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Multiple pesticides in mothers' hair samples and children's measurements at birth: results from the French national birth cohort (ELFE).

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ABSTRACT

Background: A growing body of studies now suggests that the general population is continuously and ubiquitously exposed to numerous pesticides. However, studies investigating the possible role of environmental exposure to pesticides on fetal growth have focused on a limited set of substances, despite the hundreds of modern pesticides currently available.

Aim: To explore the relation between maternal hair concentrations of 64 pesticides and metabolites and their newborns' measurements at birth, with data from the ELFE French nationwide birth cohort.

Methods: We measured 64 compounds (10 – 100% detection) in bundles of hair 9 centimeters long collected at birth from 311 women who gave birth in France in 2011. We assessed their associations with birth weight, length, and head circumference, adjusted for potential confounders, and used elastic net regularization to simultaneously select the best predictors of measurements at birth. Selected variables were multiply imputed for missing values, and unpenalized estimators were assessed by standard linear regression.

Results: We observed statistically significant associations between maternal hair concentrations of seven pesticides or pesticide metabolites and birth measurements (weight: fipronil sulfone; length: TCPy, bitertanol, DEP, and isoproturon; head circumference: tebuconazole and prochloraz). Analyses restricted to boys identified 12 additional compounds: 8 independently associated with birth weight (3Me4NP, DCPMU, DMST, fipronil, mecoprop, propoxur, fenhexamid, and thiabendazole), 2 with birth length (dieldrin and β-endosulfan), and 6 with head circumference (β-endosulfan, β-HCH, fenuron, DCPMU, propoxur, and thiabendazole).

Conclusion: Our results suggest that prenatal exposure to 19 pesticides or metabolites from various chemical families may influence measurements at birth. As with any exploratory research findings, results should be interpreted cautiously, until they are replicated or verified by further epidemiological or mechanistic studies.

Keywords: Maternal exposures; Pesticides; Fetal development; Birth weight; Environmental exposure.

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I. INTRODUCTION

Pesticides are substances used for crop protection to prevent, destroy, or control harmful organisms or diseases (European commission, 2017). Some are also used for domestic purposes, in human and animal medicine, for vector control in specific areas or to treat construction materials (Anses, 2010). During 2007, 2.4 billion kilograms of pesticides were used worldwide (Science, 2013). In 2014, France ranked as the second leading user in Europe with about 70,000 tons of pesticides applied to crops (Eurostat, 2017). This intensive pesticide use has contaminated water, soil, air, and food (Anses, 2010), and a growing body of studies conducted in industrialized countries now suggests that the general population – including pregnant women – is continuously and ubiquitously exposed to numerous banned or currently used pesticides (Dereumeaux et al., 2016; Haines et al., 2017; Osaka et al., 2016; Ramos et al., 2017).

Various studies point to a potential association between prenatal exposure to pesticides through occupational or environmental settings and adverse pregnancy outcomes, including suboptimal fetal growth (INSERM, 2013; Shirangi et al., 2011). Although birth weight (BW), birth length (BL), and head circumference at birth (HC) are not specific for a dysfunction or disease, these parameters could be early markers of an alteration in fetal development. In addition, low BW is associated with various adverse effects during later life, including obesity and type 2 diabetes (Feng et al., 2018), cardiovascular disorders (Mercuro et al., 2016), and poorer cognitive performance (Chen et al., 2016), while prenatal head growth has been associated with intelligence quotient scores in later life (Gale et al., 2006).

The epidemiological literature investigating the possible role of environmental exposure to pesticides on child health at birth often uses biomonitoring data based on blood or urine analyses, to provide more precise assessments of exposure to specific compounds. Most studies considering fetal growth have focused on organochlorine, organophosphorus (INSERM, 2013), and to a lesser extent pyrethroid pesticides (Saillenfait et al., 2015). Some associations have also been observed for some individual pesticides, such as atrazine (Goodman, 2014) and vinclozolin (Wickerham, 2012). Results remain inconsistent for most of these. Besides these specific pesticides and chemical families, very little is known about the possible effects of other pesticides on fetal growth, although about 500 active ingredients are currently approved for use in the EU (European commission, 2017). In addition, available epidemiological studies usually consider limited sets of compounds at the same time and are thus unable to control for potential coexposures within pesticide mixtures present in women's environments.

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After measuring multiple pesticides in hair with high levels of sensitivity (Beranger et al., 2018), we aimed to explore the association between hair concentrations of pesticides and measurements at birth in the French ELFE mother-child cohort, based on statistical models that simultaneously include the 64 compounds most frequently detected in the hair samples (19 chemical families).

II. METHODS

Population study

ELFE (*Etude longitudinale française depuis l'enfance*) is a multicenter, nationally representative birth cohort conducted in France, intended to explore the influence of various environmental factors on children's development, health, and socialization, from birth to age 20. The cohort design and characteristics have been presented elsewhere (Vandentorren et al., 2009; ELFE, 2017).

From the 320 maternity units that agreed to participate (59% of the eligible units in mainland France), women (≥18 years old) who gave birth to liveborn singleton at ≥ 33 weeks of gestation were included during four 4- to 8-day inclusion periods in 2011. Biological sampling was organized in the 211 participating hospitals that averaged more than 500 deliveries per year and were located <150 km from 15 biobanks distributed over mainland France. Due to delays in signing the agreement with the partner in charge of the transport and storage of biological samples, and the late receipt of approvals from French ethics committees, biological sampling was limited to women who gave birth during the last three inclusion periods (June 27–July 4, September 27–October 4, and November 28–December 5). Of the 18,040 mothers finally enrolled in the cohort (participation rate: 51%), hair samples were available for 2,866 (16%).

Among the women recruited in the southwestern (Aquitaine, Midi-Pyrénées, and Poitou-Charente) and northeastern (Champagne-Ardenne, Bourgogne, and Lorraine) regions of France, we selected all women who had hair long enough for multiresidue analysis (see below) and gave birth to singleton infants at term (37–41 weeks of pregnancy), without any identified congenital malformation (n= 311, 11%). These two regions were selected to represent different agricultural environments, both representative of the country. The southwest is characterized by vineyards, corn and sunflower crops, breeding (sheep, goats, and cattle), and fresh vegetables, and the northeast mainly by vineyards, cereals, legumes, vegetables, and cattle breeding (Agreste, 2012).

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Data and biological sample collection

Women were interviewed face-to-face at delivery by trained midwives or nurses who collected information about their socioeconomic status, lifestyle, height, and weight. Medical information about mothers and babies was collected from the medical records, including the neonates' BW, BL, and HC (hereafter summarized as “measurements at birth”). Self-administered questionnaires were also used at birth to collect information about women’s habits (alcohol, tobacco, and food intake) during pregnancy. When possible, data collected by telephone interviews two months and 1 year after birth were used to complete missing or incomplete values for variables collected at birth.

One strand of hair was collected just after delivery and before discharge (usually 3-4 days after vaginal delivery and 5-7 days after cesarean birth). Hair was cut as close as possible to the scalp, on the occipital region of the head, stapled onto an index card, placed individually in a Kraft envelope, and shipped to and stored at the biobank, all at ambient temperature. The proximal (close to the scalp) and distal parts of the strand were indicated on the index card. Assuming a growing rate of 1 cm per month, we selected the first 9 centimeters of the strand for analyses (proximal part), to cover the entire period of pregnancy. The median length of the hair sample used was 9 cm (interquartile range (IQR): 8 – 9 cm), and the median mass of the hair strands was 50.1 mg (IQR: 50.0 – 50.3). In previous analyses, we showed that observed differences in hair length or mass have negligible effects on pesticide concentrations and detection frequencies in our population (Beranger et al., 2018). The good consistency (biomarker detection) and intra-class coefficient values (biomarker concentrations) across successive hair segments suggest limited intraindividual compared with interindividual variability.

Chemical analyses

We established a list of 180 compounds selected *a priori* based on the 2011 pesticide sales data in northeastern and southwestern France (French Ministry of Agriculture, 2012); the list of priority pesticides in terms of food safety, based on the probability of exceeding the acceptable daily intake in the general French population (Nougadère et al., 2014); the prioritization list of the French general biomonitoring strategy (Fillol et al., 2014); data on indoor air/dust contamination and domestic usage of pesticides (ANSES, 2010), completed by expert assessment. The analytical method developed by the laboratory of Human Biomonitoring Research Unit at the Luxembourg Institute of Health was applied to blank hair samples supplemented with standards (parent pesticides and metabolites) for the compounds selected *a priori*. Only 113 of the 180 initially selected compounds were detectable in the final extract after undergoing through extraction, purification, and concentration. We thus did

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not test for the remaining 67 biomarkers. In addition to these prioritized compounds, the analytical method allowed the analysis of 27 additional pesticides, which were thus analyzed without any *a priori* information on the probability of exposure. Overall 111 pesticides and 29 pesticide metabolites were screened in hair samples. These have been fully described elsewhere (Béranger et al., 2018).

The analytical methods used to measure multiple pesticide residues in hair samples have previously been published in detail (Duca et al., 2014b; Hardy et al., 2015; Salquebre et al., 2012; Béranger et al., 2018). Hair samples were successively washed with sodium dodecyl sulfate (SDS) and then methanol, to remove chemicals that might have been deposited on the hair surface (Duca et al., 2014a). The samples were then pulverized with a ball mill (Retsch) and extracted overnight at 40°C in 1 mL of acetonitrile.

For parent pesticide analysis, 7.6 mL of phosphate buffer at pH 7 (1 M) was added to the 300-µL extract in a 10-mL screw cap vial fit for solid-phase microextraction (SPME) analysis. Sample analysis began with SPME (fiber exposure at 60° C for 80 min); it was followed by desorption in the GC injector at 260° C for 10 min. For metabolite analysis, the 300-µL extract was evaporated to dryness under a gentle nitrogen stream at 37° C. Next, 30 mg of potassium carbonate, 1 mL of acetonitrile, and 100 µL of PFBBr/acetonitrile (1:3, v/v) were added to the residue, before derivatization of the compounds for 30 min at 80° C. The derivatized extract was transferred to a tube without the salt and evaporated to dryness. The residue was then reconstituted in 200 µL of ethyl acetate and centrifuged for 7 min at 18,000 × g to remove any particles from the extract. After the transfer of this extract into an injection vial and its reevaporation to dryness, the residue was resuspended in a volume of 20 µL of ethyl acetate for injection into the GC–MS/MS system. The remaining supernatant was dried under a gentle stream of nitrogen and recovered in a solution of water:acetonitrile:formic acid (94.5/5/0.5, v/v/v) for analysis by ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The methods used for the analysis of the different subextracts have previously been described in more detail (Hardy et al., 2015; Duca et al., 2014a, 2014b; Salquebre et al., 2012).

Quality controls (one blank and controls supplemented at eight different concentration levels) were analyzed along with the field samples in each analytical run. The blanks controlled for the absence of cross-contamination, whereas the supplemented controls were used to quantify any possible drift in analytical response. The limit of quantification (LOQ) for each pesticide was defined by taking into account variability in the concentration of the quality controls below 25%, with accuracy between 75 and 125%. LOQ ranged from 0.02 pg/mg of hair (pyraclostrobin) to 50 pg/mg (propargite). For values

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below the LOQ, the laboratory provided the minimum detectable value, including the value for which the variability might exceed 25%. The high specificity of the tandem mass spectrometry used, however, should have prevented false-positive detection. The lowest detected values (or the limits of quantification, if lower) were thus retained as the practical limits of detection (LOD), see Table 1.

Statistical analyses

Descriptive results (detection frequency and median concentration) for the 140 pesticides and pesticide metabolites have been presented elsewhere (Beranger et al., 2018). To explore the relations between prenatal exposure to pesticides and BW, BL, and HC, we restricted our analyses to the 64 compounds detected in at least 30 subjects (10% of our study population). These compounds are listed in Table 1.

Data preparation

For chemicals with detection rates ≥ 70%, values below the LOD were imputed by random values between 0 and the LOD from a log normal probability distribution derived by a maximum-likelihood estimation method based on values above the LOD (Jin et al., 2011). Chemicals with detection rates <70% and ≥50% were categorized into three classes (below the LOD; up to the 50^{th} percentile of detected values; above the 50^{th} percentile – hereafter referred to as low, medium, and high exposure levels). Chemicals with detection rates below 50% were dichotomized (detected; not detected).

We used directed acyclic graphs (DAGs) to determine which confounders to adjust for in our different models, from the following list of variables (see supplementary files, Figures 1–3): maternal age; prepregnancy maternal weight; maternal height; gestational age at birth; parity; maternal education; tobacco consumption during pregnancy; alcohol consumption during pregnancy; fish consumption; the child's sex; period (season) of birth; and presentation at delivery (cephalic/breech). All models were adjusted for prepregnancy maternal weight (continuous); parity (nulliparous; parous); tobacco consumption during pregnancy (yes/no); and alcohol consumption during pregnancy (yes/no). For HC models, we also adjusted for fish consumption (< once a month; ≥ once a month). Height of the mother (continuous) and gestational age (continuous) were identified as two major predictors of measurements at birth and added to the models to improve the precision of the effect estimates.

In view of the data distribution, all continuous exposure variables (detection rate ≥ 70%) were natural log-transformed to reach normality, except carbendazim, which was 1/X transformed. Among

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the continuous adjustment covariates, only maternal weight required data transformation (natural log). To assess the linearity of the relations between continuous covariates and exposure variables for each outcome (BW, BL, and HC), we used restricted cubic splines (Desquilbert and Mariotti, 2010), while adjusting for potential confounders previously identified. In case of departures from linearity (*p*-value <0.05), the exposure variables were categorized into tertiles (hereafter referred to as low, medium, and high exposure levels); for adjustment covariables, the shape of the relation was assessed with a generalized additive model. All *p*-values are presented in the supplementary files, Table S1. Since all the nonlinear relations identified were quadratic, we added squared terms to the model when appropriate. Continuous exposure variables were mean-centered and scaled by twice their respective standard deviations (SD) (Gelman 2008) to give more similar prior importance in penalized regressions to continuous and binary variables and to improve the comparability of their coefficients.

Selection of best predictors

Multivariable linear regression models produce unreliable parameter estimates in cases of multicollinearity and prevent the exploration of datasets with too large numbers of dimensions (Chadeau-Hyam et al., 2013). To overcome these limitations and determine which exposures are independently associated with the outcome, we used elastic net regression modeling. Elastic net is a compromise between the lasso and ridge penalized functions (Zou and Hastie, 2005), which penalize the regression coefficient of the predictors, by limiting, respectively, the upper bound of the L^2 norm (sum of the square value of the coefficients of the model) and the L norm (sum of the coefficients). The ridge function handles multicollinearity, while the lasso function ensures the sparsity of the coefficients by shrinking them to zero for smaller values of the λ calibration parameter; it thus retains only a limited number of the models' best predictors. Elastic net regression modeling results in a balanced approach that makes it possible to select the strongest predictors, while enabling collinear predictors to be included in the model. In their comparison of different multipollutant approaches with a simulated dataset, Agier et al. (2016) suggested that an elastic net regression modeling approach performs well in our situation (i.e., environmental exposure to chemicals modestly correlated between them – see Béranger et al., 2018).

In elastic net approaches, simple imputation strategies were used for missing values of independent variables. Using the method of Jin et al. (2011) described above, we imputed continuous exposure variables (< 4% missing) from a random distribution between 0 and the maximum detected value. Continuous adjustment covariables (<1% missing) were imputed by the median. For categorical variables (0 – 12% missing), we imputed either by the mode (binary) or by the median class (tertile).

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Missing values for outcomes were not imputed. Variables categorized into 3 classes were entered in the elastic net models as two separate dummy variables. Elastic net models were built for each outcome and included all exposure variables and adjustment covariates to select the strongest predictors among exposure variables. Given that the adjustment covariates were expected to be stronger predictors of the birth outcomes than pesticide exposures, we allowed the elastic net procedure to penalize adjustment covariates to encourage selection among exposure variables of interest.

Two calibration parameters are available for the elastic net method: α and λ. The α value controls the balance between the ridge (α=0) and lasso (α =1) penalties, and the λ value (≥ 0) corresponds to the degree of penalization. We used the *optimize* function in R to draw a function of α and identify the value for which the mean square error of the model is minimum. To achieve more stable estimation, optimal α was assessed 100 times and averaged. We then used 10-fold cross-validation to define the λ values corresponding to the minimum mean square error of the model, given the averaged optimal α. Cross-validation was repeated 100 times, and the optimal λ was averaged. We then ran the elastic net regression model based on the averaged optimal α and λ to identify the best predictors of the outcomes. Elastic net regression was modeled with the GLMNET package (Friedman et al., 2010), R software V3.0.0.

Estimation of the effects of the selected predictors

Elastic net regression modeling, while optimized to select the best predictors of the outcome, produced penalized coefficients. To obtain unbiased estimates, we used multivariable linear regression models that included all the predictors selected by the elastic net regression models (Lenters et al., 2016) as well as the complete *a priori* set of adjustment covariates determined by the DAGs.

A multiple imputation strategy based on 10 imputations and a fully conditional specification method (to handle non-monotonic missing values) was used to impute missing values for both exposure and adjustment covariates. For nonlinear covariates, when at least one of the normal or squared terms was selected, both were kept in the model. We also explored possible sex-heterogeneity by stratifying models for sex. We estimated the variance inflation factor (VIF) for all coefficients of exposure variables to detect problems of multicollinearity in our linear models. Linear regressions under multiple imputations were performed with the MI and MIANALYZE procedures, SAS software V9.4 (SAS Institute).

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Sensitivity analyses

In the sensitivity analyses, we checked the influence of statistical outliers by excluding them from the statistical analyses. Outliers were defined by studentized residual values below -2.5 or above 2.5. Based on the main elastic net selections, we also stratified linear regression models by region (southwest/northeast). We then replaced the individual exposure variables in the elastic net regression models by terms summing the concentrations (weighted by the molecular mass) of each parent compound and its specific metabolites, or of common metabolites sharing biological pathways: dialkyl phosphates (diethyl and dimethyl phosphates separately); fipronil and its metabolite (fipronil sulfone); diuron and its metabolites (DCPU and DCPMU); bifenthrin and its specific metabolite (ClCF3CA); chlordanes; and DDT isomers and their metabolites.

Ethics approval

All women agreed to participate in the ELFE cohort and provided written informed consent for themselves and their babies. The ELFE maternity study, including the biological sampling and use for the assessment of environmental exposures, was approved by the appropriate ethics committees, including the French Consulting Committee for the Treatment of Information in Medical Research (n°10.623), the French National Commission for the Confidentiality of Computerized Data (n°910504), and the Committee for the Protection of Persons (n° CPP-IDF IX-11-024).

III. RESULTS

Description of the 311 study participants

Most participating women were parous (57.1 %), nonsmokers (74.4 %), ate fish at least once a month (66.2 %), and went to university (54.8%). Their mean age was 30.1 years (±5), mean height 164.5 cm (± 5.9), and mean prepregnancy weight 62.9 kg (± 13.5); they gave birth at a mean of 39.3 weeks of gestation (±1.2). The number of boys and girls was relatively well balanced (47.7 and 52.3%, respectively). The children's mean BW was 3322.3 g (± 428.7), their mean BL 49.5 cm (± 2.0), and their mean HC 34.3 cm (± 1.4). Among these 311 women, 77 gave birth in June-July (24.7%), 111 in September-October (35.7%), and 123 in November-December (39.5 %). Table 2 contains more detailed information. Population characteristics were similar in each region, except for fish consumption (at least once a month: 74% in the southwest and 61% in the northeast; see Table S2).

Exposure

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Table 1 presents the 64 pesticides and metabolites detected in the hair of more than 30 women from our population. Of these, 28 were detected in more than 70% of the hair samples, 10 were detected in 50–70%, and 26 were detected in less than half. Median concentrations ranged from 0.03 pg/mg of hair (three molecules: DMTP (di-methyl-thiophosphate), interquartile range (IQR) <LOD – 0.11; α-HCH (hexachlorocyclohexane), IQR <LOD – 0.09; metolachlor, IQR 0.01 – 0.05) to 37.93 pg/mg (permethrin, IQR 17.15 – 91.61).

Variable selection with the elastic-net approach

Overall, eight chemicals were selected as predictors of BW: β-endosulfan (decrease, i.e., BW decreases with exposure), permethrin (decrease), fipronil sulfone (increase), mecoprop (decrease), bitertanol (increase), DMST (dimethylsulftoluidide, increase), diflufenican (decrease), and imidacloprid (decrease). For BL, eight chemicals were selected: β-endosulfan (decrease), TCPy (3,5,6-trichloro-2-pyridinol; decrease), DEP (di-ethyl-phosphate; decrease), cypermethrin (decrease), dichlorprop (decrease), bitertanol (increase), fenuron (decrease), and isoproturon (increase). Finally, 10 chemicals were selected as predictors of HC: β-endosulfan (decrease), permethrin (decrease), ClCF3CA (3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylic acid; increase), carbofuran (medium intensity of exposure: increase, high: decrease), prosulfocarb (decrease), tebuconazole (increase), prochloraz (increase), and imidacloprid (decrease). Selected pesticides and metabolites are presented in Figures 1 (BW), 2 (BL), and 3 (HC).

Association between elastic net-selected exposures and birth measurements

Based on multivariable linear regression on the selected predictors, we observed statistically significantly higher BW for a medium ($\beta_{adjusted}$ = +150 g; 44, 255), but not a high level of exposure to fipronil sulfone ($\beta_{adjusted}$ = +28 g; -84, 141), compared to the group with the lowest exposure level.

We found statistically significantly lower BL associated with the intermediate ($\beta_{adjusted}$ = -0.64 cm; -1.15, -0.14), but not high level of exposure to DEP ($\beta_{adjusted}$ = -0.13 cm; -0.64, 0.38), compared to the group with the lowest exposure level, as well as with the hair concentration of TCPy (per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ = -0.42cm; -0.85, 0.00). Statistically significantly higher BL was also observed among children of women with detectable bitertanol ($\beta_{adjusted}$ = +0.60 cm; 0.09, 1.10), and isoproturon ($\beta_{adjusted}$ = +0.55 cm; 0.11, 1.00) in hair.

Finally, we observed a statistically significantly larger HC associated with prochloraz (detected vs. not: $\beta_{adjusted}$ = +0.57 cm; 0.17, 0.97) and tebuconazole (detected vs. not: $\beta_{adjusted}$ = +0.31 cm; 0.01,

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0.61). Increased HC close to statistical significance was also observed in the children of women with a medium level of ClCF3CA in the hair (compared with a low level: $\beta_{adjusted}$ = +0.39; -0.01, 0.78).

Stratification by sex

After stratification by sex (see Figures 1-3; more details in supplemental Tables S3–S5), the pesticides mentioned above were statistically significantly associated with BW among boys: medium vs. low level of fipronil sulfone: $\beta_{adjusted}$ = +216 g; 74, 357). Five additional pesticides were also associated with higher BW in boys: 3Me4NP (3-methyl-4-nitrophenol; per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ = +39 g; 9, 239), fipronil (per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ = +169 g; 24, 314), thiabendazole (per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ =+199 g; 78, 319), DCPMU (1-(3,4-dichlorophenyl)-3-methylurea , medium vs. low: $\beta_{adjusted}$ = +211 g; 70, 351), and DMST (detected vs. not: $\beta_{adjusted}$ = +135 g; 17, 254). Three additional pesticides were associated with statistically significantly lower BW in boys: mecoprop (per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ = -134 g; -249, -18), propoxur (detected vs. not: $\beta_{adjusted}$ = -261 g: -374, -147), and fenhexamid (detected vs. not: $\beta_{adjusted}$ = -256 g; -427, -86). None of these were selected as BW predictors in girls, except 3Me4NP, for which the association was not statistically significant.

Mothers' exposure to dieldrin was associated with a BL that was higher in boys (high vs. low level of exposure: $\beta_{adjusted}$ = +0.94 cm; 0.14, 1.74) and lower in girls (high vs. low level of exposure: $\beta_{adjusted}$ = -0.54 cm; -1.12, 0.03). A significant association was also observed between β-endosulfan and BL among boys (high vs. low level of exposure: $\beta_{adjusted}$ = -0.77 cm; -1.55, 0.00). DEP, TCPy, bitertanol, and isoproturon were not selected as BL predictors among either boys or girls.

HC values were statistically significantly lower in boys whose mothers had been exposed to DEP (per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ = -0.50 cm; -0.89, -0.11), β-endosulfan (high vs. low: $\beta_{adjusted}$ = -0.51 cm; -1.00, -0.02), propoxur (detected vs. not: $\beta_{adjusted}$ = -0.50 cm; -0.89, -0.10), and fenuron (detected vs. not: $\beta_{adjusted}$ = -0.46 cm; -0.90, -0.02). The opposite effect (higher HC) was observed for boys whose mothers were exposed to DCPMU (medium vs. low: $\beta_{adjusted}$ = +0.58 cm; 0.09, 1.07), β-HCH (detected vs. not: $\beta_{adjusted}$ = +0.42 cm; 0.04, 0.79), and thiabendazole (per 2-SD increase in log-transformed concentration: $\beta_{adjusted}$ = +1.03 cm; 0.56, 1.49). Among girls, detection of prochloraz in maternal hair was associated with larger HC ($\beta_{adjusted}$ = +0.72 cm; 0.18, 1.26).

Sensitivity analyses

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The associations remained similar in magnitude when statistical outliers were excluded (two to five subjects, depending on the models; see supplementary files, Table S6). In stratifying for region, we observed that associations with bitertanol (BL), fipronil sulfone (BW), DEP (BL), and prochloraz (BL) occurred mainly for offspring of women living in the northeast area (see supplementary files, Table S7).

When added to the elastic net regression models, none of the sums (parent compound and its specific metabolites, or compounds sharing common metabolites) were selected as predictors for BW, BL, or HC, except the sum of fipronil and fipronil sulfone in the model for BW (medium vs. low: $\beta_{adjusted}$ = +126 g; 19, 232; high vs. low: $\beta_{adjusted}$ = +58 g; -53, 169; see supplementary files, Table S8).

IV. DISCUSSION

We observed that maternal hair concentrations of seven pesticides or pesticide metabolites selected by elastic net regression modeling were associated with measurements at birth (BW: fipronil sulfone; BL: TCPy, bitertanol, DEP, and isoproturon; HC: tebuconazole and prochloraz); this finding suggests that prenatal exposure to pesticides belonging to five distinct chemical families may influence birth outcomes. Restriction of our analyses to boys identified 12 additional compounds: 8 were independently associated with BW (3Me4NP, DCPMU, DMST, fipronil, mecoprop, propoxur, fenhexamid, and thiabendazole), 2 with BL (dieldrin and β-endosulfan), and 6 with HC (β-endosulfan, β-HCH, fenuron, DCPMU, propoxur, and thiabendazole). The 64 pesticides and metabolites considered comprise one of the largest panels of pesticides studied simultaneously in relation to these three measurements at birth.

In our results, several organochlorine compounds were selected as predictors of these birth measurements, but the only statistically significant associations were between dieldrin and BL in boys (increase in boys, decrease in girls), between β-endosulfan and BL and HC, and between β-HCH and HC. Dieldrin was previously studied in the CHAMACOS birth cohort, which did not observe either an association with BW or any evidence of statistical interaction with sex (Fenster et al., 2006). Sharma et al. (2012) reported higher blood levels of β-HCH, but not of β-endosulfan, in mothers of children with fetal growth restriction, compared to those with normal-BW infants. Guo et al. (2014) found no statistically significant association between β-HCH and BW. According to the literature review by Vrijheid et al. (2016), numerous studies have reported associations between specific organochlorine pesticides and BW, but results between studies about the specific associated compounds and congeners remain fairly inconsistent. Of the organophosphorus compounds in our

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study, TCPy, a specific metabolite of chlorpyriphos ethyl and methyl, was associated with lower BL in our results. We also observed that DEP, a nonspecific dialkyl phosphate metabolite of organophosphorus pesticides (including chlorpyriphos ethyl) was associated with lower BL and HC (in boys only). None of these were associated with BW. Smaller measurements at birth, including BW, have previously been associated with higher maternal concentrations of urinary dialkyl phosphate metabolites, but associations remain inconsistent (INSERM, 2013). It should be noted that we did not have access to data for ethnicity or the PON1 genotype, which can produce interactions in the associations between dialkyl phosphates and fetal growth (Harley et al., 2016).

3Me4NP, another organophosphorus metabolite, was associated with increased BL and HC among boys in our results. It is specifically derived from fenitrothion, an anti-androgenic pesticide (Turner et al., 2002). To our knowledge, associations between fenitrothion/3Me4NP and measurements at birth have not previously been investigated. We also found an association between propoxur, a carbamate fungicide, and lower BW, in accordance with a previous study among minority women in New York City, based on cord blood samples collected at delivery (Whyatt et al., 2004). Our failure to observe any statistically significant association between pyrethroid pesticides and metabolites and measurements at birth is consistent with most available studies (Saillenfait et al., 2015).

To our knowledge, the other associations reported between human measurements at birth and other pesticides or pesticide families are new. We did find some animal data, including a study reporting evidence of impaired adipocyte metabolism in mice exposed to tolylfluanid, the parent compound of DMST (Neel et al., 2013). Tolylfluanid has also been associated with a reduction in insulin receptor substrate-1 levels inducing cellular insulin resistance in both mice and rats (Sargis et al., 2012). These data might support the association we observed between DMST and increased BW among boys. A study of newborn mice prenatally exposed to a mixture of 2,4D, mecroprop, dicamba and inactive pesticide ingredients reported decreased BW in the exposed mice (Cavieres et al., 2002). Although the role of mecoprop alone has not been assessed, these results were in line with the inverse correlation between maternal hair concentrations and BW among boys in our results. We also found an association between prochloraz and increased HC, especially in girls. While this association has not been reported earlier, a previous study of rat pituitary tumor cells reported that prochloraz can induce thyroid hormone disruption (Ghisari and Bonefeld-Jorgensen, 2005). Especially given that thyroid hormones have a critical role in brain development (Horn and Heuer, 2010), these findings raise questions about how this development may be influenced by prochloraz. We found no evidence in the animal or human literature to support the associations observed between the birth measurements in our study and other pesticides (fipronil and fipronil sulfone, its metabolite;

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fenhexamid; bitertanol; tebuconazole, thiabendazole; isoproturon; fenuron; DCPMU, and diuron, its main parent compound).

Several compounds, such as azole fungicides, were associated with increased BW, BL, and HC. Although lower birth anthropometric parameters have been associated with adverse health effects in later life, it must be noted that bigger neonates are not necessarily healthier neonates. Prenatal exposure to some environmental chemicals is suspected to promote obesity or to disrupt specific metabolic mechanisms in children (Liu and Peterson, 2015). Nevertheless, some pesticides might also be correlated with protective behavior or exposures, or have positive action on environmental stressors. For instance, fungicides might reduce the presence of molds, whereas aflatoxins have been associated with lower BW in human (Shuaib et al., 2010).

Given the number of compounds tested and the absence of clear prior evidence, we chose to provide results stratified for sex instead of testing for statistical interaction with sex for all chemicals. Overall, our elastic net strategy selected many more pesticides when restricted to boys, compared with girls. This might indicate a greater sensitivity to pesticides for boys. In addition, some chemicals appeared to have opposite effect depending on sex (e.g., dieldrin), which raises the question of possible statistical interactions with sex, as already suggested for some organochlorine pesticides (e.g., β-HCH and DDT; Callan 2016). Additional analyses should be conducted on individual chemicals to determine the plausibility of sex-specific effects.

We chose to measure pesticides in hair to estimate exposure during the entire pregnancy, assuming that hair would aggregate exposure over a long time period. This would present the advantage of limiting intraindividual variability, as previously suggested (Béranger et al., 2018), and of capturing sporadic exposure to chemicals with short half-lives. Nonetheless, using this biological matrix did not enable us to distinguish the pattern of exposure (low and chronic, or sporadic and intense), or to assess the point during pregnancy when the exposure occurred, or to determine whether the period of contamination corresponds to a relevant window of exposure for fetal growth. Aggregation of the exposure by the hair matrix for the entire pregnancy might thus have diluted some associations. Overall, discrepancies between our results and part of the (inconsistent) literature on organochlorine and organophosphorus compounds may be related to the differences in matrix properties (hair vs. urine or blood). There may also be some imprecision or between-operator variability in the measurement of the anthropometric variables, especially for BL and HC, and these might result in a nondifferential misclassification bias. Differences in exposure levels between populations, lack of power, and false positives are also possible explanations.

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Elastic net regression modeling presents the advantage of handling multicollinearity between pesticides, while limiting the risk of false positives due to multiple testing (Chadeau-Hyam et al., 2013; Agier et al., 2016). Correlations between the 64 compounds studied, weak in most of the situations, have previously been described (Béranger et al., 2018). Among the pesticides associated with measurements at birth in our study, we observed polychoric/tetrachoric correlation coefficients above 0.6 only for mecoprop and 2,4D; fipronil and fipronil sulfone; and isoproturon and chlortoluron. Variance inflation factors in our models were essentially below 2, and never exceeded 3.5 for exposure variables, thereby indicating an absence of multicollinearity problems. However, we cannot rule out the possibility that some results may be explained by unmeasured chemicals or behaviors associated with the selected pesticides/metabolites.

In the sensitivity analyses, considering the sum either of a parent compound and its specific metabolites or of metabolites sharing common pathways in the existing models, instead of individual metabolites, did not improve prediction of the outcomes. In the BW model, the association with the sum of fipronil and fipronil sulfone was similar to that observed with fipronil sulfone alone in the main analyses. The sum of the dialkyl phosphate metabolites was not selected in the elastic net model for BL even though the level of DEP in maternal hair was statistically significantly associated with lower BL in the main analyses. By stratifying for region, we observed some variations in the magnitude of the associations observed in the main analyses. Despite the absence of any clear differences either in population characteristics between regions, except for fish consumption (see Supplementary files, Table S2), nor in detection frequency and exposure intensity for most of the pesticides and pesticide metabolites (Beranger et al., 2018), we observed that associations were mainly driven by the women from the northeast. We have no explanation for this finding.

The frequent use of these chemicals in France, and the lack of information regarding exposure frequency, exposure intensity, or possible adverse effect in humans or animals justifies these exploratory analyses. However, in the absence of underlying mechanistic data to support the findings for most of the statistical associations reported, and considering the possibility of false positives or false negatives and the methodological choices we made, further studies should be conducted to replicate and verify our findings.

V. CONCLUSION

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By focusing on pesticides to which the French population of pregnant women is likely to be exposed, we identified 19 pesticides or pesticide metabolites that may be associated with changes in BW, BL, or HC. These compounds come from various chemical families, including the organophosphorus, urea, azole, and phenylpyrazole pesticides. To our knowledge, some of them have never previously been studied in relation to these human anthropometric parameters. Our results provide a useful basis for future human and animal research. However, like any exploratory research findings, they must be interpreted cautiously, at least until they are verified by further epidemiological or mechanistic studies.

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REFERENCES

ANSES (Agence française de sécurité sanitaire de l'environnement et du travail), 2010. Exposition de la population générale aux résidus de pesticides en France : Synthèse et recommandations du comité d'orientation et de prospective scientifique de l'observatoire des résidus de pesticides, ORP. [In French] Available: https://www.ecophyto-pro.fr/data/exposition_population_generale_pesticides_2010_vdef.pdf. Last visit: February 24th, 2017

Agier, L., Portengen, L., Chadeau-Hyam, M., Basagaña, X., Giorgis-Allemand, L., Siroux, V., Robinson, O., Vlaanderen, J., González, J.R., Nieuwenhuijsen, M.J., Vineis, P., Vrijheid, M., Slama, R., Vermeulen, R., 2016. A Systematic Comparison of Linear Regression-Based Statistical Methods to Assess Exposome-Health Associations. Environ. Health. Perspect. 124(12), 1848–1856.

Agreste, 2012. Recensement agricole 2010. [In French] Available: http://agreste.agriculture.gouv.fr/enquetes/structure-des-exploitations-964/publications-ra-2010-713/. Last visit: April 30th, 2019.

Béranger, R., Hardy, E.M., Dexet, C., Guldner, L., Zaros, C., Nougadère, A., Metten, M.A., Chevrier, C., Appenzeller, B.M.R., 2018. Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort. Environ. Int. 120, 43–53.

Callan, A.C., Hinwood, A.L., Heyworth, J., Phi, D.T., Odland, J.Ø., 2016. Sex specific influence on the relationship between maternal exposures to persistent chemicals and birth outcomes. Int. J. Hyg. Environ. Health. 219(8), 734–741.

Cavieres, M.F., Jaeger, J., Porter, W., 2002. Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size. Environ. Health. Perspect. 110(11), 1081–5.

Chadeau-Hyam, M., Campanella, G., Jombart, T., Bottolo, L., Portengen, L., Vineis, P., Liquet, B., Vermeulen, R.C., 2013. Deciphering the complex: methodological overview of statistical models to derive OMICS-based biomarkers. Environ. Mol. Mutagen. 54(7), 542–57.

Chen, J., Chen, P., Bo, T., Luo, K., 2016. Cognitive and Behavioral Outcomes of Intrauterine Growth Restriction School-Age Children. Pediatrics. 137(4), e2015386.

Dereumeaux, C., Saoudi, A., Pecheux, M., Berat, B., de Crouy-Chanel, P., Zaros, C., Brunel, S., Delamaire, C., le Tertre, A., Lefranc, A., Vandentorren, S., Guldner, L., 2016. Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. Environ. Int. 97, 56–67.

Desquilbet, L., Mariotti, F., 2010. Dose-response analyses using restricted cubic spline functions in public health research. Stat. Med. 29(9), 1037–1057.

Duca, R.C., Hardy, E., Salquèbre, G., Appenzeller, B.M., 2014a. Hair decontamination procedure prior to multi-class pesticide analysis. Drug. Test. Anal. 6 Suppl 1, 55–66.

Accepted Manuscript

Duca, R.C., Salquebre, G., Hardy, E., Appenzeller, B.M., 2014b. Comparison of solid phase- and liquid/liquid-extraction for the purification of hair extract prior to multi-class pesticides analysis. J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci. 955–956, 98–107.

Elfe, 2017. The Elfe study: How do our children grow? Available: https://www.elfe-france.fr/en/. Last visit: April 30th, 2019.

European Commission, 2017b. Pesticides. Available: http://ec.europa.eu/food/plant/pesticides_en. Last visit: April 30th, 2019.

Eurostat, 2017. https://ec.europa.eu/eurostat/web/products-datasets/-/tai02. Last visit: April 30th, 2019.

Feng, C., Osgood, N.D., Dyck, R.F., 2018. Low Birth Weight, Cumulative Obesity Dose, and the Risk of Incident Type 2 Diabetes. J. Diabetes. Res. 2018, 8435762.

Fenster, L., Eskenazi, B., Anderson, M., Bradman, A., Harley, K., Hernandez, H., Hubbard, A., Barr, D.B., 2006. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ. Health. Perspect. 114(4), 597–602.

Fillol, C., Garnier, R., Mullot, J.U., Boudet, C., Momas, I., Salmi, L.R., Vandentorren, S., 2014. Prioritization of the biomarkers to be analyzed in the French biomonitoring program. Biomonitoring. 1(1), 95–104.

French Ministry of Agriculture. 2012. [Qu'est-ce que la Banque National des Ventes distributeurs (BNV-d) ?]. Available: http://agriculture.gouv.fr/sites/minagri/files/documents/La_BNV-d_cle8978db.pdf. Last visit: April 30th, 2019.

Friedman, J., Hastie, T., Tibshirani, R., 2010. Regularization Paths for Generalized Linear Models via Coordinate Descent. Journal of Statistical Software. 33(1), 1–22.

Gale, C.R., O'Callaghan, F.J., Bredow, M., Martyn, C.N., 2006. Avon Longitudinal Study of Parents and Children Study Team. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics. 118(4), 1486–92.

Gelman, A., 2008. Scaling regression inputs by dividing by two standard deviations. Stat. Med. 27(15), 2865-73.

Ghisari, M., Bonefeld-Jorgensen, E.C., 2005. Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells. Mol. Cell. Endocrinol. 244(1–2), 31–41.

Goodman, M., Mandel, J.S., DeSesso, J.M., Scialli, A.R., 2014. Atrazine and pregnancy outcomes: a systematic review of epidemiologic evidence. Birth. Defects. Res. B. Dev. Reprod. Toxicol. 101(3), 215–36.

Accepted Manuscript

Guo, H., Jin, Y., Cheng, Y., Leaderer, B., Lin, S., Holford, T.R., Qiu, J., Zhang, Y., Shi, K., Zhu, Y., Niu, J., Bassig, B.A., Xu, S., Zhang, B., Li, Y., Hu, X., Chen, Q., Zheng, T., 2014. Prenatal exposure to organochlorine pesticides and infant birth weight in China. Chemosphere. 110, 1–7.

Haines, D.A., Saravanabhavan, G., Werry, K., Khoury, C., 2017. An overview of human biomonitoring of environmental chemicals in the Canadian Health Measures Survey: 2007-2019. Int. J. Hyg. Environ. Health. 220(2 Pt A), 13–28.

Hardy, E.M., Duca, R.D., Salquebre, G., Appenzeller, B.M.R., 2015. Multi-residue analysis of organic pollutants in hair and urine for matrices comparison. Forensic. Sci. Int. 249C, 6–19.

Harley, K.G., Engel, S.M., Vedar, M.G., Eskenazi, B., Whyatt, R.M., Lanphear, B.P., Bradman, A., Rauh, V.A., Yolton, K., Hornung, R.W., Wetmur, J.G., Chen, J., Holland, N.T., Barr, D.B., Perera, F.P., Wolff, M.S., 2016. Prenatal Exposure to Organophosphorous Pesticides and Fetal Growth: Pooled Results from Four Longitudinal Birth Cohort Studies. Environ. Health. Perspect. 124(7), 1084–92.

Horn, S., Heuer, H., 2010. Thyroid hormone action during brain development: more questions than answers. Mol. Cell. Endocrinol. 315(1–2), 19–26.

INSERM (Institut national de la santé et de la recherche médicale), 2013. Pesticides : Effets sur la santé. [in French]. Paris, France: INSERM.

Lenters, V., Portengen, L., Rignell-Hydbom, A., Jönsson, B.A., Lindh, C.H., Piersma, A.H., Toft, G., Bonde, J.P., Heederik, D., Rylander, L., Vermeulen, R., 2016. Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ. Health. Perspect. 124(3), 365–72.

Liu, Y., Peterson, K.E., 2015. Maternal Exposure to Synthetic Chemicals and Obesity in the Offspring: Recent Findings. Curr. Environ. Health. Rep. 2(4), 339–47.

Mercuro, G., Bassareo, P.P., Flore, G., Fanos, V., Dentamaro, I., Scicchitano, P., Laforgia, N., Ciccone, M.M., 2013. Prematurity and low weight at birth as new conditions predisposing to an increased cardiovascular risk. Eur. J. Prev. Cardiol. 20(2), 357–67.

Neel, B.A., Brady, M.J., Sargis, R.M., 2013. The endocrine disrupting chemical tolylfluanid alters adipocyte metabolism via glucocorticoid receptor activation. Mol. Endocrinol. 27(3), 394–406.

[No authors listed], 2013. Infographic: pesticide planet. Science. 341(6147), 730–1.

Nougadère, A., Merlo, M., Héraud, F., Réty, J., Truchot, E., Vial, G., Cravedi, J.P., Leblanc, J.C., 2014. How dietary risk assessment can guide risk management and food monitoring pogrammes: The approach results of the French Observatory on Pesticide Residues (Anses/ORP). Food. Control. 41, 32–48.

Osaka, A., Ueyama, J., Kondo, T., Nomura, H., Sugiura, Y., Saito, I., Nakane, K., Takaishi, A., Ogi, H., Wakusawa, S., Ito, Y., Kamijima, M., 2016. Exposure characterization of three major insecticide lines in

Accepted Manuscript

urine of young children in Japan-neonicotinoids, organophosphates, and pyrethroids. Environ. Res. 147, 89–96.

Ramos, J.J., Huetos, O., González, S., Esteban, M., Calvo, E., Pérez-Gómez, B., Castaño, A., Bioambient.es., 2017. Organochlorinated pesticides levels in a representative sample of the Spanish adult population: The Bioambient.es project. Int. J. Hyg. Environ. Health. 220(2 Pt A), 217–226.

Saillenfait, A.M., Ndiaye, D., Sabaté, J.P., 2015. Pyrethroids: exposure and health effects--an update. Int. J. Hyg. Environ. Health. 218(3), 281–92.

Salquebre, G., Schummer, C., Millet, M., Briand, O., Appenzeller, B.M.R., 2012. Multi-class pesticide analysis in human hair by gas chromatography tandem (triple quadrupole) mass spectrometry with solid phase microextraction and liquid injection. Anal. Chim. Acta. 710, 65–74.

Sargis, R.M., Neel, B.A., Brock, C.O., Lin, Y., Hickey, A.T., Carlton, D.A., Brady, M.J., 2012. The novel endocrine disruptor tolylfluanid impairs insulin signaling in primary rodent and human adipocytes through a reduction in insulin receptor substrate-1 levels. Biochim. Biophys. Acta. 1822(6), 952–60.

Saunders, L., Kadhel, P., Costet, N., Rouget, F., Monfort, C., Thomé, J.P., Guldner, L., Cordier, S., Multigner, L., 2014. Hypertensive disorders of pregnancy and gestational diabetes mellitus among French Caribbean women chronically exposed to chlordecone. Environ. Int. 68, 171–6.

Sharma, E., Mustafa, M., Pathak, R., Guleria, K., Ahmed, R.S., Vaid, N.B., Banerjee, B.D., 2012. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. Eur. J. Obstet. Gynecol. Reprod. Biol. 161(2), 163-9.

Shirangi, A., Nieuwenhuijsen, M., Vienneau, D., Holman, C.D., 2011. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: a review of the literature. Paediatr. Perinat. Epidemiol. 25(2), 172–91.

Shuaib, F.M., Ehiri, J., Abdullahi, A., Williams, J.H., Jolly, P.E., 2010. Reproductive health effects of aflatoxins: a review of the literature. Reprod. Toxicol. 29(3), 262–70.

Turner, K.J., Barlow, N.J., Struve, M.F., Wallace, D.G., Gaido, K.W., Dorman, D.C., Foster, P.M., 2002. Effects of in utero exposure to the organophosphate insecticide fenitrothion on androgen-dependent reproductive development in the Crl:CD(SD)BR rat. Toxicol. Sci. 68(1), 174–83.

Vandentorren, S., Bois, C., Pirus, C., Sarter, H., Salines, G., Leridon, H., 2009. Rationales, design and recruitment for the Elfe longitudinal study. BMC Pediatr. 9, 58.

Vrijheid, M., Casas, M., Gascon, M., Valvi, D., Nieuwenhuijsen, M., 2016. Environmental pollutants and child health-A review of recent concerns. Int. J. Hyg. Environ. Health. 219(4-5), 331–42.

Whyatt, R.M., Rauh, V., Barr, D.B., Camann, D.E., Andrews, H.F., Garfinkel, R., Hoepner, L.A., Diaz, D., Dietrich, J., Reyes, A., Tang, D., Kinney, P.L., Perera, F.P., 2004. Prenatal insecticide exposures and

Accepted Manuscript

birth weight and length among an urban minority cohort. Environ. Health. Perspect. 112(10), 1125–32.

Wickerham, E.L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y., Meeker, J.D., 2012. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environ. Int. 47, 80–5.

Zou, H., Hastie, T., 2005. Regularization and variable selection via the elastic net. J. R. Stat. Soc. Ser. B Stat. Methodol. 67, 301–320.

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TABLES AND FIGURES

Table 1. Pesticide concentrations in women's hair (n=311)

Compounds	CAS number	LOD pg/mg	Missing value n	Detection frequency n (%)	25th percentile pg/mg	50th percentile pg/mg	75th percentile pg/mg
ORGANOCHLORINE							
γ-HCH (Lindane)	58-89-9	0.2	0	311 (100%)	1.11	1.58	2.20
hexachlobenzene	118-74-1	0.008	0	311 (100%)	0.09	0.12	0.17
Pentachlorophenol	87-86-5	0.415	1	310 (100%)	4.37	10.03	28.47
α-endosulfan	959-98-8	0.012	0	286 (92%)	0.08	0.15	0.28
Dieldrin	60-57-1	0.054	0	213 (68%)	<LOD	0.25	0.61
β-endosulfan	33213-65-9	0.043	0	188 (60%)	<LOD	0.16	0.40
α-HCH	319-84-6	0.020	0	158 (51%)	<LOD	0.03	0.09
β-HCH	319-85-7	0.078	0	152 (49%)	<LOD	<LOD	0.42
trans-chlordane	5103-74-2	0.003	0	61 (20%)	<LOD	<LOD	<LOD
p.p'-DDT	50-29-3	1.322	0	31 (10%)	<LOD	<LOD	<LOD
ORGANOPHOSPHORUS							
p-nitrophenol	100-02-7	3.091	1	310 (100%)	8.45	13.18	20.66
TCPy	6515-38-4	0.141	2	309 (100%)	1.38	2.66	6.78
DEP	598-02-7	0.441	3	301 (98%)	3.26	7.46	23.47
DETP	2465-65-8	0.032	1	301 (97%)	0.48	0.88	1.78
IMPy	2814-20-2	0.063	1	300 (97%)	0.36	0.66	1.36
DMP	813-78-5	0.029	3	258 (84%)	0.23	0.86	3.21
3Me4NP	2581-34-2	0.055	1	255 (82%)	0.17	0.65	1.25
DMTP	1112-38-5	0.004	1	173 (56%)	<LOD	0.03	0.11
PYRETHROIDS							
3-PBA	3739-38-6	0.209	1	310 (100%)	0.76	1.69	3.76
Cl_2CA	55701-05-8	0.248	1	308 (99%)	1.37	3.51	8.06
Permethrin	52645-53-1	1.281	0	295 (95%)	17.15	37.93	91.61
Cypermethrin	52315-07-8	0.043	0	259 (83%)	0.35	1.09	2.86
ClCF3CA	72748-35-7	0.011	1	189 (61%)	<LOD	0.09	0.35
Lambda- cyhalothrin	91465-08-6	0.067	0	130 (42%)	<LOD	<LOD	1.01
4F3PBA	77279-89-1	0.005	1	107 (35%)	<LOD	<LOD	0.03
CARBAMATES							
Carbendazim	10605-21-7	0.271	1	310 (100%)	0.45	0.65	1.22
Carbofuran	1563-66-2	0.023	1	195 (63%)	<LOD	0.10	0.32
Propoxur	114-26-1	0.104	1	139 (45%)	<LOD	<LOD	0.77
DINITROANILINES							
Trifluralin	1582-09-8	0.003	0	311 (100%)	0.10	0.14	0.17
THIOCARBAMATES							
Prosulfocarb	52888-80-9	0.051	1	310 (100%)	0.17	0.31	0.62
PHENYLPYRAZOLES							
Fipronil sulfone	120068-36-2	0.039	0	307 (99%)	0.49	2.25	10.51
Fipronil	120068-37-3	0.028	0	270 (87%)	0.30	1.62	9.50
ACID HERBICIDES							
2,4-D	94-75-7	0.113	11	291 (97%)	0.44	0.68	1.16
MCPA	94-74-6	0.2	11	290 (97%)	0.45	0.82	1.30
Mecoprop	93-65-2	0.039	11	278 (93%)	0.16	0.26	0.45
Dichlorprop	120-36-5	0.070	11	85 (28%)	<LOD	<LOD	0.16
MCPB	94-81-5	0.002	11	65 (22%)	<LOD	<LOD	<LOD
AZOLES							
Thiabendazole	148-79-8	0.028	1	280 (90%)	0.20	0.68	2.42
Propiconazole	60207-90-1	0.094	1	221 (71%)	<LOD	0.67	1.44
Tebuconazole	107534-96-3	0.086	2	144 (47%)	<LOD	<LOD	0.62
Myclobutanil	88671-89-0	0.015	1	81 (26%)	<LOD	<LOD	0.02
Imazalil	35554-44-0	0.486	1	80 (26%)	<LOD	<LOD	1.63
Bitertanol	55179-31-2	0.113	2	74 (24%)	<LOD	<LOD	<LOD
Prochloraz	67747-09-5	0.028	1	51 (16%)	<LOD	<LOD	<LOD

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OXADIAZINES							
Oxadiazon	19666-30-9	0.020	0	264 (85%)	0.08	0.15	0.29
TRIAZINES/TRIAZONES							
Terbutryn	886-50-0	0.004	2	240 (78%)	0.07	0.22	0.46
AMIDE PESTICIDES							
Metolachlor	51218-45-2	0.008	1	237 (76%)	0.01	0.03	0.05
DMST	66840-71-9	0.2	1	144 (46%)	<LOD	<LOD	2.42
Fenhexamid	126833-17-8	1.221	3	45 (15%)	<LOD	<LOD	<LOD
STROBILURINS							
Azoxystrobin	131860-33-8	0.012	1	201 (65%)	<LOD	0.16	0.62
Pyraclostrobin	175013-18-0	0.014	1	96 (31%)	<LOD	<LOD	0.04
Trifloxystrobin	141517-21-7	0.017	1	60 (19%)	<LOD	<LOD	<LOD
CARBOXAMIDES							
Boscalid	188425-85-6	0.079	3	195 (63%)	<LOD	0.55	1.41
Diflufenican	83164-33-4	0.053	3	133 (43%)	<LOD	<LOD	0.19
UREA							
DCPMU	3567-62-2	0.017	0	190 (61%)	<LOD	0.06	0.21
DCPU	2327-02-8	0.277	0	188 (60%)	<LOD	0.93	2.24
Diuron	330-54-1	0.036	1	144 (46%)	<LOD	<LOD	0.28
Fenuron	101-42-8	0.009	1	136 (44%)	<LOD	<LOD	0.09
Isoproturon	34123-59-6	0.006	1	102 (33%)	<LOD	<LOD	0.02
Chlortoluron	15545-48-9	0.024	1	35 (11%)	<LOD	<LOD	<LOD
NEONICOTINOIDS							
Imidacloprid	138261-41-3	0.049	1	132 (43%)	<LOD	<LOD	0.66
ANILINO-PYRIMIDINES							
Pyrimethanil	53112-28-0	0.054	1	35 (11%)	<LOD	<LOD	<LOD
MISCELANEOUS							
Lenacil	2164-08-1	0.144	1	96 (31%)	<LOD	<LOD	0.30
Spinosyn A	131929-60-7	0.010	8	46 (15%)	<LOD	<LOD	<LOD

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 3Me4NP, 3-methyl-4-nitrophenol; 3-PBA, 3-phenoxybenzoic; 4F3PBA, 4-fluoro-3-phenoxybenzoic acid ; Cl_2CA, cis-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid; ClCF3CA, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylic acid; DCPU, 1-(3,4-dichlorophenyl)urea; DCPMU, 1-(3,4-dichlorophenyl)-3-methylurea; DDT, Dichlorodiphenyltrichloroethane; DEP, di-ethyl-phosphate; DETP, di-ethyl-thiophosphate; DMP, di-methyl-phosphate; DMTP, di-methyl-thiophosphate; DMST, dimethylsulftoluidide; HCH, hexachlorocyclohexane; IMPy, 2-isopropyl-4-methyl-6-hydroxypyrimidine; LOD, limit of detection (lowest detected value, or limit of quantification, if lower); MCPA, 4-chloro-2-methylphenoxyacetic acid; MCPB, 4-(4-chloro-2-methylphenoxy)butyric acid; TCPy, 3,5,6-trichloro-2-pyridinol.

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Table 2. Population characteristics (n=311)

Characteristics	n	%	Mean	Standard deviation
Maternal age (years)	310		30.1	± 5.0
Prepregnancy maternal weight (kg)	310		62.9	± 13.5
Maternal height (cm)	310		164.5	± 5.9
Gestational age at birth (week)	309		39.3	± 1.2
Parity				
Nulliparous	133	42.9 %		
Parous	177	57.1 %		
Missing	1			
Educational level				
High school or lower level	140	45.2 %		
University	170	54.8 %		
Missing	1			
Tobacco consumption during pregnancy				
No	230	74.4 %		
Yes	79	25.6 %		
Missing	2			
Fish consumption during pregnancy				
< 1 time per month	93	33.8 %		
At least once a month	182	66.2 %		
Missing	36			
Sex of the children				
Boys	148	47.7 %		
Girls	162	52.3 %		
Missing	1			
Cesarean section				
No	264	87.1 %		
Yes	39	12.9 %		
Missing	8			
Recruitment period				
June 27 – July 4	77	24.7 %		
September 27 – October 4	111	35.7 %		
November 28 – December 5	123	39.5 %		
Birth weight (g)	**300**		**3322.3**	**± 428.7**
Birth length (cm)	**301**		**49.5**	**± 2.0**
Head circumference at birth (cm)	**304**		**34.3**	**± 1.4**

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Figure 1. Associations between pesticides and birth weight, for the entire population (n= 300) and by sex.

Abbreviations: AC, Acid herbicides; AM, Amides; AN, Anilino-pyrimidines; AZ, Azoles; b, binary variable; c, continuous variable; CA, Carbamates; CX, Carboxamides; DIN, Dinitroanilines; MIS, Miscellaneous; NEO, Neonicotinoids; OC, Organochlorines; OP, Organophosphorus; OX, Oxadiazins; PH, Phenylpyrazoles; PYR, Pyrethroids; STR, Strobilurins; TH, Thiocarbamates; TRI, Triazines/triazones; UR, Ureas. Empty dots correspond to values not selected as predictors of the outcome (elastic net regression modeling). Filled dots represent estimators and their 95% confidence intervals estimated by multivariable linear regression. Results in black, blue, and pink represent findings observed for the entire population (n= 300), for boys (n= 148) and for girls (n= 152), respectively. Multivariable models were adjusted for parity, prepregnancy maternal weight, tobacco and alcohol consumption, and other selected exposure variables.

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Figure 2. Associations between pesticides and birth length, for the entire population (n= 301) and by sex.

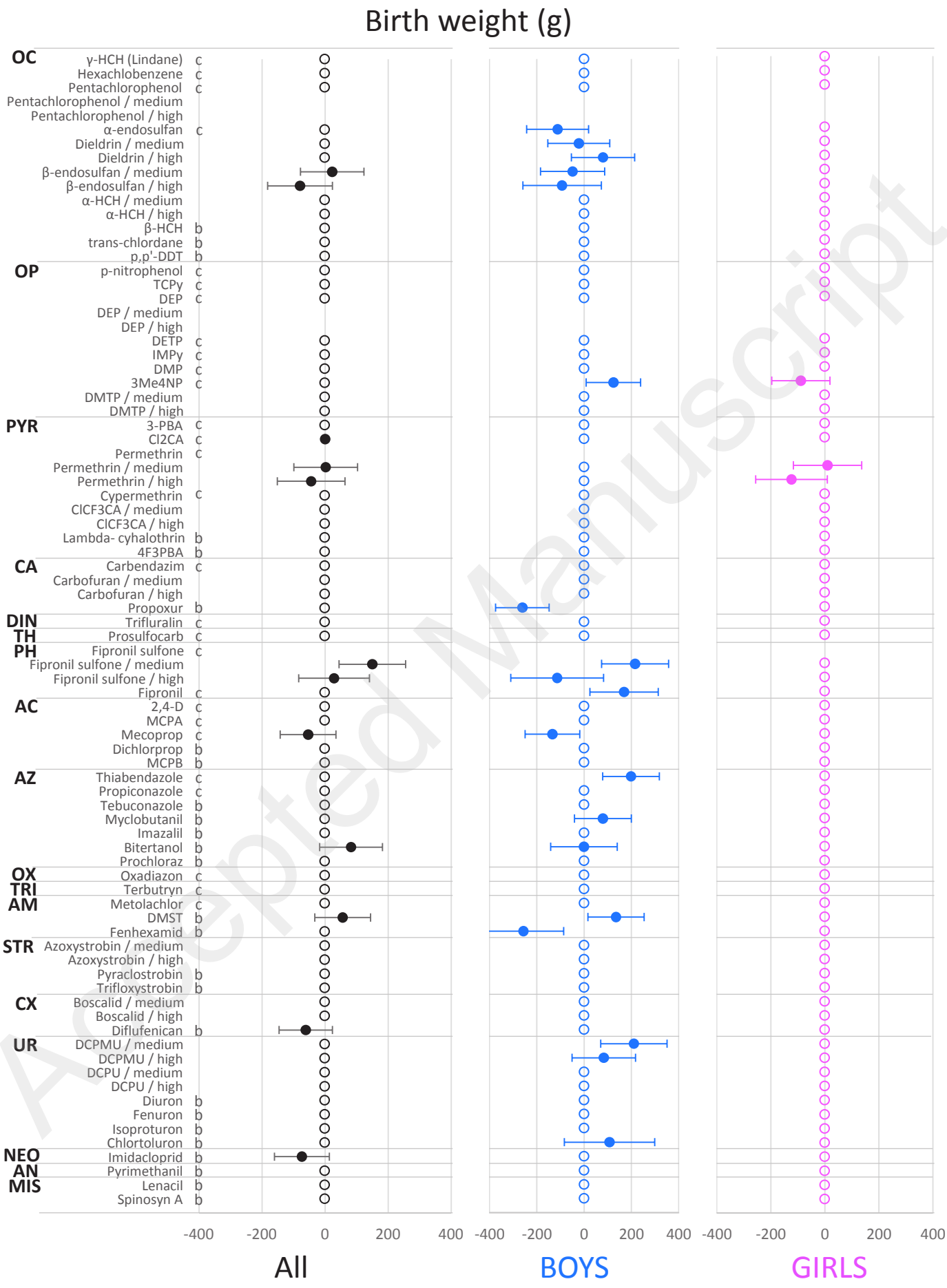
Abbreviations: AC, Acid herbicides; AM, Amides; AN, Anilino-pyrimidines; AZ, Azoles; b, binary variable; c, continuous variable; CA, Carbamates; CX, Carboxamides; DIN, Dinitroanilines; MIS, Miscellaneous; NEO, Neonicotinoids; OC, Organochlorines; OP, Organophosphorus; OX, Oxadiazins; PH, Phenylpyrazoles; PYR, Pyrethroids; STR, Strobilurins; TH, Thiocarbamates; TRI, Triazines/triazones; UR, Ureas. Empty dots correspond to values not selected as predictors of the outcome (elastic net regression modeling). Filled dots represent estimators and their 95% confidence intervals estimated by multivariable linear regression. Results in black, blue, and pink represent findings observed for the entire population (n= 301), for boys (n= 141), and for girls (n= 160), respectively. Multivariable models were adjusted for parity, prepregnancy maternal weight (squared), tobacco and alcohol consumption, and other selected exposure variables.

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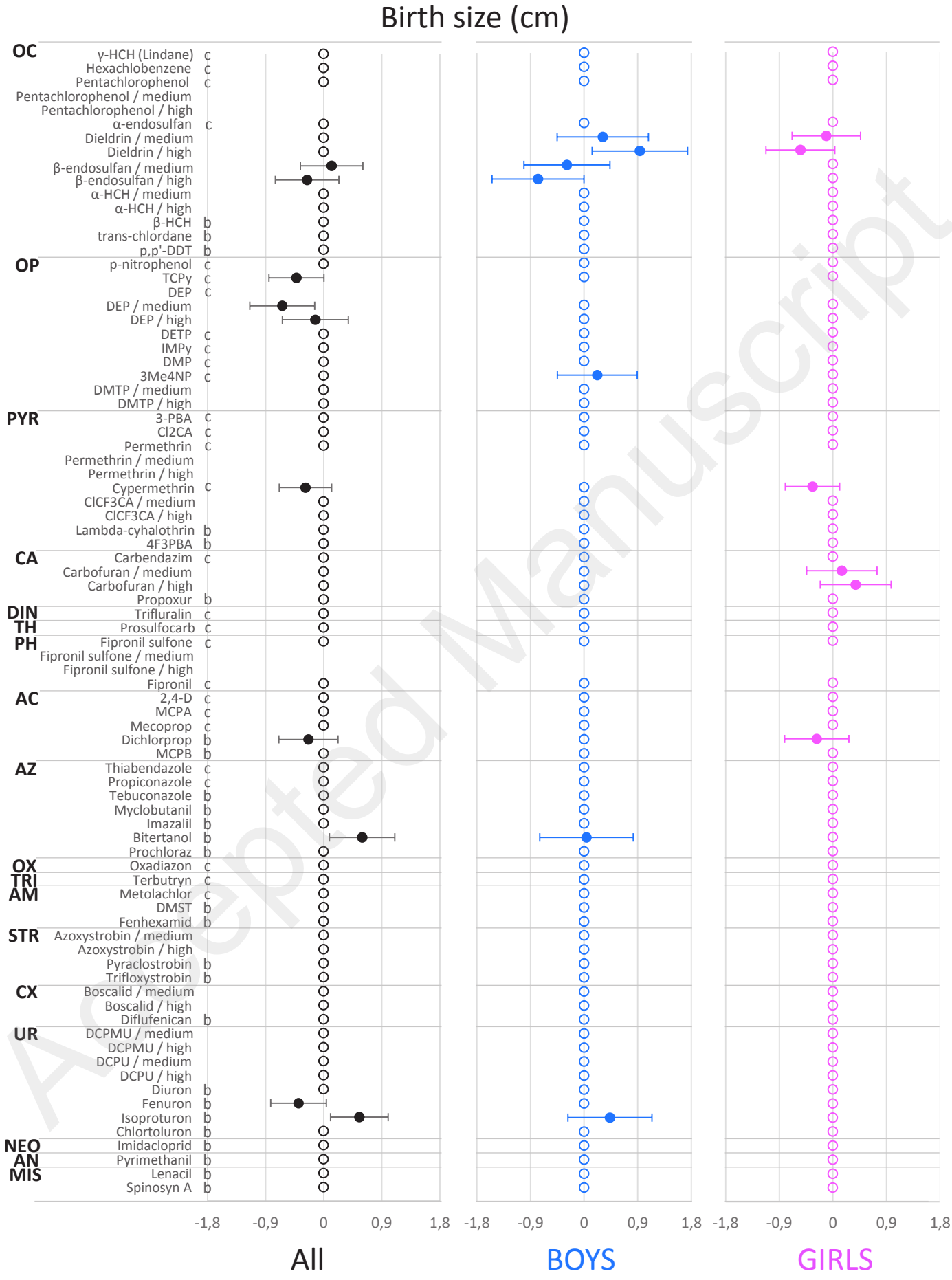
Figure 3. Associations between pesticides and head circumference at birth, for the entire population (n= 304) and by sex.

Abbreviations: AC, Acid herbicides; AM, Amides; AN, Anilino-pyrimidines; AZ, Azoles; b, binary variable; c, continuous variable; CA, Carbamates; CX, Carboxamides; DIN, Dinitroanilines; MIS, Miscellaneous; NEO, Neonicotinoids; OC, Organochlorines; OP, Organophosphorus; OX, Oxadiazins; PH, Phenylpyrazoles; PYR, Pyrethroids; STR, Strobilurins; TH, Thiocarbamates; TRI, Triazines/triazones; UR, Ureas. Empty dots correspond to values not selected as predictors of the outcome (elastic net regression modeling). Filled dots represent estimators and their 95% confidence intervals estimated by multivariable linear regression. Results in black, blue, and pink represent findings observed for the whole (n= 304), for boys (n= 144) and for girls (n= 160), respectively. Multivariable models were adjusted for parity, prepregnancy maternal weight, fish consumption, tobacco and alcohol consumption, and other selected exposure variables.

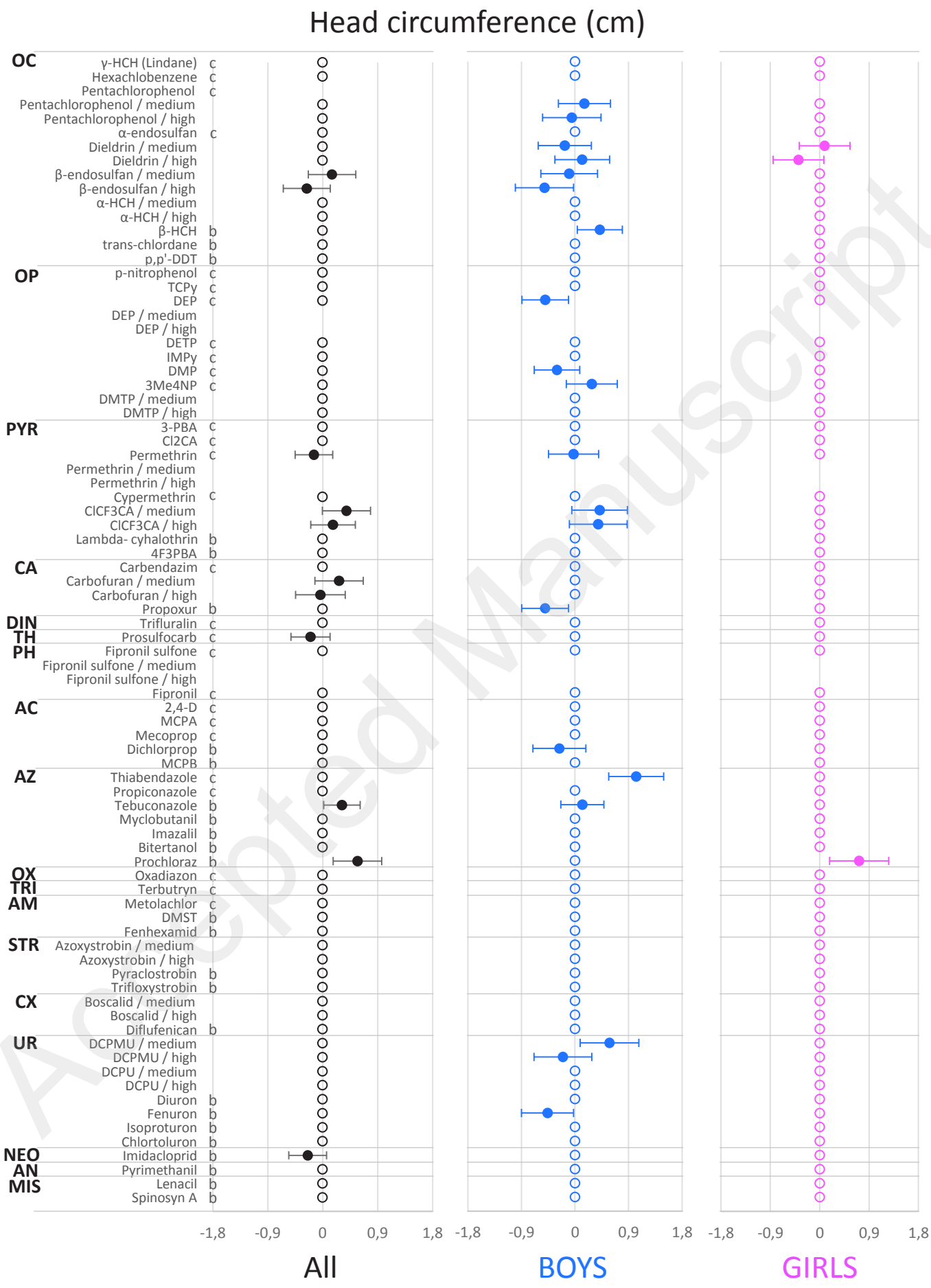
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