more relevant.

In sensitivity analyses, we reran the models restricting the analysis to the maternal–children dyads ($n = 425$ for the 1-y and 418 for the 2-y visits) for whom maternal urine samples were obtained prior to delivery (see Table S3). Overall, the trends in associations in this restricted and full sample (Table 6) were similar, with some results slightly attenuated (e.g., Social-Emotional and *cis*- and *trans*-DCCA and 3PBA) and others slightly strengthened (e.g., *cis*-DBCA and language measures). However, significant inverse associations emerge between *cis*- and *trans*-DCCA and 3PBA and fine motor scores at the 1-y visit but were no longer apparent at the 2-y visit, and between *trans*-DCCA and Language Composite at the 2-y visit, primarily driven by Expressive Communication. In other sensitivity analyses, removing the covariate for preterm status from the models (see Tables S4–S5) or including variables in the regression models for maternal HIV status (see Tables S6–S7), child lead levels (see Tables S8–S11), or child hemoglobin levels (see Tables S12–S15) did not alter the above results.

Table 8 presents the PIP results from the BKMR analysis indicating the relative ranking of exposure variable importance within the DDT/DDE and pyrethroid mixture. At the 1-y visit, pyrethroid-group PIPs ranked higher than DDT/DDE-group PIPs for the study outcomes, with the exception of Cognitive scores. At the 2-y visit, there was no consistent pattern of which set of

Table 5. Sex-stratified adjusted linear regression β coefficient and 95% confidence interval for the association between maternal prenatal *p,p′*-DDT and *p,p′*-DDE serum concentrations (log_{10}-transformed; lipid-adjusted) and children's performance on the Bayley Scales of Infant Development (3rd edition) at the 1- and 2-y visits, VHEMBE, Limpopo, South Africa.

	p,p′-DDT			*p,p′*-DDE		
BSID Measure	Boys β (95% CI)	Girls β (95% CI)	p_{Int}[a]	Boys β (95% CI)	Girls β (95% CI)	p_{Int}[a]
At 1-y visit ($n = 689$)						
Cognitive	0.25 (0.01, 0.48)*	0.08 (−0.18, 0.34)	0.36	0.36 (0.11, 0.61)*	0.16 (−0.14, 0.46)	0.35
Receptive Communication	−0.07 (−0.27, 0.13)	0.05 (−0.18, 0.28)	0.39	−0.08 (−0.33, 0.18)	0.09 (−0.20, 0.37)	0.37
Expressive Communication	−0.07 (−0.29, 0.16)	−0.13 (−0.42, 0.17)	0.77	0.03 (−0.24, 0.30)	−0.12 (−0.47, 0.24)	0.55
Fine Motor	0.05 (−0.25, 0.36)	−0.09 (−0.42, 0.24)	0.50	0.10 (−0.20, 0.40)	0.01 (−0.38, 0.39)	0.69
Gross Motor	0.28 (−0.04, 0.60)	−0.01 (−0.36, 0.34)	0.33	0.29 (−0.11, 0.69)	−0.08 (−0.48, 0.32)	0.26
Language Composite	−0.43 (−1.33, 0.48)	−0.23 (−1.53, 1.08)	0.77	−0.13 (−1.27, 1.01)	−0.07 (−1.69, 1.55)	0.88
Motor Composite	0.99 (−0.42, 2.40)	−0.35 (−2.05, 1.35)	0.28	1.16 (−0.49, 2.82)	−0.24 (−2.19, 1.72)	0.32
Social-Emotional	0.11 (−0.32, 0.54)	0.07 (−0.31, 0.46)	0.92	0.27 (−0.24, 0.78)	0.25 (−0.20, 0.71)	0.92
At 2-y visit ($n = 681$)						
Cognitive	−0.01 (−0.21, 0.19)	−0.06 (−0.28, 0.15)	0.98	0.01 (−0.24, 0.27)	0.09 (−0.19, 0.36)	0.56
Receptive Communication	0.09 (−0.11, 0.29)	−0.11 (−0.33, 0.11)	0.21	0.21 (−0.05, 0.46)	−0.17 (−0.45, 0.10)	0.05
Expressive Communication	0.06 (−0.24, 0.37)	−0.21 (−0.53, 0.11)	0.27	0.30 (−0.10, 0.69)	−0.23 (−0.64, 0.19)	0.08
Fine Motor	0.04 (−0.21, 0.29)	−0.23 (−0.45, −0.01)*	0.16	0.11 (−0.21, 0.43)	−0.14 (−0.40, 0.13)	0.29
Gross Motor	0.13 (−0.07, 0.32)	−0.09 (−0.29, 0.11)	0.13	0.19 (−0.04, 0.42)	−0.07 (−0.32, 0.18)	0.14
Language Composite	0.49 (−0.83, 1.81)	−0.93 (−2.33, 0.47)	0.18	1.51 (−0.18, 3.21)	−1.17 (−2.93, 0.59)	0.03
Motor Composite	0.52 (−0.56, 1.59)	−1.00 (−1.99, 0.00)	0.07	0.92 (−0.45, 2.29)	−0.65 (−1.85, 0.55)	0.11

Note: Coefficients indicate the change in scaled BSID score associated with a 10-fold increase in maternal serum DDT/DDE concentrations. Models adjusted for maternal education, age, marital status, poverty status at delivery, risk for depression (CES-D) and Raven's Coloured Progressive Matrices score (at 1-y visit); food insecurity (USDA Food Security Survey); HOME score; preterm delivery; and psychometrician at the time of exam. BSID, Bayley Scales of Infant Development (3rd edition); CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HOME, Home Observation Measurement of the Environment; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.
[a]p-Value for sex interaction. Interactions were considered statistically significant at $p < 0.1$. *$p < 0.05$.

Table 6. Adjusted linear regression β coefficient and 95% confidence interval for the association between maternal prenatal urinary pyrethroid metabolite concentration (specific-gravity adjusted) and children's performance on the Bayley Scales of Infant Development (3rd edition) at the 1- and 2-y visits, VHEMBE study, Limpopo, South Africa.

BSID Measure	*cis*-DBCA [β (95% CI)]	*cis*-DCCA [β (95% CI)]	*trans*-DCCA [β (95% CI)]	3PBA[a] [β (95% CI)]
At 1-y visit ($n = 681$)				
Cognitive	0.05 (−0.26, 0.36)	0.01 (−0.30, 0.33)	0.02 (−0.26, 0.30)	0.03 (−0.31, 0.37)
Receptive Communication	0.13 (−0.12, 0.38)	0.04 (−0.24, 0.32)	−0.03 (−0.29, 0.23)	0.08 (−0.23, 0.40)
Expressive Communication	−0.21 (−0.51, 0.08)	−0.06 (−0.35, 0.23)	−0.08 (−0.33, 0.16)	−0.10 (−0.41, 0.20)
Fine Motor	0.09 (−0.25, 0.42)	−0.08 (−0.47, 0.30)	−0.08 (−0.42, 0.27)	−0.01 (−0.42, 0.39)
Gross Motor	0.10 (−0.29, 0.50)	0.36 (−0.10, 0.81)	0.24 (−0.16, 0.64)	0.28 (−0.20, 0.76)
Language Composite	−0.26 (−1.50, 0.99)	−0.08 (−1.39, 1.22)	−0.33 (−1.49, 0.83)	−0.07 (−1.48, 1.34)
Motor Composite	0.59 (−1.15, 2.33)	0.84 (−1.22, 2.90)	0.51 (−1.33, 2.35)	0.81 (−1.35, 2.97)
Social-Emotional[b]	−0.19 (−0.64, 0.26)	−0.63 (−1.14, −0.12)*	−0.48 (−0.92, −0.05)*	−0.58 (−1.11, −0.06)*
At 2 years ($n = 671$)				
Expressive Communication	−0.40 (−0.77, −0.04)*	0.01 (−0.40, 0.41)	−0.22 (−0.58, 0.13)	−0.22 (−0.66, 0.22)
Fine Motor	0.16 (−0.12, 0.44)	0.31 (−0.02, 0.64)	0.17 (−0.13, 0.47)	0.30 (−0.05, 0.65)
Gross Motor	−0.19 (−0.43, 0.05)	0.08 (−0.22, 0.39)	−0.04 (−0.31, 0.22)	0.03 (−0.30, 0.36)
Language Composite	−1.74 (−3.34, −0.13)*	0.23 (−1.61, 2.08)	−0.91 (−2.50, 0.69)	−0.79 (−2.74, 1.16)
Motor Composite	−0.11 (−1.37, 1.16)	1.19 (−0.38, 2.76)	0.37 (−1.04, 1.78)	0.98 (−0.69, 2.66)

Note: Coefficients show the change in scaled BSID score associated with a 10-fold increase in maternal urinary pyrethroid metabolite concentrations. Models adjusted for maternal education, age, marital status, poverty status at delivery, risk for depression (CES-D) and Raven's Coloured Progressive Matrices score (at 1-y visit); food insecurity (USDA Food Security Survey); HOME score; preterm delivery; psychometrician; and time of urine collection (before or after delivery). 3PBA, 3-phenoxybenzoic acid; BSID, Bayley Scales of Infant Development (3rd edition); CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; DBCA, (2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; DCCA, (2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; HOME, Home Observation Measurement of the Environment; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.

[a]Due to one missing value, models with 3PBA had 680 participants at the 1-y visit and 670 participants at the 2-y visit.

[b]Social-Emotional outcome models had 688 participants for *cis*-DBCA, *cis*-DCCA, and *trans*-DCCA exposures, and 687 participants for 3PBA. *$p < 0.05$.

group PIPs were highest. DDT/DDE did not reach the PIP group inclusion of 50% for any of the analyses at the 1- or 2-y visit. The pyrethroids did reach the group PIP inclusion criterion of 50% at the 1-y visit for Receptive Communication ($PIP_{pyrethroid} = 54\%$), with 3PBA resulting in the highest conditional PIP; for Fine Motor ($PIP_{pyrethroid} = 52\%$), with 3PBA having the highest conditional PIP; and for Social-Emotional domain ($PIP_{pyrethroid} = 67\%$), with *cis*-DCCA resulting in the highest conditional PIP ranking. No associations of the pyrethroid group for the 2-y assessment reached PIP inclusion criterion. Based upon review of bivariate plots of exposure responses for each chemical, the BKMR models showed no evidence for interaction between DDT or DDE and any of the pyrethroid metabolites (see Figures S2–S14).

Discussion

We examined the relationship between *in utero* exposure to insecticides used in IRS for malaria control in Limpopo, South Africa, and neurodevelopment of children at 1- and 2-y follow-up as assessed on the BSID. Maternal peripartum serum concentrations of *p,p′*-DDT or *p,p′*-DDE were not associated with statistically significant decrements in BSID subscales at either time point in boys and girls combined, though fine motor scores at the 2-y visit were significantly lower in association with *p,p′*-DDT in girls. A few significant positive associations were also present (for cognitive scores at the 1-y visit and *p,p′*-DDE in boys and girls combined, and *p,p′*-DDT and *p,p′*-DDE in boys). In contrast, maternal urinary metabolites of certain pyrethroids—specifically *cis*-DCCA, *trans*-DCCA, and 3PBA levels—were associated with lower Social-Emotional scores at the 1-y visit, and *cis*-DBCA levels were associated with lower Language Composite scores (particularly driven by Expressive Communication) at the 2-y visit. Other associations with pyrethroids were less consistent. Some pyrethroids are potential endocrine disruptors that may influence *in utero* development through sex-specific mechanisms (Brander et al. 2012), and there was some evidence that maternal pyrethroid exposure was associated with poorer motor skills in girls but better skills in boys at 2 y of age.

The results of this study on DDT/DDE were somewhat unexpected, given our previous findings in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort study (Eskenazi et al. 2006) as well as those of others (Bahena-Medina et al. 2011; Ribas-Fitó et al. 2006). The *p,p′*-DDT levels averaged more than three times higher in the VHEMBE cohort (GM = 69.0 ng/g lipid) than in CHAMACOS (GM = 22.0 ng/g lipid) (Eskenazi et al. 2006). However, the null findings of DDT and neurodevelopment in the VHEMBE study are in agreement with results from the Collaborative Perinatal Project (CPP), which was conducted in the early 1960s when maternal serum levels of *p,p′*-DDT were an order of magnitude higher than in the VHEMBE study (median = 1,100 ng/g lipid in CPP vs. 55.3 ng/g lipid in VHEMBE). The results are also in line with the null findings found with older children in the CPP and CHAMACOS studies (Gaspar et al. 2015; Jusko et al. 2012).

Despite pyrethroids being widely used in agriculture and IRS (Hlongwana et al. 2013), there have been relatively few studies examining the relationship of early life exposure to this class of insecticides with child neurodevelopment. Studies from NYC (Horton et al. 2011) and France (Viel et al. 2015) reported no relationship of maternal exposure with cognitive development on the infant BSID-II scales and on the WISC Verbal Comprehension and Working Memory, respectively. However, studies from China (Xue et al. 2013) and Mexico (Watkins et al. 2016) reported decreases in infant and toddler cognition with even lower levels of maternal pyrethroid metabolites than in the current study (e.g., 3PBA GM = 0.26 ng/mL in Mexico vs. 0.71 ng/mL in VHEMBE). We did not observe a relationship of maternal pyrethroid exposure and child cognition, but did observe associations of *cis*-DBCA and language development. The VHEMBE children should be followed to older ages to determine if maternal pyrethroid exposure continues to be related to language and/or cognitive development.

Although the French study reported no relationship of maternal prenatal pyrethroid exposure with cognitive development in 6-y-olds (Viel et al. 2015), in a subsequent analysis in the same children they reported a relationship for *cis*-DCCA and 3PBA with behavioral problems, and this latter finding was confirmed in a recent NYC study (Furlong et al. 2017). Although the children in our study were considerably younger than in previous studies, our finding of a relationship between maternal pyrethroid metabolites (*cis*-

Table 7. Sex-stratified adjusted linear regression β coefficient and 95% confidence interval for the association between maternal prenatal urinary pyrethroid metabolite concentration (specific-gravity adjusted) and children's performance on the Bayley Scales of Infant Development (3rd edition) at the 1- and 2-y visits, VHEMBE study, Limpopo, South Africa.

	cis-DrBCA			*cis*-DCCA			*trans*-DCCA			3PBA		
BSID Measure	Boys [β (95% CI)]	Girls [β (95% CI)]	p_{Int}[a]	Boys [β (95% CI)]	Girls [β (95% CI)]	p_{Int}[a]	Boys [β (95% CI)]	Girls [β (95% CI)]	p_{Int}[a]	Boys [β (95% CI)]	Girls [β (95% CI)]	p_{Int}[a]
At 1-y visit ($n=681$)												
Cognitive	−0.09 (−0.48, 0.31)	0.22 (−0.26, 0.70)	0.28	0.00 (−0.42, 0.42)	0.04 (−0.45, 0.52)	0.97	0.11 (−0.27, 0.49)	−0.05 (−0.46, 0.36)	0.55	−0.02 (−0.48, 0.43)	0.13 (−0.41, 0.66)	0.76
Receptive Communication	0.45 (0.07, 0.83)*	−0.19 (−0.50, 0.12)	0.02	0.12 (−0.31, 0.56)	−0.08 (−0.44, 0.29)	0.43	0.15 (−0.24, 0.54)	−0.23 (−0.58, 0.11)	0.17	0.25 (−0.23, 0.73)	−0.10 (−0.48, 0.28)	0.26
Expressive Communication	0.02 (−0.35, 0.38)	−0.43 (−0.89, 0.02)	0.17	−0.02 (−0.43, 0.38)	−0.14 (−0.59, 0.31)	0.61	0.05 (−0.29, 0.39)	−0.25 (−0.66, 0.17)	0.23	0.02 (−0.36, 0.40)	−0.25 (−0.78, 0.27)	0.31
Fine Motor	0.04 (−0.42, 0.49)	0.19 (−0.32, 0.71)	0.61	0.02 (−0.50, 0.54)	−0.14 (−0.74, 0.46)	0.64	0.08 (−0.39, 0.56)	−0.18 (−0.72, 0.35)	0.51	0.11 (−0.43, 0.65)	−0.05 (−0.69, 0.59)	0.65
Gross Motor	0.03 (−0.56, 0.61)	0.20 (−0.35, 0.75)	0.60	0.29 (−0.37, 0.95)	0.32 (−0.33, 0.98)	0.90	0.35 (−0.25, 0.94)	0.05 (−0.53, 0.62)	0.55	0.35 (−0.33, 1.04)	0.13 (−0.59, 0.85)	0.65
Language Composite	1.30 (−0.41, 3.01)	−1.82 (−3.56, −0.07)*	0.02	0.29 (−1.58, 2.16)	−0.66 (−2.56, 1.24)	0.40	0.59 (−1.05, 2.23)	−1.40 (−3.17, 0.38)	0.10	0.76 (−1.16, 2.69)	−1.07 (−3.18, 1.05)	0.16
Motor Composite	0.17 (−2.26, 2.60)	1.25 (−1.29, 3.78)	0.49	0.91 (−1.94, 3.75)	0.60 (−2.48, 3.69)	0.89	1.27 (−1.31, 3.85)	−0.33 (−3.08, 2.43)	0.47	1.39 (−1.53, 4.30)	0.30 (−3.03, 3.63)	0.60
Social-Emotional	−0.19 (−0.85, 0.46)	−0.33 (−0.94, 0.28)	0.75	−0.83 (−1.55, −0.11)*	−0.51 (−1.22, 0.20)	0.48	−0.68 (−1.27, −0.09)*	−0.41 (−1.08, 0.26)	0.52	−0.82 (−1.55, −0.08)*	−0.48 (−1.24, 0.29)	0.43
At 2-y visit ($n=671$)												
Cognitive	0.08 (−0.28, 0.43)	−0.08 (−0.39, 0.23)	0.46	−0.04 (−0.52, 0.44)	0.17 (−0.17, 0.50)	0.60	0.06 (−0.35, 0.46)	−0.10 (−0.40, 0.21)	0.52	0.06 (−0.39, 0.51)	0.05 (−0.32, 0.42)	0.78
Receptive Communication	−0.10 (−0.45, 0.24)	−0.30 (−0.66, 0.05)	0.33	0.17 (−0.22, 0.55)	−0.07 (−0.49, 0.34)	0.40	0.02 (−0.30, 0.35)	−0.26 (−0.63, 0.11)	0.27	0.10 (−0.29, 0.49)	−0.26 (−0.71, 0.19)	0.18
Expressive Communication	−0.44 (−0.96, 0.09)	−0.40 (−0.93, 0.12)	0.93	0.12 (−0.44, 0.69)	−0.21 (−0.84, 0.43)	0.46	−0.03 (−0.51, 0.46)	−0.55 (−1.10, 0.00)*	0.16	0.03 (−0.57, 0.64)	−0.60 (−1.26, 0.07)	0.14
Fine Motor	0.48 (0.05, 0.91)*	−0.18 (−0.49, 0.14)	0.01	0.50 (−0.03, 1.04)	0.03 (−0.31, 0.37)	0.09	0.28 (−0.19, 0.75)	−0.01 (−0.33, 0.30)	0.23	0.51 (−0.03, 1.04)	0.04 (−0.34, 0.42)	0.08
Gross Motor	−0.06 (−0.40, 0.27)	−0.34 (−0.70, 0.01)	0.17	0.41 (−0.02, 0.83)	−0.34 (−0.77, 0.09)	0.02	0.36 (−0.02, 0.73)	−0.56 (−0.92, −0.21)*	<0.01	0.37 (−0.07, 0.81)	−0.42 (−0.89, 0.06)	0.01
Language Composite	−1.52 (−3.86, 0.81)	−2.02 (−4.29, 0.26)	0.62	0.90 (−1.65, 3.46)	−0.85 (−3.58, 1.87)	0.37	0.04 (−2.14, 2.22)	−2.36 (−4.76, 0.04)	0.15	0.45 (−2.21, 3.11)	−2.48 (−5.39, 0.43)	0.11
Motor Composite	1.24 (−0.67, 3.15)	−1.59 (−3.19, 0.02)	0.02	2.75 (0.27, 5.23)*	−0.95 (−2.64, 0.74)	0.01	1.93 (−0.23, 4.09)	−1.76 (−3.27, −0.25)*	0.01	2.64 (0.21, 5.07)*	−1.16 (−3.10, 0.78)	0.01

Note: Coefficients show the change in scaled BSID score associated with a 10-fold increase in in maternal urinary pyrethroid metabolite concentrations. Models adjusted for maternal education, age, marital status, poverty status at delivery, risk for depression (CES-D) and Raven's Coloured Progressive Matrices score (at 1-y visit); food insecurity (USDA Food Security Survey); HOME score; preterm delivery; psychometrician at the time of exam; and urine sample collection before or after delivery. 3PBA, 3-phenoxybenzoic acid; BSID, Bayley Scales of Infant Development (3rd edition); CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; DBCA, (2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; DCCA, (2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; HOME, Home Observation Measurement of the Environment; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.
[a]p-Value for sex interaction. Interactions were considered statistically significant at $p<0.1$. *$p<0.05$.

Table 8. Posterior inclusion probabilities for group inclusion into models and conditional inclusion into models using Bayesian kernel machine regression to describe relative ranking of variable "importance."

	Group PIP[a]		Conditional PIP[b]					
BSID Measure	DDT/DDE	Pyrethroid	*p,p′*-DDT	*p,p′*-DDE	*cis*-DBCA	*cis*-DCCA	*trans*-DCCA	3PBA
At 1-y visit								
Cognitive	0.30	0.06	0.22	0.78	0.15	0.24	0.40	0.21
Receptive Communication	0.03	0.54[c]	0.47	0.53	0.01	0.17	0.39	0.42
Expressive Communication	0.06	0.06	0.73	0.27	0.35	0.17	0.22	0.25
Fine Motor	0.07	0.52[c]	0.73	0.27	0.03	0.20	0.23	0.54
Gross Motor	0.10	0.10	0.51	0.49	0.15	0.43	0.24	0.18
Language Composite	0.02	0.04	0.54	0.46	0.05	0.11	0.33	0.50
Motor Composite	0.02	0.05	0.49	0.51	0.11	0.42	0.28	0.19
Social-Emotional	0.35	0.67[c]	0.25	0.75	0.05	0.45	0.27	0.23
At 2-y visit								
Cognitive	0.11	0.10	0.38	0.62	0.17	0.54	0.19	0.10
Receptive Communication	0.08	0.11	0.38	0.62	0.60	0.14	0.16	0.11
Expressive Communication	0.25	0.19	0.16	0.84	0.72	0.06	0.13	0.09
Fine Motor	0.15	0.24	0.32	0.68	0.11	0.40	0.23	0.26
Gross Motor	0.11	0.08	0.52	0.48	0.42	0.31	0.14	0.14
Language Composite	0.07	0.08	0.16	0.84	0.79	0.06	0.10	0.05
Motor Composite	0.06	0.04	0.40	0.60	0.02	0.66	0.19	0.14

Note: Models adjusted for maternal education, age, marital status, poverty status at delivery, risk for depression (CES-D) and Raven's Coloured Progressive Matrices score (at 1-y visit); food insecurity (USDA Food Security Survey); HOME score; psychometrician; preterm delivery; and time of urine collection (before or after delivery). 3PBA, 3-phenoxybenzoic acid; BKMR, Bayesian kernel machine regression; BSID, Bayley Scales of Infant Development (3rd edition); CES-D, Center for Epidemiologic Studies Depression Scale; DBCA, (2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; DCCA, (2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HOME, Home Observation Measurement of the Environment; PIP, posterior inclusion probability; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment.

[a]Group PIPs indicate the posterior probability that an exposure grouping (e.g., pyrethroids) was included into the "true" model from the multiple iterations (25,000) of the Markov Chain Monte Carlo (MCMC) sampler. For example, the pyrethroid grouping was included 67% of the time across all models for the Social-Emotional outcome at the 1-y visit.

[b]Conditional PIPs indicate the posterior probability that a particular chemical exposure (e.g., *cis*-DCCA) within an exposure grouping (e.g., pyrethroids) was included into the "true" model from the multiple iterations (25,000) of the MCMC sampler, conditional on the exposure grouping being included. For example, within the pyrethroid grouping, *cis*-DCCA was included 45% of the time across all models that included pyrethroids for the Social-Emotional measure at the 1-y visit. Note that within a group, the conditional PIPs will total 1.0.

[c]Group PIP with probability exceeding the threshold of 0.5 (the median probability model). Emphasis should be placed on the relative ranking across groups (pyrethroids or DDT/DDE).

trans-DCCA and 3PBA) and poorer socio-emotional development in the 1-y-olds are in line with the results of these two studies. Unfortunately, we did not administer the Social Emotional subtest at 2 y of age and therefore, cannot confirm this finding at 2 y of age.

Because of the long half-life of DDT/DDE in humans, measurements made in serum collected late in pregnancy or immediately postpartum likely reflect exposure throughout pregnancy (Verner et al. 2015). However, a single measure of pyrethroids, as measured by urinary metabolites, may reflect only recent exposure due to their short half-life (Barlow et al. 2001). In the current study, we aimed to collect urine from women when they entered the hospital for admission, but in more than a third the urine sample was collected postdelivery. Women who had the urine sample collected predelivery were more likely to deliver by Cesarean section, but did not differ in any other obvious ways. Because we were concerned that measurements in postdelivery urine samples reflected exposure occurring in the hospital instead of in the home (from IRS), we controlled for timing of the sample collection in the main analysis and conducted sensitivity analyses restricting the sample to women who provided specimens predelivery. Although the findings were not substantially different when restricting analysis to the smaller sample, three of the four pyrethoid metabolite levels were higher in the predelivery samples. Serial urine samples collected over the course of pregnancy would have yielded a more accurate picture of exposure to pyrethroids. Thus, the risk of exposure misclassification of pyrethroids around the critical period of brain development is a limitation of this study.

To our knowledge, this is the first study to examine the relationship of insecticides used in IRS and neurodevelopment. Previous studies have considered the neurodevelopmental effects of organochlorine or pyrethroid insecticides, but in populations unlike those that are currently exposed to these pesticides in the context of IRS. Our population is economically impoverished, food insecure, and has fairly high rates of HIV—all factors that may potentially render this population more vulnerable to chemical exposure. Although exposures are generally higher than in other cohorts, the VHEMBE cohort is not uniformly highly exposed. Spraying practices vary between demographically similar villages based largely on minor differences in altitude, which impact malaria transmission risk. This yielded a wide range of exposures in an otherwise relatively demographically homogeneous population. However, findings on the effects of these insecticides on neurodevelopment in this impoverished rural African population may not be generalizable to other worldwide populations that continue to use pyrethroids in homes and fields and who may have only residual body burdens of DDT/DDE.

Another limitation of the study is that we found a lack of consistency between associations at the 1-y and 2-y visits, and hence the longitudinal measures were deemed not relevant. It is possible that the assessment at 1 y of age was less reliable (given infant behavior) than at 2 y of age. Likewise, we do not have a ready explanation for the positive association of DDT/DDE and cognition. The fact that this finding no longer exists at 2 y of age suggests that it may be due to chance. Data from future visits in this ongoing longitudinal study should give us an indication whether the associations persist over time.

Previous studies have not considered the joint effects of the two pesticide classes, although both classes of pesticides are widely used in malaria-endemic areas in South Africa. A strength of this study is the use of BKMR to consider individual chemical exposure responses with co-occurring insecticides and their interaction without numerous comparison testing. The results of the BKMR models did not support interactions between DDT/DDE and any of the pyrethroid metabolites. From the perspective of relative ranking of PIPs, we found that pyrethroids as a group ranked higher than DDT/DDE for most of the study outcomes, with the strength of the evidence highest for social-emotional outcomes, which is consistent with the finding in the single-pollutant models.

Conclusions

The challenge of this study was to disentangle the effects of various IRS insecticides and to consider concurrent conditions, such as poverty, malaria, AIDS, poor nutrition—all of which are possible determinants of delays in children's physical and mental development. We found that prenatal exposure to DDT/DDE in the context of IRS was not associated with adverse neurodevelopmental outcomes in infants and toddlers, controlling for these other sociodemographic factors. In contrast, pyrethoid exposure measured in the peripartum period was associated with statistically significant decrements in social-emotional and language development in this age range, as well as lower motor function scores in girls but higher motor function scores in boys. Given pyrethroids' widespread use worldwide in both agriculture and indoor pest control, these findings deserve further investigation. Although IRS has been instrumental in the prevention of malaria in sub-Saharan Africa, these benefits must be considered against possible neurotoxic effects on young children.

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