

# UC Berkeley

## UC Berkeley Previously Published Works

### Title

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

### Permalink

<https://escholarship.org/uc/item/2jk464d8>

### Journal

Environmental Health Perspectives, 126(5)

### ISSN

0091-6765

### Authors

Mora, Ana María  
Córdoba, Leonel  
Cano, Juan Camilo  
[et al.](#)

### Publication Date

2018-05-21

### DOI

10.1289/ehp1955

Peer reviewed

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

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

<sup>1</sup>Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, Heredia, Costa Rica

<sup>2</sup>Center for Environmental Research and Children's Health (CERCH), School of Public Health, University of California at Berkeley, Berkeley, California, USA

<sup>3</sup>Environmental Health Department, National Institute of Public Health, Mexico City, Mexico

<sup>4</sup>Division of Research in Community Interventions, National Institute of Perinatology, Mexico City, Mexico

<sup>5</sup>Department of Microbiology and Environmental Toxicology, University of California at Santa Cruz, Santa Cruz, California, USA

<sup>6</sup>Faculty of Pharmacy, Federal University of Bahia, Bahia, Brazil

<sup>7</sup>Centre for Interdisciplinary Research Centre on Health, Well-being, Society and Environment (CINBIOSE), University of Quebec in Montreal, Montreal, Quebec, Canada

<sup>8</sup>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 [ $\beta$  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](mailto:ana.mora.mora@una.cr)

Supplemental Material is available online (<https://doi.org/10.1289/EHP1955>).

The authors declare they have no actual or potential competing financial interests.

Received 27 March 2017; Revised 10 April 2018; Accepted 24 April 2018; Published 29 May 2018.

**Note to readers with disabilities:** *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact [ehponline@niehs.nih.gov](mailto:ehponline@niehs.nih.gov). Our staff will work with you to assess and meet your accessibility needs within 3 working days.

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  $\geq 15$  y old,  $< 33$  wk of gestation, and living  $\leq 5$  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  $-20^{\circ}\text{C}$  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 ( $\mu\text{g/L}$ ),  $ETU$  is the observed ETU concentration ( $\mu\text{g/L}$ ),  $SG$  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  $\mu\text{g/L}$ ).

**Hair Mn measurements.** Hair samples ( $\sim 20$ – $30$  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  $20$ – $25^{\circ}\text{C}$  (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  $\mu\text{g/L}$ ); their values were set at  $\text{LOD}/\sqrt{2}$ .

**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  $-20^{\circ}\text{C}$ . 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  $\chi^2$  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 ( $\leq 36$  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  $p_{\text{INT}} < 0.15$ .

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 ( $\geq 20$  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  $\leq 18$  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

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

| Characteristics                                   | $n$ (%)    | $n$ (%) Imputed <sup>a</sup> |
|---|------------|------------------------------|
| <b>Maternal/household</b>                         |            |                              |
| Age (y) <sup>b</sup>                              |            |                              |
| <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) <sup>c</sup>          |            |                              |
| ≤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                            |
| <b>Child</b>                                      |            |                              |
| 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.

<sup>a</sup>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.

<sup>b</sup>Modeled as a continuous variable in regression models.

<sup>c</sup>Modeled as a dichotomous variable (≤6th grade and >6th grade) in regression models.

samples collected during pregnancy were 3.3 (2.4–4.9)  $\mu\text{g/L}$ , 1.7 (0.9–4.1)  $\mu\text{g/g}$ , and 24.0 (20.3–28.0)  $\mu\text{g/L}$ , 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 ( $\pm$  SD) BSID-III cognitive, language, motor, and social-emotional standardized composite scores were  $98.2 \pm 9.5$ ,  $90.1 \pm 7.1$ ,  $97.3 \pm 8.9$ , and  $90.3 \pm 11.9$  points, respectively (Table 2). Among boys, we observed lower language (mean  $\pm$  SD =  $88.6 \pm 6.8$  points) and social-emotional ( $88.8 \pm 11.6$  points) standardized composite scores than among girls ( $91.7 \pm 7.1$  and  $91.7 \pm 12.2$  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 [ $\beta$  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 [ $\beta$  per 10-fold increase =  $-4.6$  points (95% CI:  $-8.5$ ,  $-0.8$ )] but not among girls [ $\beta = -0.2$  points (95% CI:  $-4.2$ ,  $3.9$ );  $p_{\text{INT}} = 0.17$ ]. Additionally, higher averaged prenatal hair Mn concentrations were associated with lower cognitive scores among girls [ $\beta = -3.0$  points (95% CI:  $-6.1$ ,  $0.1$ )] but not among boys [ $\beta = 2.5$  points (95% CI:  $-0.4$ ,  $5.4$ );  $p_{\text{INT}} = 0.01$ ]. We also observed that higher averaged specific gravity–corrected urinary ETU concentrations were marginally associated with lower social-emotional composite scores among girls [ $\beta$  per 10-fold increase =  $-7.4$  points (95% CI:  $-15.2$ ,  $0.4$ )] but not among boys [ $\beta = 0.0$  points (95% CI:  $-6.9$ ,  $7.0$ );  $p_{\text{INT}} = 0.11$ ]. 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  $\sim 10$   $\mu\text{g/g}$  (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 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                                       | <i>n</i> | <i>k</i> | Mean $\pm$ SD   | Min  | Percentile |       |       | Max   | $\sigma^2_{\text{btw}}$ | $\sigma^2_{\text{within}}$ | ICC  |
|--|----------|----------|-----------------|------|------------|-------|-------|-------|-------------------------|----------------------------|------|
|  |          |          |                 |      | 25th       | 50th  | 75th  |       |                         |                            |      |
| Prenatal biomarkers of exposure <sup>a</sup>                 |          |          |                 |      |            |       |       |       |                         |                            |      |
| Urinary ETU <sub>SG</sub> ( $\mu\text{g/L}$ ) <sup>b,c</sup> | 764      | 355      | 4.4 $\pm$ 7.1   | 0.8  | 2.4        | 3.3   | 4.9   | 127.4 | 0.02                    | 0.08                       | 0.17 |
| Hair Mn ( $\mu\text{g/g}$ ) <sup>c,d</sup>                   | 661      | 355      | 3.7 $\pm$ 5.4   | 0.1  | 0.9        | 1.7   | 4.1   | 53.3  | 0.16                    | 0.11                       | 0.59 |
| Blood Mn ( $\mu\text{g/L}$ ) <sup>e</sup>                    | 571      | 349      | 24.4 $\pm$ 6.2  | 9.1  | 20.3       | 24.0  | 28.0  | 50.6  | 19.92                   | 26.45                      | 0.43 |
| Neurodevelopmental outcomes <sup>f</sup>                     |          |          |                 |      |            |       |       |       |                         |                            |      |
| BSID-III composite scores                                    |          |          |                 |      |            |       |       |       |                         |                            |      |
| Cognitive  | —        | 355      | 98.2 $\pm$ 9.5  | 70.0 | 90.0       | 100.0 | 105.0 | 130.0 | —                       | —                          | —    |
| Language   | —        | 346      | 90.1 $\pm$ 7.1  | 68.0 | 86.0       | 90.0  | 94.0  | 109.0 | —                       | —                          | —    |
| Motor  | —        | 338      | 97.3 $\pm$ 8.9  | 76.0 | 91.0       | 97.0  | 103.0 | 124.0 | —                       | —                          | —    |
| Social-emotional   | —        | 352      | 90.3 $\pm$ 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<sub>SG</sub>, 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.

<sup>a</sup>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.

<sup>b</sup>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.

<sup>c</sup>Between- and within-woman variances and ICCs were calculated and reported for log<sub>10</sub>-transformed specific gravity-adjusted urinary ETU and hair Mn concentrations.

<sup>d</sup>Mn was measured in 661 hair samples collected from 355 study participants: 306 women provided two hair samples, and 49 provided only one.

<sup>e</sup>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.

<sup>f</sup>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 [ $\beta$  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 [ $\beta$  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 [ $\beta = -0.31$  (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 [ $\beta$  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 [ $\beta = -2.5$  (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 [ $\beta$  per 10-fold increase =  $-8.6$  (95% CI:  $-20.5, 3.3$ ); see Table S7 and Figure S2] than during the second half [ $\beta = -2.7$  (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<sub>SG</sub> ( $\mu\text{g/L}$ ), logMnH ( $\mu\text{g/g}$ ), and MnB ( $\mu\text{g/L}$ ) 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), Infantes' Environmental Health (Infantes y Salud Ambiental, ISA) study.

| Neurodevelopmental outcomes <sup>a</sup> | All children |                        | Boys     |                         | Girls    |                         | <i>P</i> <sub>INT</sub> |
|--|--------------|------------------------|----------|-------------------------|----------|-------------------------|-------------------------|
|  | <i>n</i>     | $\beta$ (95% CI)       | <i>n</i> | $\beta$ (95% CI)        | <i>n</i> | $\beta$ (95% CI)        |                         |
| Cognitive                                |              |                        |          |                         |          |                         |                         |
| LogETU <sub>SG</sub>                     | 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 <sub>SG</sub>                     | 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 <sub>SG</sub>                     | 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 <sub>SG</sub>                     | 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<sub>SG</sub>, log<sub>10</sub>-transformed and specific gravity-adjusted urinary ethylenethiourea; logMnH, log<sub>10</sub>-transformed hair manganese; MnB, blood manganese; *n*, number of samples.

<sup>a</sup>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; Skróder 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; Skróder 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.

## Acknowledgments

We gratefully acknowledge the Infantes' Environmental Health Study (Infantes y Salud Ambiental, ISA) families, staff, and community partners. We would also like to thank T. Jursa, M. Maxe, and M. Faniband for their analytical assistance, and M. Torres-Calapiz for her training on the neurodevelopmental tests' administration.

A.M.M. is a former scholar of the Ministry of Science, Technology, and Telecommunications of Costa Rica (MICITT). This publication was made possible by research supported by grant numbers PO1 105296-001 [International Development Research Centre (IDRC)], 6807-05-2011/7300127 (Health Canada), 2010-1211 and 2009-2070 (Swedish Research Council Formas), and D43 ES018745 and R01 ES015572 [National Institutes of Health(NIH)/ National Institute of Environmental Health Sciences (NIEHS)]. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the funders.

## References

- Bayley N. 2006. *Bayley Scales of Infant Development*. 3rd edition. San Antonio, TX: Pearson.
- Bickel G, Nord M, Price C, Hamilton W, Cook J. 2000. Guide to measuring household food security. <https://fnsp-wd.azureedge.net/sites/default/files/FSGuide.pdf> [accessed 17 February 2017].
- Bouchard M, Laforest F, Vandelay L, Bellinger D, Mergler D. 2007. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. *Environ Health Perspect* 115(1):122–127, PMID: 17366831.
- Bouchard MF, Sauvé S, Barbeau B, Legrand M, Brodeur ME, Bouffard T. 2011. Intellectual impairment in school-age children exposed to manganese from drinking water. *Environ Health Perspect* 119(1):138–143, PMID: 20855239, <https://doi.org/10.1289/ehp.1002321>.
- Bravo Durán V, de la Cruz Malavass E, Herrera Ledezma G, Ramírez Muñoz F. 2013. Uso de plaguicidas en cultivos agrícolas como herramienta para el monitoreo de peligros en salud [in Spanish]. *Uniciencia* 27(1):351–376.
- Caldwell BM, Bradley RH. 1984. *Home Observation for Measurement of the Environment*. Little Rock:University of Arkansas.
- Carvalho CF, Menezes-Filho JA, de Matos VP, Bessa JR, Coelho-Santos J, Viana GF, et al. 2014. Elevated airborne manganese and low executive function in school-aged children in Brazil. *Neurotoxicology* 45:301–308, PMID: 24308913, <https://doi.org/10.1016/j.neuro.2013.11.006>.
- Chojnacka K, Górecka H, Górecki H. 2006. The effect of age, sex, smoking habit and hair color on the composition of hair. *Environ Toxicol Pharmacol* 22(1):52–57, PMID: 21783686, <https://doi.org/10.1016/j.etap.2005.11.006>.
- Chung SE, Cheong HK, Ha EH, Kim BN, Ha M, Kim Y, et al. 2015. Maternal blood manganese and early neurodevelopment: the Mothers and Children's Environmental Health (MOCEH) study. *Environ Health Perspect* 123(7):717–722, PMID: 25734517, <https://doi.org/10.1289/ehp.1307865>.
- Claus Henn B, Bellinger DC, Hopkins MR, Coull BA, Ettinger AS, Jim R, et al. 2017. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. *Environ Health Perspect* 125(6):067020, PMID: 28665786, <https://doi.org/10.1289/EHP925>.
- Claus Henn B, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, et al. 2010. Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology* 21(4):433–439, PMID: 20549838, <https://doi.org/10.1097/EDE.0b013e3181d8e52>.
- Coetzee DJ, McGovern PM, Rao R, Harnack LJ, Georgieff MK, Stepanov I. 2016. Measuring the impact of manganese exposure on children's neurodevelopment: advances and research gaps in biomarker-based approaches. *Environ Health* 15:91, PMID: 27576472, <https://doi.org/10.1186/s12940-016-0174-4>.
- Coles C, Crawford J, McClure PR, Roney N, Todd GD, SRC, Inc. 2012. *Toxicological Profile for Manganese*. Atlanta, GA:Agency for Toxic Substances and Disease Registry.
- Cromwell EA, Dube Q, Cole SR, Chirambo C, Dow AE, Heyderman RS, et al. 2014. Validity of U.S. norms for the Bayley Scales of Infant Development-III in Malawian children. *Eur J Paediatr Neurol* 18(2):223–230, PMID: 24423629, <https://doi.org/10.1016/j.ejpn.2013.11.011>.
- Doerge DR, Takazawa RS. 1990. Mechanism of thyroid peroxidase inhibition by ethylenethiourea. *Chem Res Toxicol* 3(2):98–101, PMID: 2130946, <https://doi.org/10.1021/tx00014a003>.
- Domico LM, Cooper KR, Bernard LP, Zeevalk GD. 2007. Reactive oxygen species generation by the ethylene-bis-dithiocarbamate (EBDC) fungicide mancozeb and its contribution to neuronal toxicity in mesencephalic cells. *Neurotoxicology* 28(6):1079–1091, PMID: 17597214, <https://doi.org/10.1016/j.neuro.2007.04.008>.
- Donders J, Hunter SJ. 2010. *Principles and Practice of Lifespan Developmental Neuropsychology*. Cambridge, UK:Cambridge University Press.
- Dorman DC, McManus BE, Marshall MW, James RA, Struve MF. 2004. Old age and gender influence the pharmacokinetics of inhaled manganese sulfate and manganese phosphate in rats. *Toxicol Appl Pharmacol* 197(2):113–124, PMID: 15163547, <https://doi.org/10.1016/j.taap.2004.02.010>.
- Eastman RR, Jursa TP, Benedetti C, Lucchini RG, Smith DR. 2013. Hair as a biomarker of environmental manganese exposure. *Environ Sci Technol* 47(3):1629–1637, PMID: 23259818, <https://doi.org/10.1021/es3035297>.
- Ekman E, Maxe M, Littorin M, Jönsson BA, Lindh CH. 2013. High-throughput method for the analysis of ethylenethiourea with direct injection of hydrolysed urine using online on-column extraction liquid chromatography and triple quadrupole mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 934:53–59, PMID: 23896430, <https://doi.org/10.1016/j.jchromb.2013.06.035>.
- Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T, Robertson RT. 2007. Prenatal manganese levels linked to childhood behavioral disinhibition. *Neurotoxicol Teratol* 29(2):181–187, PMID: 17079114, <https://doi.org/10.1016/j.ntt.2006.09.020>.
- FAO (Food and Agriculture Organization of the United Nations). 1980. FAO tentative specifications for plant protection products: Mancozeb: complex of zinc and maneb containing 20% Mn and 2.5% Zn. [http://www.fao.org/fileadmin/templates/agphome/documents/Pests\\_Pesticides/Specs/Old\\_specs/Mancozeb.pdf](http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Old_specs/Mancozeb.pdf) [accessed 17 February 2017].
- Gunier RB, Arora M, Jerrett M, Bradman A, Harley KG, Mora AM, et al. 2015. Manganese in teeth and neurodevelopment in young Mexican-American children. *Environ Res* 142:688–695, PMID: 26381693, <https://doi.org/10.1016/j.envres.2015.09.003>.
- Gunier RB, Bradman A, Jerrett M, Smith DR, Harley KG, Austin C, et al. 2013. Determinants of manganese in prenatal dentin of shed teeth from CHAMACOS children living in an agricultural community. *Environ Sci Technol* 47(19):11249–11257, PMID: 24053404, <https://doi.org/10.1021/es4018688>.
- Gwiazda R, Campbell C, Smith D. 2005. A noninvasive isotopic approach to estimate the bone lead contribution to blood in children: implications for assessing the efficacy of lead abatement. *Environ Health Perspect* 113(1):104–110, PMID: 15626656, <https://doi.org/10.1289/ehp.7241>.
- Haynes EN, Sucharew H, Kuhnell P, Alden J, Barnas M, Wright RO, et al. 2015. Manganese exposure and neurocognitive outcomes in rural school-age



- children: the Communities Actively Researching Exposure Study (Ohio, USA). *Environ Health Perspect* 123(10):1066–1071, PMID: 25902278, <https://doi.org/10.1289/ehp.1408993>.
- He P, Liu DH, Zhang GQ. 1994. Effects of high-level-manganese sewage irrigation on children's neurobehavior [in Chinese]. *Zhonghua Yu Fang Yi Xue Za Zhi* 28(4):216–218, PMID: 7842882.
- Hernández-Bonilla D, Schilman A, Montes S, Rodríguez-Agudelo Y, Rodríguez-Dozal S, Solís-Vivanco R, et al. 2011. Environmental exposure to manganese and motor function of children in Mexico. *Neurotoxicology* 32(5):615–621, PMID: 21871921, <https://doi.org/10.1016/j.neuro.2011.07.010>.
- Jacobsen PR, Axelstad M, Boberg J, Isling LK, Christiansen S, Mandrup KR, et al. 2012. Persistent developmental toxicity in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. *Reprod Toxicol* 34(2):237–250, PMID: 22677472, <https://doi.org/10.1016/j.reprotox.2012.05.099>.
- Kempson IM, Lombi E. 2011. Hair analysis as a biomonitor for toxicology, disease and health status. *Chem Soc Rev* 40(7):3915–3940, PMID: 21468435, <https://doi.org/10.1039/c1cs15021a>.
- Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, et al. 2012. Manganese exposure from drinking water and children's academic achievement. *Neurotoxicology* 33(1):91–97, PMID: 22182530, <https://doi.org/10.1016/j.neuro.2011.12.002>.
- Kim Y, Kim BN, Hong YC, Shin MS, Yoo HJ, Kim JW, et al. 2009. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. *Neurotoxicology* 30(4):564–571, PMID: 19635390, <https://doi.org/10.1016/j.neuro.2009.03.012>.
- Kjeldsen LS, Ghisari M, Bonefeld-Jørgensen EC. 2013. Currently used pesticides and their mixtures affect the function of sex hormone receptors and aromatase enzyme activity. *Toxicol Appl Pharmacol* 272(2):453–464, PMID: 23871939, <https://doi.org/10.1016/j.taap.2013.06.028>.
- Lin CC, Chen YC, Su FC, Lin CM, Liao HF, Hwang YH, et al. 2013. *In utero* exposure to environmental lead and manganese and neurodevelopment at 2 years of age. *Environ Res* 123:52–57, PMID: 23578827, <https://doi.org/10.1016/j.envres.2013.03.003>.
- Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect* 112(17):1691–1696, PMID: 15579415, <https://doi.org/10.1289/ehp.7199>.
- Lucchini RG, Guazzetti S, Zoni S, Donna F, Peter S, Zacco A, et al. 2012a. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. *Neurotoxicology* 33(4):687–696, PMID: 22322213, <https://doi.org/10.1016/j.neuro.2012.01.005>.
- Lucchini RG, Zoni S, Guazzetti S, Bontempi E, Micheletti S, Broberg K, et al. 2012b. Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents. *Environ Res* 118:65–71, PMID: 22925625, <https://doi.org/10.1016/j.envres.2012.08.003>.
- Madison JL, Wegrzynowicz M, Aschner M, Bowman AB. 2011. Gender and manganese exposure interactions on mouse striatal neuron morphology. *Neurotoxicology* 32(6):896–906, PMID: 21641932, <https://doi.org/10.1016/j.neuro.2011.05.007>.
- McGraw K, Wong S. 1996. Forming inferences about some intraclass correlation coefficients. *Psychol Methods* 1(1):30–46, <https://doi.org/10.1037/1082-989X.1.1.30>.
- Menezes-Filho JA, de Carvalho-Vivas CF, Viana GF, Ferreira JR, Nunes LS, Mergler D, et al. 2014. Elevated manganese exposure and school-aged children's behavior: a gender-stratified analysis. *Neurotoxicology* 45:293–300, PMID: 24121006, <https://doi.org/10.1016/j.neuro.2013.09.006>.
- Menezes-Filho JA, Novaes C de O, Moreira JC, Sarcinelli PN, Mergler D. 2011. Elevated manganese and cognitive performance in school-aged children and their mothers. *Environ Res* 111(1):156–163, PMID: 20943219, <https://doi.org/10.1016/j.envres.2010.09.006>.
- Menezes-Filho JA, Paes CR, Pontes AM, Moreira JC, Sarcinelli PN, Mergler D. 2009. High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxicology* 30(6):1207–1213, PMID: 19336889, <https://doi.org/10.1016/j.neuro.2009.04.005>.
- Miranda-Contreras L, Dávila-Ovalles R, Benítez-Díaz P, Peña-Contreras Z, Palacios-Prü E. 2005. Effects of prenatal paraquat and mancozeb exposure on amino acid synaptic transmission in developing mouse cerebellar cortex. *Brain Res Dev Brain Res* 160(1):19–27, PMID: 16198425, <https://doi.org/10.1016/j.devbrainres.2005.08.001>.
- Mora AM, Arora M, Harley KG, Kogut K, Parra K, Hernández-Bonilla D, et al. 2015. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. *Environ Int* 84:39–54, PMID: 26209874, <https://doi.org/10.1016/j.envint.2015.07.009>.
- Mora AM, van Wendel de Joode B, Mergler D, Córdoba L, Cano C, Quesada R, et al. 2014. Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in Costa Rica. *Environ Sci Technol* 48(6):3467–3476, PMID: 24601641, <https://doi.org/10.1021/es404279r>.
- Nascimento S, Baierle M, Göethel G, Barth A, Brucker N, Charão M, et al. 2016. Associations among environmental exposure to manganese, neuropsychological performance, oxidative damage and kidney biomarkers in children. *Environ Res* 147:32–43, PMID: 26844420, <https://doi.org/10.1016/j.envres.2016.01.035>.
- Oulhote Y, Mergler D, Barbeau B, Bellinger DC, Bouffard T, Brodeur MÈ, et al. 2014a. Neurobehavioral function in school-age children exposed to manganese in drinking water. *Environ Health Perspect* 122(12):1343–1350, PMID: 25260096, <https://doi.org/10.1289/ehp.1307918>.
- Oulhote Y, Mergler D, Bouchard MF. 2014b. Sex- and age-differences in blood manganese levels in the U.S. general population: National Health and Nutrition Examination Survey 2011–2012. *Environ Health* 13:87, PMID: 25342305, <https://doi.org/10.1186/1476-069X-13-87>.
- Racette BA, Aschner M, Guilarte TR, Dydak U, Criswell SR, Zheng W. 2012. Pathophysiology of manganese-associated neurotoxicity. *Neurotoxicology* 33(4):881–886, PMID: 22202748, <https://doi.org/10.1016/j.neuro.2011.12.010>.
- Radloff LS. 1977. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* 1(3):385–401, <https://doi.org/10.1177/014662167700100306>.
- Rahman SM, Kippler M, Tofail F, Bölte S, Derakhshani Hamadani J, Vahter M. 2017. Manganese in drinking water and cognitive abilities and behavior at 10 years of age: a prospective cohort study. *Environ Health Perspect* 125(5):057003, PMID: 28564632, <https://doi.org/10.1289/EHP631>.
- Riojas-Rodríguez H, Solís-Vivanco R, Schilman A, Montes S, Rodríguez S, Ríos C, et al. 2010. Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. *Environ Health Perspect* 118(10):1465–1470, PMID: 20936744, <https://doi.org/10.1289/ehp.0901229>.
- Rodrigues EG, Bellinger DC, Valeri L, Hasan MO, Quamruzzaman Q, Golam M, et al. 2016. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. *Environ Health* 15:44, PMID: 26968381, <https://doi.org/10.1186/s12940-016-0127-y>.
- Rosenfeld CS, Trainor BC. 2014. Environmental health factors and sexually dimorphic differences in behavioral disruptions. *Curr Environ Health Rep* 1(4):287–301, PMID: 25705580, <https://doi.org/10.1007/s40572-014-0027-7>.
- Semrud-Clikeman M, Ellison PAT. 2009. *Child Neuropsychology: Assessment and Interventions for Neurodevelopmental Disorders*. New York, NY:Springer.
- Skröder H, Kippler M, Nermell B, Tofail F, Levi M, Rahman SM, et al. 2017. Major limitations in using element concentrations in hair as biomarkers of exposure to toxic and essential trace elements in children. *Environ Health Perspect* 125(6):067021, PMID: 28669939, <https://doi.org/10.1289/EHP1239>.
- Smith D, Gwiazda R, Bowler R, Roels H, Park R, Taicher C, et al. 2007. Biomarkers of Mn exposure in humans. *Am J Ind Med* 50(11):801–811, PMID: 17924418, <https://doi.org/10.1002/ajim.20506>.
- Soldin OP, Aschner M. 2007. Effects of manganese on thyroid hormone homeostasis: potential links. *Neurotoxicology* 28(5):951–956, PMID: 17576015, <https://doi.org/10.1016/j.neuro.2007.05.003>.
- Spencer A. 1999. Whole blood manganese levels in pregnancy and the neonate. *Nutrition* 15(10):731–734, PMID: 10501283, [https://doi.org/10.1016/S0899-9007\(99\)00144-6](https://doi.org/10.1016/S0899-9007(99)00144-6).
- Takser L, Lafond J, Bouchard M, St-Amour G, Mergler D. 2004. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. *Environ Res* 95(2):119–125, PMID: 15147916, <https://doi.org/10.1016/j.envres.2003.11.002>.
- Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. 2003. Manganese, monoamine metabolite levels at birth, and child psychomotor development. *Neurotoxicology* 24(4–5):667–674, PMID: 12900080, [https://doi.org/10.1016/S0161-813X\(03\)00058-5](https://doi.org/10.1016/S0161-813X(03)00058-5).
- U.S. EPA (U.S. Environmental Protection Agency). 2005. "Reregistration Eligibility Decision for Mancozeb." EPA 738-R-04-012. Washington, DC:U.S. Environmental Protection Agency. [https://archive.epa.gov/pesticides/reregistration/web/pdf/mancozeb\\_red.pdf](https://archive.epa.gov/pesticides/reregistration/web/pdf/mancozeb_red.pdf) [accessed 17 February 2017].
- Vaccari A, Saba P, Mocchi I, Ruiu S. 1999. Dithiocarbamate pesticides affect glutamate transport in brain synaptic vesicles. *J Pharmacol Exp Ther* 288(1):1–5, PMID: 9862745.
- van Wendel de Joode B, Barbeau B, Bouchard MF, Mora AM, Skytt A, Córdoba L, et al. 2016a. Manganese concentrations in drinking water from villages near banana plantations with aerial mancozeb spraying in Costa Rica: results from the Infants' Environmental Health Study (ISA). *Environ Pollut* 215:247–257, PMID: 27208757, <https://doi.org/10.1016/j.envpol.2016.04.015>.
- van Wendel de Joode B, Mora AM, Córdoba L, Cano JC, Quesada R, Faniband M, et al. 2014. Aerial application of mancozeb and urinary ethylene thiourea (ETU) concentrations among pregnant women in Costa Rica: the Infants' Environmental Health study (ISA). *Environ Health Perspect* 122(12):1321–1328, PMID: 25198283, <https://doi.org/10.1289/ehp.1307679>.
- van Wendel de Joode B, Mora AM, Lindh CH, Hernández-Bonilla D, Córdoba L, Wesseling C, et al. 2016b. Pesticide exposure and neurodevelopment in

- children aged 6–9 years from Talamanca, Costa Rica. *Cortex* 85:137–150, PMID: [27773359](https://pubmed.ncbi.nlm.nih.gov/27773359/), <https://doi.org/10.1016/j.cortex.2016.09.003>.
- Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, et al. 2006. Water manganese exposure and children's intellectual function in Araihaaz, Bangladesh. *Environ Health Perspect* 114:124–129, PMID: [16393669](https://pubmed.ncbi.nlm.nih.gov/16393669/), <https://doi.org/10.1289/ehp.8030>.
- WHO (World Health Organization). 1988. *Dithiocarbamate Pesticides, Ethylenethiourea, and Propylenethiourea: A General Introduction*. Geneva, Switzerland:World Health Organization.
- Wright RO, Amarasiwardena C, Woolf AD, Jim R, Bellinger DC. 2006. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. *Neurotoxicology* 27(2):210–216, PMID: [16310252](https://pubmed.ncbi.nlm.nih.gov/16310252/), <https://doi.org/10.1016/j.neuro.2005.10.001>.
- Yu X, Chen L, Wang C, Yang X, Gao Y, Tian Y. 2016. The role of cord blood BDNF in infant cognitive impairment induced by low-level prenatal manganese exposure: LW birth cohort, China. *Chemosphere* 163:446–451, PMID: [27565312](https://pubmed.ncbi.nlm.nih.gov/27565312/), <https://doi.org/10.1016/j.chemosphere.2016.07.095>.