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Exposure to common-use pesticides, manganese, lead, and thyroid function among pregnant women from the Infants' Environmental Health (ISA) study, Costa Rica

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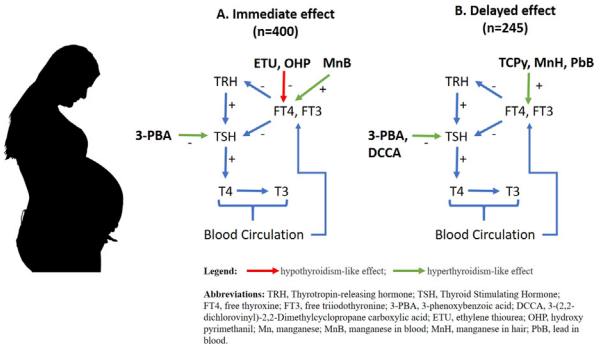
HIGHLIGHTS

- Pesticides and metals may alter thyroid function.
- Few studies have been performed among pregnant women from agricultural villages.
- The fungicides mancozeb and pyrimethanil may inhibit thyroid hormones among pregnant women in Costa Rica.
- Chlorpyrifos, synthetic pyrethroids, manganese and lead may produce a hyperthyroidism-like effect.
- Some pesticides seemed to cause an immediate effect, others a delayed effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Pesticides and metals may disrupt thyroid function, which is key to fetal brain development.
Objectives: To evaluate if current-use pesticide exposures, lead and excess manganese alter free thyroxine (FT4), free triiodothyronine (FT3), and thyroid stimulating hormone (TSH) concentrations in pregnant women from the Infants' Environmental Health Study (ISA).
Methods: At enrollment, we determined women's ($n = 400$) specific-gravity corrected urinary pesticide (μg/L) metabolite concentrations of mancozeb (ethylene thiourea (ETU)), pyrimethanil, thiabendazole, chlorpyrifos, synthetic pyrethroids, and 2,4-D. We also measured manganese hair (MnH) (μg/g) and blood (MnB) (μg/L), and blood lead (PbB) (μg/L) concentrations. To detect an immediate and late effect on thyroid homeostasis, we determined TSH, FT4 and FT3 in serum obtained at the same visit ($n = 400$), and about ten weeks afterwards

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($n = 245$). We assessed associations between exposures and outcomes with linear regression and general additive models, Bayesian multivariate linear regression, and Bayesian kernel machine regression.
Results: About 80%, 94%, and 100% of the women had TSH, FT4, and FT3 within clinical reference ranges, respectively. Women with higher urinary ETU, and pyrimethanil-metabolites, had lower FT4: $\beta = -0.79$ (95%CI $= -1.51, -0.08$) and $\beta = -0.29$ (95%CI $= -0.62, -0.03$), respectively, for each tenfold increase in exposure. MnB was positively associated with FT4 ($\beta = 0.04$ (95%CI $= 0.00, 0.07$ per 1 μg/L increase), and women with high urinary pyrethroid-metabolite concentrations had decreased TSH (non-linear effects). For the late-effect analysis, metabolites of pyrethroids and chlorpyrifos, as well as MnH, and PbB were associated decreased TSH, or increased FT4 and/or FT3.
Discussion: Mancozeb (ETU) and pyrimethanil may inhibit FT4 secretion (hypothyroidism-like effect), while chlorpyrifos, pyrethroids, MnB, MnH, PbB and Mn showed hyperthyroidism-like effects. Some effects on thyroid homeostasis seemed to be immediate (mancozeb (ETU), pyrimethanil, MnB), others delayed (chlorpyrifos, MnH, PbB), or both (pyrethroids), possibly reflecting different mechanisms of action.

1. Introduction

Maternal thyroid homeostasis during pregnancy, with optimal serum concentrations of free thyroxine (FT4), triiodothyronine (FT3), and thyroid stimulating hormone (TSH), is essential for fetal brain development (Boas et al., 2012; Jansen et al., 2019). Low FT4 in early pregnancy has been associated with a lower IQ across several birth cohorts (Levie et al., 2018). Thyroid function is regulated by a complex feedback system of circulating hormones at the hypothalamic and pituitary levels (Fig. 1) (Boas et al., 2012). Alterations in these hormonal concentrations among others may reflect an overactive thyroid function (hyperthyroidism), characterized by low TSH, high FT4 and/or FT3, whilst an underactive thyroid function (hypothyroidism) is reflected by high TSH, low FT4 and/or FT3 (Dayan, 2001).

Environmental chemicals may disrupt thyroid function in a variety of ways including: 1) at the gland itself by interfering with TSH receptors or synthesis of thyroid hormones; 2) alter the hypothalamic pituitary thyroid (HPT) axis; 3) modify binding of the thyroid transport proteins; liver enzyme induction leading to increased inactivation and further excretion of thyroid hormones from the body, and; 5) up- or down regulate expression of thyroid hormone regulated genes (Boas et al., 2012; Du et al., 2010; Goldner et al., 2013; Hernández et al., 2020; Maranghi et al., 2013). For example, increased blood lead (PbB) concentrations in women from China and Kosovo have been associated with increased TSH, decreased FT4 and increased thyroid peroxidase (TPO) antibodies (Kahn et al., 2014; Nie et al., 2017), yet underlying mechanisms are poorly understood. Exposure to pesticides are also of concern as about 2 million tons of pesticides are used annually, but the effects of common-use pesticides on pregnant women's thyroid function have hardly been studied (Leemans et al., 2019; Sharma et al., 2019; Zhang et al., 2013).

Globally, one of the most used pesticides is the ethylene bis dithiocarbamate (EBDC) fungicide mancozeb, registered for use in more than 120 countries (Gullino et al., 2010). In Costa Rica, mancozeb is weekly sprayed with light aircrafts on bananas grown for export purposes (Bravo Durán et al., 2013). EBDC formulations are commonly contaminated with its metabolite ethylene thiourea (ETU) (Epa and of Pesticide Programs, 2005). Suspected EDBC thyroid toxicity in humans is thought to be mainly caused by ETU; ETU-exposed rats showed hypothyroidism reflected by decreased T4 (Axelstad et al., 2011) and increased TSH (Maranghi et al., 2013). Studies in workers also showed hypothyroidism-like effects, as rubber manufacturing workers exposed to ETU had decreased T4 (Smith, 1984), and agricultural workers exposed to EDBC had increased TSH serum concentrations (Panganiban et al., 2004; Steenland et al., 1997). Mancozeb also contains 20% (w/w) manganese (Mn), an essential element that in excess may be toxic

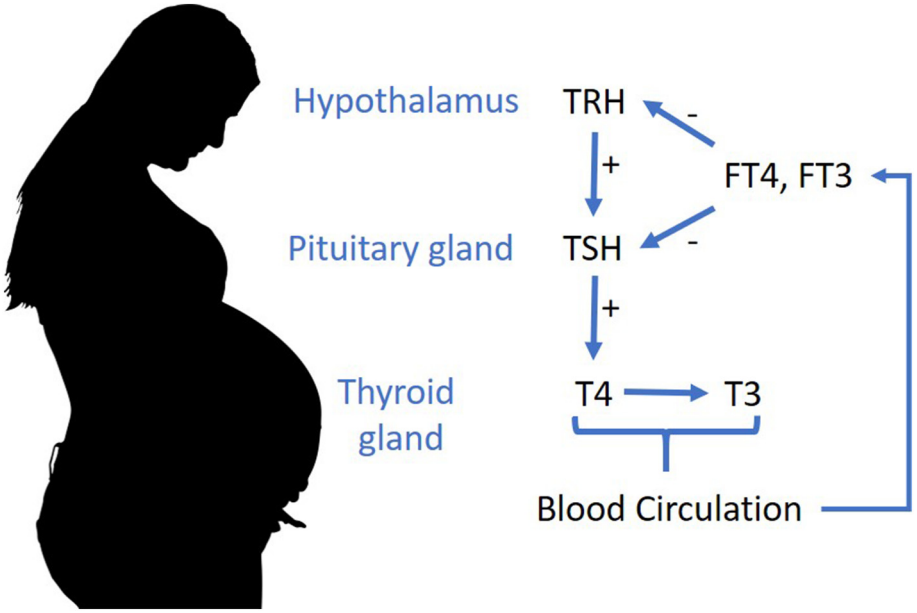

Fig. 1. Mechanisms of regulation of thyroid hormone homeostasis in the hypothalamic-pituitary-thyroid axis.

(Food and Agriculture Organization of the United Nations, 1980; Mora et al., 2018). Memon et al. (2015) found both higher and lower mean Mn serum concentrations in adult women were associated with hyper- and hypothyroidism, respectively. Nevertheless, no study reported both the effect of ETU and Mn on thyroid function.

In addition to mancozeb, other fungicides are used in banana production that may also influence thyroid homeostasis. The anilinopyrimidine fungicide pyrimethanil is broadly applied in agriculture and aerially sprayed on bananas (Córdoba Gamboa et al., 2020). Rats and mice (sub)chronically exposed to pyrimethanil showed increased TSH and decreased T4 serum concentrations (Brucker-Davis et al., 2011; Hurley et al., 1998). Thiabendazole is generally used as a pre-planting and post-harvest fungicide (Ekman et al., 2013) and applied post-harvest on bananas (Córdoba Gamboa et al., 2020). Rats exposed to the fungicide thiabendazole showed alterations in thyroid hormones, thyroid volume, and thyroid follicular cell adenomas formation (EFSA, 2014; US-EPA, 2002).

Insecticides and herbicides also may alter thyroid function, but evidence from epidemiological studies is scarce and inconclusive. Exposure to the insecticide chlorpyrifos among adult men has been associated with inhibition and among adult women with stimulation of thyroid function (Fortenberry et al., 2012; Meeker et al., 2006). In Costa Rica, chlorpyrifos is used in bags to protect the bananas from insects during growth (van Wendel de Joode et al., 2012). Synthetic pyrethroid insecticides, commonly used both in agriculture and home environments, may disrupt thyroid function by binding to hormone receptors due to similarity in chemical structures as measured by thyroid hormone receptor binding ability (Du et al. (2010)). Finally, the herbicide 2,4-D is used to control broad-leafed weeds in pasture and sometimes soccer fields. Knopp (1994) reported no effects on T4, T3 and TSH in 2,4-D exposed factory workers, but specific associations were not presented. Results from animal studies showed rats and ewes exposed to 2,4-D had lower T3 and T4 (Kobal et al., 2000; Rawlings et al., 1998).

The Infants' Environmental Health Study (ISA, for its acronym in Spanish) is a community-based birth cohort study situated in a banana growing area in the Northern Caribbean of Costa Rica, designed to evaluate the effects of pesticide exposure and excess manganese on children's and women's health (Mora et al., 2014, 2015, 2020, 2018; van Wendel de Joode et al., 2014, 2016). The study population is exposed to pesticides used in agriculture, vector control and at home; to manganese from mancozeb and natural sources; and to very low levels of lead (probably from old paint and water pipes) (Supplementary material Table S1). In this study, we evaluated if exposure to common-use pesticides, manganese and lead was associated with altered serum TSH, FT4 and FT3 hormone concentrations in pregnant women from the ISA birth cohort.

2. Materials and methods

2.1. Study population

Between March 2010 and June 2011, we enrolled 451 women aged >15 years with a gestational age of <33 weeks, who lived in Matina county, Limón, Costa Rica (van Wendel de Joode et al., 2014). All women gave written informed consent prior to participating. For women aged <18 years, their legal representative also gave informed consent. All study activities were approved by the Scientific Ethics Committee of the Universidad Nacional in Costa Rica (CECUNA-11-2009). We obtained both urine and blood samples from women at enrollment or shortly after. For the current study, we included 400 out of the 451 (89%) pregnant women with data on exposure metabolites and TSH, FT4, and FT3 serum concentrations. We excluded two women with hypothyroidism confirmed by a physician. For 245 out of these 400 women, we obtained a second blood sample about ten weeks after the first sample (mean 10.7 weeks, SD = 4.1).

2.2. Maternal interviews, gestational age, and pre-pregnancy body mass index

Data collection during pregnancy, post-partum and at the child-age of 1 year has been described in detail previously (van Wendel de Joode et al., 2014; Mora et al., 2015, 2018). We obtained information about sociodemographic and occupational variables, pesticide use at work and home, medical history, and lifestyle habits, among others. We calculated gestational age (GA) using information about the first day of the last menstrual period, ultrasound (<14 weeks of gestation) or medical records (Mora et al., 2015). In addition, for six women we used information from symphysis-fundus height measurement. We calculated pre-pregnancy body mass index (BMI) by weight in kilograms / (height in meters)^2, using maternal pre-pregnancy weight (if available) or weight at the first prenatal care visit (if <14 weeks gestation) abstracted from medical records, and height measured by the study interviewers.

2.3. Thyroid hormones sampling and analysis

TSH, FT4 and FT3 were measured in serum obtained from whole blood samples, collected with 9 mL glass Vacuette® Serum Clot Activator Tubes. We allowed blood to clot and then centrifuged at 1000 × gravitational units (g), and separated serum following a standardized procedure (WHO 2012). We then stored samples at −20 °C until shipment to Sweden (Department of Clinical Chemistry, Malmö University Hospital, Malmö, Sweden). We analyzed TSH, FT4 and FT3 with a two-step competitive electrochemiluminescence immunoassay method (ECLIA), using Elecsys and Cobas-e immunoassay analyzers (Cobas ® 2017 TSH, FT3, FT4 Roche Diagnostics, Mannheim, Germany). Limit of detections (LODs) were: TSH = 0.005 mIU/L, FT4 = 0.5 pmol/L and FT3 = 0.6 pmol/L.

2.4. Urinary pesticide metabolites sampling and analysis

Urine samples were collected and stored as described previously (van Wendel de Joode et al., 2014). To analyze urine samples for pesticide metabolites, we used a two-dimensional liquid chromatography mass spectrometer (LC/MS/MS; UFLCRX; Shimadzu Corporation, Kyoto, Japan) with a triple quadrupole linear ion trap (QTRAP 5500; AB Sciex, Foster City, CA. USA) (Mora et al., 2020; Norén et al., 2020). We measured the following specific pesticide metabolites (Supplemental Table S1): ETU for mancozeb, hydroxy pyrimethanil (OHP) for pyrimethanil, 5-hydroxythiabendazole (OHT) for thiabendazole, 3,5,6-trichloro-2-pyridinol (TCPy) for chlorpyrifos, and 2,4-D for 2,4-D (Ekman et al., 2014, 2013; Faniband et al., 2019). We also analyzed two non-specific metabolites for synthetic pyrethroids: 3-(2,2-dichlorovinyl)-2,2-Dimethylcyclopropane carboxylic acid (DCCA) for, among others, alpha-cypermethrin, deltamethrin, beta-cyfluthrin, lambda-cyhalothrin, and permethrin, and 3-phenoxybenzoic acid (3-PBA) for, among others, alpha-cypermethrin, beta-cyfluthrin and permethrin. The laboratory at Lund University is part of an inter-laboratory control program for TCP and 3-PBA (Erlangen, Germany). All concentrations of ETU, TCPy, and 3PBA were above the LOD; OHP, OHT and DCCA were detected in 87%, 66% and 99%, respectively. Urinary pesticide metabolite concentrations that were below the LOD but above LOD/2 were imputed with the value indicated by the analytical equipment; samples below LOD/2 were set at LOD/2. Specific gravity (kg/L) (sg) of each urine sample was measured with a hand refractometer, and pesticide metabolite concentrations were corrected for dilution by: $M_{sg} = M * [(1.017–1)/(SG-1)]$, where M_{sg} is the specific gravity-corrected metabolite concentration (μg/L), M is the observed metabolite concentration (μg/L), SG is the specific gravity of the urine sample, and 1.017 kg/L is the average specific gravity for all urine samples included in this study ($n = 400$).

2.5. Manganese in hair sampling and analysis

Hair samples (~223C;20–30 strands) were collected from the occipital region, cutting from 2 mm from the scalp, stored in plastic sampling bags at room temperature (20–25 °C) and sent to the Federal University of Bahia Brazil for analysis (Mora et al., 2014). Briefly, the one centimeter of strands closest to the scalp were analyzed thought to represent last three-weeks' excess Mn. Mn was measured using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120; Varian, Inc. (Menezes-Filho et al., 2009). Samples below the LOD (0.1 μg/g, $n = 2$) were set at LOD/2.

2.6. Manganese and lead in blood sampling and analysis

Blood samples were collected by venipuncture and collected into metal-free EDTA tubes (ref. number 454036, Greiner Bio-One Vacuette, Monroe, NC) and frozen at −20 °C until shipment to the University of California, Santa Cruz, for their analysis (Mora et al., 2014). Samples were analyzed for Mn and Pb with a high-resolution inductively coupled plasma mass spectrometry (Finnigan XR ICP-MS) (Smith et al. 2007). All samples had detectable Mn (>0.003 μg/L) and Pb (>0.0016 μg/L) (Mora et al., 2018).

2.7. Statistical analysis

We used descriptive statistics and distributional plots to explore all variables. We tested whether continuous variables followed a normal distribution (Shapiro–Wilk W-test). As urinary pesticide metabolite concentrations, manganese hair, PbB and TSH followed a lognormal distribution, we used log10-transformed concentrations in statistical analysis. We evaluated associations between biomarkers of exposure, covariates, TSH, FT4, and FT3 with Pearson's Chi-square test for categorical measures, Student's *t*-test and Tukey's Honest Significant Difference test, Spearman's r correlation coefficient, and ran bivariate regression analyses.

Based on literature, we identified the following potential confounders and known predictors of thyroid hormone and TSH serum concentrations during pregnancy: gestational age at sampling (weeks); maternal age (years); more than primary education (yes/no); parity (0/≥1); pre-pregnancy BMI (weight/(height)^2); family income (below/above poverty line); maternal agricultural work (yes/no), paternal agricultural work (yes/no); smoking (yes/no); cotinine detected in urine sample (yes/no); consumption of fish (yes/no), coffee (yes/no), vitamin supplements (yes/no); and vomiting during pregnancy resulting in loss-of-weight, or requiring hospitalization or intravenous hydration (yes/no). Variables with missing values (<10%) were imputed by randomly selecting a variable from the subset of observations with known values of the covariate: gestational age at sampling ($n = 4$), parity ($n = 6$), family income ($n = 9$), and vomiting (n = 4). For 31 women, data on pre-pregnancy weight was missing, and we predicted the value of this variable by a regression model including maternal weight at children's age of 1-year and parity (0/≥1) ($R^2 = 0.84$) for 17 of the women. For the remaining 14 women, who were missing information about their weight at the 1-year visit, we predicted pre-pregnancy weight by a regression model including maternal education and maternal age at enrollment ($R^2 = 0.12$).

We ran separate multivariate linear regression models to evaluate cross-sectional associations between urinary pesticide metabolites, MnH, MnB or PbB and TSH, FT4 and FT3 ($n = 400$). To evaluate a possible additive effect of pesticides, we calculated summed values of 3-PBA and DCCA (both metabolites of pyrethroids) as well as ETU and OHP (both fungicides). We included maternal age a priori as a covariate, and we also included gestational age at sampling, cotinine (detected yes/no), pre-pregnancy BMI, and severe vomiting during pregnancy, as these changed the estimated beta of effect with 10% or more. We used Rosner's test to identify outliers of metabolites, TSH, FT4 and FT3 and subsequently excluded them from analysis as the beta-of-effect estimates changed more than 10% in most models. For MnB we calculated percent difference in TSH concentration associated with a 1-unit increase in MnB concentration while all other variables in the model are held constant, by $[10\hat{}(\beta) - 1] \times 100$ (Halvorsen and Palmquist, 1980). In addition, for exposure metabolites that were log10-transformed, we calculated % change in TSH for each 10% increase in exposure metabolite concentration by $[(1.10^{\beta}) - 1] * 100$ (UCLA: Statistical Consulting Group, 2020 (online). We also estimated change in FT4 or FT3 for each 1-unit increase in MnB; while for the other exposure metabolites, β-estimates reflect the change in FT4 or FT3 for each ten-fold increase in exposure. In addition, to detect a possible late effect of exposure, we reran all models using as an outcome the second thyroid hormone or TSH concentrations and the first measures of exposure measure as a predictor of effect ($n = 245$). As a sensitivity analysis, to be able to compare results from cross-sectional and late-effect analysis for the same women, we also reran all cross-sectional analyses limited to women with a second measured of TSH, FT4, or FT3 (n = 245). For all linear regression models, we tested residuals models for normality (Shapiro-Wilk test), autocorrelation (Durbin-Watson) and multicollinearity (variance inflation factors).

We explored non-linear associations with covariate-adjusted generalized additive models (GAMs) with penalized spline smooth terms for continuous exposure (constrained to a maximum of 4 knots) and visually explored plots. We considered regression models non-linear if estimated degrees of freedom (edf) > 2 and p-value$_{GAM}$ < 0.05.

Finally, we examined two types of models which include all metabolites simultaneously. We first implemented a Bayesian multivariate linear regression that employs stochastic search variable selection

Table 1
Characteristics of pregnant women from the ISA birth cohort study with at least one serum sample ($n = 400$), at enrollment 2010–2011.

Variable	Value	n (%)
Trimester of pregnancy at enrollment[a,b]	First	95 (24%)
	Second	213 (54%)
	Third	88 (22%)
Maternal age at enrollment (years)[a]	<18	72 (18%)
	18–24	192 (48%)
	25–29	69 (17%)
	≥ 30	67 (17%)
Prepregnancy BMI (kg/m^2)[a,d]	Underweight: <18.5	13 (3%)
	Normal: 18.5–24.9	196 (49%)
	Overweight: 25.0–29.9	106 (27%)
	Obese: 30 or more	85 (21%)
Parity (≥1)[b]		247 (63%)
Married/living as married		305 (76%)
Family income below poverty line[b]		235 (60%)
Woman works in agriculture[c]		33 (8%)
Partner works in agriculture[d]		233 (60%)
Severe vomiting during pregnancy		58 (15%)
Smoking during pregnancy		15 (4%)
Cotinine detected in urine during pregnancy (>1 μg/L)		42 (10%)
Fish consumption during pregnancy		264 (66%)
Coffee consumption during pregnancy		288 (72%)
Vitamin supplement consumption during pregnancy		90 (22%)
Residential distance to banana plantations at enrollment <50 m		102 (26%)

Abbreviations: n, number of participants; GM, geometric mean; GSD, geometric standard deviation; SD, standard deviation; BMI, body mass index.

[a] Modeled as a continuous variable in linear regression models.

[b] Missing values for: gestational age at sampling $n = 4$; parity $n = 8$; family income $n = 9$ and $n = 22$ filled with information obtained when children aged 1 and 5 years; severe vomiting n = 4; prepregnancy BMI $n = 31$.

[c] Of whom 7.5% ($n = 30$) at banana plantations.

[d] For 11 single and 1 married women information on work of partner was missing.

Table 2
Distribution of pregnant women's biomarkers of exposure obtained at same visit as first serum sample, ISA study ($n = 400$).

Metabolite (μg/L)[a]	n	Min	p10	p25	p50	p75	p90	Max
ETU	400	0.58	1.60	2.14	3.08	4.70	7.39	251
OHP	400	0.02	0.06	0.16	0.41	0.95	2.16	946
OHT	400	0.01	0.01	0.03	0.07	0.28	1.49	491
TCPy	400	0.28	0.72	1.04	1.60	2.4	4.08	50.0
DCCA	400	0.06	0.30	0.57	1.09	2.04	3.83	45.8
3-PBA	400	0.07	0.24	0.38	0.69	1.25	2.12	32.6
2,4-D	400	0.04	0.12	0.17	0.28	0.45	1.04	39.7
Hair Mn (μg/g)[b]	384	0.05	0.45	0.86	1.62	3.49	7.87	53.2
Blood Mn (μg/L)[c]	344	8.94	15.1	19.4	23.4	27.4	31.1	50.6
Blood Pb (μg/L)[c]	344	2.03	4.11	5.17	6.66	9.08	12.11	34.3

Abbreviations: ETU, ethylene thiourea; OHP, hydroxypyrimethanil; OHT, 5-hydroxythiabendazole; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid; 2,4-D, dichlorophenoxiacetic acid; Mn, manganese; Pb, lead.

[a] Unless otherwise indicated, urinary metabolite concentrations (ETU, OHP, 5-OH-TBZ, TCPy, DCCA, 3-PBA, 2,4-D) were corrected for specific-gravity; all metabolites but MnB, that was normally distributed, followed a lognormal distribution.

[b] Data were available for a subsample of 384 women.

[c] Data were available for a subsample of 344 women.

(Edward and McCulloch, 1993). This model examines additive effects of all exposures on the response, while assigning probabilities that each variable is included in the true model. We also, explored more general exposure-response relationships using a Bayesian kernel machine regression that can accommodate interaction and non-linear effects (Bobb et al., 2018). For the linear models these probabilities are recovered using the standard spike-and-slab prior, where the prior distributions are scaled to the response and the predictive variables are scaled a priori (Edward and McCulloch, 1993). Computational details are provided in the supplementary material (Text S 1).

Statistical analysis was performed with R (version 3.5.3; R Development Core Team).

3. Results

The 400 women who provided samples were young, most had attended only primary school, and 60% lived below the Costa Rica poverty line (Table 1). Few women (8%) worked in agriculture during pregnancy, whilst 60% of their partners, mostly on banana plantations. About a quarter of the women lived <50 m from a banana plantation. All urinary pesticide metabolite concentrations, MnH and PbB were right skewed, only MnB was normally distributed (Table 2). Overall, only DCCA and 3-PBA, partially metabolites of the same pyrethroids, were correlated ($r = 0.80$). The other metabolites did not, or were only weakly, correlated ($r \leq 0.30$) (Table S2). Highest median urinary pesticide metabolite concentrations were observed for ETU, followed by TCPy, DCCA and 3-PBA (Table S3). OHT showed lowest median concentrations; but women working in agriculture ($n = 33$, 8%) of whom most in packing plants of banana plantations ($n = 30$ out of 33, 7.5%), had considerably higher OHT compared to women who did not: geometric mean (GM), 95%CI = 0.65 (0.28, 1.54) versus GM, 95%CI = 0.09 (0.07, 0.11) μg/L, respectively.

As expected, women who donated their sample during the first trimester of pregnancy had lower TSH and higher FT4 and FT3 compared to women with samples in the second or third trimester (Table 3). About 20% of the women had TSH outside the trimester-specific reference ranges of the Elecsys Thyroid Tests and American Thyroid Association, while only 6% of the women had FT4 higher than expected and FT3 concentrations were all within normal range (Roche©, 2009). Furthermore, in bivariate analyses, women who worked in agriculture during pregnancy had lower FT4 (Table S4), and still tended to be lower after adjustment for age, gestational age at sampling, cotinine, pre-pregnancy BMI, and severe vomiting during pregnancy: $\beta = -0.69$; 95% CI = −1.44, 0.05. Women with lower FT4 also had higher BMIs, were older, were parous, and consumed fish. Lower TSH was associated with detected cotinine. Higher FT3 was associated with increased BMI and severe vomiting.

With respect to the cross-sectional analysis of pesticide exposure and thyroid function, TSH and FT3 were not influenced by any of the fungicides (Fig. 2[A–C] and Table S5). Yet, for FT4, we found women with higher ETU had lower FT4 ($\beta = -0.79$; 95% CI = −1.51, −0.08; $R^2 = 0.25$) for each tenfold increase in urinary ETU. Furthermore, for the fungicide pyrimethanil, we found higher concentrations of OHP were associated with lower FT4 ($\beta = -0.29$; 95% CI = −0.62, 0.03; $R^2 = 0.25$). Summed ETU and OHP showed a stronger association ($\beta = -0.96$; 95% CI = −1.65, −0.27; $R^2 = 0.26$) than ETU or OHP alone, suggesting an additive effect. The metabolite of thiabendazole (OHT) was not associated with FT4. We did not observe non-linear or late effects for exposure to fungicides (Fig. 3[A–C], Table S6).

For insecticides, exposure to chlorpyrifos did not explain any of the thyroid hormones in the cross-sectional analysis (Fig. 2[A–C], Table S5), yet increased exposure to chlorpyrifos (TCPy) was associated with both higher FT4 and FT3 in serum obtained ten weeks after exposure: $\beta = 0.75$; 95% CI = −0.03, 1.53; $R^2 = 0.09$) and $\beta = 0.30$; 95%CI: 0.09, 0.52; $R^2 = 0.12$), respectively, for each tenfold increase in TCPy (Fig. 3[B, C] Table S6). Furthermore, results from both cross-sectional and late effect analyses showed increased exposure to pyrethroids (3-PBA, DCCA, and 3PBA + DCCA) was non-linearly associated with lower TSH (Fig. 4). The herbicide 2,4-D showed null associations with thyroid hormones and TSH for all analyses.

Regarding metals, results from cross-sectional analysis showed women with higher MnB concentrations had both higher FT4 ($\beta = 0.04$; 95% CI = 0.00, 0.07; $R^2 = 0.24$) and FT3 ($\beta = 0.01$; 95% CI = 0.00, 0.02; $R^2 = 0.24$), respectively (Table S5), and no late effect was

Table 3
Distribution of pregnant women's ($n = 400$) TSH, FT4 and FT3 concentrations in their first serum sample obtained during pregnancy, ISA birth cohort study.

Hormone	Trimester	*n*	Mean	SD	Min	P10	P25	P50	P75	P90	Max	Reference Elecsys[a]
TSH (mIU/L)	All	400	1.67	1.01	0.01	0.64	1.06	1.47	2.08	2.93	7.66	
	I	96	1.48	1.19	0.02	0.35	0.77	1.26	1.85	2.67	7.66	0.33–4.59
	II	216	1.73	0.90	0.01	0.74	1.10	1.53	2.14	3.16	4.50	0.35–4.10
	III	88	1.75	1.06	0.01	0.74	1.12	1.63	2.08	2.82	6.08	0.21–3.15
Free T4 (pmol/L)	All	400	14.4	2.37	8.81	11.8	13.0	14.1	15.7	17.2	26.0	
	I	96	15.8	2.34	10.0	13.4	14.0	15.6	17.2	18.4	22.4	12.1–19.6
	II	216	14.3	2.03	8.81	12.0	13.0	14.0	15.3	16.7	26.0	9.63–17.0
	III	88	13.1	1.94	9.00	10.6	11.9	13.0	14.4	15.6	17.9	8.39–15.6
Free T3 (pmol/L)	All	400	4.68	0.61	3.28	3.96	4.29	4.60	5.06	5.44	7.10	
	I	96	5.03	0.57	3.28	4.38	4.65	5.05	5.31	5.61	6.72	3.78–5.97
	II	216	4.62	0.60	3.28	3.97	4.20	4.55	4.95	5.30	7.10	3.21–5.45
	III	88	4.43	0.51	3.32	3.80	4.09	4.47	4.71	5.07	5.87	3.09–5.03

Abbreviations: n, number of samples; Min, Minimum; Max, Maximum; SD, standard deviation, TSH, Thyroid Stimulating Hormone.

[a] Trimester-specific reference values Elecsys Thyroid Tests Germany (Roche 2009).

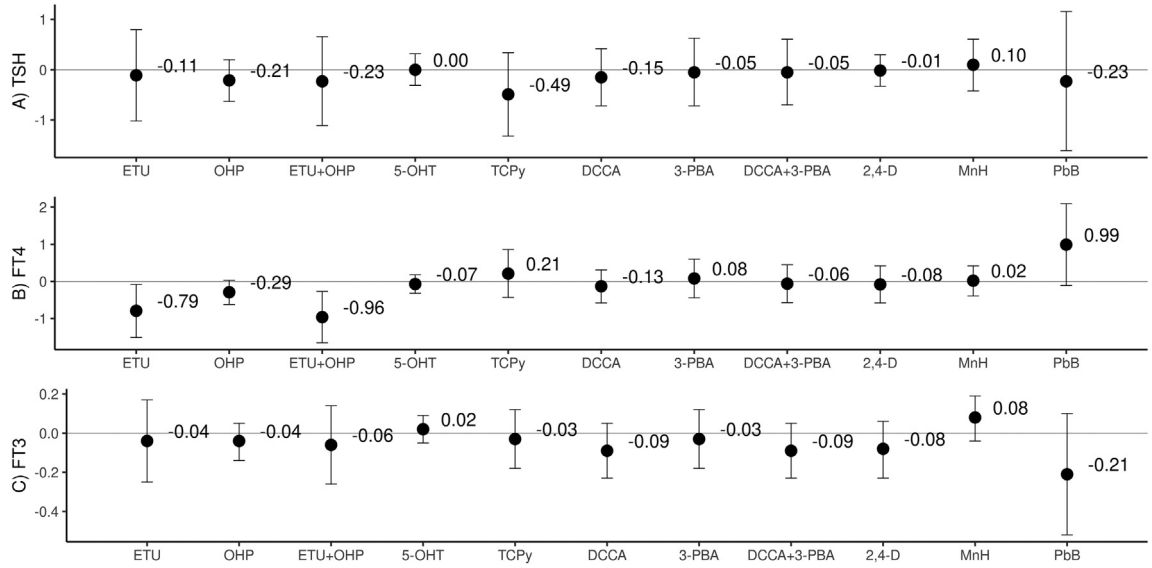

Fig. 2. [A–C]. Adjusted[a] beta coefficients with 95% confidence intervals estimated from linear regression models of pregnant women's biomarkers of exposure with thyroid hormone and TSH, excluding outliers, ISA cohort ($n = 400$). [a]Adjusted for women´s age (years), gestational age (weeks), cotinine detection (yes/no), pre-pregnancy BMI (kg/m^2), and severe vomiting during pregnancy (yes/no).

observed (Table S5). In contrast, MnH was associated with a late positive effect was observed for FT3 ($\beta_{\text{per 10-fold increase}} = 0.16$; 95% CI = 0.03, 0.28, (Table S6), but not in the cross-sectional analysis ($\beta_{\text{per 10-fold increase}} = 0.08$; 95% CI = −0.04, 0.19) (Fig. 2[C], Table S5). Finally, for lead, women with higher PbB concentrations had higher FT4 both in the cross-sectional and the late analysis (Figs. 2[B] and 3[B], Table S5 and S6), but we observed null associations for TSH and FT3.

In general, results of the cross-sectional analysis of exposure metabolites and FT4, FT3, and TSH for all women ($n = 400$) and late-effect analysis ($n = 245$) were similar, although estimates were attenuated because of the smaller sampling size (Table S7). The estimates from the Bayesian multivariate linear regressions with stochastic search variable selection that include all exposures are shown in Table S8. The effect sizes in these multiple exposure models generally were small. Nonetheless, consistent with the single-exposure models, women with higher ETU + OHP had lower serum FT4 concentrations [$\beta = -0.14$ (95%CI = −0.45, 0.02)] and women with higher BPb had slightly higher FT4 [$\beta = 0.09$ (95% credible interval − 0.18, 0.71)]. Table S9 shows the posterior inclusion probabilities for all variables in both the Bayesian multivariate linear regression models with stochastic search variable selection, as well as the Bayesian kernel machine regression models. With respect to the Bayesian multivariate linear models, results generally agreed with the results from the single-exposure linear regression models (Fig. 2[A–C] and Table S5); we observed highest probabilities of inclusion in the FT4 model for (ETU + OHP), MnB, and PbB with inclusion probabilities of 62%, 62%, and 50% respectively. This indicates moderate evidence of relationships with FT4, after adjusting for other exposures. The posterior probabilities of inclusion of the Bayesian kernel machine regressions (BKMR) were consistently smaller than those of the linear regressions; all probabilities were between 1 and 35%

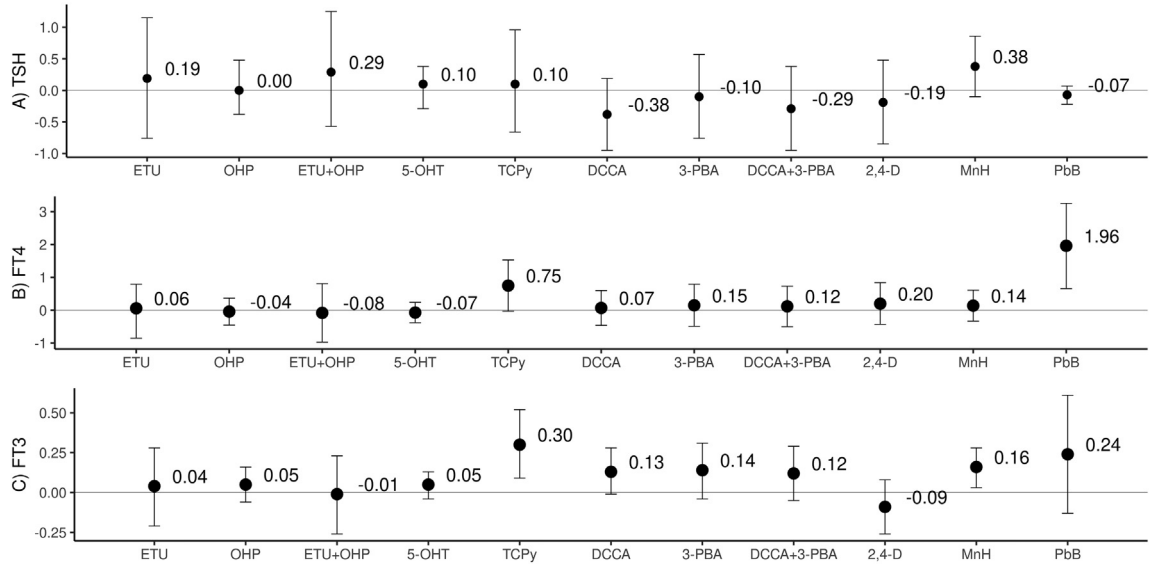

Fig. 3. [A–C]. Adjusted beta coefficients with 95% confidence intervals estimated from linear regression models of pregnant women's biomarkers of exposure with thyroid hormone and TSH measured about ten weeks after exposure, excluding outliers a priori, ISA cohort ($n = 245$).
[a]Adjusted for women´s age (years), gestational age (weeks), cotinine detection (yes/no), pre-pregnancy BMI (kg/m^2), and severe vomiting during pregnancy (yes/no).

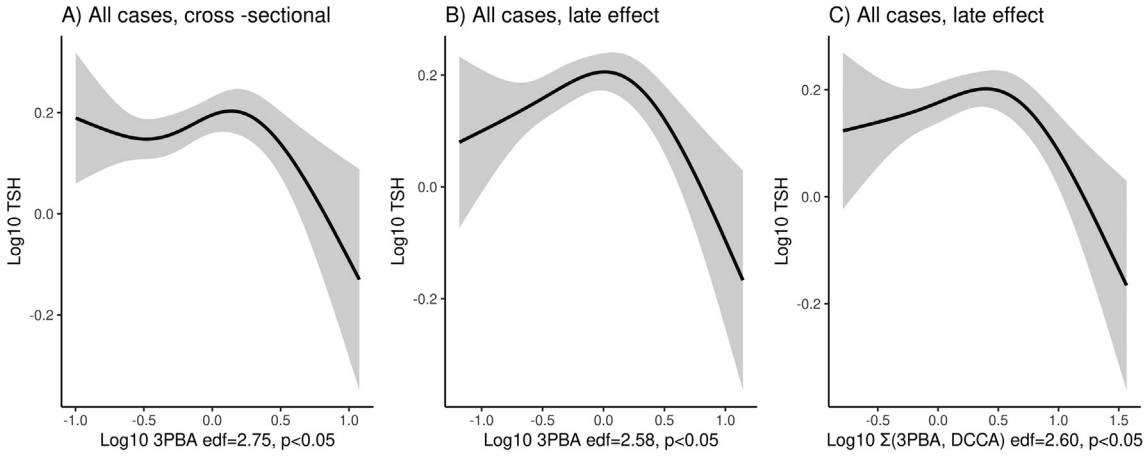

Fig. 4. Significant (edf ≥ 2, $p < 0.05$) smoothed adjusted non-linear associations for prenatal exposure metabolites with thyroid hormones and TSH, excluding outliers a priori, ISA cohort study. Abbreviations: TSH, Thyroid Stimulating Hormone; FT4, free thyroxine; FT3, free triiodothyronine; DCCA, 3-(2,2-dichlorovinyl)-2,2-Dimethylcyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid; Mn, manganese; MnB, manganese in blood; MnH, manganese in hair.

(Table S8). This shows the BKMR model did not improve the data-fit; and therefore, our mixture analysis relied on the estimates of Bayesian multivariate linear regressions with stochastic search variable selection.

4. Discussion

The results of this study show exposure to fungicides, insecticides, manganese, and lead were associated with changes in thyroid hormone concentrations in pregnant women from the ISA study, particularly FT4. In the cross-sectional analysis, we observed lower FT4 in women with increased exposure to mancozeb/ETU and pyrimethanil, and higher FT4 in women with increased MnB and PbB. In the subset of women with a second serum sample, FT3 was increased among women with higher chlorpyrifos exposure and higher MnH at enrollment; these were late effects, about ten weeks after exposure was measured. Consistently lower TSH concentrations were observed in women with high pyrethroid exposure in both the cross-sectional and late-effect analyses; these associations were non-linear.

The lower levels of FT4 observed in pregnant women with elevated urinary ETU in the current study, concurs with previous studies in human beings that reported hypothyroidism-like effects in relation to ETU and EDBC-exposures. ETU-exposed workers from a rubber manufactory had lower T4 than non-exposed workers (Smith, 1984), and EDBC-exposed Mexican and Philippian agricultural workers had increased TSH serum concentrations (Steenland et al., 1997; Panganiban et al., 2004). Smith et al. (1984) reported decreased T4 among workers exposed to ETU during rubber manufacturing activities, as compared to workers with lower exposures, but only one worker had altered TSH. Steenland et al. (1997) and Panganiban et al. (2004) reported increased TSH, but no decreased T4, in EBDC-exposed agricultural workers. In addition, a cross-sectional study among farm residents in Brazil found men, but not women, with more days of EDBC use during their lifetime, had increased TSH and decreased FT4 (Piccoli et al., 2016). In contrast, results from a cross-sectional study among male Spanish adolescents (Freire et al., 2021) showed null associations for urinary ETU with TSH, FT4, and total T3, but their median urinary ETU concentrations were about six-times lower than those observed among the pregnant women of this study [0.5 versus 2.9 μg/g creatinine (van Wendel de Joode et al., 2014), respectively]. Finally, female spouses of pesticide applicators exposed to EBDC fungicides had an increased risk on both hypo- and hyperthyroidism (Goldner et al., 2010; Shrestha et al., 2018). The inhibiting effect of mancozeb and ETU on thyroid functioning maybe due interference with active iodide uptake mediated by the sodium/iodide symporter (NIS) and thus directly interfere with thyroid hormone synthesis (Hallinger et al., 2017; Hurley et al., 1998; Kackar et al., 1997). This hypothesis is supported by results from a study in mancozeb-exposed workers in Italy where increased urinary iodine excretion (>250 μg/L) was observed more frequently found among higher exposed workers (urinary ETU > 20 μg/L) (Medda et al., 2017). The effect observed in our study seems immediate as it was observed in the cross-sectional analysis only; nevertheless, this finding may still be of biological relevance as even minor changes in the thyroid homeostasis may affect fetal neurological development (Boas et al., 2012).

With respect to the additional fungicides, our finding of higher concentrations of the metabolite of pyrimethanil being associated with decreased FT4 in our cross-sectional analysis, albeit not statistically significant, partly coincides with results from studies in pyrimethanil-exposed rats that had lower serum T4 (Hurley et al., 1998). Pyrimethanil is thought to cause liver enzyme induction leading to increased inactivation and further excretion of thyroid hormones from the body (Hernández et al., 2020). The stronger association of summed ETU and OHP concentrations suggests an additive effect when being exposed to both mancozeb/ETU and pyrimethanil. Furthermore, in our study, urinary metabolites of the fungicide thiabendazole did not explain thyroid function, although studies in rats exposed to thiabendazole reported altered thyroid hormones and thyroid volume, and, in part of the studies, also thyroid follicular cell adenomas formation (US-EPA, 2002; Efsa, 2014). A possible explanation for this difference is women participating in the ISA cohort generally had low exposures to thiabendazole, except from the 8% of the women who worked in agriculture, mainly at banana plantations (7.5 out of 8%). Thiabendazole is used as a post-harvest fungicide on bananas, prior to packing them for shipment; as it is not aerially sprayed, exposure to thiabendazole is mainly occupational. The women working in agriculture during pregnancy had about a ten-fold higher urinary thiabendazole metabolite concentrations than women who did not have this condition, and, interestingly, women who worked in agriculture during pregnancy also tended to have lower FT4; possibly, higher exposures to thiabendazole inhibit thyroid hormones, yet as relatively few women had these relatively high thiabendazole exposure we may have been underpowered to observe this effect in all women ($n = 400$), or possibly the women were exposed to other pesticides that we did not measure but are used on packing plants, like for example imazilil. Also, pregnant women working in agriculture had higher ETU concentrations (van Wendel de Joode et al., 2014).

Regarding insecticides, our finding of chlorpyrifos metabolite TCPy being associated with higher FT3 measured approximately ten weeks later, suggest a late thyroid-stimulating effect of chlorpyrifos on thyroid function, while our cross-sectional analysis showed null associations. Results from cross-sectional studies in men and women from the general population were inconclusive; some suggested stimulating effects

on FT4 and TSH, with others suggested inhibiting effects or null associations (Meeker et al. 2009; NHANES 1999-2002; Fortenberry et al., 2012; Suárez et al., 2021). None of these studies have addressed late effects in pregnant women. In addition, for exposure to synthetic pyrethroid insecticides, we observed consistently lower TSH concentrations in women with high pyrethroid exposure (these associations were non-linear). A cohort study in Tokyo did not observe effects of pyrethroid exposure on thyroid function in pregnant women or neonates, yet the exposure levels (median = 0.36 μg/L) were about half as low (Zhang et al., 2013) as compared levels observed in our study (median = 0.69 μg/L). Furthermore, our findings contrast with results from a South African birth cohort study in which maternal urinary pyrethroid metabolite concentrations during pregnancy were associated with increased TSH in newborns (Chevrier et al., 2019). These different findings might be because Chevier et al. (2019) measured TSH in newborns (median 8 days post-partum) whilst we measured TSH in pregnant women. Also, our associations were non-linear, whilst Chevier et al. reported linear associations after transforming both pyrethroid metabolites and TSH to log10-scale. The pyrethroid exposure levels in Chevier et al.'s study and our study were similar. Pyrethroids have similar structures as thyroid hormones T3 and T4 may therefore disrupt thyroid homeostasis, causing modifications in the binding of thyroid transport proteins and interfering with thyroid hormone signaling pathways (Du et al., 2010; Zhang et al., 2013; Hernández et al., 2020); but these effects may be different for low and high pyrethroid exposures, and non-linear. Our finding of pyrethroid exposure being associated with hyperthyroidism-like effects coincides with results from a recent study in Sprague-Dawley rats and rat thyroid cells that indicated hyperthyroidism induced by the pyrethroid cypermethrin (Ha et al., 2021).

We observed null associations for the herbicide 2,4-D which is consistent with a small study in 21 male and 18 female workers from herbicide production plants for whom no notable abnormalities on thyroid function were reported during a five-year period, but associations of urinary 2,4-D with T4, T3 and TSH were not presented (Knopp, 1994). In our study, 2.4-D concentrations were relatively low, although frequently detected.

Manganese is an essential element and both lack of and excess Mn may affect thyroid homeostasis, although the mechanism is unknown (Memon et al., 2015). In our study, manganese seemed to have a stimulating effect on thyroid function, as MnB was associated with increased FT4 and FT3, and MnH with increased FT3 (late effect); but we did not observe consistent results for Mn in both blood and hair. MnB and MnH reflect measures of Mn, and did not correlate ($r = -0.04$, Table S2). MnB, being Mn an essential element, is homeostatically regulated by different systems in the body, while MnH is thought to reflect excess Mn during the past three weeks (Mora et al., 2018). Furthermore, even though both ETU and Mn are metabolites of mancozeb, we found opposite results for ETU and Mn, so their mechanisms of action are likely to differ. In our study excess manganese is likely to occur from drinking Mn-containing well water and manganese from mancozeb exposure applied with light aircrafts (Mora et al., 2014; van Wendel de Joode et al., 2016). The increased FT3 with MnH partly agrees with results from previous studies. Memon et al. (2015) found women in Pakistan with hyperthyroidism had higher mean serum Mn concentrations as compared women with normal thyroid function, while women with hypothyroidism had lower mean serum Mn concentrations.

According to our results, PbB also may stimulate thyroid function as women with higher PbB concentrations had higher FT4 both in the cross-sectional and the late-effect analysis. The PbB levels in our population were low (median = 7, p90 = 12), similar to pregnant women from National Health and Nutrition Evaluation Survey (NHANES) in 2003–2004: median = 6, p95 = 18 μg/L (Woodruff et al., 2011). Like in this study, results from an analysis of NHANES in 2007–2010 among women showed increased PbB was associated with increased FT4, but not associated with TSH or FT3 (Luo and Hendryx, 2014). In contrast, results from a study in pregnant women from Kosovo (mean PbB = 200 ± 70 μg/L showed higher PbB was associated with lower FT4 concentrations (Kahn et al., 2014), and a study in China (median PbB = 38, p25–75 = 25–55 μg/L) (Nie et al., 2017) found higher PbB was associated with increased TSH, decreased FT4 and increased thyroid peroxidase (TPO) antibodies). Yet, PbB concentrations in these studies were 6 to 30-fold higher than the concentrations measured in our study. The exact mechanism of action of lead on thyroid function is unknown (ATSDR 2019), nevertheless, Pb may interfere with 5′-deiodinase causing an imbalance in the hypothalamic-pituitary-thyroid axis, reflected by an increase in serum T4 (Erfurth et al. 1987; Gustafson et al. 1989).

This study has some limitations. First, the response time of thyroid hormones and hypothalamic-pituitary-thyroid axis to environmental exposures in pregnant women is unknown; for example, after a reduction of FT4 by thyroid peroxidase inhibition it is unclear when TSH concentrations will modify (Giray et al., 2010). Second, most of the previous studies correspond to exposed workers, healthy volunteers and sometimes different methods were used to evaluate exposures and effects on thyroid function (Hartfort-Nielsen et al. 2011), limiting comparisons between. Third, we only obtained a second sample of thyroid function for a subsample of the women, not allowing a direct comparison between the cross-sectional and late-effect analyses. Fourth, as we performed multiple comparisons, some of the associations we found may be due chance. Yet, as the models that included multiple exposures showed similar findings as the models that included one exposure metabolite at a time, this is unlikely. Last, some studies have included iodine as a confounding variable because iodine deficiency affect thyroid function (Giray et al., 2010; Zhang et al., 2013). Nevertheless, the women of our study are not expected to have iodine deficiency because salt for human consumption has been iodinated since some decades in Costa Rica (UNICEF (Fondo de las Naciones Unidas para la Infancia), 2013).

A strength of our study is that were able to adjust for a wide range of potential confounders and our sample size was larger than most studies published so far on pesticide metabolite concentrations and thyroid function (Smith, 1984; Knopp, 1994; Zhang et al., 2013; Kahn et al., 2014). We used biomonitoring to measure exposure to contaminants that provided us with measures of exposures that reflect uptake from all routes of exposure (Fenske, 2005). To our knowledge, this is one of the first studies among pregnant women to evaluate effects of pesticides and metals on thyroid function.

5. Conclusions

The results of this study show that exposure during pregnancy to the fungicides mancozeb, and/or its metabolite ETU, pyrimethanil, and possibly thiabendazole, was associated with an inhibition of FT4, a thyroid-inhibiting effect. In contrast, exposure to chlorpyrifos, pyrethroids, lead, and excess manganese, seemed to stimulate thyroid function, as we observed higher FT4, FT3, or decreased TSH. Most associations were observed for FT4, and future research is needed to understand the underlying biological mechanisms that may explain our findings.

Although changes in FT4, FT3 and TSH were mostly within clinical ranges, our findings are of concern as even subtle changes in pregnant women's thyroid function may affect fetal development and child development during early life (Escobar et al. 2004; Hartford-Nielsen et al. 2011; Zhang et al., 2013). Following the precautionary principal, we recommend implementing measures to reduce exposure to pesticides, lead, and excess manganese.

CRediT authorship contribution statement

Andrea Corrales Vargas: Data Curation, Formal Analysis, Writing-Original draft preparation.

Jorge E. Peñaloza Casteñeda: Data Curation, Formal Analysis, Writing-Reviewing.

Emelie Rietz Liljedahl: Methodology, Formal Analysis, Writing-Reviewing.

Ana M. Mora: Methodology, Investigation, Writing-Reviewing and Editing.

Jose Antonio Menezes-Filho: Methodology, Writing-Reviewing.

Donald R. Smith: Methodology, Writing-Reviewing.

Donna Mergler: Methodology Investigation, Funding Acquisition, Reviewing and Editing.

Brian Reich: Formal Analysis, Reviewing and Editing.

Andrew Giffin: Formal Analysis, Writing-Reviewing.

Jane A. Hoppin: Methodology, Reviewing and Editing, Funding Acquisition, Project Administration.

Christian Lindh: Conceptualization, Methodology, Reviewing and Editing, Funding Acquisition, Project Administration.

Berna van Wendel de Joode: Conceptualization, Methodology, Investigation, Supervision of formal analysis, Writing-Original draft preparation, reviewing and Editing, Funding Acquisition, Supervision, Project Administration.

Declaration of competing interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151288.

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