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Research report

Pesticide exposure and neurodevelopment in children aged 6–9 years from Talamanca, Costa Rica

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ABSTRACT

Certain pesticides may affect children's neurodevelopment. We assessed whether pesticide exposure was associated with impaired neurobehavioral outcomes in children aged 6–9 years.

We conducted a cross-sectional study in 140 children living near banana plantations and plantain farms in the Talamanca County, Costa Rica and assessed their neurobehavioral performance. Exposure was determined by analyzing urinary metabolites of chlorpyrifos (3,5,6-trichloro-2-pyridinol, TCPy), mancozeb (ethylenethiourea, ETU), and pyrethroids (3-phenoxybenzoic acid, 3-PBA). Repeated urine samples were obtained for 36 children. We estimated associations of pesticide concentrations with neurobehavioral outcomes using multivariable linear and logistic regression models.

Median (25th–75th percentiles) TCPy, ETU, and 3-PBA concentrations were 1.4 (.7–3.1), 1.2 (.7–3.0), and .8 (.5–1.5) μg/L, respectively. Intraclass correlation coefficients (ICC) ranged between .32 and .67. After adjustment for potential confounders, higher urinary TCPy concentrations were associated with poorer working memory in boys ($n = 59$) (β per 10-fold increase in TCPy concentrations = −7.5, 95% CI: −14.4, −.7); poorer visual motor coordination (β = −1.4, 95% CI: −2.7, −.1); increased prevalence of parent-reported cognitive problems/inattention (adjusted OR per 10-fold increase in urinary concentrations = 5.8, 95% CI: 1.6, 22.9), oppositional disorders (aOR = 3.9, 95% CI: 1.0, 16.0), and ADHD (aOR = 6.8, 95% CI: 1.8, 28.6), and; decreased ability to discriminate colors (aOR = 6.6, 95% CI: 1.6, 30.3; the higher the score the worse). Higher ETU concentrations were associated with poorer

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verbal learning outcomes (β = −7.0, 95% CI: −12.7, −1.3). Higher 3-PBA concentrations were associated with poorer processing speed scores, particularly in girls (β = −8.8, 95% CI: −16.1, −1.4).

Our findings indicate that children living near banana and plantain plantations are exposed to pesticides that may affect their neurodevelopment, which for certain domains may differ between boys and girls. We recommend the implementation of measures to reduce pesticide exposure in children living nearby banana plantations.

1. Introduction

There is a rapidly growing body of knowledge regarding the harmful effects of environmental toxins on children's neurodevelopment that differ between boys and girls (Mergler, 2012). The developing brain is particularly vulnerable to toxic substances. Early life exposures to lead, methylmercury, polychlorinated biphenyls, arsenic, toluene, and the insecticide chlorpyrifos, may cause learning disabilities, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders, developmental delays, and emotional and behavioral problems (Bjørling-Poulsen, Andersen, & Grandjean, 2008; Grandjean & Landrigan, 2006).

Birth cohort studies conducted in the United States (U.S.) have reported associations of prenatal organophosphate (OP) exposures, including the insecticide chlorpyrifos, with delayed mental development, lower intelligence quotient (IQ), and ADHD (Bouchard et al., 2011; Engel et al., 2011; Eskenazi et al., 2006; Marks et al., 2010; Rauh et al., 2011, 2006). For example, higher prenatal, but not postnatal, urinary OP metabolite concentrations were associated with impaired cognitive development in children aged 2 (Eskenazi et al., 2006) and 7 years (Bouchard et al., 2011) living near agricultural fields in California. Similarly, a birth cohort study of inner-city children in New York City showed that higher chlorpyrifos concentrations in cord blood were associated with developmental delays at 3 years (Rauh et al., 2006) and poorer Full-Scale and Working Memory IQ scores at 7 years (Rauh et al., 2011). In contrast, cross-sectional studies on urinary OP pesticide metabolites and neurobehavioral problems in children have reported inconsistent findings, possibly due to methodological differences including sample size (Fiedler et al., 2015; Lu et al., 2009; Sánchez Lizardi, O'Rourke, & Morris, 2008) and methods used to assess neurobehavioral outcomes (e.g., direct assessments *vs* parental-reported outcomes) (Bouchard, Bellinger, Wright, & Weisskopf, 2010; Oulhote & Bouchard, 2013).

The mechanistic pathways by which pesticides can impair children's neurodevelopment are largely unknown (Bjørling-Poulsen et al., 2008). The associations described in epidemiological studies for OP pesticides may possibly be explained by non-cholinergic mechanisms, such as oxon-metabolites that disrupt neurobehavioral processes at doses lower than acetylcholinesterase inhibition (Flaskos, 2014; Yang et al., 2011).

Neurodevelopmental effects of non-OP pesticides have been hardly studied (Bjørling-Poulsen et al., 2008). Pyrethroids can target the nervous system by disrupting sodium channels in axonal membranes of neurons and induce apoptosis of nerve cells (Bjørling-Poulsen et al., 2008; Saillenfait, Ndiaye, & Sabaté, 2015). Animal studies have also shown that early life exposure to pyrethroids can decrease the density of muscarinic cholinergic receptors (Eriksson & Fredriksson, 1991) and affect the dopaminergic system (Richardson et al., 2015) leading to behavioral disorders later in life. Two prospective studies of pregnant women from China (Xue et al., 2013) and the U.S. (Horton et al., 2011) observed associations of urinary and personal air pyrethroid (or pyrethroid synergists) concentrations with poorer mental development in infants and toddlers. In addition, two cross-sectional studies conducted in Canada and the U.S. reported increased parent-reported behavioral problems in school-age children with higher pyrethroid concentrations in urine (Oulhote et al., 2014; Wagner-schuman et al., 2015). Conversely, a few cross-sectional studies found null associations between pyrethroid exposure and neurobehavioral outcomes in school-age children (Fiedler et al., 2015; Lu et al., 2009; Quirós-Alcalá, Mehta, & Eskenazi, 2014).

Finally, to our knowledge, no epidemiological studies have examined the neurobehavioral effects of mancozeb. Mancozeb is a commonly-used dithiocarbamate fungicide registered for use in almost 120 countries throughout the world (Gullino et al., 2010). *In vitro* and *in vivo* studies suggest that the neurotoxicity of mancozeb and/or its metabolite ethylenethiourea (ETU) may be mediated by generation of reactive oxygen species (Domico, Cooper, Bernard, & Zeevalk, 2007), interference of the vesicular transport of glutamate (Vaccari, Saba, Mocci, & Ruiu, 1999), and inhibition of thyroid peroxidase (Doerge & Takazawa, 1990).

In tropical countries, pesticides are extensively used in monocultures, such as banana and plantain plantations, resulting in elevated environmental exposures to those living nearby (Rodríguez et al., 2006; van Wendel de Joode et al., 2012, 2014). Chlorpyrifos-treated bags are wrapped around bananas and plantains to protect their skin (Barraza, Jansen, van Wendel de Joode, & Wesseling, 2011) and elevated urinary 3,5,6-trichloro-2-pyridinol (TCPy) concentrations, a specific biomarker of chlorpyrifos exposure, have been observed in children living near these plantations (van Wendel de Joode et al., 2012). Additionally, children living near these banana and plantain crops are likely to be exposed to other pesticides such as mancozeb, which is used in Costa Rican banana plantations on a weekly basis and applied by light aircraft

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(Barraza et al., 2011; Bravo-Durán et al., 2013; van Wendel de Joode et al., 2014). Elevated concentrations of urinary ETU, a biomarker of mancozeb exposure, have been reported in pregnant women living near banana plantations in Costa Rica (van Wendel de Joode et al., 2014). Children may also be exposed to pesticides used inside and around their homes, including chemical pyrethroids (used to control indoor pests, treat head lice and scabies in humans and fleas in pets, and control vector borne diseases) (ATDSR, 2003). In the present study, we assessed whether children's exposure to chlorpyrifos, mancozeb, and chemical pyrethroids was associated with impaired cognitive, motor, behavior, and sensory outcomes.

2. Materials and methods

2.1. Study population

We conducted a cross-sectional study in the Talamanca County, Costa Rica, between February and August 2007. The Talamanca County, located in the Caribbean coast, has large-scale banana and small-scale plantain production and is one of the counties with the lowest Human Development Index in the country (PNUD-UCR, 2012). A detailed description of the study area (Barraza et al., 2011), study population, and children's urinary TCPy concentrations (van Wendel de Joode et al., 2012) has been published elsewhere. In short, we included children from three villages within a 40 kilometer's distance, surrounded by: (1) large-scale banana plantations with extensive pesticide use (Daytonia) that included weekly aerial spraying of mancozeb and use of chlorpyrifos-treated bags to protect the fruits (see **Graphical abstract**); (2) small-holders who grew plantains using pesticides by, for example, ground-level applications of mancozeb and use of chlorpyrifos-treated bags (Shiroles, 97% of the interviewed mothers reported pesticide use on farms); or (3) small-holders who grew mainly organically (Amubrë, 12% of interviewed reported pesticide use on farms) (van Wendel de Joode et al., 2012). Chemical type–II–pyrethroids (i.e., deltamethrin, cypermethrin, cyfluthrin), that metabolize to 3-PBA were not used in agriculture, but were used indoors and for vector control in the three villages.

Children were eligible to participate in the study if they were 6–9 years-old, attending elementary school, had been living in their community for ≥1 year, and had no history of pre- and perinatal problems, diabetes, head trauma, and neurological or psychiatric disorders. Eligible children ($n = 190$) and their parents were invited to participate in the study through meetings held in the schools and home visits. The parents of 26 (14%) children could not be located and four (2%) refused to participate. Written informed consent was obtained from parents of 160 (84%) children and verbal assent was obtained from their children. Of these, 140 provided ≥1 urine sample and completed the neurobehavioral test battery. Children included in data analysis ($n = 140$) were similar to the entire study population ($n = 160$) with respect to child's age, parental age and education, number of siblings, and bilingualism (yes/no). All study activities were approved by the Scientific Ethics Committee of the Universidad Nacional in Costa Rica (CECUNA-03-2007).

2.2. Maternal interviews

Structured questionnaires were administered to the children's mothers to collect information on socio-demographic characteristics (e.g., maternal age, education, marital status, number of years living in the village), medical history of the child, and pesticide exposure-related factors (e.g., parental occupation and pesticide use). Mothers were also instructed on how to collect urine samples from their children.

2.3. Pesticide exposure assessment

We assessed children's ($n = 140$) exposure to pesticides by measuring pesticide metabolites in urine. To assess temporal reliability of these metabolites, we obtained repeat urine samples from 40 children as follows: 35 children with two, 1 child with five, 1 child with six, two children with eight, and 1 child with ten samples. On average, the time between the first and second urine sample was 3.3 months (standard deviation = 1.3 months). For the five children with additional repeats, three to eight additional samples were obtained during the days following the second urine sample collection. In total, 207 urine samples were obtained from the 140 children.

2.4. Urinary sampling and analysis

Children were given 100 mL plastic beakers (Vacuette®, sterile) and asked to bring a first-morning urine sample on the day of their neurobehavioral assessment. Five percent of the children ($n = 6$) did not bring a first-morning urine sample and therefore provided a spot sample. Urine samples were stored at 4 °C during the assessments. At the end of the day, samples were aliquoted and stored at −20 °C until their shipment to the Department of Occupational and Environmental Medicine at Lund University Hospital, Sweden.

Analysis of TCPy and 3-PBA in urine were performed as described elsewhere (Lindh, Littorin, Amilon, & Jönsson, 2008; Elfman, Hogstedt, Engvall, Lampa, & Lindh, 2009) using liquid chromatography triple quadrupole mass spectrometry (LC–MS/MS; API 3000; AB Sciex, Foster City, CA, USA), with some modifications. Briefly, urine was acidified and hydrolyzed overnight, and thereafter extracted using solid-phase extraction columns. For the analysis of TCPy, a pseudo-MS/MS transition with both quadrupoles scanning at m/z 196 was used. The compound [$^{13}C_6$]-3-PBA was used as the internal standard for the analysis of 3-PBA (Lindh, Littorin, Amilon, et al., 2008; Elfman, Hogstedt, Engvall, Lampa, & Lindh, 2009). The sample preparation for the ETU analyses in urine was performed using a single-step extractive derivatization procedure and processed samples were analyzed using LC–MS/MS (Lindh, Littorin, Amilon, et al., 2008), with [$^2H_4$]-ETU as the internal standard. All urine samples were analyzed in duplicates. The coefficients of variance calculated from duplicate determinations were 10%, 11%, and 13% for TCPy, 3-PBA, and ETU, respectively. The limits of detection (LOD) for urinary TCPy, 3-PBA, and ETU, were 1, .1, and .1 μg/L, respectively. Creatinine levels (g/L) were analyzed using an enzymatic

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method and used to adjust for urine dilution (Mazzachi, Peake, & Ehrhardt, 2000). All processed samples were analyzed in duplicates. We detected pesticide metabolites in 57% (TCPy), 96% (ETU), and 94% (3-PBA) of urine samples. Values below the LOD were replaced with the LOD divided by the square root of 2 (Hornung and Reed, 1990).

2.5. *Medical examination*

All children had a physical exam conducted by a physician. Height and weight were registered and used to calculate body mass index [BMI = weight in kilograms/(height in meters)^2]. Visual acuity was assessed using a Snellen E chart at 20 feet in adequate light.

2.6. *Neurobehavioral assessments*

2.6.1. *Test administration*

Assessments were completed by three psychometricians, who were trained and supervised by an experienced neuropsychologist (DH). Each psychometrician administered a specific subset of tests to all study participants. All assessments were conducted in Spanish and took place in a school classroom or a library.

2.6.2. *Intellectual ability*

The Mexican–Spanish version of the Wechsler Intelligence Scale for Children 4th edition (WISC-IV), was used to assess children's cognitive abilities (Wechsler, 2007). Scores for three indexes were calculated: Perceptual Reasoning (non-verbal and fluid reasoning, tests: Block Design, Picture Concepts, and Matrix Reasoning), Working Memory (tests: Digit Span and Letter-Number Sequencing), and Processing Speed (Speed of Information Processing, tests: Coding, Symbol Search, Cancellation). A Full-Scale intelligence quotient (IQ) was not calculated because the Verbal Comprehension subscale was not administered as the concurrent validity was of concern in our study population. The WISC-IV has not been standardized for use in Costa Rica, and therefore scores were standardized against Mexican population-based norms (mean = 100 ± 10) (Wechsler, 2007).

2.6.3. *Behavioral problems*

The Spanish version of the Conner's Parent Rating Scale-Revised Short Version (CPRS-R) (Keith Conners, Sitarenios, Parker, & Epstein, 1998) was administered to the children's mothers. This test is a commonly used 27-item standardized questionnaire that measures children's behavioral problems at home and takes 5–10 min to complete. CPRS-R standardized T-scores (mean ± SD = 50 ± 10) for four subscales (Oppositional, Cognitive Problems/Inattention, Hyperactivity, and ADHD Index) were calculated according to the manual (Conners, 1997).

2.6.4. *Sensory function (color discrimination)*

The Lanthony Desaturated D-15 (LDD-15) test (Geller, 2001) was used to assess color discrimination as a measure of visual sensory function. The test was completed separately for each eye and conducted under standardized conditions (i.e., a small table covered with black cloth and a desktop fluorescent lamp). In this test, children were shown a rectangular box containing 16 colored caps arranged in chromatic order. The psychometrician then scrambled the caps in front of the child, correctly positioned the first cap, and asked the child to order the remaining caps in a regular color series. When the child finished, the box was flipped, and the cap order was documented. A total color distance score (TCDS) was calculated as the sum of the published perceptual distances between each pair of caps in the order placed by participants. A color confusion index (CCI) was calculated according to Bowman (1982) as the ratio of each child's TCDS to the TCDS associated with a perfect performance such that a CCI of 1.0 indicates a perfect score. Since results were similar for the right and left eye, the mean CCI for both eyes was calculated.

2.6.5. *Perception and memory*

2.6.5.1. VISUOSPATIAL CONSTRUCTION AND VISUAL MEMORY. To assess visuospatial constructional abilities, a card with the Rey–Osterrieth Complex Figure (ROCF) was placed on the table in front of the child, who was instructed to copy the design on a piece of paper (copy trial) (Fig. 1). Colored markers were provided to the child so that his/her strategy could be discerned more easily. To assess visual memory, 30 min after the card with the ROCF was shown to the children, they were asked to draw as much of the figure as possible from memory (delayed recall trial). Scoring of the ROCF was based on the criteria defined by Galindo y Villa according to which the figure is partitioned into 18 structural units (Cortés, Galindo, Villa, & Salvador, 1997; Knight, 2003). A four-point scale ranging from 0 to 2 was assigned to each unit based on the degree to which the units were correctly drawn and placed and a total raw sum score was calculated.

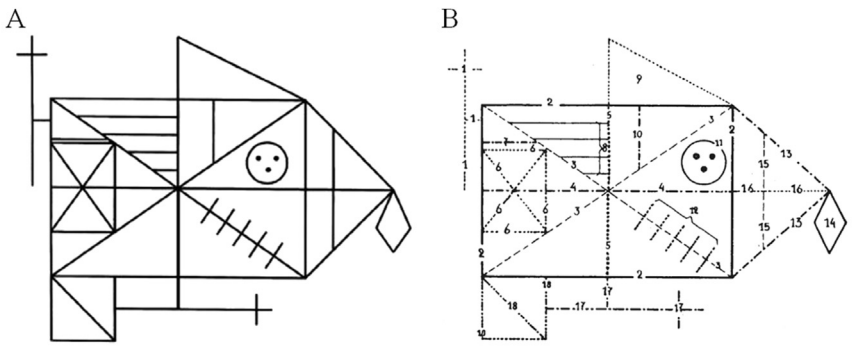

Fig. 1 – Rey–Osterrieth Complex Figure. A. Original stimulus; B. Perceptual units.

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2.6.5.2. Verbal memory and learning abilities. We used the Spanish version of the Children's Auditory Verbal Learning Test 2nd edition (CAVLT-2), to assess children's verbal learning and memory abilities (Talley, 1997; Torres-Agustín et al., 2013). This test is comprised of a 16-item word list that is administered across five trials, and the child is asked to recall the words after each trial. A different set of words is then presented and the child is asked to recall the items from the new list (interference trial). Following the interference list, the child is instructed to recall as many items as possible from the initial list (immediate recall). Fifteen minutes later, the study participant is asked to recall the initial list one more time (delayed recall). Finally, the child is presented with a 32-item word list and asked to recognize the words from the initial list (recognition trial). To reduce the number of outcomes, we calculated standardized scores for three subscales (Jones et al., 2011; Torres-Agustín et al., 2013) according to the manual (Talley, 1997): Immediate Memory Span (short-term memory function: sum of first trial and interference trial), Delayed Recall (long-term memory functioning and retrieval ability, score of delayed recall trial), and Level of Learning (rate of learning: sum of trial 3, 4, and 5).

2.6.6. *Motor function*

2.6.6.1. Visual-motor coordination. We evaluated children's visual-motor coordination using the Eye-Hand Coordination subtest of the Frostig Developmental Test of Visual Perception, 2nd edition (DTVP-2) (Hammill, Pearson, Voress, & Alvarado Guerrero, 1995). In this subtest, children are required to draw precise straight or curved lines to connect two objects (e.g., mouse and cheese, dog and bone) in accordance with visual boundaries. Age-standardized scores were calculated based on how well the child drew between the boundaries and if he/she broke the continuity of the line.

2.6.6.2. Fine motor functioning. Children were administered the Wide Range Assessment of Visual Motor Ability (WRAVMA) pegboard test to assess fine motor dexterity (Adams & Shelow, 1995). In this test, children are asked to insert as many pegs as possible within 90 s into a nearly square pegboard (70 holes) using first their dominant hand and then their non-dominant hand. Age-standardized scores (mean = 100 ± 15) were calculated according to the test manual (Adams & Shelow, 1995).

2.6.6.3. Psychomotor speed. Attention was assessed using the Reaction Time Test (RTT), a task designed to measure the speed of response to a visual stimulus (Lezak, Howieson, & Loring, 2004). In this test, a square appears on the screen and the child is instructed to press a button (with their dominant hand) as fast as possible to make it disappear. A total of 64 trials were conducted and the mean response latency score (in milliseconds) was calculated.

2.7. *Statistical analysis*

Urinary metabolite concentrations were skewed, so we $\log_{10}$-transformed TCPy, ETU, and 3-PBA concentrations for all statistical analyses. To calculate intra-class correlation coefficients (ICC), an indicator of biomarkers' temporal reliability, we used variance components models with random intercepts for each child with repeated urine samples (Rosner, 2006) including urinary creatinine levels as a fixed factor (Barr et al., 2005).

We examined the association of TCPy, ETU, and 3-PBA urinary concentrations measured on the day of the assessment (independent variable) with neurobehavioral outcomes as continuous dependent variables, using multivariable linear regression models. Because pesticides might have different relationships to the outcomes, we built a separate model for each pesticide metabolite and each test outcome. For RTT, CPRS-R, and LDD-15 higher scores reflect worse performance. As residuals of linear models for RTT, CPRS-R, and LDD-15 were not normally distributed, we dichotomized these outcomes using the 50th and 75th percentile as cut-off points and subsequently used logistic regression models to estimate odds ratios.

We selected our main covariates based on previous reports in literature (Bouchard et al., 2011; Oulhote & Bouchard, 2013; Riojas-Rodríguez et al., 2010) and model fit. Child sex and age at assessment (continuous). Maternal education (categorical variable: ≤3 years, 3–6, and >6 years) was included because it is a well-known predictor of cognitive development in children. Having repeated a school year (yes/no) was also included as it may be an outcome of both neurodevelopmental problems or pesticide exposure. Number of siblings (continuous) and child BMI (continuous) (Oulhote & Bouchard, 2013; Riojas-Rodríguez et al., 2010) were retained in multivariate models because they were associated with the exposure and at least one outcome of interest in the bivariate analyses ($p < .10$). Visual acuity (dichotomous, 20/20 *vs* other) was included in all models, with the only exception of CAVLT-2 because this test did not have a visual component. Number of languages spoken at home (dichotomous, 1 *vs* >1 language) was considered as a potential confounder; however, since children's mothers from bilingual families also had lower educational attainment (t-ratio = −3.4, $p < .001$), it was not retained in the multivariate models. To correct for urinary dilution, creatinine was included as a covariate in all models (Barr et al., 2005).

For continuous outcomes, we evaluated differences of the associations between urinary pesticide concentrations and neurobehavioral outcomes by child sex using cross-term products (sex*pesticide concentration). Interaction by sex was regarded notable for those with $p < .20$. We subsequently presented results stratified by sex. Due to limited statistical power, we did not stratify the logistic regression models by child sex.

We defined outliers as studentized residuals (residuals divided by the model standard error) greater than three standard units, and assessed the influence of outliers on the effect estimates by running models with and without those outliers. Outliers that changed the magnitude of the effect estimates by >30% were excluded from our analyses as indicated in the footnotes of Tables 4 and 5.

Main effects were considered significant with $p < .05$ based on two-tailed tests. All analyses were conducted using JMP 8 (SAS Institute, Cary, NC).

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Table 1 – Characteristics of children aged 6–9 years and their urinary pesticide concentrations measured on the day of the neurobehavioral testing, Talamanca County, Costa Rica, 2007 ($n = 140$).

Characteristic	*n* (%)	TCPy	ETU	3-PBA
		GM (95% CI)	GM (95% CI)	GM (95% CI)
Child characteristics				
Sex				
Boy	69 (49)	1.7 (1.4, 2.0)	1.2 (.9, 1.6)	.8 (.7, 1.0)
Girl	71 (51)	1.4 (1.2, 1.7)	1.2 (1.0, 1.6)	.9 (.7, 1.1)
Age at assessment (years)[a]				
6.5–7.5	43 (31)	1.7 (1.4, 2.2)	1.4 (1.0, 2.0)	1.2 (.9, 1.6)*
≥7.5–8.5	47 (34)	1.5 (1.2, 1.8)	1.3 (.9, 1.8)	.8 (.6, 1.1)
≥8.5–9.3	50 (36)	1.5 (1.2, 1.8)	1.1 (.8, 1.5)	.7 (.5, .9)
Nutritional status[a,b]				
Underweight	9 (6)	1.2 (.7, 2.1)	1.8 (.8, 3.7)	.7 (.4, 1.3)
Normal	105 (75)	1.6 (1.4, 1.9)	1.3 (1.0, 1.6)	.9 (.7, 1.1)
Overweight or obese	26 (19)	1.3 (.9, 1.7)	1.0 (.6, 1.5)	.8 (.6, 1.2)
Normal visual acuity[c]				
Yes	81 (58)	1.5 (1.3, 1.8)	1.3 (1.0, 1.6)	.8 (.7, 1.0)
No	59 (42)	1.6 (1.3, 2.0)	1.2 (.9, 1.6)	.9 (.7, 1.2)
Number of siblings[a]				
0	10 (7)	1.5 (.9, 2.5)	.7 (.3, 1.4)#	.8 (.5, 1.5)
≥1	130 (93)	1.5 (1.3, 1.8)	1.3 (1.1, 1.6)	.9 (.7, 1.0)
Repeated grade				
Yes	16 (11)	1.5 (1.0, 2.1)	.9 (.5, 1.6)	.5 (.3, .7)**
No	124 (89)	1.6 (1.4, 1.8)	1.3 (1.1, 1.6)	.9 (.8, 1.1)
Maternal characteristics				
Age (years)				
20–30	58 (41)	1.5 (1.2, 1.8)	1.2 (.9, 1.7)	.9 (.7, 1.1)
≥30–40	58 (41)	1.7 (1.4, 2.0)	1.3 (.9, 1.7)	.8 (.6, 1.0)
≥40	22 (16)	1.5 (1.1, 2.1)	1.1 (.7, 1.8)	1.1 (.7, 1.7)
Education				
≤3rd grade	33 (24)	2.0 (1.5, 2.6)*	1.7 (1.2, 2.5)	.6 (.4, .9)*
3–6th grade	76 (54)	1.5 (1.3, 1.8)	1.1 (.9, 1.5)	.9 (.7, 1.1)
>6th grade	31 (22)	1.2 (.9, 1.6)	1.1 (.7, 1.6)	1.1 (.8, 1.6)
Married or living as married				
Yes	101 (72)	1.5 (1.3, 1.8)	1.4 (1.1, 1.7)	.9 (.7, 1.0)
No	39 (28)	1.6 (1.2, 2.0)	1.0 (.7, 1.4)	.9 (.7, 1.2)
Household characteristics				
Village or study site				
Daytonia (banana)	38 (27)	2.2 (1.7, 2.7)**	3.4 (2.6, 4.6)**	.7 (.5, 1.0)**
Shiroles (plantain)	48 (34)	1.9 (1.6, 2.3)	1.2 (.9, 1.6)	1.1 (.9, 1.5)
Amubrë (organic)	54 (39)	1.0 (.8, 1.2)	.6 (.5, .8)	.9 (.7, 1.1)

Abbreviations: GM, geometric mean; CI, confidence interval; TCPy, 3,5,6-trichloro-2-pyridinol; ETU, ethylenethiourea; and 3-PBA, 3-phenoxybenzoic acid.

#$p < .10$; *$p < .05$; **$p < .01$; *p*-value of F-ratio of one-way analysis of variance on log-transformed concentrations.

[a] Modeled as a continuous variable in multivariable models.

[b] Nutritional status based on child body mass index (BMI): underweight = BMI < 5th percentile, normal = BMI 5th < 85th percentile, overweight or obese = BMI ≥ 85th percentile.

[c] Abnormal visual acuity = ≥20/30 in Snellen E chart in at least one eye.

3. Results

3.1. *Characteristics of study population and pesticide concentrations*

Table 1 presents summary statistics for the study population. Briefly, children were 6.5–9.3 years old (mean age = 7.9 ± .8 years at assessment) and most of them had a normal weight (75%), and more than one sibling (93%). Surprisingly, only 58% of the children had normal visual acuity. Their mothers were relatively young (mean age = 32.4 ± 6.9 years at children's enrollment), mainly married or living as married (72%), and with low educational attainment (78% with primary school or lower level).

TCPy concentrations were higher in children who lived in the banana and plantain villages and whose mothers had the lowest educational level (≤3 years of education), whereas 3-PBA concentrations were lower in children whose mothers had lowest educational level. ETU concentrations were higher in children living in the banana village, followed by those living in the plantain community. Younger children had higher 3-PBA concentrations compared to older children, whereas children who had repeated a school year had lower concentrations compared to those who had not. Also, concentrations of 3-PBA were higher in children living in the plantain village compared to the banana and organic village. No statistically significant differences in pesticide metabolite

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Table 2 – Description of urinary pesticide concentrations (μg/L) in children aged 6–9 years, Talamanca County, Costa Rica, 2007.

Pesticide metabolite	Samples collected on the day of neurodevelopment assessment ($n = 140$)			Repeated samples ($n = 207$, children = 140)			
	% > LOD[a]	Median (P25–P75)	Max	% > LOD[a]	Median (P25–P75)	Max	ICC
TCPy	57	1.4 (.7–3.1)	9.6	62	1.6 (.7–3.2)	26.8	.52
ETU	96	1.2 (.7–3.0)	11.0	97	1.6 (.8–3.8)	34.5	.67
3-PBA	94	.8 (.5–1.5)	20.4	94	.7 (.4–1.3)	20.4	.32

Abbreviations: LOD, limit of detection; ICC, intraclass correlation coefficient; TCPy, 3,5,6-trichloro-2-pyridinol; ETU, ethylenethiourea; 3-PBA, 3-phenoxybenzoic acid.
[a] LOD for TCPy = 1.0 μg/L; and for ETU and 3-PBA = .1 μg/L.

Table 3 – Distribution of neurobehavioral outcomes in children aged 6–9 years, Talamanca County, Costa Rica, 2007 ($n = 140$).

Outcomes	Mean ± SD	Median (P25–P75)
Intellectual ability		
WISC-IV Scales (standardized scores)		
Working Memory	85 ± 8.8	86 (80–91)
Processing Speed	81 ± 11	80 (74–88)
Perceptual Reasoning	81 ± 10	79 (74–86)
Behavioral problems		
CPRS-R (T-scores)[a]		
Cognitive Problems/Inattention	53 ± 10	50 (44–59)
Oppositional Disorder	50 ± 10	47 (43–57)
Hyperactivity	58 ± 10	56 (50–66)
ADHD Index	53 ± 9.3	51 (46–58)
Sensory function (color discrimination)		
LDD-15 (mean CCI)[a]	1.4 ± .27	1.3 (1.2–1.5)
Perception and memory		
Visuospatial construction and visual memory		
Rey–Osterrieth Complex Figure		
Copy	13 ± 3.6	14 (11–16)
Delayed recall	7.5 ± 3.3	8.0 (6.0–9.5)
Verbal learning abilities and memory		
CAVLT-2 (standardized scores)[b]		
Immediate Recall	90 ± 18	91 (76–102)
Delayed Recall	96 ± 15	96 (86–107)
Level of Learning	99 ± 15	101 (92–108)
Motor function		
Visual-motor coordination		
Eye-Hand Coordination (DTVP-2) (mean standardized scores)	6.1 ± 2.4	6.0 (4.0–8.0)
Fine motor		
WRAVMA Pegboard (standardized scores)	108 ± 14	110 (100–118)
Psychomotor speed		
Reaction Time Test (raw scores)[a,c]		
Mean response latency (ms)	.68 ± .24	.63 (.51–.81)

Abbreviations: WISC-IV, Wechsler Intelligence Scale for Children 4th edition; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; and WRAVMA, Wide Range Assessment of Visual Motor Ability; CPRS-R, Conner's Parent Rating Scale-Revised; ADHD, attention deficit/hyperactivity disorder; LDD-15, Lanthony Desaturated D-15 test; and CCI, color confusion index.
[a] Higher scores reflect worse performance.
[b] Data were missing for 1 child.
[c] Data were missing for 5 children.

concentrations were observed for the additional variables presented in Table 1.

The distribution of pesticide metabolite concentrations in children's urine ($n = 140$) is shown in Table 2. Median (25th–75th percentiles) TCPy, ETU, and 3-PBA concentrations were 1.4 (.7–3.1), 1.2 (.7–3.0), and .8 (.5–1.5) μg/L, respectively. Pesticide metabolite concentrations were weakly correlated [range of Spearman correlation coefficients: .21–.32 ($p < .01$)]. ICCs were .52 for TCPy, .67 for ETU, and .32 for 3-PBA, indicating moderate temporal reliability (Table 2).

3.2. *Pesticide exposures and neurobehavioral outcomes*

Summary statistics for the children's performance on the various neurobehavioral tests are presented in Table 3. With the exception of the RTT, CPRS-R, and LDD-15 tests, the outcomes followed a normal distribution and fell within the normal range. Notably, mean WISC-IV Perceptual Reasoning, Working Memory, and Processing Speed Indexes were below average (Wechsler, 2007), whereas Fine Motor scores were above average.

3.2.1. *Intellectual ability*

As shown in Table 4, overall, we found null associations between urinary TCPy and ETU concentrations and intellectual ability outcomes. However, in sex-stratified analyses, we observed that higher TCPy concentrations were associated with poorer Working Memory Indexes in boys (β per 10-fold increase in TCPy concentrations = −7.5, 95% CI: −14.4, −.7), but not in girls (β = 1.9, 95% CI: −4.7, 8.4; Table 5 and Fig. 2). With respect to 3-PBA, higher concentrations were associated with poorer Processing Speed Indexes (Table 4). This association was stronger in girls (β = −8.8, 95% CI: −16.4, −1.4) than in boys (β = −1.9, 95% CI: −8.5, 4.6; Table 5).

3.2.2. *Behavioral problems*

Table 6 shows TCPy concentrations were associated with increased odds using the 75th percentile as cut-off points for parent-reported cognitive problems/inattention (aOR = 5.8, 95% CI: 1.6, 22.9), oppositional disorders (aOR = 3.9, 95% CI: 1.0, 16.0), and ADHD Index (aOR = 6.8, 95% CI: 1.8, 28.6). We observed null associations of urinary ETU and 3-PBA concentrations and parent-reported behavioral problems.

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Table 4 – Effect estimates (β, and 95% CI) of adjusted[a,b] linear regression models for neurobehavioral outcomes in children aged 6–9 years per 10-fold increase in urinary pesticide concentrations (μg/L), Talamanca County, Costa Rica, 2007 ($n = 140$).

Outcomes[c]	TCPy	ETU	3-PBA
Intellectual ability			
WISC-IV Scales			
Working Memory	−2.1 (−6.6, 2.5)	.3 (−2.7, 3.4)	−.2 (−4.1, 3.7)
Processing Speed	−2.0 (−7.9, 4.0)	−.1 (−4.1, 4.0)	−5.3 (−10.3, −.2)*
Perceptual Reasoning	3.0 (−1.9, 8.0)	1.6 (−1.7, 4.9)	−.4 (−4.7, 3.9)
Perception and memory			
Visuospatial construction and visual memory			
Rey–Osterrieth Figure			
Copy	−.6 (−2.5, 1.2)	.1 (−1.1, 1.3)	−.2 (−1.7, 1.4)
Delayed recall	−1.2 (−3.0, .5)	.2 (−1.0, 1.4)	.4 (−1.1, 1.9)
Verbal learning abilities and memory			
CAVLT-2[d]			
Immediate Recall	−3.9 (−14.0, 6.1)	−3.0 (−9.8, 3.7)	−3.2 (−11.8, 5.5)
Delayed Recall	−.7 (−9.4, 7.9)	−.9 (−6.7, 4.9)	−.8 (−8.2, 6.7)
Level of Learning	−4.1 (−12.8, 4.6)	−7.0 (−12.7, −1.3)*	−2.8 (−10.2, 4.6)
Motor function			
Visual-motor coordination			
Eye-Hand Coordination (DTVP-2) (mean)[d]	−1.4 (−2.7, −.1)*	.0 (−.9, .8)	−.3 (−1.4, .8)
Fine motor coordination			
WRAVMA Pegboard	−.4 (−9.1, 8.3)	.2 (−5.6, 6.1)	.2 (−7.1, 7.5)

Abbreviations: TCPy, 3,5,6-trichloro-2-pyridinol; ETU, ethylenethiourea; 3-PBA, 3-phenoxybenzoic acid; CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; and WRAVMA, Wide Range Assessment of Visual Motor Ability.

#$p < .10$; *$p < .05$.

[a] Models adjusted for maternal education, child's sex, age at assessment, body mass index, number of siblings, children who had repeated a school year, urinary creatinine levels (measured in the same samples in which pesticide levels were quantified), and visual acuity impairment (CAVLT-2 models were not adjusted for the latter variable).

[b] Excluding outliers with studentized residuals > |3|: WISC-IV Working Memory ($n = 2$), Processing Speed (n = 1), Perceptual Reasoning ($n = 2$), Rey–Osterrieth Figure Copy ($n = 1$).

[c] Outcomes are expressed in standardized scores, except for the Rey–Osterrieth Figure test for which raw scores were analyzed.

[d] Data missing for CAVLT-2 and Eye-Hand Coordination (DTVP-2) for one girl.

3.2.3. *Sensory function (color discrimination)*

Table 6 illustrates that higher TCPy concentrations were associated with a poorer ability to discriminate color: the probability of having a mean CCI ≥ 1.5 (75th percentile) increased 6.6 times (95% CI: 1.6, 30.3) per 10-fold increase in TCPy concentrations. No statistically significant associations were found between ETU or 3-PBA concentrations and color discrimination.

3.2.4. *Perception and memory*

Table 4 shows children with higher urinary ETU concentrations had poorer verbal learning abilities (β = −7.0, 95% CI: −12.7, −1.3; Table 4). Children with increased TCPy concentrations tended to have delayed visual memory scores (β per 10-fold increase in TCPy concentrations = −1.2, 95% CI: −3.0, .5), but associations were not statistically significant. Associations were similar for boys and girls. Children's 3-PBA concentrations were not associated with perception and memory outcomes (Table 5).

3.2.5. *Motor function*

Children with higher TCPy concentrations had worse visual motor coordination scores (β = −1.4, 95% CI: −2.7, −.1; Table 4). In contrast, we found null associations of ETU and 3-PBA concentrations with motor outcomes (Table 4). Except for 3PBA, associations were similar for boys and girls (Table 5).

Children with higher urinary TCPy and 3-PBA concentrations tended to have poorer psychomotor speed, as shown by their longer mean reaction times, compared to those with lower concentrations [adjusted odds ratio (aOR) (using 75th percentile used as cut-off point) per 10-fold increase in TCPy and 3-PBA urinary concentrations = 2.1, 95% CI: .5, 8.5; and aOR = 1.5, 95% CI: .6, 4.3; respectively], although results were not statistically significant (Table 6).

4. Discussion

Our results indicate that children living near banana plantations and plantain farms are exposed to pesticides that may affect their neurodevelopment. More specifically, we observed that exposure to chlorpyrifos may impair visual motor coordination, behavior, and color discrimination abilities in both girls and boys, and working memory in boys. In addition, we found that exposure to mancozeb and/or its metabolite ETU may affect negatively children's verbal learning. We also found that exposure to chemical pyrethroids may impair children's processing speed, particularly in girls.

Our findings contrast with results of the cross-sectional studies by Fiedler et al. (2015) and Lu et al. (2009). Although urinary TCPy and 3-PBA concentrations measured in this study were lower than those reported by Fiedler et al. (2015),

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Table 5 – Effect estimates (β, and 95% CI) of adjusted[a,b] linear models for neurobehavioral outcomes in boys ($n = 69$) and girls ($n = 71$) aged 6–9 years, per 10-fold increase in urinary pesticide concentrations (μg/L), Talamanca County, Costa Rica, 2007.

Outcomes[c]	TCPy			ETU			3-PBA		
	Boys	Girls	p_{int}	Boys	Girls	p_{int}	Boys	Girls	p_{int}
Intellectual ability									
WISC-IV Scales									
Working Memory[d]	−7.5 (−14.4, −.7)*	1.9 (−4.7, 8.4)	.15	−3.6 (−8.4, 1.1)	3.2 (−.8, 7.3)	.04	−.8 (−6.4, 4.9)	1.0 (−4.5, 6.5)	.62
Processing Speed[e]	.4 (−7.5, 8.3)	−3.3 (−12.5, 5.8)	.13	−.2 (−5.3, 5.8)	.4 (−5.4, 6.2)	.45	−1.9 (−8.5, 4,6)	−8.8 (−16.1, −1.4)*	.04
Perceptual Reasoning[f]	5.9 (−1.8, 13.6)	.3 (−6.9, 7.5)	.35	.3 (−5.2, 5.9)	2.6 (−1.9, 7.0)	.49	3.8 (−2.7, 10.3)	−3.9 (−9.8, 2.0)	.08
Perception and memory									
Visuospatial construction and visual memory									
Rey–Osterrieth Figure									
Copy[g]	−1.5 (−3.9, 1.0)	−.3 (−3.1, 2.5)	.94	−.8 (−2.6, .9)	1.0 (−.8, 2.7)	.46	−.6 (−2.7, 1.5)	.3 (−2.1, 2.6)	.60
Delayed recall	−1.1 (−4.0, 1.7)	−1.7 (−4.0, .7)	.41	.4 (−1.6, 2.4)	.3 (−1.2, 1.8)	.53	1.1 (−1.3, 3.4)	−.2 (−2.2, 1.8)	.06
Verbal learning abilities and memory									
CAVLT-2[f]									
Immediate Recall	.4 (−14.0, 14.7)	−10.1(-25.3, 5.2)	.12	−6.2 (−16.2, 3.9)	−2.2 (−11.9, 7.5)	.80	2.1 (10.0, 14.2)	−7.4 (−20.1, 5.4)	.35
Delayed Recall	1.8 (−10.5, 14.2)	−3.9 (−16.8, 9.0)	.40	−3.9 (−12.7, 4.8)	−.5 (−7.7, 8.6)	.81	7.4 (−2.9, 17.7)	−8.1 (−18.7, 2.6)	.09
Level of Learning	−9.0 (−21.9, 4.0)	−1.1 (−13.2, 11.0)	.61	−8.0 (−17.1, 1.2)	−5.6 (−13.1, 1.9)	.59	.5 (−10.6, 11.7)	−4.8 (−14.8, 5.3)	.27
Motor function									
Visual motor coordination									
Eye-Hand Coordination (DTVP-2) (mean)[f]	−.9 (−2.9, 1.0)	−2.2 (−4.1, −.3)*	.62	.1 (−1.3, 1.5)	−.2 (−1.4, 1.0)	.89	.4 (−1.2, 2.1)	−1.1 (−2.7, .5)	.48
Fine motor									
WRAVMA Pegboard	−3.2 (−16.0, 9.7)	3.4 (−7.5, 14.4)	.65	−.5 (−9.6, 8.6)	.7 (−6.2, 7.7)	.95	5.8 (−4.9, 16.5)	−3.8 (−13.0, 5.3)	.17

Abbreviations: TCPy, 3,5,6-trichloro-2-pyridinol; ETU, ethylenethiourea; 3-PBA, 3-phenoxybenzoic acid; CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; and WRAVMA, Wide Range Assessment of Visual Motor Ability.

#$p < .10$; *$p < .05$.

[a] Models adjusted for maternal education, child's age at assessment, body mass index, number of siblings, urinary creatinine levels (measured in the same samples in which pesticide levels were quantified), and visual acuity impairment (CAVLT-2 models were not adjusted for this variable).

[b] Excluding outliers with studentized residuals > |3|.

[c] Outcomes are expressed in standardized scores, except for the Rey–Osterrieth Figure test for which raw scores were analyzed.

[d] For boys, two outliers with studentized residuals > |3| was excluded from analysis.

[e] For boys, one outlier with studentized residuals > |3| was excluded from analysis.

[f] For girls, two outliers with studentized residuals > |3| were excluded from analysis.

[g] For girls, one outlier with studentized residuals > |3| was excluded from analysis.

[h] Data were missing for CAVLT-2 and Eye-Hand Coordination (DTVP-2) for one girl.

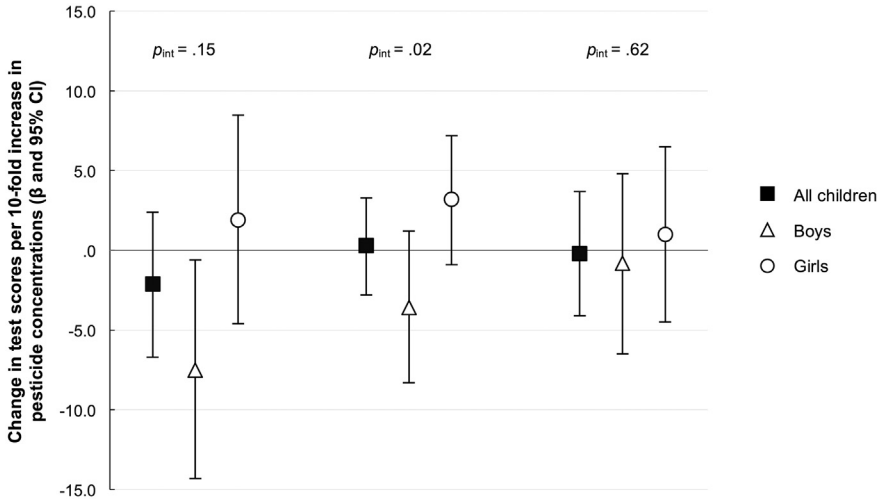

Fig. 2 – Adjusted linear models for WISC-IV Working Memory IQ at age 6–9 years per 10-fold increase in urinary pesticide concentrations (μg/L) stratified by child sex. Models adjusted for maternal education, child's age at neurobehavioral assessment; body mass index, number of siblings, urinary creatinine levels, and visual acuity impairment. *Abbreviations*: TCPy, 3,5,6-trichloro-2-pyridinol; ETU, ethylenethiourea; 3-PBA, 3-phenoxybenzoic acid; WISC-IV, Wechsler Intelligence Scale for Children 4th edition.

we found that children's urinary pesticide metabolite concentrations were associated with neurobehavioral outcomes, whereas Fiedler et al. (2015) and Lu et al. (2009) observed null associations. A study by Sánchez Lizardi et al. (2008) reported that children with higher non-specific urinary OP dialkylphosphate (DAP) metabolites had worse cognitive performance; yet associations lost statistical significance after the exclusion of two extreme urinary DAP concentrations. Possibly, the studies by Fiedler et al. (2015), Lu et al. (2009) and Sánchez Lizardi et al. (2008) lacked statistical power as they included only 53 children or less.

Several of our results are similar to those reported by a few prospective studies (Rauh et al., 2011; Bouchard et al., 2011; Fortenberry et al., 2014). For instance, a U.S. study found that prenatal exposure to chlorpyrifos was associated with poorer Working Memory and Full-Scale IQ scores in children at age 7 (Rauh et al., 2011), whereas, in our study, we observed a negative association between urinary TCPy concentrations and Working Memory IQ scores but only among boys. Unfortunately, the prospective study conducted in the U.S. did not examine associations for postnatal chlorpyrifos exposure or interaction by child sex. In a different cohort study of mother–child pairs living near agricultural fields in California (CHAMACOS), prenatal OP exposure, estimated using DAP metabolites, was also associated with poorer Working Memory IQ scores in school-age children (Bouchard et al., 2011), but in this study, null associations were observed for postnatal OP exposures, and no sex differences were found.

A third cohort study conducted in Mexico (n = 187) by Fortenberry et al. (2014) found borderline significant associations of urinary TCPy concentrations measured during the third trimester of pregnancy with increased parent-reported ADHD indexes in boys and attention problems in girls at age 6–11 years. In our study, we observed that higher TCPy concentrations were associated with increased risks of children's oppositional disorder, cognitive problems/inattention and ADHD as reported by their mothers, but we could not examine sex differences due to limited statistical power. Notably, although urinary TCPy concentrations were comparable between the Mexican study and ours, the temporal reliability of TCPy concentrations was higher in our study (Fortenberry et al., 2014). Results from our study are also consistent with findings from U.S. cohort studies that observed that higher prenatal chlorpyrifos and OP exposures were associated with ADHD/attention problems in children aged 3–5 years (Marks et al., 2010; Rauh et al., 2006).

Our findings on the associations between pyrethroid exposure and neurodevelopment are somewhat consistent with the few epidemiological studies that have been published to date. For example, one cross-sectional study that analyzed data from NHANES 1999–2002 reported null associations of urinary 3-PBA concentrations with parental report of ADHD in children 6–15 years of age (Quirós-Alcalá et al., 2014). Conversely, another study that also used NHANES 2011–2012 data, found that higher urinary 3-PBA concentrations were associated with increased odds of an ADHD diagnosis and number of ADHD symptoms in boys, but not girls, aged 8–15 years (Wagner-schuman et al., 2015). A study of 6–11 years old Canadian children found that postnatal pyrethroid exposure was associated with more frequent parent report of conduct problems that were more pronounced in girls compared to boys (Oulhote & Bouchard, 2013). In our study, we did not find any associations between 3-PBA concentrations and parent-reported behavioral problems, but we did observe children with higher urinary 3-PBA concentrations tended to have poorer attention outcomes, reflected by their longer (mean) reaction times, compared to those with lower concentrations.

To our knowledge, this is the first study to report on the potential neurobehavioral effects of mancozeb and its metabolite ETU. Therefore, we cannot compare our findings to those from previous studies. Additionally, this is the first study to report on children's pesticide exposure and possible

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Table 6 – Odds ratios (and 95% CI) of adjusted[a] logistic models for neurobehavioral outcomes in children aged 6–9 years, per 10-fold increase in urinary pesticide concentrations (μg/L), Talamanca County, Costa Rica, 2007 ($n = 124$).

Outcomes[b]	TCPy	ETU	3-PBA
Sensory function (color discrimination)			
LDD-15 (mean CCI ≥ P50)	3.5 (1.0, 12.9)*	1.2 (.5, 2.9)	.8 (.3, 2.2)
LDD-15 (mean CCI ≥ P75)	6.6 (1.6, 30.3)*	1.1 (.4, 3.1)	1.7 (.5, 5.8)
Behavioral problems			
CPRS-R (T-scores ≥ P50)			
Cognitive Problems/ Inattention	3.2 (.9, 11.8)#	1.0 (.4, 2.4)	.5 (.2, 1,4)
Oppositional Disorder	3.8 (1.2, 13.5)*	.6 (.2, 1.3)	1.6 (.6, 4.5)
Hyperactivity	1.9 (.6, 6.3)	1.4 (.6, 4.3)	1.1 (.0, 10.1)
ADHD Index	2.0 (.6, 6.7)	.7 (.3, 1.4)	.6 (.2, 1.6)
CPRS-R (T-scores ≥ P75)			
Cognitive Problems/ Inattention	5.8 (1.6, 22.9)**	1.0 (.4, 2.4)	.7 (.2, 2.0)
Oppositional Disorder	3.9 (1.0, 16.0)*	.7 (.3, 189)	1.5 (.5, 5.0)
Hyperactivity	2.6 (.6, 10.8)	1.1 (.4, 2.8)	.8 (.2, 2.6)
ADHD Index	6.8 (1.8, 28.6)**	.9 (.4, 2.2)	.8 (.2, 2.6)
Psychomotor speed			
Reaction time test (raw scores ≥ P50)[c]	2.4 (.7, 8.9)	1.3 (.6, 3.1)	.4 (.1, 1.3)
Reaction time test (raw scores ≥ P75)[c]	2.1 (.5, 8.5)	1.5 (.6, 4.3)	.7 (.2, 2.6)

Abbreviations: TCPy, 3,5,6-trichloro-2-pyridinol; ETU, ethylenethiourea; 3-PBA, 3-phenoxybenzoic acid; OR, odds ratio; CI, confidence interval; CPRS-R, Conner's Parent Rating Scale-Revised; ADHD, attention deficit/hyperactivity disorder; LDD-15, Lanthony Desaturated D-15 test; and CCI, color confusion index.

#$p < .10$; *$p < .05$; **$p < .01$.

[a] Models adjusted for maternal education, child's sex, age at assessment, body mass index, number of siblings, urinary creatinine levels (measured in the same samples in which pesticide concentrations were quantified), and visual acuity impairment.

[b] Reference category for all outcomes: raw scores, T-scores, or mean ICC < 50 or 75th percentile, respectively.

[c] Data missing for 5 children.

effects on visual motor coordination and sensory function such as color discrimination abilities. Nevertheless, consistent with our findings, a study in chlorpyrifos-applicators used the same test that we did (LDD-15) and found that increased urinary TCPy concentrations were associated with poorer color discrimination (Dick, Steenland, Krieg, & Hines, 2001).

As exposures, overall, were similar for boys and girls, neurobehavioral effects may differ between boys and girls. Chlorpyrifos, mancozeb, and chemical pyrethroids are suspected neuroendocrine disruptors that may lead to sexually dimorphic behavioral differences in children (Rosenfeld & Trainor, 2014; Venerosi, Ricceri, Tait, & Calamandrei, 2012). Yet, a limitation of this study is its relatively small sample size ($n = 140$), further reduced in the stratified analyses, which limited our statistical power. Therefore, the interactions by sex that were identified as statistically significant should be interpreted with caution. Nevertheless, our study population was more than twice as large as those reported by previous cross-sectional studies on pesticide exposure and neurodevelopment in school-age children (Sánchez Lizardi et al., 2008; Lu et al., 2009; Fiedler et al., 2015).

A strength of our study is that pesticide exposures were estimated using biomonitoring, which is considered the gold standard for exposure assessment because it takes into account all routes of exposure, and reflects uptake, absorption, and metabolism (Fenske, Bradman, Whyatt, Wolff, & Barr, 2005). An additional strength is that, for more than a quarter of our study population ($n = 40$), we obtained one or more repeated urine samples that allowed us to understand whether urinary concentrations represented longer-term exposures (Rosner, 2006), as the pesticide metabolites that were measured have relatively short biological half-lives.

The inferences that can be drawn from the present study are limited by its relatively small sampling size that is reflected by the wide confidence intervals for some of the odds ratios. Another limitation is its cross-sectional study design. However, we can almost certainly assume that exposures started already during pregnancy for most children. Most children (86%) had lived in the same village during their whole life, where pesticide use has been similar during the last decade. In addition, we observed an acceptable temporal reliability for pesticide concentrations, which suggests that measured concentrations predict chronic exposures at least to some extent. Nevertheless, we do not know what period of exposure the urinary pesticide metabolites reflect, and whether the observed associations are a result of prenatal or postnatal exposure, chronic or acute, or a combination of those mentioned. Another limitation of our study is that some the tests that were administered may be influenced by cultural differences and have not been standardized for Costa Rican populations. We therefore *a priori* decided to exclude the tests of WISC-IV's Verbal Comprehension Index from the assessment. Although remaining WISC indexes were below average, variability in test scores was still large enough to detect statistically significant associations for increasing pesticide metabolite concentrations. Also, since we compared children with different gradients of pesticide exposure who lived in similar socio-economic and cultural contexts, and all received similar education, effects on test results due to cultural differences would affect all children similarly, independently of their exposure status.

5. Conclusions

Our results indicate that children aged 6–9 years living in the vicinity of banana and plantain plantations in Talamanca County, Costa Rica, have higher levels of exposure to chlorpyrifos and mancozeb compared to children living near organic farms. In contrast, the somewhat lower urinary 3-PBA concentrations seemed to reflect residential exposure or exposure through food, as concentrations did not differ between villages. Our findings indicate that exposure to chlorpyrifos may impair children's cognition, behavior, and motor and sensory function; mancozeb and pyrethroids may also affect some of their cognitive abilities. In addition, we observed some sex differences in the associations between pesticide exposure and neurobehavioral outcomes that merit more research. In view of these findings, we recommend

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implementing measures to reduce pesticide exposure in children living nearby banana and plantain plantations to prevent possible neurobehavioral effects due to these exposures.

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