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Prenatal current-use pesticide exposure and children's neurodevelopment at one year of age in the Infants' Environmental Health (ISA) birth cohort, Costa Rica

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ABSTRACT

Background: Pesticide exposure may affect young children's neurodevelopment, but only few cohort studies have addressed possible effects of non-organophosphate pesticides.
Objective: We evaluated associations between prenatal current-use pesticide exposure and neurodevelopmental outcomes among 1-year-old children from the Infants' Environmental Health (ISA) birth cohort.
Methods: To determine prenatal pesticide exposure, we measured biomarkers of pyrimethanil, chlorpyrifos, synthetic pyrethroids, and 2,4-D in urine samples among 355 women, 1–3 times during pregnancy. One-year post-partum, we evaluated children's neurodevelopment with the Bayley Scales of Infant and Toddler Development 3rd edition (BSID-III). We assessed associations between exposures and neurodevelopmental outcomes (composite and z-scores) using single-chemical linear regression models adjusted for possible confounders (maternal education, parity, sex, gestational age at birth, child age, HOME-score, location of assessment, biomarkers of mancozeb), and studied effect-modification by sex. We evaluated non-linear associations of multiple pesticide exposures with Bayesian kernel machine regression (BKMR).
Results: We found higher prenatal urinary 2,4-D concentrations were associated with lower language ($\beta_{\text{per ten-fold increase}} = -2.0$, 95 % confidence interval (CI) = −3.5, −0.5) and motor ($\beta_{\text{per ten-fold increase}} = -2.2$, 95 %CI = −4.2, −0.1) composite scores among all children. Also, higher chlorpyrifos exposure [measured as urinary 3,5,6-trichloro-2-pyridinol (TCPy)] was associated with lower cognitive composite scores ($\beta_{\text{per ten-fold increase}} = -1.9$, 95 %CI = −4.7, 0.8), and lower motor composite scores among boys ($\beta_{\text{per ten-fold increase}} = -3.8$, 95 % CI = −7.7, 0.1) but not girls ($\beta_{\text{per ten-fold increase}} = 2.3$, 95 %CI = −1.6, 6.3, pINT = 0.11). Finally, higher pyrimethanil was associated with lower language abilities among girls, but not boys. Pyrethroid metabolite concentrations did not explain variability in BSID-III composite scores. Associations were similar for BSID-III z-scores, and we found no evidence for non-linear associations or mixture effects.
Discussion: Prenatal exposure to common-use pesticides may affect children's neurodevelopment at 1-year of age, some effects may be sex-specific.

1. Introduction

Pesticide use in agriculture, vector control and at home, may expose people in both their work and living environment (Mora et al., 2020; van Wendel de Joode et al., 2012, 2014). Prenatal pesticide exposures may affect children's neurodevelopment, as many pesticides can cross the

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placenta and the blood-brain barrier (Bradman et al., 2003) and the neurodevelopmental system is particularly vulnerable during pregnancy and early life (Eskenazi et al., 2007; Watkins et al., 2016).

To date, several birth cohorts have studied organophosphate (OP) pesticide exposures and young (≤3 years of age) children's neurodevelopment (Roberts et al., 2012). Results of these studies have shown exposure to OPs, including chlorpyrifos, may affect young children's cognitive (*e.g.* working memory, language) and motor development, and may also contribute to behavioral problems (Eskenazi et al., 2007; Guo et al., 2019, 2007; V. A. Rauh et al., 2006). With respect to pyrethroid insecticides, Fluegge et al. (2016) reported elevated 3-phenoxy benzoic acid (3-PBA), but not dichlorovinyl-dimethylcyclopropane carboxylic acid (DCCA), was associated with decreased mental scores (Bayley-II) among infants aged 3 months, while An et al. (2022) showed that cis-DBCA and 3-PBA were associated with affective disorders and externalizing behaviors, respectively, among two-year-old children. A recent Chinese cohort study showed that increased prenatal urinary 3PBA concentrations were associated with poorer language and cognitive development among infants at one year of age (Qi et al., 2022).

Fungicides such as mancozeb and pyrimethanil may also affect children's neurodevelopment, possibly through alteration of the thyroid function (Bernabò et al., 2017; Jansen et al., 2019). Results from the Infants' Environmental Health (ISA, for its acronym in Spanish) cohort showed mancozeb and pyrimethanil were associated with hypothyroidism-like effects (Corrales Vargas et al., 2022). Furthermore, prenatal exposure to mancozeb, determined by maternal urinary ethylene thiourea (ETU) and manganese hair (MnH) and blood (MnB) concentrations during pregnancy, was associated with poorer socioemotional development at one year of age among girls, but not boys (Mora et al., 2018). Additionally, increased prenatal MnH concentrations were associated with poorer cognitive development among girls, as well as poorer socioemotional development among boys at 1-year of age (Mora et al., 2018). Excess Mn may be partly due to mancozeb exposure, as mancozeb contains 20 % w/w Mn (van Wendel de Joode et al., 2016).

Regarding to the herbicide 2,4-D, results from a previous study showed prenatal 2,4-D exposure was associated with slower auditory response times among newborns at six weeks of age (Silver et al., 2019) and results from animal studies have associated 2,4-D exposure with impaired recognition memory (Ueda et al., 2021), hyperactivity, and stereotypic movements (Bortolozzi et al., 1999). Underlying biological mechanisms may involve disruption of motor neurons (Gaaied et al., 2020), alteration of cholinesterase activity (Gaaied et al., 2019), and myelin deficits in the brain (Duffard et al., 1996).

Therefore, to expand knowledge on the potential neurodevelopmental effects of exposure to current-use pesticides in infants, we evaluated whether increased maternal prenatal urinary biomarkers of exposure to 2,4-D, synthetic pyrethroid insecticides, the fungicide pyrimethanil, and chlorpyrifos were associated with decreased neurodevelopmental outcomes in 1-year-olds from the ISA birth cohort.

2. Methods

2.1. Study population

ISA is a community-based prospective birth cohort study situated in the Costa Rica Caribbean, in a rural area with large-scale banana plantations (Mora et al., 2014; van Wendel de Joode et al., 2014). The study population is exposed to pesticides used in agriculture (*e.g.* mancozeb, pyrimethanil, chlorpyrifos), on pasture (*e.g.* 2,4-D), and at home or governmental vector control programs (*e.g.* synthetic pyrethroids). They are also exposed to excess Mn from the fungicide mancozeb as well as from natural sources (Mora et al., 2014; van Wendel de Joode et al., 2016). Detailed methods for the ISA study have been described previously (Mora et al., 2014, 2015, 2018; van Wendel de Joode et al., 2014). In short, from March 2010 to June 2011, we enrolled 451 women (94 % response rate) who were >15 years old, <33 weeks pregnant, and lived at ≤5 km from a banana plantation. We identified women through meetings in local schools, communal groups, advertisements, and referral. All women gave written informed consent prior to participation; for women <18 years of age, their legal representative also gave written informed consent. All study activities were approved by the Scientific Ethics Committee of Universidad Nacional in Costa Rica (CECUNA, for its acronym in Spanish; CECUNA-11-2009).

Of the 451 women originally enrolled in the study, 22 (5 %) had a miscarriage or stillbirth and 69 (15 %) were lost to follow-up before the one-year visit. Of the remaining 360 mother-child pairs, 355 (99 %) had at least one maternal urine sample measured during pregnancy and their children had completed the administration of one or more neurodevelopment scales at 1 year of age (Mora et al., 2018). This population (n = 355) did not differ significantly from the initial cohort (n = 451; van Wendel de Joode et al., 2014) with respect to age, marital status, maternal education, parity, family income, work status at enrollment, and prenatal specific gravity-corrected urinary ETU, MnH, and blood Mn (MnB) concentrations (Mora et al., 2018).

2.2. Maternal interviews

We visited women 1–3 times during pregnancy, depending on their gestational age at enrollment: 26 % of mothers were interviewed three times; 63 % two times and 11 % once (van Wendel de Joode et al., 2014). Median gestational age at each visit during pregnancy was: 19, 30, and 33 weeks of gestation, respectively. We subsequently visited women-infant pairs ~7 weeks after delivery and ~1 year postpartum (Mora et al., 2015, 2018). We collected information on sociodemographic, occupational, socioeconomic, dietary characteristics, lifestyle habits, pesticide use, and medical history at each visit using structured questionnaires. Data were also abstracted from medical records given to the mothers by hospital/clinic personnel. We estimated gestational age at birth using the last menstrual period date, information from early ultrasounds (<14 weeks of gestation), and medical records.

2.3. Neurodevelopment assessment at one year of age

We administered a Spanish-translated version of the Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III; Bayley, 2006) to children aged 13.2 months (range = 10.9–19.4 months) at the ISA field office (80 %) or alternative quiet and private space (*e.g.*, church, community center, school or day care, 20 %) (Mora et al., 2018). The BSID-III includes four domains: cognition, motor function (fine and gross motor subtests), language (receptive and expressive language subtests), and social-emotional development. All tests were administered directly to the infant except for the social-emotional scale that was assessed through a maternal questionnaire. We calculated standardized composite scores for the four domains [derived from the sum of age-corrected subtest scaled scores (language and motor domains) or equivalents to scaled scores (cognitive and social-emotional domains); mean ± SD = 100 ± 15, range = 40–160], using the norms of a sample representative of the U.S. population for children between 1 and 42 months of age (Bayley, 2006). We also calculated BSID-III z-scores (mean ± SD = 0 ± 1), standardizing the raw scores for each subtest (i.e., cognitive, fine motor, gross motor, receptive language, expressive language, and socio-emotional) within our study population.

Mothers were also administered the Infant-Toddler Home Observation for Measurement of the Environment (HOME) inventory short form (Bradley and Caldwell, 1984), the U.S. Department of Agriculture Food Security Scale (six-item short form; Bickel et al., 2000), and the Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977), as these factors may influence infants' neurodevelopmental outcomes.

2.4. Urinary pesticide metabolite measurements

During pregnancy, we collected 1–3 samples of maternal urine

(during the same visit as the pregnancy interviews) using 100 mL beakers (Vacuette ®, sterile), aliquoted into 15 mL tubes (PerformRTM Centrifuges tubes, Labcon ®, sterile) and then stored at −20 °C until shipment (−4 °C) to Lund University, Sweden, for their analysis (van Wendel de Joode et al., 2014). A total of 93 women (26 %) provided three samples during pregnancy, 222 women (63 %) provided two samples and 40 (11 %) provided only one (total n = 763).

Urine samples were analyzed for the following biomarkers of pesticide exposure: hydroxypyrimethanil [OH-PYR, metabolite of pyrimethanil, limit of detection (LOD) =] 0.06 μg/L, hydroxythiabendazole (5-OH-TBZ, metabolite of thiabendazole, LOD = 0.03 μg/L), 3,5,6-trichloro-2-pyridinol (TCPy, metabolite of chlorpyrifos, LOD = 0.06 μg/L); DCCA (metabolite of *e.g.* permethrin, cypermethrin, and cyfluthrin, LOD = 0.04 μg/L), 3PBA (metabolite of *e.g.* permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, and fenvalerate, LOD = 0.03 μg/L), and dichlorophenoxiacetic acid (2,4-D, parent compound, LOD = 0.02 μg/L) using liquid chromatography mass spectrometry (LC/MS/MS; QTRAP 5500; Sciex, Framingham, MA, USA; Ekman et al., 2013; Mora et al., 2020; Norén et al., 2020; van Wendel de Joode et al., 2014). The laboratory participates successfully in the German External QUality Assessment Scheme (G-EQUAS) coordinated by University of Erlangen-Nuremberg, Germany for TCPy and 3PBA analysis (see Supplemental S1).

Concentrations of TCPy, 3PBA, DCCA and 2,4-D were detected in 100 %, 99.7 %, 99.5 % and 99.7 % of the 763 samples, respectively, whilst OHP was detected in 87 % of samples. 5-OH-TBZ was detected in 65 % of the samples. Urinary pesticide metabolite concentrations <LOD and >0.5 * LOD were imputed with the value indicated by the analytical equipment, whilst samples <0.5 * LOD were set at 0.5 * LOD. Specific gravity (kg/l) (sg) of each urine sample was measured with a hand refractometer, and pesticide metabolite concentrations were corrected for dilution using the formula: $M_{sg} = M * [(1.017\text{-}1)/(SG\text{-}1)]$, where M_{sg} is the specific gravity-corrected metabolite concentration (μg/l), M is the observed metabolite concentration (μg/l), SG is the specific gravity of the urine sample, and 1.017 kg/l is the average specific gravity for all urine samples included in this study.

2.5. Reduction of bias

We attempted to reduce information bias by using well standardized protocols for data collection and pilot testing of all procedures; applying structured questionnaires, previously piloted among women with similar educational background; training interviewers to obtain information in a standardized way; training of the psychometrist (JCC) at both National Institute of Perinatology, Mexico City, Mexico and at Berkeley, applying BSID-III among Mexican infants of similar age, and subsequently piloting application among Costa Rica children of the same age who were not part of the ISA cohort. The psychometrist was closely supervised by an experienced neuropsychologist (DH) during the full period of data collection, all tests were applied by the same person who was blind to the maternal and infant's exposure status.

2.6. Statistical analyses

We limited our statistical analysis to biomarkers of exposure that were detected in at least 85 % of the urine samples, resulting in the exclusion of 5-OH-TBZ (65 % of the 763 samples had detectable concentrations), and calculated the average of the urinary specific gravity-adjusted concentrations for each biomarker during pregnancy. We calculated intraclass correlation coefficients (ICC) for pesticide metabolites using mixed-effects models (McGraw and Wong, 1996). As the distributions of urinary pesticide biomarkers were skewed, we $\log_{10}$ transformed concentrations prior to statistical analysis. We generated distributional plots and descriptive statistics for all exposure, outcome, and co-variables. We ran bivariate linear regressions models between each BSID-III score and all the biomarkers of exposure and covariables. We then ran separate multivariate regression models for each exposure biomarker with each of the BSID-III composite and z-scores (receptive language, expressive language, fine motor abilities, gross motor abilities, cognitive, and socioemotional abilities), controlling for potential confounders.

To reduce confounding bias, we included a priori potential confounders and known predictors of child neurodevelopment identified in a previous publication of the ISA cohort in which we reported associations of exposures to mancozeb and excess manganese with BSID-III scores by Mora et al. (2018): maternal education (≤6/>6 years), parity (0/≥1), sex (girl/boy), gestational age at birth (<37/≥37 weeks), child age (months), HOME-score [≤ median (=8)/>median], location of assessment at 1-year visit (field office/other) (see Supplemental S2 for directed acyclic graph), as well as biomarkers of mancozeb exposure and excess manganese $\log_{10}$MnH, $\log_{10}$MnB, $\log_{10}$ETU. Although BSID-III composite scores are corrected for prematurity and the child's age, we still included these variables in our models because of using U.S. norms to calculate composite scores, as no Latin American norms have been published to date. Furthermore, for all children, we checked if associations were different for exposures during the first (<20 weeks of gestation) and second (≥20 weeks of gestation) half of pregnancy. We also analyzed possible effect modification by sex, by adding an interaction term for each metabolite by sex and subsequently ran stratified analyses. We considered effect modification possible if p-value of the interaction term was <0.2. We checked if models met assumptions statistical criteria of validity (normality of residuals, lack of multicollinearity, homoscedasticity, Cook's distance). We ran a sensitivity analysis including additional covariables to the model: poverty (below/above poverty line), breast feeding (</≥6 months), and country of birth (Costa Rica/other).

Finally, we examined two additional models including all metabolites simultaneously. First, Bayesian multivariate linear regression models employing stochastic search variable selection (SSVS) were implemented for each BSID-III score. These models examine additive effect estimates of all exposures on the response, while also assigning probabilities that each variable is included in the final model. Second, a more general model that explored exposure-response relations using a Bayesian kernel machine regression (BKMR) was implemented, accommodating interaction and non-linear associations (Bobb et al., 2018). The standard spike-and-slab prior was used in the linear models to recover the probabilities of inclusion (George and McCulloch, 1993).

Multiple imputation was used to address missing data in the covariables. The Multiple Imputation Chain Equation package for R was employed to run the models (Buuren and Groothuis-Oudshoorn, 2011). This method is preferred than the merely substitution of the missing values by the mean or any other estimate (Molenberghs and Verbeke, 2005) and performs adequately with databases that have variables measured in different scales (*e.g.*, nominal and continuous), as the method allows the use of different models to impute variables depending on variables' scale. All statistical analyses were performed in R-4.3.0 (R Core Team, 2022).

3. Results

3.1. Sociodemographic characteristics and neurobehavioral performance

Table 1 shows that the children's mean age at time of assessment was 13.8 months (SD = 1.6 months). Half of the infants were girls, and most were born at term (94 %), weighed more than 2500 g (97 %) at birth, and were breastfed for 6 months or more (77 %). Mothers were young (mean age 24.1 years, SD = 6.6 years; 64 % ≤ 24 years) and had low educational attainment (51 % had primary school or less). Most of the women were born in Costa Rica (84 %), married/living as married (74 %), multiparous (66 %), and living below the Costa Rican poverty line (66 %). More than a third of the mothers (38 %) were food insecure and about a quarter reported depressive symptoms (CES-D scale, ≥24

Table 1
Characteristics of mother-infant pairs at the child-age of 1-year (mean = 13.8, sd = 1.6 months), ISA birth cohort, Costa Rica.

Characteristics	Value	n (%)
Child age in months at evaluation[1,2]	≤13.3	183 (51.5 %)
	>13.3	172 (48.4 %)
Child sex	Girl	177 (49.9 %)
	Boy	178 (50.1 %)
Low birth weight[3]	No	345 (97.2 %)
	Yes	10 (2.8 %)
Preterm birth[3]	No	332 (93.5 %)
	Yes	23 (6.5 %)
Breast-feeding	≥6 months	273 (76.9 %)
	<6 months	82 (23.1 %)
Maternal age in years at enrollment	<18	60 (16.9 %)
	18–24	165 (46.5)
	25–29	64 (18.0 %)
	30–35	36 (10.1 %)
	30 or more	30 (8.4 %)
Maternal Education	≤6 years	180 (50.7 %)
	>6 years	175 (49.3 %)
Country of birth	Costa Rica	297 (83.7 %)
	Other	58 (16.3 %)
Marital status	Married/living as married	264 (74.4 %)
	Other	91 (25.6 %)
Parity[3]	0	230 (64.6 %)
	1 or more	125 (35.4)
Gestational anemia	No	209 (58.9 %)
	Yes	146 (41.1 %)
Poverty status[3]	< poverty line	235 (66.2 %)
	≥ poverty line	120 (33.8 %)
Food security status	Very low	34 (9.6 %)
	Low	101 (28.4 %)
	High/marginal	220 (62.0 %)
Location of assessment[3]	Field office	285 (80.3 %)
	other	70 (19.7 %)
Home-score[1-4]	≤8	221 (62.3 %)
	>8	134 (37.7 %)
Social support score	Low	167 (47.0 %)
	High	188 (53.0 %)
Maternal depression (CES-D[3,5] score)	No < 24	262 (74.0 %)
	Yes ≥24	93 (26.2 %)

[1] variable shown in two groups, ≤ and > median value.
[2] variable included as continuous in the regression model.
[3] Missing observations: low birth weight n = 5; preterm birth n = 7; parity n = 1; poverty status n = 36; location of assessment n = 1; depression n = 6.
[4] = Home Observation for the Measurement of the Environment scale, scored out of 12.
[5] CES-D, Center for Epidemiologic Studies Depression Scale.

points).

Table 2 shows the BSID-III composite scores and their mean and standard deviations for all children, and stratified by sex. Mean motor and cognitive scores were near 100, 97.3 and 98.2, respectively, whilst language and socioemotional scores were lower, 90.1 and 90.3, respectively. Girls had somewhat higher language and socioemotional composite scores as compared to boys. Results from simple linear regression models showed known predictors of neurodevelopment were associated with outcomes in the expected directions (Table S3).

Table 2
Bayley-III composite scores at the child-age of 1-year (mean = 13.8, sd = 1.6 months), ISA birth cohort, Costa Rica.

Dimension	All infants		Girls		Boys	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Language	346	90.1 (7.1)	173	91.6 (7.0)	173	88.6 (6.8)
Motor	338	97.3 (8.9)	172	97.9 (9.2)	166	96.6 (8.5)
Cognitive	355	98.2 (9.5)	177	98.2 (9.7)	178	98.2 (9.4)
Socioemotional	352	90.3 (11.9)	175	91.7 (12.1)	177	88.8 (11.6)

3.2. Concentrations of prenatal urinary pesticide biomarkers

Table 3 shows that temporal variability in biomarkers of exposure measured within pregnant women was substantial, as intraclass correlations ranged from 0.26 to 0.37. Highest median (25th-75th percentiles) specific gravity-corrected urinary concentrations were observed for TCPy = 1.75 μg/L (1.31–2.54), followed by DCCA = 1.30 μg/L (0.78–2.30); 3PBA = 0.79 μg/L (0.50–1.31); OHP = 0.53 μg/L (0.21–1.32); and 2.4-D = 0.33 μg/L (0.23–0.53). The distributions of all biomarkers were skewed to the right (Fig. 1).

3.3. Neurodevelopmental outcomes and prenatal pesticide exposure

Fig. 2 (A-D) shows beta coefficients with 95 % confidence intervals adjusted for maternal education, parity, sex, gestational age at birth, child age, HOME-score, location of assessment at 1-year visit, log10MnH, MnB and log10ETU. The figures present results from separate models of associations of biomarkers with BSID-III composite scores for all children as well as stratified by sex (see Table S4 for unadjusted associations and Table S5 for adjusted associations). Among all children, higher prenatal urinary 2,4-D concentrations were associated with lower language ($\beta_{\text{per ten-fold increase}} = -2.0$, 95 % confidence interval (CI) = −3.5, −0.5) and motor ($\beta_{\text{per ten-fold increase}} = -2.2$, 95 %CI = −4.2, −0.1) composite scores. Also, higher TCPy was associated with lower cognitive composite scores ($\beta_{\text{per ten-fold increase}} = -1.9$, 95 %CI = −4.7, 0.8) among all children, and lower motor composite scores among boys ($\beta_{\text{per ten-fold increase}} = -3.8$, 95 % CI = −7.7, 0.1) but not girls ($\beta_{\text{per ten-fold increase}}$ = 2.3, 95 %CI = −1.6, 6.3, pINT = 0.11). Furthermore, higher urinary OHP concentrations were associated with lower scores of girls' language abilities ($\beta_{\text{per ten-fold increase}}$ = -1.7, 95 % CI = −3.5, 0.1) but not boys' ($\beta_{\text{per ten-fold increase}} = 0.01$, 95 % CI = −1.6, 1.5, pINT = 0.14). Pyrethroid metabolite concentrations did not explain variability in BSID-III composite scores. For all children, models that included exposure biomarkers concentrations obtained during the first versus second half of pregnancy showed similar beta coefficients (data not shown). Beta coefficients of biomarkers of exposure were also similar for models that also included the covariables poverty, breast feeding, and country of birth (data not shown).

Fig. 3 (A-F) shows the adjusted associations between biomarkers of exposure and BSID-III z-scores among all children and stratified by sex (see also Table S6 for unadjusted results and Table S for adjusted results). In general, associations for z-scores were similar to those of composite scores. For example, among all children, higher prenatal 2,4-D was associated with: 1) lower receptive ($\beta_{\text{per ten-fold increase}} = -0.24$, 95 % CI = −0.44, −0.04) and expressive ($\beta_{\text{per ten-fold increase}} = -0.20$, 95 % CI = −0.51, 0.11) language abilities; 2) lower fine motor abilities ($\beta_{\text{per ten-fold increase}} = -0.17$, 95 % CI = −0.37, 0.03), but also; 3) lower socioemotional abilities ($\beta_{\text{per ten-fold increase}} = -0.15$, 95 % CI = −0.33, 0.02). Consistent with results from composite scores, higher TCPy was associated with somewhat lower cognitive scores ($\beta_{\text{per ten-fold increase}} = -0.21$, 95 % CI = −0.49, 0.07), and lower fine motor z-scores among boys ($\beta = -0.40$, 95 %CI = −0.86, 0.08) but not girls ($\beta_{\text{per ten-fold increase}} = 0.13$, 95 %CI = −0.19, 0.44; pINT = 0.06). Also, higher levels of OHP were associated with lower receptive language z-scores among girls ($\beta_{\text{per ten-fold increase}} = -.15$, 95 %CI = −0.33, 0.04) but not boys ($\beta_{\text{per ten-fold increase}} = -0.03$, 95 %CI = −0.23, 0.18) although the interaction-term was not significant pINT = 0.31. Finally, biomarkers of pyrethroid exposure were not associated with behavioral outcomes expressed as z-scores.

The results from the Bayesian multivariate linear regressions of composite scores with SSVS including all exposures are presented in Table S8. In general, the estimates from these models were consistent with the single-exposure regression models, as 2,4D showed strongest inverse associations for language $\beta_{\text{per ten-fold increase}} = -0.98$ (−3.14, 0.13) and motor abilities $\beta_{\text{per ten-fold increase}} = -0.75$ (−3.21, 0.35), although beta estimates were attenuated. Also, the models that included

Table 3
Distribution of specific-gravity corrected maternal urine biomarkers concentrations (μg/L) and ICCs during pregnancy, ISA study (n = 763 urinary samples from 355 women), ISA birth cohort, Costa Rica.

		Individual concentrations (n = 763, 355 women)		Averaged concentrations (μg/L, n = 355)						
Mother compound	Biomarker	%>LOD	ICC	Min	p10	P25	P50	P75	P90	Max
2,4-D	2,4-D	99.7 %	0.28	0.09	0.17	0.23	0.33	0.53	1.13	79.76
Synthetic Pyrethroids	3PBA	99.7 %	0.28	0.10	0.30	0.50	0.79	1.31	2.46	16.96
	DCCA	99.5 %	0.26	0.15	0.50	0.78	1.30	2.30	3.75	23.56
Pyrimethanil	OHP	87.0 %	0.28	0.03	0.12	0.21	0.53	1.32	3.10	368.55
Thiabendazole	OHT	65.0 %	0.39	0.01	0.02	0.03	0.10	0.62	2.48	339.00
Chlorpyrifos	TCPy	100 %	0.37	0.41	0.95	1.31	1.75	2.54	4.51	62.96

Note. LOD = level of detection; ICC = Intraclass correlation coefficient.

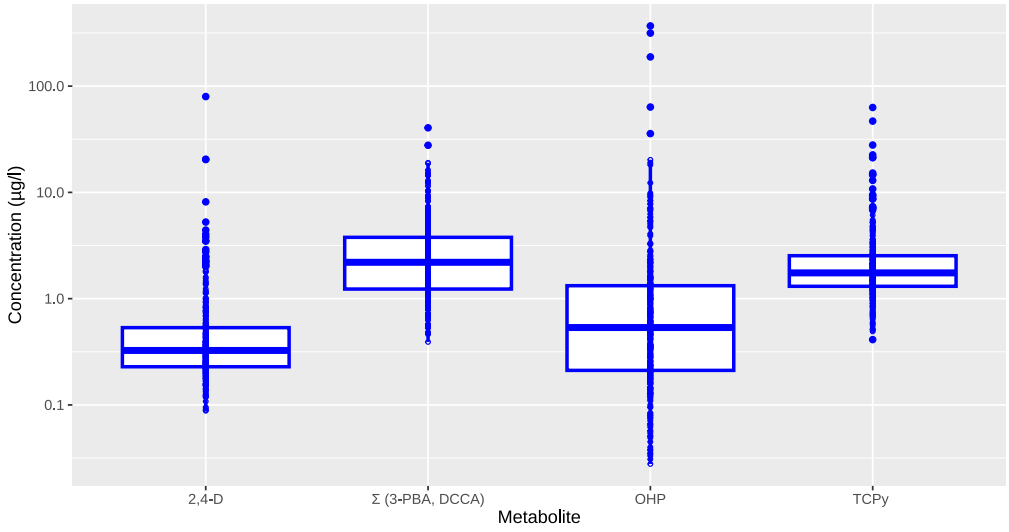

Fig. 1. Distribution of prenatal averaged prenatal urinary pesticide metabolite concentrations with >85 % of samples > LOD, ISA birth cohort, Costa Rica.

z-scores (Table S9) showed negative associations for 2,4-D and receptive language abilities (β = −0.07, 95 %CI = −0.31, 0.03), fine motor abilities (β = −0.03, 95 %CI = −0.20, 0.04), as well as a negative association between TCPy and cognitive abilities (β = −0.04, 95 %CI = −0.26, 0.08).

Tables S10 and S11 show the posterior inclusion probabilities (PIPs) of all biomarkers of exposure in both the Bayesian multivariate linear regression models with SSVS and BKMR for composite and z-scores, respectively. Regarding the SSVS models, these probabilities are congruent with the results from the single-exposure regression models as 2,4-D is considered the best predictor for language abilities (PIP = 76 % and 64 % for language composite scores and receptive z-scores, respectively) and motor abilities (PIP = 63 % and 54 % for motor composite scores and fine motor z-scores, respectively). This, while TCPy was identified as the best predictor for cognitive abilities among both composite and z-scores (PIP = 54 % and 51 %, respectively) after adjusting for other exposures. The PIPs for the BKMR models were consistently lower than the linear regressions, indicating BKMR modelling did not improve data fit. Nevertheless, also for BKMR models, 2,4-D was the best predictor for both language composite scores and receptive language z-scores after adjusting for other exposures.

4. Discussion

The results of our birth cohort study on prenatal pesticide exposure and infants' neurodevelopment at 1-year of age showed higher prenatal urinary 2,4-D concentrations were consistently associated with poorer language and motor abilities among all children. Further examination showed these inverse associations were stronger for receptive as compared to expressive language abilities. Higher prenatal 2,4-D exposure was also associated with lower motor abilities, driven by poorer fine motor scores. Higher prenatal chlorpyrifos exposure (measured by urinary TCPy) was associated with lower cognitive (all children) and motor (boys) abilities, albeit associations were not statistically significant. Lastly, pyrimethanil exposure was associated with lower language abilities, particularly among girls.

To date, to our knowledge, only one previous study has addressed 2,4-D exposure and infants' neurodevelopment. Results from this previous study showed prenatal 2,4-D exposure was associated with slower auditory response times among newborns at six weeks of age (Silver et al., 2019). This seems consistent with our finding of 2,4-D being associated with lower receptive language abilities among infants at 1-year of age, as Antinmaa et al. (2020) found that a slower auditory processing capacity is associated with a smaller receptive lexicon at 1 year of age. In animal studies, exposure to 2,4-D has been associated with several neurodevelopmental outcomes, that include impaired recognition memory (Ueda et al., 2021), hyperactivity, and stereotypic movements (Bortolozzi et al., 1999).

Our finding that higher prenatal TCPy concentrations were associated with lower cognitive (all children) and motor development (boys), albeit associations were not statistically significant, is consistent with a robust body of research that suggests chlorpyrifos, and other OP insecticides, negatively impact children's neurodevelopment (Burke et al., 2017; Chiu et al., 2021; Dalsager et al., 2019; Fluegge et al., 2016; Guo et al., 2019; Marks et al., 2010). Specifically, a small cohort study in Ohio found lower mental functioning at three months of age in infants who had increased chlorpyrifos exposure at 2 months of age (Fluegge et al., 2016). This, whilst other birth cohort studies have documented negative associations between chlorpyrifos exposure and psychomotor developmental delays at 3 years of age (Rauh et al., 2006), and attention

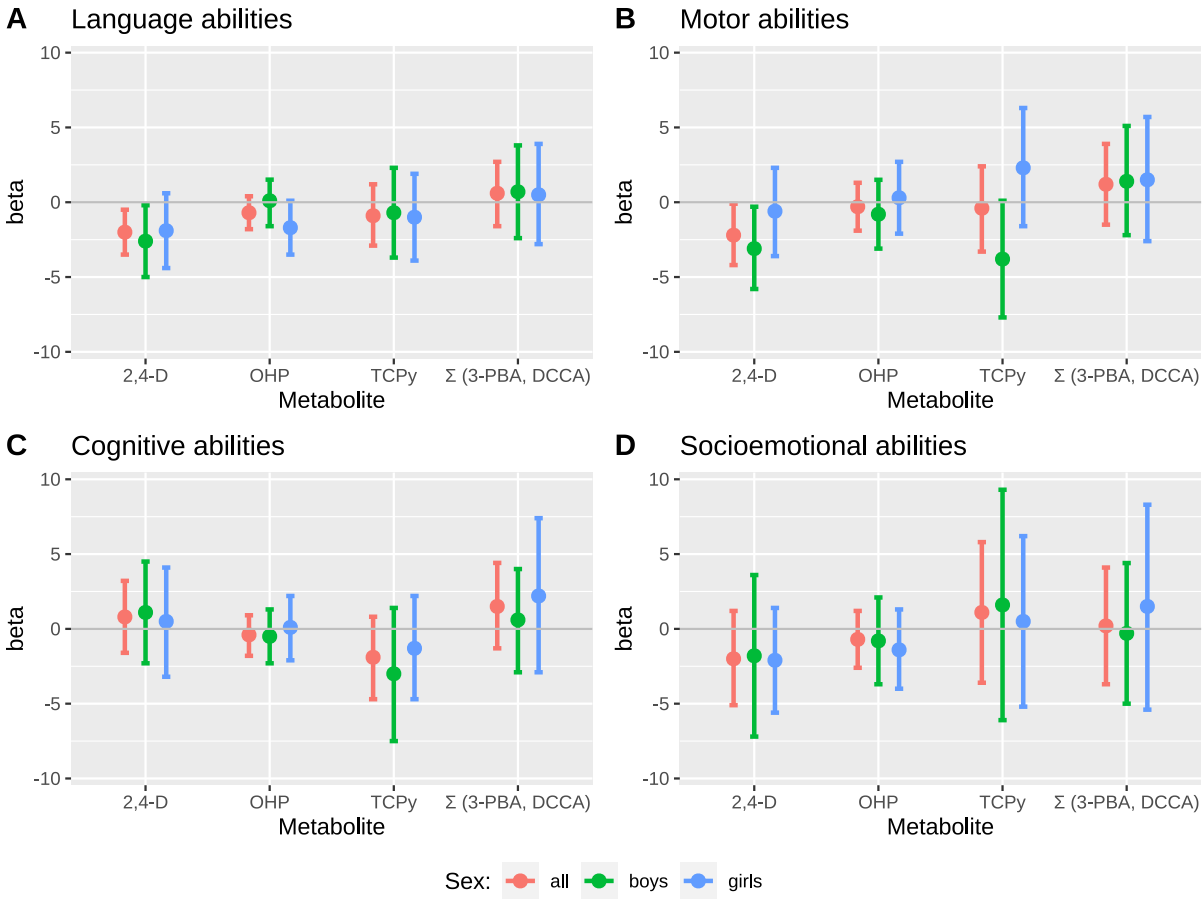

Fig. 2. Adjusted beta estimates with 95 %CI estimated from separate multivariate linear regression models of biomarkers with BSID-III composite scores at the child-age of 1 year (n = 355); adjusted a priori for maternal education, parity, sex, gestational age at birth, and child age, HOME-score, location of assessment at 1-year visit, log10MnH, MnB, log10ETU; also results from sex-stratified analyzes are presented.

deficits at 5 years of age (Marks et al., 2010). Also, postnatal exposure to chlorpyrifos at age 3 has been related to lower performance in motor and social abilities among boys, but not girls (Guo et al., 2019). Results from in vitro studies have shown mechanisms by which chlorpyrifos may impair neurodevelopment. For example, chlorpyrifos has been described to disrupt axonal transport and outgrowth controlled by tubulin and other structural proteins (Prendergast et al., 2007). Consequently, exposure to chlorpyrifos during neurodevelopment can cause abnormal patterns of neural connectivity, which in turn induce neurobehavioral alterations (Burke et al., 2017) such as the ones suggested by this study regarding motor and cognitive development.

With respect to the association between higher exposure to pyrimethanil and lower levels of receptive language specially for girls, it is worth noting that previous research from the ISA cohort has shown pyrimethanil exposure during pregnancy was associated with a hypothyroidism-like effect, which in turn may affect young children's neurodevelopment (Corrales Vargas et al., 2022). Finally, in contrast to previous studies, we found null associations for prenatal pyrethroid exposure and neurodevelopment at 1-year of age. This finding we cannot explain, as exposure levels measured in this study were like those reported in these previous studies, and Qi et al. (2022) evaluated children at the same age, using the same scale, as in our study. Yet, the South-African cohort evaluated behavioral and emotional problems at 2 years (An et al., 2022), so at an older age and evaluating a different neurobehavioral outcome as in our study.

One of the strengths of this study is its prospective design with repeated measures of urinary exposure biomarkers in different periods during pregnancy. We explored if beta estimates for all children differed for concentrations of biomarkers of exposure measured during the first versus second half of pregnancy, yet results were similar. Also, the repeated exposure measures enabled us to have a better overall estimate of each biomarker of pesticide exposure during pregnancy, as compared to studies that only collected a single sample during pregnancy (Watkins et al., 2016). The latter is particularly true for biomarkers with relatively short half-lives (Kalkbrenner et al., 2014). Nevertheless, to obtain unattenuated beta estimates more than ten measurements would be needed for each participant (Perrier et al., 2016), which was unfeasible. Another strength of our study is that the longitudinal analysis enabled us to study neurobehavioral outcomes taking into account information about possible confounding factors during pregnancy and at birth, as we obtained detailed information on sociodemographic, occupational, environmental, and exposure variables and health outcomes since the pregnant women were enrolled.

A limitation of our study is that we conducted the neurodevelopmental assessment at 1-year-of-age, which could be too early to detect lasting effects; for example, Bouchard et al. (2011), Rauh et al. (2006, 2011) studied infants at 3 years of age or older. Also, our statistical approach studied the association of pesticides for each neurodevelopmental outcome separately, not considering the relationships between different components of neurodevelopment. With respect to bias, we addressed potential sources of bias during study design, data collection and data analysis. In case information bias occurred, we expect misclassifications of neurobehavioral outcomes to be equally distributed among high and low exposed infants (non-differential misclassification) resulting in an attenuation of exposure-outcome associations towards the null. Furthermore, we attempted to mitigate

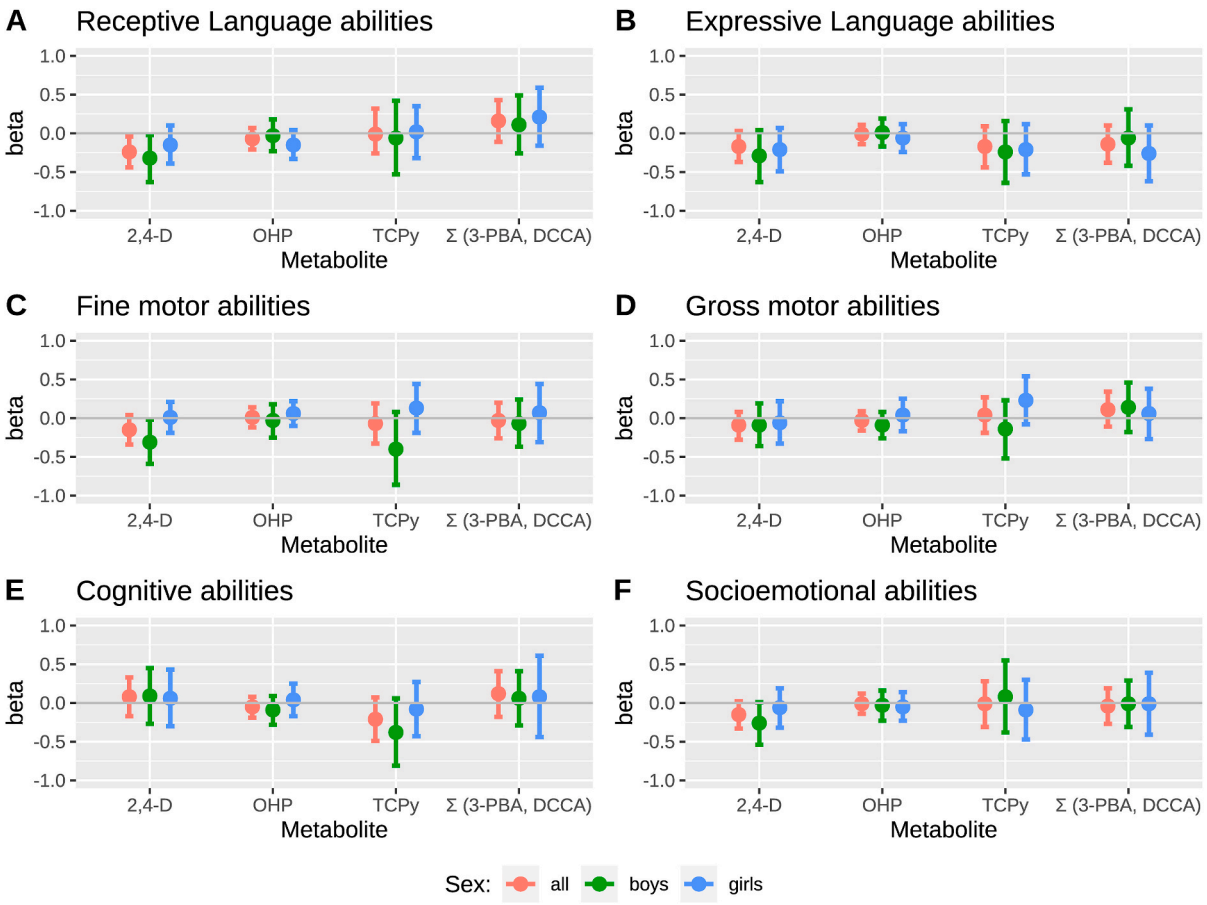

Fig. 3. Adjusted beta estimates with 95 %CI estimated from separate multivariate linear regression models of with BSID-III z-scores at the child-age of 1 year (n = 355); adjusted a priori for maternal education, parity, sex, gestational age at birth, and child age, HOME-score, location of assessment at 1-year visit, log_{10}MnH, MnB, log_{10}ETU; also results from sex-stratified analyzes are presented.

possible bias by confounding, as we adjusted our estimates for confounding variables described in literature. Finally, using a prospective cohort design, we cannot rule out selection bias due to loss-to-follow-up. Yet, substantial selection bias seems unlikely as retainment since the visit performed shortly after birth was 92 %, and general exposure characteristics of women who still participated 1-year post-partum did not differ from women who dropped out since enrollment.

Our findings about possible neurodevelopmental toxicity of current-use pesticides are important, particularly for 2,4-D, as previous studies on 2,4-D exposure and neurodevelopment are almost inexistent. We expect our findings can be generalized to agricultural populations with similar socioeconomic status. Given the increasing exposure of the general population to 2,4-D, Freisthler et al. (2022) recently stressed the importance of understanding the health effects 2,4-D exposure in humans, and especially in children. Together with glyphosates, 2,4-D is one of the most used herbicides in the world (Islam et al., 2018). It is broadly used to control broad-leaved weeds for agricultural and non-agricultural purposes on pasture, turf, lawns, rights-of-way, aquatic sites, forestry sites, and a variety of fruit and vegetable crops (Environmental Protection Agency, 2005); in our study area it was also reported to be used on soccer fields. Between 2002 and 2012, the use of 2,4-D worldwide increased by 40 % (Islam et al., 2018; USDA, 2013). With respect to chlorpyrifos, this insecticide is still widely being used on banana plantations in plastic bags applied to banana bunches to protect the fruits from insects, despite evidence of its neurodevelopmental effects that lead to non-renewal of its use in Europe and the restriction of its use in the United States of America. The fungicide pyrimethanil, a relatively new fungicide, is being sprayed by light aircraft on banana plantations in Costa Rica (Giffin et al., 2022). About half of the infant-mother pairs of the ISA cohort lives at less than 200 m from these plantations, which explain the about five times higher urinary hydroxy pyrimethanil concentrations in our population compared to Swedish adolescents (Norén et al., 2020).

The results from the current and previous studies illustrate the need to reduce children's pesticide exposure, following a precautionary principle. In addition, efforts should be made to alleviate its possible negative impact on children's development, by promoting the stimulation of children's language skills. This is particularly important as children exposed to pesticides, often live in a context with additional environmental and socio-economical stressors that may impair neurodevelopment like low family income per capita, low food security, being born from a mother with little scholarity, whilst language abilities are key to school achievement (Lurie et al., 2021).

Concluding, our findings indicate that prenatal exposure to 2,4-D, chlorpyrifos and pyrimethanil may affect children's neurodevelopment during their first year of age. These results expand the body of research that has reported neurotoxic effects of these pesticides in animal models (Bernabò et al., 2017; Bortolozzi et al., 1999), and add to previous findings among 1-year old children from the ISA cohort that showed prenatal exposure to mancozeb/ETU and excess manganese was associated with lower socioemotional and cognitive development (Mora et al., 2018). Our results are of concern as these pesticides are widely used in both agricultural and non-agricultural settings.

CRediT authorship contribution statement

L. Diego Conejo-Bolaños: Writing – original draft, Methodology, Investigation, Formal analysis. **Ana M. Mora:** Writing – review &

editing, Methodology, Investigation, Data curation. **David Hernández-Bonilla:** Writing – review & editing, Supervision, Methodology, Data curation. **Juan Camilo Cano:** Writing – review & editing, Methodology, Investigation. **José A. Menezes-Filho:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Brenda Eskenazi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Christian H. Lindh:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Berna van Wendel de Joode:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118222.

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