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Prenatal Mancozeb Exposure, Excess Manganese, and Neurodevelopment at 1 Year of Age in the Infants' Environmental Health (ISA) Study

Ana María Mora,[1,2] Leonel Córdoba,[1] Juan Camilo Cano,[1] David Hernandez-Bonilla,[3] Larissa Pardo,[1] Lourdes Schnaas,[4] Donald R. Smith,[5] José A. Menezes-Filho,[6] Donna Mergler,[7] Christian H. Lindh,[8] Brenda Eskenazi,[2] and Berna van Wendel de Joode[1]

[1]Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, Heredia, Costa Rica
[2]Center for Environmental Research and Children's Health (CERCH), School of Public Health, University of California at Berkeley, Berkeley, California, USA
[3]Environmental Health Department, National Institute of Public Health, Mexico City, Mexico
[4]Division of Research in Community Interventions, National Institute of Perinatology, Mexico City, Mexico
[5]Department of Microbiology and Environmental Toxicology, University of California at Santa Cruz, Santa Cruz, California, USA
[6]Faculty of Pharmacy, Federal University of Bahia, Bahia, Brazil
[7]Centre for Interdisciplinary Research Centre on Health, Well-being, Society and Environment (CINBIOSE), University of Quebec in Montreal, Montreal, Quebec, Canada
[8]Division of Occupational and Environmental Medicine, Institute of Laboratory Medicine, Lund University, Lund, Sweden

Background: Although growing evidence suggests that early-life excess manganese (Mn) impairs neurodevelopment, data on the neurodevelopmental effects of mancozeb, a fungicide containing Mn, and its main metabolite ethylenethiourea (ETU) are limited.

Objective: We examined whether prenatal mancozeb exposure and excess Mn were associated with neurodevelopment in 355 1-y-old infants living near banana plantations with frequent aerial mancozeb spraying in Costa Rica.

Methods: We measured urinary ETU, hair Mn, and blood Mn concentrations in samples collected 1–3 times during pregnancy from mothers enrolled in the Infants' Environmental Health (ISA) study. We then assessed neurodevelopment in their 1-y-old infants using the Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III). We estimated exposure–outcome associations using linear regression models adjusted for maternal education, parity, gestational age at birth, child age, Home Observation for Measurement of the Environment score, and location of neurodevelopmental assessment.

Results: Median (P25–P75) urinary ETU, hair Mn, and blood Mn measured during pregnancy were 3.3 μg/L (2.4–4.9; specific gravity–corrected), 1.7 μg/g (0.9–4.1), and 24.0 μg/L (20.3–28.0), respectively. Among girls, higher ETU was associated with lower social-emotional scores [β per 10-fold increase = −7.4 points (95% CI: −15.2, 0.4)], whereas higher hair Mn was associated with lower cognitive scores [−3.0 (−6.1, 0.1)]. Among boys, higher hair Mn was associated with lower social-emotional scores [−4.6 (−8.5, −0.8)]. We observed null associations for blood Mn, language, and motor outcomes.

Conclusions: Our findings indicate that maternal exposure to mancozeb and excess Mn during pregnancy may have adverse and sex-specific effects on infant neurodevelopment. https://doi.org/10.1289/EHP1955

Introduction

Manganese (Mn) ethylene bis-dithiocarbamate (EBDC) fungicides, such as mancozeb and maneb, are widely used in agriculture and professional turf management (U.S. EPA 2005). EBDCs contain approximately 21% Mn by weight (FAO 1980), and recent studies suggest that their use may constitute a source for elevated ethylenethiourea (ETU) (van Wendel de Joode et al. 2014), EBDCs' main metabolite, and Mn (Gunier et al. 2013; Mora et al. 2014; van Wendel de Joode et al. 2016a) in agricultural communities. Naturally occurring Mn in groundwater may also be a source of excess Mn for these communities, whose water supply is mainly from artesian wells (van Wendel de Joode et al. 2016a).

Animal studies have shown that early-life exposure to Mn-containing fungicides and/or to ETU impairs neurodevelopment (Jacobsen et al. 2012; Miranda-Contreras et al. 2005). However, the few epidemiological studies that have examined this association in children have found inconsistent results (Gunier et al. 2015; Mora et al. 2015; van Wendel de Joode et al. 2016b). A cross-sectional study of children 6–9 y old living near banana and plantain plantations in Talamanca County, Costa Rica, observed that higher urinary ETU concentrations were associated with poorer verbal learning outcomes (van Wendel de Joode et al. 2016b). In addition, a study from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) that measured Mn in deciduous teeth from children living near agricultural fields in the Salinas Valley, California, reported an association between higher postnatal dentin Mn levels and decreased mental development scores on the Bayley Scales of Infant Development, 2nd Edition (BSID-II) at 6 and 12 mo of age, but not at 24 mo of age (Gunier et al. 2015). Another study within CHAMACOS found associations of higher prenatal and postnatal dentin Mn levels with poorer behavioral outcomes in school-age boys and girls (7–10.5 y old), but better cognitive, memory, and/or motor function abilities in boys (Mora et al. 2015).

Multiple prospective and cross-sectional studies have assessed the neurodevelopmental effects of excess Mn from sources other than Mn-containing fungicides. Most of these studies have consistently linked exposure to airborne or waterborne Mn with impaired cognitive abilities (Bouchard et al. 2011; Carvalho et al. 2014; do Nascimento et al. 2016; Haynes et al. 2015; Kim et al. 2009; Menezes-Filho et al. 2011; Rahman et al. 2017; Riojas-Rodríguez et al. 2010; Wasserman et al. 2006; Wright et al. 2006), behavioral problems (Bouchard et al. 2007; Khan et al. 2012; Menezes-Filho et al. 2014; Oulhote et al. 2014a; Rahman et al. 2017), and poor memory outcomes (Carvalho et al. 2014; He et al. 1994; Hernández-Bonilla et al. 2011; Lucchini et al. 2012a; Oulhote et al. 2014a) in

Address correspondence to A.M. Mora, Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, P.O. Box 86-3000 Heredia, Costa Rica. Telephone: 506 2277-3677. Email: ana.mora.mora@una.cr

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school-age children, adolescents, or in both. Findings from studies in infants have not been as consistent as those conducted in older children. For example, prenatal Mn exposure has been associated with impaired mental, psychomotor, and/or language skills at 1 and 2 y of age (Claus Henn et al. 2017; Lin et al. 2013; Yu et al. 2016); and with poorer behavior (Ericson et al. 2007) and nonverbal memory at 3 y of age (Takser et al. 2003). Some studies have also reported inverted U-shaped associations of prenatal or early postnatal Mn exposure with BSID-II mental and/or psychomotor developmental scores at 6 mo (Chung et al. 2015) and at 1 y (Claus Henn et al. 2010), and with Bayley Scales of Infant Development, 3rd edition (BSID-III) fine motor scores at 2–3 y of age (Rodrigues et al. 2016).

The Infants' Environmental Health Study (Infantes y Salud Ambiental, ISA) is a community-based birth cohort study examining the health effects of pesticides and Mn in pregnant women and their children living near banana plantations with extensive aerial spraying of Mn-containing fungicides (Bravo Durán et al. 2013) in Matina County, Costa Rica. Previous reports on the ISA cohort have shown elevated urinary ETU concentrations among women who lived close to banana plantations, who washed agricultural work clothes, and who worked in agriculture during pregnancy (van Wendel de Joode et al. 2014). Higher hair Mn concentrations were observed in women who lived close to plantations, who worked in agriculture before pregnancy, or who had elevated Mn concentrations in drinking water, whereas higher blood Mn concentrations were observed in women who lived in crowded houses and in houses made of permeable and difficult-to-clean materials (Mora et al. 2014). Higher drinking-water Mn concentrations in the ISA study were found in houses located close to banana plantations (van Wendel de Joode et al. 2016a).

In the present study, we examined the association of prenatal mancozeb exposure and excess Mn, indicated by urinary ETU and hair and blood Mn concentrations measured in maternal samples collected during pregnancy, with neurodevelopmental outcomes in 1-y-old infants from the ISA study.

Methods

Study Population

Detailed methods for the ISA study have been described elsewhere (Mora et al. 2014; van Wendel de Joode et al. 2014). Briefly, between March 2010 and June 2011, we recruited pregnant women through meetings in local schools, community groups, advertisements, and friends' referrals. Eligible women were ≥15 y old, <33 wk of gestation, and living ≤5 km from a banana plantation in Matina County. A total of 451 women were enrolled in the ISA study. For the present study, we included 355 (79%) children who completed the administration of one or more neurodevelopmental scales at 1 y of age and whose mothers provided at least one urine, hair, or blood sample during pregnancy. Mother–child pairs included in these analyses ($n = 355$) did not differ significantly from the initial cohort ($n = 451$) on their attributes, including maternal education, parity, household income, and prenatal specific gravity–corrected urinary ETU, hair Mn, and blood Mn concentrations (Mora et al. 2014; van Wendel de Joode et al. 2014).

Written informed consent was obtained from all women before participation. Additional informed consent was obtained from parents or legal guardians of participants <18 y of age. The human subjects committee of the Universidad Nacional in Costa Rica (CECUNA) approved all study materials and procedures.

Maternal Interviews

Women were interviewed at their homes one to three times during pregnancy depending on their gestation age at enrollment (median at the first, second, and third visit = 19, 30, and 33 wk gestation, respectively), after delivery (median = 7 wk postpartum), and when children were 1 y old (median = 1.1 year). Sociodemographic and occupational information, including maternal age, education, parity, and household income, was collected at the baseline interview. Data on birth outcomes were abstracted from prenatal and delivery medical records completed by hospital/clinic personnel and were provided to the study participants.

At the 1-y visit, mothers and their children were interviewed and/or assessed at a house that was rented for the ISA study (80%) or at another community facility (e.g., church, community center, school or day care facility; 20%). During this study visit, mothers were administered the Infant-Toddler Home Observation for Measurement of the Environment (HOME) inventory short form (Caldwell and Bradley 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). The questionnaire for the social-emotional scale of the Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III), Spanish version (Bayley 2006), was also completed by the mothers at the 1-y visit.

Urinary ETU Measurements

Maternal urine samples were collected one to three times during pregnancy (at the same time as the pregnancy interviews). Specimens were aliquoted and were stored at −20°C until their shipment to Lund University, Sweden. Samples were then analyzed for ETU using a two-dimensional liquid chromatography mass spectrometer (LC-MS/MS; UFLCRX; Shimadzu Corporation) with a triple quadrupole linear ion trap (QTRAP 5500; AB Sciex) (Ekman et al. 2013). Urinary specific gravity (kg/L) was determined using a hand refractometer, and ETU concentrations were normalized for dilution using the formula $ETU_{SG} = ETU \times [(1.017–1)/(SG - 1)]$, where ETU_{SG} is the specific gravity–corrected ETU concentration (μg/L), ETU is the observed ETU concentration (μg/L), SG is the specific gravity of the urine sample, and 1.017 kg/L is the average specific gravity for our study population. All processed urine samples were analyzed in duplicate with a coefficient of variance (CV) of 10%. Details of urine collection, analysis, and quality control procedures are described elsewhere (van Wendel de Joode et al. 2014). All measured urinary ETU concentrations were above the analytical limit of detection (LOD = 0.08 μg/L).

Hair Mn measurements. Hair samples (~20–30 strands) were collected from the occipital region, within 2 mm from the scalp, at one or two pregnancy visits (conducted at the same time as the pregnancy interviews and the urine sample collection). Samples were stored at 20–25°C (room temperature) and were shipped to the Federal University of Bahia, Brazil. The one-centimeter closest to the scalp from each hair sample was cleaned as described elsewhere (Menezes-Filho et al. 2009) and was analyzed for Mn using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120; Varian, Inc.). Processed hair samples and reference materials were analyzed in duplicate and had CVs that ranged between 1.5 and 7.3%. Only two hair samples had Mn concentrations below the analytical LOD (0.1 μg/L); their values were set at $\mathrm{LOD}/\sqrt{2}$.

Blood Mn measurements. Venous blood samples were collected at one or two pregnancy visits (at the same time as pregnancy interviews and urine and hair sample collection) and were immediately frozen at −20°C. Samples were shipped to the University of California, Santa Cruz, where they were analyzed for Mn using high-resolution inductively coupled plasma mass spectrometry (Finnigan XR ICP-MS) (Smith et al. 2007). The CV of the blood Mn measurements, based on triplicate samples analyzed with each analytical batch, was 3.8%. Blood sample

collection, analysis, and quality control procedures have been described elsewhere (Mora et al. 2014). All blood Mn concentrations were above the analytical LOD (0.003 μg/L).

In addition, lead was measured in all maternal blood samples collected during pregnancy (in the same specimens as those used for Mn quantification) using high-resolution inductively coupled plasma mass spectrometry (Gwiazda et al. 2005; Lucchini et al. 2012b). All blood lead concentrations were above the analytical LOD (0.0016 μg/L).

Neurodevelopmental Outcomes

We assessed child neurodevelopment at the 1-y study visit using a Spanish-translated version of the BSID-III (Bayley 2006). The English version was translated to Spanish by child developmental psychologists (L.S. and her team) at the National Institute of Perinatology in Mexico and was revised by Costa Rican researchers to ensure that the vocabulary was appropriate for our study population. Four domains were evaluated: cognition, motor function (composed of the fine and gross motor subtests), language (receptive and expressive language subtests), and social-emotional development. The first three domains were tested through direct child assessment, and the fourth domain was assessed using a maternal questionnaire. A single psychometrician, who was trained and supervised by a pediatric neuropsychologist, conducted all of the assessments. Quality assurance measures included extensive pilot testing and review of videotaped assessments. Standardized composite scores [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] for the four domains were calculated using norms based on a sample representative of the U.S. population for infants 1 mo through 42 mo of age (Bayley 2006). BSID-III z-scores (mean ± SD = 0 ± 1) were also calculated by standardizing raw scores for each subtest (i.e., cognitive, fine motor, gross motor, receptive language, expressive language, and social-emotional) within our study population.

Statistical Analyses

Distributional plots were generated and descriptive statistics were calculated for all variables. Bivariate associations between biomarkers of exposure, outcomes, and covariates were estimated using t-tests for continuous variables and χ^2 tests for categorical variables. Correlations between specific gravity–corrected urinary ETU, hair Mn, and blood Mn concentrations were estimated using Spearman's correlation coefficients (r_s). To assess the within- and between-woman variability and reproducibility of urinary ETU, hair Mn, and blood Mn concentrations, we calculated intraclass correlation coefficients (ICCs) using mixed-effects models (McGraw and Wong 1996).

We averaged specific gravity–corrected urinary ETU, hair Mn, and blood Mn concentrations across the repeated samples collected for each woman throughout pregnancy. We then examined associations of averaged prenatal urinary ETU, hair Mn, and blood Mn concentrations with BSID-III standardized composite scores using multivariable linear regression models. Because biomarkers were only weakly correlated [r_s ranged between −0.10 and 0.21; strongest correlation for urinary ETU and hair Mn], we simultaneously included urinary ETU, blood Mn, and hair Mn concentrations parameterized as continuous variables in the models. Specific gravity–corrected urinary ETU and hair Mn concentrations were transformed to the $\log_{10}$ scale to normalize the residuals and to reduce the influence of outliers.

We identified potential confounders and known predictors of child neurodevelopment [i.e., maternal education, parity, gestational age at birth, and child age and HOME (raw) scores at the 1-y visit] using directed acyclic graphs and included them *a priori* in our regression models. Although BSID-III composite scores were corrected for prematurity (≤36 weeks gestation) and child's age at assessment during the scoring process, we decided to adjust our models for both variables because composite scores were standardized using U.S. norms given that no Latin American norms have been published to date. We assessed other potential confounders [i.e., maternal age, breastfeeding duration, household income, maternal depression (CES-D scores), food security status at the 1-y study visit, and location of neurodevelopmental assessment] by adding them, one at a time, to the final models (models with *a priori* covariates). Additional covariates (i.e., location of assessment) were included in the final model if they materially changed the magnitude of one or more exposure coefficients (>10%). Missing values (<10%) for covariates were imputed by randomly selecting a value from the subset of observations with known values of the covariate (Lubin et al. 2004).

We evaluated effect modification of the exposure–outcome associations by child sex using cross-product terms and stratifying by sex. We also assessed two-way interaction terms between the three biomarkers (urinary ETU, blood Mn, and hair Mn concentrations) to determine if the effect estimates of a single biomarker differed by varying concentrations of another biomarker. Interactions were considered significant if $p_{INT} < 0.15$.

We conducted several sensitivity analyses to assess the robustness of our results. First, we fitted covariate-adjusted generalized additive models with penalized spline smooth terms for continuous exposures (constrained to a maximum of 3 knots), and we visually assessed plotted splines for evidence of nonlinear exposure–outcome associations. Second, we ran our linear regression models *a*) adjusting for covariates with missing values that were imputed using simple imputation [either assigning the category with the largest number of observations for categorical variables (i.e., parity and location of assessment) or the mean of the distribution for continuous variables (i.e., gestational age at birth and child age at the time of assessment)] and *b*) using complete cases only and compared results with those using the randomly imputed covariates. Third, we fitted multivariate linear regression models using subtests' BSID-III z-scores instead of standardized composite scores, given that reliance on U.S. norm-based standardized composite scores may result in misclassification of the neurodevelopmental scores of Costa Rican children (Cromwell et al. 2014). Fourth, to explore whether our findings were sensitive to differences in the developmental stages of the central nervous system (Donders and Hunter 2010; Semrud-Clikeman and Ellison 2009) or to timing of the exposure measurements (Mora et al. 2014), we reran our analyses using specific gravity–corrected urinary ETU, blood Mn, and hair Mn concentrations for the first (<20 weeks gestation) and second (≥20 weeks) halves of pregnancy. Fifth, we examined the confounding effect of averaged prenatal blood lead concentrations by adding this variable to the final models. All statistical analyses were performed using Stata (version 14.2; StataCorp LLC) and R (version 3.1.2; R Development Core Team).

Results

ISA mothers were relatively young at the time of enrollment (mean age ± SD = 22.4 ± 6.6 y; 17% were ≤18 y old) and predominantly Costa Rican–born (84%), married or cohabitating with their partner (74%), and multiparous (65%; Table 1). Approximately half had completed primary school (51%). Only 9% of mothers worked in agriculture during pregnancy (8% of all mothers included in these analyses worked in banana plantations during pregnancy), whereas 24% did at the 1-y study visit (22% worked in banana plantations). Approximately 65% of the families lived

below the Costa Rican poverty line, and 25% of the mothers were "at risk" for depression at the 1-y study visit (Table 1). Most children were born at term (94%) and had a birth weight >2,500 g (97%). The median age of the children at the time of the neurodevelopmental assessment was 1.1 y (range = 0.9–1.6). Covariate data were complete for the majority of the participants, with household income at the 1-y visit having the largest number of missing values before imputation ($n = 36$, 10%; Table 1).

Median (25th–75th percentiles) specific gravity–corrected urinary ETU, hair Mn, and blood Mn concentrations in maternal samples collected during pregnancy were 3.3 (2.4–4.9) μg/L, 1.7 (0.9–4.1) μg/g, and 24.0 (20.3–28.0) μg/L, respectively (Table 2). Urinary ETU, hair Mn, and blood Mn concentrations were similar for boys and girls (data not shown). Averaged urinary ETU concentrations were weakly correlated with hair Mn ($r_s = 0.21$, $p < 0.01$) and were not correlated with averaged blood Mn concentrations ($r_s = -0.10$, $p = 0.06$); averaged blood Mn and hair Mn concentrations were not correlated ($r_s = -0.06$, $p = 0.29$). Urinary ETU and blood Mn concentrations varied more within than between women (ICC = 0.17 and 0.43, respectively), whereas hair Mn concentrations varied more between women (ICC = 0.59; Table 2).

Mean (±SD) BSID-III cognitive, language, motor, and social-emotional standardized composite scores were 98.2 ± 9.5, 90.1 ± 7.1, 97.3 ± 8.9, and 90.3 ± 11.9 points, respectively (Table 2). Among boys, we observed lower language (mean ± SD = 88.6 ± 6.8 points) and social-emotional (88.8 ± 11.6 points) standardized composite scores than among girls (91.7 ± 7.1 and 91.7 ± 12.2 points, respectively; $p < 0.05$; see Table S1). Boys also had lower fine motor, receptive language, and expressive language z-scores than girls ($p < 0.01$), but social-emotional z-scores were similar for boys and girls (see Table S1).

Although most associations of averaged prenatal urinary ETU, hair Mn, and blood Mn concentrations with BSID-III standardized composite scores among all children hovered around the null, we observed consistently lower social-emotional composite scores at higher hair Mn concentrations [β per 10-fold increase = −2.3 points (95% confidence interval (CI): −5.0, 0.4)] after simultaneously adjusting for urinary ETU and blood Mn concentrations (Table 3). When we stratified by child sex, we found that higher averaged prenatal hair Mn concentrations were associated with lower social-emotional scores among boys [β per 10-fold increase = −4.6 points (95% CI: −8.5, −0.8)] but not among girls [β = −0.2 points (95% CI: −4.2, 3.9); $p_{INT} = 0.17$]. Additionally, higher averaged prenatal hair Mn concentrations were associated with lower cognitive scores among girls [β = −3.0 points (95% CI: −6.1, 0.1)] but not among boys [β = 2.5 points (95% CI: −0.4, 5.4); $p_{INT} = 0.01$]. We also observed that higher averaged specific gravity–corrected urinary ETU concentrations were marginally associated with lower social-emotional composite scores among girls [β per 10-fold increase = −7.4 points (95% CI: −15.2, 0.4)] but not among boys [β = 0.0 points (95% CI: −6.9, 7.0); $p_{INT} = 0.11$]. We observed null associations for blood Mn, language, and motor outcomes (Table 3) and did not find evidence of interaction between biomarkers (see Table S2).

When we fitted our covariate-adjusted generalized additive models with penalized spline smooth terms for continuous exposures, we observed that most exposure–outcome associations were linear (see Figure S1). We found a few nonlinear penalized spline model estimates (i.e., estimated degrees of freedom >1), but these were generally consistent with linear regression model effect estimates. For example, the overall association between prenatal hair Mn concentrations and lower social-emotional scores among boys was statistically significant in our generalized additive models ($p = 0.02$), but this association was inverse over most of the exposure range and was only null for exposures above ∼10 μg/g (see Figure S1B).

When we adjusted our regression models for covariates imputed using simple imputation and using complete cases only (see Table S3), we observed similar results to those from the analyses that included randomly imputed data. When we fitted separate regression models for urinary ETU, blood Mn, and hair Mn concentrations (see Table S4), we found similar exposure–outcome associations to those from the main models (Table 3). When we ran our models for BSID-III subtest z-scores (standardized within our study population) for boys and girls combined (see Table S5), we observed similar

Table 1. Characteristics of study population, Infants' Environmental Health (Infantes y Salud Ambiental, ISA) study ($n = 355$).

Characteristics	n (%)	n (%) Imputed[a]
Maternal/household		
Age (y)[b]		
<18	60 (16.9)	60 (16.9)
18–24	165 (46.5)	165 (46.5)
25–29	64 (18.0)	64 (18.0)
30–34	36 (10.1)	36 (10.1)
≥35	30 (8.5)	30 (8.5)
Education (years completed)[c]		
≤6th grade	180 (50.7)	180 (50.7)
7th–11th grade	165 (46.5)	165 (46.5)
Completed high school	10 (2.8)	10 (2.8)
Parity		
0	121 (35.1)	123 (34.7)
≥1	224 (64.9)	232 (65.3)
Missing	10	0
Smoking during pregnancy		
No	336 (94.7)	336 (94.7)
Yes	19 (5.3)	19 (5.3)
Agricultural work at 1-y visit		
No	270 (76.1)	270 (76.1)
Yes	85 (23.9)	85 (23.9)
Depression at 1-y visit (CES-D score)		
No (<24)	257 (73.6)	262 (73.8)
Yes (≥24)	92 (26.4)	93 (26.2)
Missing	6	0
Household income at 1-y visit		
Above poverty line	107 (33.6)	120 (33.8)
Below poverty line and above extreme poverty line	144 (45.1)	156 (43.9)
Below extreme poverty line	68 (21.3)	79 (22.3)
Missing	36	0
Food security status at 1-y visit		
High or marginal	220 (62.0)	220 (62.0)
Low	101 (28.4)	101 (28.4)
Very low	34 (9.6)	34 (9.6)
Location of neurodevelopmental assessment		
Field office	285 (80.5)	285 (80.3)
Other	69 (19.5)	70 (19.7)
Missing	1	0
Child		
Child's sex		
Boy	178 (50.1)	178 (50.1)
Girl	177 (49.9)	177 (49.9)
Low birth weight (<2,500 g)		
No	340 (97.1)	345 (97.2)
Yes	10 (2.9)	10 (2.8)
Missing	5	0
Preterm birth (<37 wk)		
No	326 (93.7)	333 (93.8)
Yes	22 (6.3)	22 (6.2)
Missing	7	0

Note: CES-D, Center for Epidemiologic Studies Depression Scale; n, number of participants.
[a]Number of observations for each characteristic after imputing missing values by randomly selecting from observed data for each covariate. Imputed data were used in analytic models.
[b]Modeled as a continuous variable in regression models.
[c]Modeled as a dichotomous variable (≤6th grade and >6th grade) in regression models.

Table 2. Distribution of prenatal biomarkers of exposure and child neurodevelopmental outcomes in the study population, Infants' Environmental Health (Infantes y Salud Ambiental, ISA) study ($k = 355$ mother–child pairs).

Exposures and outcomes	n	k	Mean ± SD	Min	Percentile 25th	Percentile 50th	Percentile 75th	Max	σ^2_{btw}	σ^2_{within}	ICC
Prenatal biomarkers of exposure[a]											
Urinary ETU_{SG} (μg/L)[b,c]	764	355	4.4 ± 7.1	0.8	2.4	3.3	4.9	127.4	0.02	0.08	0.17
Hair Mn (μg/g)[c,d]	661	355	3.7 ± 5.4	0.1	0.9	1.7	4.1	53.3	0.16	0.11	0.59
Blood Mn (μg/L)[e]	571	349	24.4 ± 6.2	9.1	20.3	24.0	28.0	50.6	19.92	26.45	0.43
Neurodevelopmental outcomes[f]											
BSID-III composite scores											
Cognitive	—	355	98.2 ± 9.5	70.0	90.0	100.0	105.0	130.0	—	—	—
Language	—	346	90.1 ± 7.1	68.0	86.0	90.0	94.0	109.0	—	—	—
Motor	—	338	97.3 ± 8.9	76.0	91.0	97.0	103.0	124.0	—	—	—
Social-emotional	—	352	90.3 ± 11.9	60.0	80.0	90.0	100.0	130.0	—	—	—

Note: —, no information was collected at that particular examination point. BSID-III, Bayley Scales of Infant and Toddler Development, 3rd edition; ETU_{SG}, specific gravity–adjusted ethylenethiourea; ICC, intraclass correlation coefficient; k, number of women; Max, maximum; Min, minimum; Mn, manganese; n, number of samples; SD, standard deviation.
[a]Descriptive analyses were conducted with averaged concentrations across pregnancy samples, whereas ICC analyses were conducted with the nonaveraged (individual) concentrations. In the women for whom only one Mn or ETU measurement was available, the single measurement was used in lieu of the average.
[b]ETU was measured in 764 urine samples collected from 355 study participants: 93 women provided three urine samples during pregnancy, 223 provided two samples, and 39 provided one sample.
[c]Between- and within-woman variances and ICCs were calculated and reported for $\log_{10}$-transformed specific gravity–adjusted urinary ETU and hair Mn concentrations.
[d]Mn was measured in 661 hair samples collected from 355 study participants: 306 women provided two hair samples, and 49 provided only one.
[e]Whole-blood Mn was measured in 571 blood samples collected from 349 women: A total of 222 women provided two samples during pregnancy, and 127 provided only one.
[f]Not all 355 infants were administered all four BSID scales. For example, only 346 infants were administered the language scale, whereas only 338 were administered the motor scale.

associations to those observed using U.S. norm–based standardized composite scores. Nevertheless, we found that among girls, the inverse association between hair Mn concentrations and cognitive scores was somewhat strengthened [β per 10-fold increase = −0.40 (95% CI: −0.70, −0.09)], whereas the association between urinary ETU concentrations and social-emotional scores was attenuated [β per 10-fold increase = −0.28 (95% CI: −0.78, 0.22)] compared with the associations observed in the main models. Among boys, higher averaged prenatal hair Mn concentrations remained associated with lower social-emotional scores [β = −0.31 (95% CI: −0.57, −0.06)].

When we included averaged prenatal urinary ETU, hair Mn, and blood Mn concentrations for the first or second halves of pregnancy in the adjusted models instead of the averaged concentrations for the entire pregnancy, we observed that the associations of hair Mn concentrations with lower social-emotional scores in boys and lower cognitive scores in girls were stronger during the second half of pregnancy [β per 10-fold increase = −3.9 (95% CI: −7.6, −0.2) and −2.3 (95% CI: −5.7, 1.1), respectively; see Table S6 and Figure S2] than during the first half [β = −2.5 (95% CI: −8.1, 3.1) and −0.7 (95% CI: −4.4, 3.1), respectively; see Table S7 and Figure S2]. In contrast, we found that the association between urinary ETU and lower social-emotional development scores in girls, although imprecise, was somewhat stronger during the first half of pregnancy [β per 10-fold increase = −8.6 (95% CI: −20.5, 3.3); see Table S7 and Figure S2] than during the second half [β = −2.7 (95% CI: −10.9, 5.6); see Table S6 and Figure S2]. Lastly, the addition of prenatal blood lead concentration to the models did not change the point estimates observed in the main analyses (see Table S8), most likely because of the low concentrations

Table 3. Adjusted associations of prenatal $logETU_{SG}$ (μg/L), logMnH (μg/g), and MnB (μg/L) concentrations with BSID-III standardized composite scores at 1 year of age for all children and stratified by child sex (models simultaneously adjusted for all three biomarkers), Infants' Environmental Health (Infantes y Salud Ambiental, ISA) study.

Neurodevelopmental outcomes[a]	All children n	All children β (95% CI)	Boys n	Boys β (95% CI)	Girls n	Girls β (95% CI)	p_{INT}
Cognitive							
$LogETU_{SG}$	349	−1.1 (−4.9, 2.8)	174	−1.8 (−7.1, 3.4)	175	−1.4 (−7.3, 4.5)	0.60
LogMnH	349	−0.2 (−2.3, 1.8)	174	2.5 (−0.4, 5.4)	175	−3.0 (−6.1, 0.1)	0.01
MnB	349	0.1 (−0.1, 0.2)	174	0.1 (−0.1, 0.3)	175	0.0 (−0.2, 0.2)	0.33
Language							
$LogETU_{SG}$	340	−0.6 (−3.6, 2.5)	169	−1.3 (−5.4, 2.9)	171	0.1 (−4.5, 4.6)	0.79
LogMnH	340	0.0 (−1.7, 1.6)	169	0.8 (−1.5, 3.1)	171	−0.6 (−2.9, 1.8)	0.46
MnB	340	0.0 (−0.1, 0.1)	169	0.0 (−0.2, 0.1)	171	0.1 (−0.1, 0.3)	0.26
Motor							
$LogETU_{SG}$	332	−0.3 (−4.3, 3.7)	162	1.2 (−4.3, 6.6)	170	−2.4 (−8.6, 3.8)	0.34
LogMnH	332	0.6 (−1.6, 2.7)	162	0.3 (−2.8, 3.4)	170	1.4 (−1.8, 4.6)	0.99
MnB	332	0.0 (−0.1, 0.2)	162	0.1 (−0.1, 0.3)	170	0.0 (−0.3, 0.2)	0.52
Social-emotional							
$LogETU_{SG}$	346	−3.9 (−9.0, 1.2)	173	0.0 (−6.9, 7.0)	173	−7.4 (−15.2, 0.4)	0.11
LogMnH	346	−2.3 (−5.0, 0.4)	173	−4.6 (−8.5, −0.8)	173	−0.2 (−4.2, 3.9)	0.17
MnB	346	0.1 (−0.1, 0.3)	173	0.1 (−0.2, 0.3)	173	0.1 (−0.2, 0.4)	0.89

Note: Models were adjusted for maternal education, parity, gestational age at birth, and child age, Home Observation for Measurement of the Environment (HOME) score, location of assessment at 1-year visit, and all three biomarkers of exposure. BSID-III, Bayley Scales of Infant and Toddler Development, 3rd edition; $logETU_{SG}$, $\log_{10}$-transformed and specific gravity–adjusted urinary ethylenethiourea; logMnH, $\log_{10}$-transformed hair manganese; MnB, blood manganese; n, number of samples.
[a]Sample sizes vary between neurodevelopmental domains and with respect to Table 2 because not all infants completed all four BSID-III scales, and not all of their mothers contributed urine, hair, and blood samples during pregnancy. For example, 355 infants completed the administration of the BSID-III cognitive scale, and all of them had a maternal urine sample collected during pregnancy (see Table 2), but only 349 of these infants had maternal hair and blood samples.

detected in our study population (median = 6.5 μg/L; 25th–75th percentiles = 5.0–8.6).

Discussion

In a prospective cohort of mother–child pairs living near banana plantations in Costa Rica, we observed that higher prenatal Mn, as indicated by hair Mn concentrations measured in women during pregnancy, was associated with lower cognitive scores among girls and lower social-emotional scores among boys at 1 y of age. We also found that higher prenatal urinary ETU concentrations were associated with lower social-emotional scores among girls. We observed no associations for prenatal blood Mn concentrations, language, or motor function.

Our findings are consistent with studies on early-life excess Mn that have reported inverse associations with cognitive and behavioral outcomes in infants (Claus Henn et al. 2017; Ericson et al. 2007; Lin et al. 2013). However, unlike some studies that have observed biphasic dose–response relationships of prenatal and early postnatal Mn exposure with child neurodevelopment (Chung et al. 2015; Claus Henn et al. 2010; Rodrigues et al. 2016), we found linear exposure–outcome associations. Recently, a birth cohort study of 232 Korean mother–child pairs measured Mn concentrations in maternal blood samples collected at delivery and reported inverse U-shaped associations with both mental and psychomotor development indexes in 6-mo-old children (Chung et al. 2015). A cross-sectional study of 270 12-mo-old Mexican children reported an inverted U-shaped association between blood Mn concentrations and mental development (Claus Henn et al. 2010). Furthermore, a study in 524 2–3 y old Bangladeshi children reported an inverse U-shaped relationship between drinking-water Mn concentrations and concurrent fine motor scores (Rodrigues et al. 2016). Discrepancies between the findings of these studies and our own may be due to the fact that Mn concentrations in our study population could be within the range at which Mn acts as a neurotoxicant rather than as a nutrient with a beneficial capacity, thus resulting in impaired neurodevelopmental outcomes. Blood Mn concentrations in ISA mothers were higher than those reported in Korean (Chung et al. 2015) and French (Takser et al. 2003) mothers, but similar to those observed in mothers living near a Superfund site in Oklahoma (Claus Henn et al. 2017); hair Mn concentrations in ISA mothers were higher than those reported in French mothers (Takser et al. 2003). Inconsistent findings between previous studies and ours, including null associations of prenatal excess Mn with motor function and language, may also be due to differences in sources and pathways of excess Mn [e.g., airborne in Mexico (Claus Henn et al. 2010) vs. mainly waterborne in Costa Rica] and in the timing at which neurodevelopmental assessments were completed [e.g., language and motor outcomes were mostly assessed at 2 and/or 3 y of age in other studies (Lin et al. 2013; Takser et al. 2003; Yu et al. 2016) vs. at 1 y in the ISA study].

In our analyses, we observed sex- and domain-specific differences in the associations of urinary ETU, hair Mn, and blood Mn concentrations with infant neurodevelopment. These differences in exposure–outcome associations, as well as the lack of correlation between biomarkers, could be explained by several factors: *a*) differences in exposure sources [excess Mn is likely to originate from both mancozeb spraying and naturally occurring Mn in groundwater (Mora et al. 2014; van Wendel de Joode et al. 2016a), whereas urinary ETU only originates from mancozeb/ETU spraying (van Wendel de Joode et al. 2014); ETU is not naturally present in the environment]; *b*) differences in exposure pathways [excess Mn is likely due to ingestion of waterborne Mn and inhalation of airborne Mn (Mora et al. 2014; van Wendel de Joode et al. 2016a), whereas urinary ETU is thought to reflect inhalation exposures (van Wendel de Joode et al. 2014)]; or *c*) differences in exposure windows [hair Mn concentrations measured in the 1-cm hair sample closest to the scalp reflect exposures during the last 30 d (Eastman et al. 2013; Skröder et al. 2017); blood Mn and urinary ETU concentrations reflect exposures during the last few days (Coles et al. 2012; WHO 1988)]. Domain-specific differences between biomarkers could also be present because ETU and Mn may have different biological mechanisms for their neurotoxic effects and, consequently, could affect neurodevelopmental domains in different ways. Studies have shown that the neurotoxicity of mancozeb and/or ETU may be mediated by oxidative stress (Domico et al. 2007), interference of the vesicular transport of glutamate (Vaccari et al. 1999), and thyroid homeostasis dysfunction (Doerge and Takazawa 1990). In contrast, some studies have suggested that Mn neurotoxicity may be mediated by dopaminergic dysfunction (Racette et al. 2012) and by disruption of thyroid homeostasis (Soldin and Aschner 2007). Lastly, it is possible that both Mn-containing fungicides and excess Mn could lead to sexually dimorphic neurodevelopmental differences in children. For example, human and animal studies indicate that there might be biological sex differences in response to Mn (Dorman et al. 2004; Madison et al. 2011), such as metabolic variations in the homeostatic mechanisms that regulate Mn concentrations in the human body (Oulhote et al. 2014b). In addition, several pesticides, including EBDCs, are suspected endocrine disruptors that may lead to a differential pattern of association with neurodevelopment for boys and girls (Kjeldsen et al. 2013; Rosenfeld and Trainor 2014). Further toxicological and epidemiological studies are needed to determine how Mn-containing fungicides and excess Mn target different neurodevelopmental domains and whether they cause sex-specific effects.

To date, there is no consensus on which is the best biomarker to assess human exposure to Mn (Coetzee et al. 2016). Hair Mn concentrations could be affected by external contamination (Eastman et al. 2013; Skröder et al. 2017) and by interindividual variability resulting from differences in hair characteristics and personal habits (Chojnacka et al. 2006; Kempson and Lombi 2011). Notably, concentrations of Mn in blood are homeostatically regulated by the hepatic portal system (ATSDR 2012) and increase throughout pregnancy (Mora et al. 2014; Spencer 1999; Takser et al. 2004); therefore, they may not be the best surrogate for maternal Mn load or fetal exposure. Additional research is needed to better understand the connections between environmental sources and internal dose of these biomarkers, as well as the reliability of biomarkers of maternal exposure to Mn-containing fungicides and excess Mn for assessing prenatal exposure to the fetus.

Our study has several limitations. First, we cannot rule out the possibility that selection bias could have arisen from loss to follow-up. Second, we recognize that we conducted multiple comparisons, which could have led to statistically significant associations by chance, but we tried to look for patterns in our results rather than to highlight isolated findings. Third, we assessed child neurodevelopment using a test that has not been standardized in Latin American populations; nevertheless, our standardized composite scores were within the expected range (Bayley 2006), and we observed similar exposure–outcome associations using U.S. norm-based standardized composite and *z*-scores in our models. Fourth, because social-emotional development scores were based on maternal report, we cannot exclude the possibility that there might have been information bias in the mothers' responses; however, we would not expect reporting differences between mothers who had high ETU or Mn concentrations during pregnancy and those who had low concentrations because mothers were not aware of their prenatal concentrations at the time of the 1-y study visit. Fifth, it is possible that 1 y of age may be too early to identify long-

lasting neurodevelopmental effects of prenatal exposures to environmental toxicants; at the present time, further neurodevelopmental assessments of the ISA study participants are being conducted to determine if exposure–outcome associations observed in infancy persist until mid- and late childhood.

This is among the first studies to examine the potential neurodevelopmental impact of exposure to Mn-containing fungicides in children living in agricultural communities. It is also one of the few prospective studies to assess the association of prenatal excess Mn with neurodevelopment, which strengthens the growing body of literature on this topic. In our analyses, we were able to examine and adjust for several important prenatal and postnatal factors such as lead exposure, socioeconomic indicators, maternal education, and child stimulation.

Conclusion

Our present findings indicate that prenatal exposure to mancozeb and excess Mn, as measured by urinary ETU and hair Mn concentrations during pregnancy, may be associated with poorer cognitive abilities in girls and with worse social-emotional development in boys and girls at 1 y of age. In view of these results and the precautionary principle, we recommend improving the infrastructure and management of water sources to reduce elevated Mn concentrations in drinking water (van Wendel de Joode et al. 2016a) and implementing measures to reduce environmental exposure to mancozeb/ETU [e.g., increasing the distance between banana plantations and residential areas, washing work clothes at the workplace (Mora et al. 2014; van Wendel de Joode et al. 2014; van Wendel de Joode et al. 2016a)] in pregnant women and children living near banana plantations to prevent further neurodevelopmental effects and other adverse health outcomes resulting from these exposures.

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