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Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort

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ABSTRACT

Background: A growing body of evidence suggests that prenatal exposure to pesticides might impair fetal development. Nonetheless, knowledge about pesticide exposure of pregnant women, especially in Europe, is largely restricted to a limited panel of molecules.
Aim: To characterize the concentration of 140 pesticides and metabolites in hair strands from women in the ELFE French nationwide birth cohort.
Methods: Among cohort members who gave birth in northeastern and southwestern France in 2011, we selected those with a sufficient available mass of hair (n = 311). Bundles of hair 9 cm long were collected at delivery. We screened 111 pesticides and 29 metabolites, including 112 selected a priori based on their reported usage or detection in the French environment. The bundles of hair from 47 women were split into three segments to explore the intraindividual variability of the exposure. Intraclass correlation coefficients (ICCs) were computed for the chemicals with a detection frequency > 70%.
Results: We detected a median of 43 chemicals per woman (IQR 38–47). Overall, 122 chemicals (> 20 chemical families) were detected at least once, including 28 chemicals detected in 70–100% of hair samples. The highest median concentrations were observed for permethrin (median: 37.9 pg/mg of hair), *p*-nitrophenol (13.2 pg/mg), and pentachlorophenol (10.0 pg/mg). The ICCs for the 28 chemicals studied ranged from 0.59 to 0.94.
Conclusion: Pregnant women are exposed to multiple pesticides simultaneously from various chemical families, including chemicals suspected to be reproductive toxicants or endocrine disruptors. The ICCs suggest that the intraindividual variability of pesticide concentrations in hair is lower than its interindividual variability.

1. Introduction

The European Commission has defined pesticides as products that prevent, destroy, or control pests or diseases, or protect plants or plant products (European Commission, 2017). Pesticides are thus used in agriculture, mostly as insecticides, fungicides, or herbicides, but other

Abbreviations of the names of pesticides and pesticide metabolites: 2-ClBA, 2-(4-chlorophenyl)-3-methylbutyric acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DB, 2,4-dichlorophenoxybutyric acid; 3Me4NP, 3-methyl-4-nitrophenol; 3-PBA, 3-phenoxybenzoic; 4F3PBA, 4-fluoro-3-phenoxybenzoic acid; Br2CA, cis-3-(2,2dibromovinyl)-2,2-dimethylcyclopropane-carboxylic acid; Cl2CA, 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; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DDD, dichlorodiphenyldichloroethane; DEDTP, di-ethyl-di-thiophosphate; DEP, di-ethyl-phosphate; DETP, di-ethyl-thiophosphate; DMDTP, di-methyl-di-thiophosphate; DMP, di-methyl-phosphate; DMTP, di-methyl-thiophosphate; DMST, di-methylsulftoluidide; HCH, hexachlorocyclohexane; IMPy, 2-isopropyl-4-methyl-6-hydroxypyrimidine; 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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usages are also reported, including in domestic settings, for medical applications (ANSES, 2010), and for vector control in specific areas. Pesticide contamination has been reported in water, soil, outdoor and indoor air, and food in a large body of studies and results in potential exposure of the general population by ingestion, inhalation, or skin contact (ANSES, 2010). Biomonitoring studies of general populations have reported detectable pesticide residues all across Europe (Dereumeaux et al., 2016; Fréry et al., 2013; Heudorf et al., 2006; Koureas et al., 2016; Ramos et al., 2017; Saoudi et al., 2014; Ye et al., 2008).

Because several currently used and banned pesticides are suspected of promoting prematurity (Ferguson et al., 2013), increasing the risk of congenital malformations (Nieuwenhuijsen et al., 2013), impairing fetal and child neurodevelopment (Vrijheid et al., 2016), or acting as endocrine disruptors (WHO, 2013), pregnant women and their fetuses are particularly vulnerable to these chemicals. In 2018, there are around 500 active substances from different chemical families approved in the European Union according to Regulation (EC) No 1107/2009 on plant protection products (European Commission, 2017). With 75,000 tons of pesticides used for agricultural purposes in 2014, France was the second largest pesticide consumer in Europe (Eurostat, 2017). Existing biomonitoring of pesticide exposure of pregnant women has investigated only a few of them, however. The data on pesticides currently used in France are limited to organophosphorus, pyrethroid and carbamate insecticides, triazine herbicides, and glyphosate; they come from urine samples of pregnant women in the PELAGIE mother-child cohort (Chevrier et al., 2009; 44 molecules) and the ELFE national birth cohort (Dereumeaux et al., 2016; 30 molecules). PELAGIE included about 3500 pregnant women between 2002 and 2006 in Brittany (northwestern France), and ELFE 18,000 women who gave birth in metropolitan France in 2011.

Although biological fluids such as urine and blood have long been the preferred matrices for biomonitoring pesticide exposure, interest in hair has been increasing in recent years (Appenzeller, 2015; Appenzeller and Tsatsakis, 2012). Hair is commonly used in forensics to detect various drugs or poisons because of its ability to archive long periods of exposure (provided the hair is long enough) (Pragst and Balikova, 2006). Because many pesticides in current use have short half-lives, this ability might be seen as a strength compared to single samples of urine or blood, which can be strongly affected by the intraindividual variability of exposure over time (Attfield et al., 2014). Recent analytical developments allow the measurement of a wide range of pesticides in hair by multiresidue analyses (Duca et al., 2014b) for a reasonable cost in terms of time, matrix consumed, and money.

Our aim was to use hair samples from pregnant women in the ELFE French national birth cohort to describe the concentrations of 140 pesticides and metabolites known to be present in diverse compartments of the French environment. We also assessed the variability of these chemical concentrations over pregnancy by using individual bundles of hair strands cut horizontally into three segments to represent trimesters of pregnancy.

2. Methods

2.1. Study design

The ELFE birth cohort ("*Etude longitudinale française depuis l'enfance*") was designed to explore the influence of the environment on child development, health, and socialization. The cohort design has been presented elsewhere (Vandentorren et al., 2009). More information can also be found on the website: https://www.elfe-france.fr/en/.

Briefly, ELFE is a multicenter, nationally representative cohort, based on a two-stage random stratified sampling design (stratified for maternity units and pregnant women). Of the 542 maternity wards in continental France with more than one delivery per day, 349 were randomly selected, and 320 agreed to participate. Among them, 211 units that averaged > 500 deliveries per year and were located < 150 km from biobanks participated in the biological sample collection at birth (samples of maternal urine, blood, milk, and hair, as well as newborn cord blood and meconium).

2.2. Study participants

The ELFE cohort enrolled 18,040 women at delivery, in 2011, in participating maternity units. Women met the inclusion criteria if they were at least 18 years old, had a liveborn singleton or twins, and gave birth at ≥ 33 weeks of gestation. Women were recruited during four inclusion periods, one during each quarter of 2011 (to reflect environmental conditions that differed with the time of year). Each inclusion period was 4 to 8 days long. The participation rate was 51%. Biological samples were collected only from women enrolled during the last three inclusion periods (from June 27 to July 4, from September 27 to October 4, and from November 28 to December 5). Data about the mothers' sociodemographic, socioeconomic, and medical characteristics were collected through self-reported questionnaires during the maternity stay (before discharge) and medical records.

Overall, hair samples were available for 2866 mothers. Among these, we selected all women who had a sufficient amount of hair for analysis, gave birth to singleton infants without any congenital malformation, and were living in either southwestern (Aquitaine, Midi-Pyrénées, and Poitou-Charentes) or northeastern (Champagne-Ardenne, Bourgogne, and Lorraine) France (n = 311, 11%). These regions were chosen to cover two different agricultural environments representative of the country. Agricultural activities of the Southwest are characterized by vineyards, corn, and sunflower crops, breeding (ovine, caprine, and bovine), and fresh vegetables. In the Northeast, crops were mostly vineyards, cereals, legumes, vegetables, and bovine breeding (Agreste, 2012). Women were selected regardless of whether they were living in an urban or rural area.

2.3. Choice of pesticides of interest

We focused on pesticides widely used in agriculture in these regions, or used domestically in France, or frequently detected in the environment, including food. Overall, about 180 pesticides and/or metabolites were selected a priori, based on (see Table S1): 1/sales data (in tons, for the southwestern and northeastern regions), provided by pesticide suppliers, as required since 2008 (French Ministry of Agriculture, 2012); 2/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); 3/data on domestic uses or indoor environment contamination (air, dust) in France, indexed in a summary by the national observatory of pesticide residues (ANSES, 2010), completed by an expert assessment based on more recent data; 4/the international expert assessment conducted in 2010 to guide the French biomonitoring strategy (Fillol et al., 2014).

Of these chemicals, 112 could be measured by a multiresidue method with acceptable sensitivity by the laboratory of the Human Biomonitoring Research Unit at the Luxembourg Institute of Health. In addition, 28 pesticides of similar physicochemical properties were measurable and were thus added to the list without any a priori information about the probability of exposure. Overall, 140 chemicals were screened: 111 pesticides and 29 pesticide metabolites from 25 distinct chemical families (see Table S1).

2.4. Biological sample collection and preparation

Hair samples were collected at the maternity unit after delivery and before discharge. For each mother, one bundle of hair strands was cut off the occipital region of the head, as close as possible to the scalp, and then stapled onto a paper card. Orientation was indicated on the card to identify the proximal (close to the root) and distal parts of the strands.

Each sample was placed individually in an envelope and shipped to and stored at the biobank at ambient temperature.

To cover the period of pregnancy, and assuming a hair growth rate of 1 cm per month, only the first 9 cm (proximal) of the strands were used for analyses. Among the 311 hair samples, 47 samples ≥ 9 cm were cut into 3 segments of 3 cm (141 segments) and analyzed separately to represent the three trimesters of pregnancy. The other 264 samples were analyzed in one piece. The median length of the 311 bundles of strands was 9 cm (interquartile range (IQR): 8–9 cm), 28 were < 6 cm. The median mass of the samples analyzed was 50.1 mg (IQR 50.0–50.3); 23 of the 264 one-piece samples and 40 of the 141 3-cm segments were < 40 mg. Neither the length nor the mass of the samples had more than a limited influence on the detection frequency or concentration for most of the chemicals we assessed (see Tables S2 and S3).

2.5. Chemical analyses

The analytical method to measure multiple pesticide residues in hair samples was similar to those previously published (Duca et al., 2014b; Hardy et al., 2015; Salquebre et al., 2012). Briefly, to remove possible external deposits on the hair surface, the samples were successively washed with sodium dodecyl sulfate (SDS) and then methanol, as fully detailed in a previous work (Duca et al., 2014a). As previously demonstrated, this washing procedure enabled the efficient removal of pesticides deposited on the hair surface without removing those incorporated in the bulk matrix, which were presumably incorporated through biological mechanisms. After washing, hair samples were pulverized with a ball mill (Retsch). Fifty milligrams of pulverized hair was then 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 mL extract in a 10-mL screw cap vial fitted to solid-phase microextraction (SPME) analysis. The sample was analyzed with a first step of SPME (fiber exposure at 60 °C for 80 min), and then desorption in the GC injector at 260 °C for 10 min. For metabolite analysis, the 300 mL extract was evaporated to dryness under a gentle nitrogen stream at 37 °C. Thirty milligrams of potassium carbonate, 1 mL of acetonitrile, and 100 mL of PFBBr acetonitrile (1:3, v/v) were added to the residue, and the compounds were derivatized 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 mL of ethyl acetate and centrifuged for 7 min at 18,000 ×*g* to remove any particles from the extract. After this extract was transferred into an injection vial and evaporated to dryness once again, the residue was resuspended in a volume of 20 mL 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 ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The methods used for the analysis of the different sub-extracts were described in more details in previous works (Hardy et al., 2015; Duca et al., 2014a; Duca et al., 2014b; Salquebre et al., 2012). In each analytical run, quality controls (consisting of one blank and controls supplemented at eight different concentrations) were analyzed along with the field samples. The blanks allowed us to confirm the absence of cross-contamination and the supplemented controls let us monitor any possible drift in the analytical response. The limit of quantification (LOQ) was based on the variability in the concentration of quality controls below 25% and accuracy between 75 and 125%. LOQ ranged from 0.02 pg/mg for pyraclostrobin to 50 pg/mg for propargite.

The laboratory provided the lowest possible detectable values, including values below the limit of quantification and for which variability might exceed 25%. We thus retained the lowest detected values (see Table 1) as the practical LOD.

2.6. Statistical analyses

For the chemicals detected in ≥70% of the 141 3-cm hair bundle segments, values below LOD were imputed by random values between 0 and the LOD from a log-normal probability distribution derived by a maximum-likelihood estimation method (Jin et al., 2011). When the detection rate was below 70%, we imputed the value as the LOD divided by the square root of two. We then averaged the three 3-cm hair bundle segment values to obtain one value per woman, representing the pregnancy period. Because some of these average values were below the LOD defined according to the 311 hair samples, those concentrations were considered to be below the LOD.

We explored possible co-exposures or exposure profiles by firstly using the Spearman correlation calculation for the pesticides with a detection frequency ≥70%, after imputation of values below the LOD (see Fig. 1). Next, we categorized all chemicals detected in at least 30 women into 2, 3, or 4 classes according to their detection rates and then estimated their tetrachoric and polychoric correlations (Olsson, 1979). Pesticides detected in < 50% of hair strand bundles were placed in 2 classes (not detected; detected), those with a detection frequency between 50 and 70%, 3 classes (< LOD; below and above the median of the detected values), and for those with a detection frequency ≥70%, 4 classes (first quartile, or value < LOD if detection rate 70–75%; second, third and fourth quartile). Correlations were described by presenting the strongest correlation coefficients (Robinson et al., 2015). *P-values* were not provided.

The variability of the hair concentrations across the three 3-cm segments was assessed with a linear mixed model for pesticides with a detection frequency ≥70%. We estimated the intraclass correlation coefficient (ICC) for each pesticide, defined as the proportion of the total variability explained by the interindividual variability. A higher ICC would indicate that the intraindividual variability between hair segments, which might reflect temporal variability across trimesters of pregnancy and analytical variability, is lower than the interindividual variability. For pesticides with detection rates between 30% and 70%, we assessed the agreement in pesticide detection between the three 3-cm hair-segments with the Fleiss' Kappa, an extension of Cohen's Kappa adapted for multiple raters (Warrens, 2010). Variability for pesticides detected in < 30% of the samples ($n < 14$) was not assessed because of the small number of samples.

All statistics were analyzed with SAS software V9.4. Fleiss' Kappas were estimated with the MKAPPA macro (Chen et al., 2005). Figures were made with R software V3.0.0, and specifically the Corrplot (Wei and Simko, 2016) and Circlize packages (Gu et al., 2014).

2.7. Ethical issues

All participants agreed to participate and provided written informed consent. This study 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).

3. Results

3.1. Population characteristics

The 311 women were 30.1 years old on average, mainly parous (57.1%), living with a partner (97.4%), and employed at the beginning of pregnancy (83.1%). Most were office, sales, and service workers (52.7%) or involved in intermediate occupations (25.1%); only one woman worked in farming. In all, 58.5% of the women were from the northeastern region, and 39.5% were included during the late November–early December inclusion period (Table 2).

Table 1
Detection frequencies and concentrations for the 140 pesticides and metabolites screened in hair.

Compounds	CAS number	Analytical method	LOQ pg/mg	Lowest detected value pg/mg	Missing value *n*	Number of detections *n* (%)	P25 pg/mg	P50 pg/mg	P75 pg/mg	Usage in 2011
Organochlorine										
γ-HCH (lindane)	58-89-9	SPME	0.2	0.248	0	311 (100%)	1.11	1.58	2.20	None
Hexachlorobenzene	118-74-1	SPME	0.1	0.008	0	311 (100%)	0.09	0.12	0.17	None
Pentachlorophenol[a]	87-86-5	SPME	0.5	0.415	1	310 (100%)	4.37	10.03	28.47	None
α-Endosulfan	959-98-8	SPME	0.04	0.012	0	286 (92%)	0.08	0.15	0.28	None
Dieldrin	60-57-1	SPME	0.5	0.054	0	213 (68%)	< LOD	0.25	0.61	None
β-Endosulfan	33213-65-9	SPME	0.1	0.043	0	188 (60%)	< LOD	0.16	0.40	None
α-HCH	319-84-6	SPME	0.2	0.020	0	158 (51%)	< LOD	0.03	0.09	None
β-HCH	319-85-7	SPME	1	0.078	0	152 (49%)	< LOD	< LOD	0.42	None
Trans-chlordane	5103-74-2	SPME	0.1	0.003	0	61 (20%)	< LOD	< LOD	< LOD	None
p.p′-DDT	50-29-3	SPME	2	1.322	0	31 (10%)	< LOD	< LOD	< LOD	None
p.p′-DDE[a]	72-55-9	SPME	2	0.312	0	27 (9%)	< LOD	< LOD	< LOD	None
Endrin	72-20-8	SPME	0.5	0.033	0	14 (5%)	< LOD	< LOD	< LOD	None
o.p′-DDE[a]	3424-82-6	SPME	5	0.046	0	12 (4%)	< LOD	< LOD	< LOD	None
o.p′-DDT	789-02-6	SPME	5	1.682	0	8 (3%)	< LOD	< LOD	< LOD	None
Heptachlor	76-44-8	SPME	1	0.063	0	7 (2%)	< LOD	< LOD	< LOD	None
ε-HCH	6108-10-7	SPME	0.5	0.035	0	6 (2%)	< LOD	< LOD	< LOD	None
Oxy-chlordane[a]	27304-13-8	SPME	0.5	0.026	0	6 (2%)	< LOD	< LOD	< LOD	None
Metazachlor	67129-08-2	UPLC	0.1	0.020	1	6 (2%)	< LOD	< LOD	< LOD	A
δ-HCH	319-86-8	SPME	1	0.233	0	5 (2%)	< LOD	< LOD	< LOD	None
Cis-chlordane	5103-71-9	SPME	0.2	0.013	0	5 (2%)	< LOD	< LOD	< LOD	None
Aldrin	309-00-2	SPME	0.2	0.195	0	3 (10%)	< LOD	< LOD	< LOD	None
o.p′-DDD[a]	53-19-0	SPME	5	3.427	0	2 (1%)	< LOD	< LOD	< LOD	None
p.p′-DDD[a]	72-54-8	SPME	5	8.003	0	2 (1%)	< LOD	< LOD	< LOD	None
Heptachlor-*endo*-epoxide[a]	28044-83-9	SPME	0.5	0.383	0	2 (1%)	< LOD	< LOD	< LOD	None
Heptachlor-*exo*-epoxide[a]	1024-57-3	SPME	0.5.	0.653	0	1 (< 1%)	< LOD	< LOD	< LOD	None
Isodrin	465-73-6	SPME	1		0	0	< LOD	< LOD	< LOD	None
Organophosphorus										
p-Nitrophenol[a]	100-02-7	GC	5	3.091	1	310 (100%)	8.45	13.18	20.66	None
TCPy[a]	6515-38-4	GC	0.2	0.141	2	309 (100%)	1.38	2.66	6.78	A + D
DEP[a]	598-02-7	GC	5	0.441	3	301 (98%)	3.26	7.46	23.47	A + D
DETP[a]	2465-65-8	GC	0.5	0.032	1	301 (97%)	0.48	0.88	1.78	A + D
IMPy[a]	2814-20-2	UPLC	0.4	0.063	1	300 (97%)	0.36	0.66	1.36	A + D
DMP[a]	813-78-5	GC	20	0.029	3	258 (84%)	0.23	0.86	3.21	A + D
3Me4NP[a]	2581-34-2	GC	0.3	0.055	1	255 (82%)	0.17	0.65	1.25	D
DMTP[a]	1112-38-5	GC	0.5	0.004	1	173 (56%)	< LOD	0.03	0.11	A + D
DMDTP[a]	756-80-9	GC	5	0.041	1	16 (5%)	< LOD	< LOD	< LOD	A + D
DEDTP[a]	298-06-6	GC	5	1.920	0	1 (< 1%)	< LOD	< LOD	< LOD	None
Dimethoate	60-51-5	UPLC	0.1		1	0	< LOD	< LOD	< LOD	A
Malathion	121-75-5	GC	1		1	0	< LOD	< LOD	< LOD	None
Pyrethroids										
3-PBA[a]	3739-38-6	GC	0.5	0.209	1	310 (100%)	0.76	1.69	3.76	A + D
Cl_2CA[a]	55701-05-8	GC	0.5	0.248	1	308 (99%)	1.37	3.51	8.06	A + D
Permethrin	52645-53-1	GC	10	1.281	0	295 (95%)	17.15	37.93	91.61	D
Cypermethrin	52315-07-8	GC	0.4	0.043	0	259 (83%)	0.35	1.09	2.86	A + D
ClCF3CA[a]	72748-35-7	GC	10	0.011	1	189 (61%)	< LOD	0.09	0.35	A + D
Lambda-cyhalothrin	91465-08-6	GC	1	0.067	0	130 (42%)	< LOD	< LOD	1.01	None
4F3PBA[a]	77279-89-1	GC	0.1	0.005	1	107 (35%)	< LOD	< LOD	0.03	A + D
Bifenthrin	82657-04-3	GC	2	0.140	0	23 (7%)	< LOD	< LOD	< LOD	A + D
Br_2CA[a]	53179-78-5	GC	0.2	0.041	1	21 (7%)	< LOD	< LOD	< LOD	A + D
Deltamethrin	52918-63-5	GC	2	0.651	0	11 (4%)	< LOD	< LOD	< LOD	A + D
Cyfluthrin	68359-37-5	GC	0.4	0.517	0	8 (3%)	< LOD	< LOD	< LOD	A + D
Fenvalerate	51630-58-1	GC	3	3.078	0	2 (1%)	< LOD	< LOD	< LOD	D
2-ClBA[a]	2012-74-0	GC	0.2	0.977	1	2 (1%)	< LOD	< LOD	< LOD	None
Carbamates										
Carbendazim	10605-21-7	UPLC	0.5	0.271	1	310 (100%)	0.45	0.65	1.22	None
Carbofuran	1563-66-2	UPLC	0.2	0.023	1	195 (63%)	< LOD	0.10	0.32	None
Propoxur	114-26-1	UPLC	0.3	0.104	1	139 (45%)	< LOD	< LOD	0.77	D
Iprovalicarb	140923-17-7	UPLC	0.5	0.115	1	5 (2%)	< LOD	< LOD	< LOD	A
Fenoxycarb	72490-01-8	UPLC	0.5	1.572	2	3 (1%)	< LOD	< LOD	< LOD	A
Methomyl	16752-77-5	UPLC	0.1	4.971	1	1 (< 1%)	< LOD	< LOD	< LOD	D
Carbaryl	63-25-2	UPLC	0.5		1	0	< LOD	< LOD	< LOD	D
Oxamyl	23135-22-0	UPLC	0.2		1	0	< LOD	< LOD	< LOD	A
Promecarb	2631-37-0	UPLC	0.1		1	0	< LOD	< LOD	< LOD	None
Dinitroanilines										
Trifluralin	1582-09-8	SPME	0.1	0.003	0	311 (100%)	0.10	0.14	0.17	None
Pendimethalin	40487-42-1	UPLC	2	0.588	8	2 (1%)	< LOD	< LOD	< LOD	A + D
Thiocarbamates										
Prosulfocarb	52888-80-9	UPLC	0.5	0.051	1	310 (100%)	0.17	0.31	0.62	A
Phenylpyrazoles										

(*continued on next page*)

Table 1 (*continued*)

Compounds	CAS number	Analytical method	LOQ pg/mg	Lowest detected value pg/mg	Missing value *n*	Number of detections *n* (%)	P25 pg/mg	P50 pg/mg	P75 pg/mg	Usage in 2011
Fipronil sulfone[a]	120068-36-2	UPLC	0.5	0.039	0	307 (99%)	0.49	2.25	10.51	D
Fipronil	120068-37-3	UPLC	0.5	0.028	0	270 (87%)	0.30	1.62	9.50	D
Acid herbicides										
2,4-D	94-75-7	GC	0.2	0.113	11	291 (97%)	0.44	0.68	1.16	A + D
MCPA	94-74-6	GC	0.2	0.210	11	290 (97%)	0.45	0.82	1.30	A + D
Mecoprop	93-65-2	GC	0.5	0.039	11	278 (93%)	0.16	0.26	0.45	A + D
Dichlorprop	120-36-5	GC	1	0.070	11	85 (28%)	< LOD	< LOD	0.16	D
MCPB	94-81-5	GC	0.5	0.002	11	65 (22%)	< LOD	< LOD	< LOD	A
2,4-DB	94-82-6	GC	0.5		10	0	< LOD	< LOD	< LOD	A
Azoles										
Thiabendazole	148-79-8	UPLC	0.3	0.028	1	280 (90%)	0.20	0.68	2.42	A
Propiconazole	60207-90-1	UPLC	0.5	0.094	1	221 (71%)	< LOD	0.67	1.44	A + D
Tebuconazole	107534-96-3	UPLC	0.5	0.086	2	144 (47%)	< LOD	< LOD	0.62	A + D
Myclobutanil	88671-89-0	UPLC	0.5	0.015	1	81 (26%)	< LOD	< LOD	0.02	A + D
Imazalil	35554-44-0	UPLC	10	0.486	1	80 (26%)	< LOD	< LOD	1.63	A
Bitertanol	55179-31-2	UPLC	0.5	0.113	2	74 (24%)	< LOD	< LOD	< LOD	A
Prochloraz	67747-09-5	UPLC	0.05	0.028	1	51 (16%)	< LOD	< LOD	< LOD	A
Difenoconazole	119446-68-3	UPLC	0.1	0.012	1	26 (8%)	< LOD	< LOD	< LOD	A + D
Flusilazole	85509-19-9	UPLC	0.1	0.021	2	15 (5%)	< LOD	< LOD	< LOD	A
Cyproconazole	94361-06-5	UPLC	0.5	0.072	1	13 (4%)	< LOD	< LOD	< LOD	A + D
Fenbuconazole	114369-43-6	UPLC	0.5	0.102	2	7 (2%)	< LOD	< LOD	< LOD	A + D
Epoxiconazole	133855-98-8	UPLC	0.1	0.205	1	6 (2%)	< LOD	< LOD	< LOD	A + D
Penconazole	66246-88-6	UPLC	0.1	0.018	1	5 (2%)	< LOD	< LOD	< LOD	A + D
Triadimenol	55219-65-3	UPLC	2	0.503	1	3 (1%)	< LOD	< LOD	< LOD	A
Tetraconazole	112281-77-3	UPLC	0.5	1.410	1	1 (< 1%)	< LOD	< LOD	< LOD	A + D
Oxadiazins										
Oxadiazon	19666-30-9	SPME	0.5	0.020	0	264 (85%)	0.08	0.15	0.29	A + D
Indoxacarb	173584-44-6	UPLC	0.1	0.210	3	2 (1%)	< LOD	< LOD	< LOD	A
Triazines/triazones										
Terbutryn	886-50-0	UPLC	0.2	0.004	2	240 (78%)	0.07	0.22	0.46	None
Sebuthylazine	7286-69-3	UPLC	0.1	0.086	1	9 (3%)	< LOD	< LOD	< LOD	None
Prometryn	7287-19-6	UPLC	0.1	0.033	2	4 (1%)	< LOD	< LOD	< LOD	None
Propazine	139-40-2	UPLC	0.5	0.091	1	3 (1%)	< LOD	< LOD	< LOD	None
Terbuthylazine	5915-41-3	UPLC	0.1	0.168	1	2 (1%)	< LOD	< LOD	< LOD	None
Atrazine	1912-24-9	UPLC	0.5	0.225	1	1 (< 1%)	< LOD	< LOD	< LOD	None
Atrazine desethyl	6190-65-4	UPLC	2	0.280	2	1 (< 1%)	< LOD	< LOD	< LOD	None
Simazine	122-34-9	UPLC	0.5	0.194	1	1 (< 1%)	< LOD	< LOD	< LOD	None
Metamitron	41394-05-2	UPLC	1		1	0	< LOD	< LOD	< LOD	A
Metribuzin	21087-64-9	UPLC	5		1	0	< LOD	< LOD	< LOD	A + D
Chloridazon	1698-60-8	UPLC	0.2		1	0	< LOD	< LOD	< LOD	A
Amide pesticides										
Metolachlor	51218-45-2	UPLC	0.03	0.008	1	237 (76%)	0.01	0.03	0.05	None
DMST[a]	66840-71-9	UPLC	0.2	0.297	1	144 (46%)	< LOD	< LOD	2.42	None
Fenhexamid	126833-17-8	UPLC	5	1.221	3	45 (15%)	< LOD	< LOD	< LOD	A + D
Dimethachlor	50563-36-5	UPLC	0.5	0.002	1	18 (6%)	< LOD	< LOD	< LOD	A
Alachlor	15972-60-8	UPLC	0.5		1	0	< LOD	< LOD	< LOD	None
Strobilurins										
Azoxystrobin	131860-33-8	UPLC	0.2	0.012	1	201 (65%)	< LOD	0.16	0.62	A + D
Pyraclostrobin	175013-18-0	UPLC	0.02	0.014	1	96 (31%)	< LOD	< LOD	0.04	A
Trifloxystrobin	141517-21-7	UPLC	0.1	0.017	1	60 (19%)	< LOD	< LOD	< LOD	A + D
Kresoxim-methyl	143390-89-0	UPLC	0.5	0.002	1	7 (2%)	< LOD	< LOD	< LOD	A
Carboxamides										
Boscalid	188425-85-6	UPLC	0.3	0.079	3	195 (63%)	< LOD	0.55	1.41	A
Diflufenican	83164-33-4	UPLC	0.1	0.053	3	133 (43%)	< LOD	< LOD	0.19	A + D
Urea										
DCPMU[a]	3567-62-2	UPLC	0.5	0.017	0	190 (61%)	< LOD	0.06	0.21	A + D
DCPU[a]	2327-02-8	UPLC	5	0.277	0	188 (60%)	< LOD	0.93	2.24	D
Diuron	330-54-1	UPLC	0.5	0.036	1	144 (46%)	< LOD	< LOD	0.28	D
Fenuron	101-42-8	UPLC	0.2	0.009	1	136 (44%)	< LOD	< LOD	0.09	None
Isoproturon	34123-59-6	UPLC	0.5	0.006	1	102 (33%)	< LOD	< LOD	0.02	A
Chlortoluron	15545-48-9	UPLC	0.3	0.024	1	35 (11%)	< LOD	< LOD	< LOD	A
Methabenzthiazuron	18691-97-9	UPLC	0.1	0.125	1	3 (1%)	< LOD	< LOD	< LOD	None
Chloroxuron	1982-47-4	UPLC	0.5	1.660	1	3 (1%)	< LOD	< LOD	< LOD	None
Metoxuron	19937-59-8	UPLC	1	0.008	1	2 (1%)	< LOD	< LOD	< LOD	None
Metobromuron	3060-89-7	UPLC	0.2	3.038	1	1 (< 1%)	< LOD	< LOD	< LOD	None
3,4-Dichloroaniline[a]	95-76-1	UPLC	5	24.165	1	1 (< 1%)	< LOD	< LOD	< LOD	None
Monolinuron	1746-81-2	UPLC	0.5		1	0	< LOD	< LOD	< LOD	None
Linuron	330-55-2	UPLC	0.5		1	0	< LOD	< LOD	< LOD	A
Neonicotinoids										
Imidacloprid	138261-41-3	UPLC	0.5	0.049	1	132 (43%)	< LOD	< LOD	0.66	A + D

(*continued on next page*)

Table 1 (*continued*)

Compounds	CAS number	Analytical method	LOQ pg/mg	Lowest detected value pg/mg	Missing value *n*	Number of detections *n* (%)	P25 pg/mg	P50 pg/mg	P75 pg/mg	Usage in 2011
Acetamiprid	135410-20-7	UPLC	2	0.010	16	28 (9%)	< LOD	< LOD	< LOD	A + D
Thiacloprid	111988-49-9	UPLC	1	0.014	16	19 (6%)	< LOD	< LOD	< LOD	A + D
Clothianidin	210880-92-5	UPLC	0.5	0.399	1	4 (1%)	< LOD	< LOD	< LOD	A + D
Thiamethoxam	153719-23-4	UPLC	0.2	0.109	1	3 (1%)	< LOD	< LOD	< LOD	A + D
Dinotefuran	165252-70-0	UPLC	1		1	0	< LOD	< LOD	< LOD	None
Anilino-pyrimidines										
Pyrimethanil	53112-28-0	UPLC	0.2	0.054	1	35 (11%)	< LOD	< LOD	< LOD	A + D
Cyprodinil	121552-61-2	UPLC	0.5	0.175	1	4 (1%)	< LOD	< LOD	< LOD	A + D
Benzamides										
Zoxamide	156052-68-5	UPLC	0.1	0.036	3	3 (1%)	< LOD	< LOD	< LOD	A
Propyzamide	23950-58-5	UPLC	0.5		3	0	< LOD	< LOD	< LOD	A
Miscellaneous										
Lenacil	2164-08-1	UPLC	0.8	0.144	1	96 (31%)	< LOD	< LOD	0.30	A
Spinosyn A	131929-60-7	UPLC	3.8	0.010	8	46 (15%)	< LOD	< LOD	< LOD	A + D
Aclonifen	74070-46-5	UPLC	10	10.924	1	2 (1%)	< LOD	< LOD	< LOD	A + D
Iprodione	36734-19-7	UPLC	5	29.165	2	1 (< 1%)	< LOD	< LOD	< LOD	A
Crimidine	535-89-7	UPLC	0.5		1	0	< LOD	< LOD	< LOD	D
Fenarimol	60168-88-9	UPLC	0.2		1	0	< LOD	< LOD	< LOD	None
Propargite	2312-35-8	UPLC	50		8	0	< LOD	< LOD	< LOD	A

[a] Pesticide metabolites (note: pentachlorophenol and oxy-chlordane are simultaneously pesticides and pesticide metabolites). Pesticide metabolites were classified as A and/or D if at least one of the parent compounds had agricultural and/or domestic usage in 2011. Abbreviations: A, authorized in agriculture; D, possibly used for domestic purposes; GC, derivatization – gas chromatography – tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; SPME, solid-phase microextraction – gas chromatography – mass spectrometry; UPLC, ultra-performance liquid chromatography – tandem mass spectrometry.

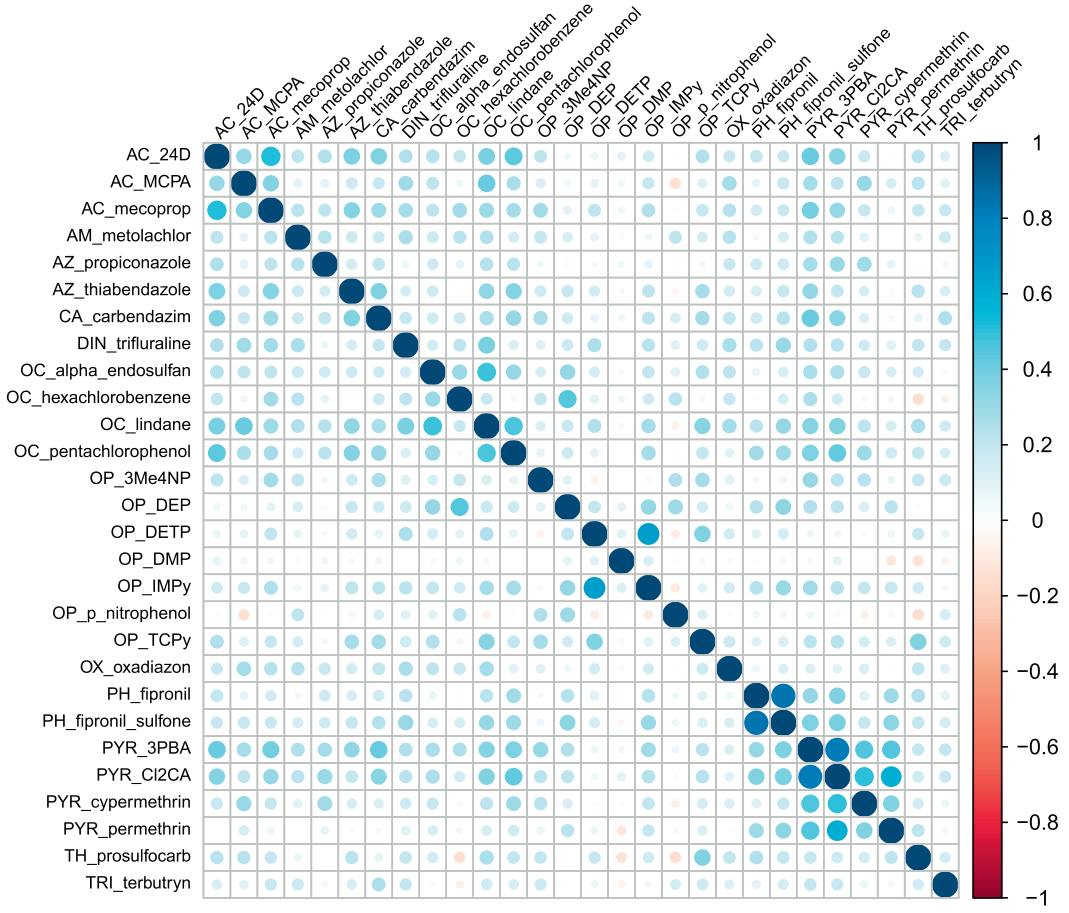

Fig. 1. Correlation matrix of the 28 compounds detected in > 70% of women's hair strands.
The intensity and size of the dot is proportional to the value of the Spearman correlation coefficient between the two compounds (blue for positive coefficients, red for negative coefficients). Values < LOD were randomly imputed between 0 and the lowest detected value. The first letters correspond to the chemical family of the molecules: AC, acid herbicide; AZ, Azole; CA, carbamate; DIN, dinitroaniline; PH, phenylpyrazole; PYR, pyrethroid; OC, organochlorine; OP, Organophosphate; OX, oxadiazin; TH, thiocarbamate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Population characteristics.

Characteristics	Study population (n = 311)	
	N (%)	Mean ± STD
Maternal age at birth	310	30.1 ± 5.0
Parity		
0	133 (42.9%)	
≥1	177 (57.1%)	
Missing	1	
Educational level		
High school or lower	140 (45.2%)	
University level	170 (54.8%)	
Missing	1	
Family situation		
Couple	301 (97.4%)	
Alone	8 (2.6%)	
Missing	2	
Socio-professional category		
1. Farmers	1 (0.3%)	
2. Small businesses and self-employed workers	12 (3.9%)	
3. Higher managerial and professional occupations	37 (11.9%)	
4. Intermediate occupations	78 (25.1%)	
5. Office, sales, and service workers	164 (52.7%)	
6. Manual workers	10 (3.2%)	
7. Retired	1 (0.3%)	
8. Unemployed	3 (1.0%)	
9. Unknown	5 (1.6%)	
Missing	0	
Working status at the beginning of the pregnancy		
Employed	256 (83.1%)	
Unemployed, parental leave, student, retired, or other	52 (16.9%)	
Missing	3	
Period of sampling		
June/July	77 (24.8%)	
September/October	111 (35.7%)	
November/December	123 (39.5%)	
Living area		
Southwestern France	129 (41.5%)	
Northeastern France	182 (58.5%)	

3.2. Descriptive results

Overall, the number of chemicals detected per women ranged from 25 to 65, with a median of 43 (IQR 38–47). The detection rate and median concentration of each chemical are presented by chemical families in Table 1. Among the 140 chemicals analyzed, 20 had detection rates ≥90%; 8 between 70 and 90%, and 23 between 30 and 70%; 71 were detected in < 30% of the hair samples, and 18 compounds were not detected at all (substances with 0 detections in Table 1). Overall, 46 of the 112 compounds selected a priori (41%), and 5 (terbutryn, pyraclostrobin, fenuron, lenacil, and dimethylsulftoluidide (DMST, a metabolite of tolylfluanid)) of the 28 compounds selected without an evidentiary basis (19%) were detected in > 30% of hair samples. Of the 25 chemical families screened, 22 were detected at least once in hair. Fourteen of these chemical families included at least one compound detected in at least 50% of samples.

Table S4 presents the 20 most frequently detected chemicals, sorted by detection frequency (90–100%). These included four organochlorine pesticides (lindane (γ-HCH), 100%; hexachlorobenzene, 100%; pentachlorophenol, 100%; and α-endosulfan, 92%), five metabolites of organophosphorus pesticides (*p*-nitrophenol, 100%; TCPy (3,5,6-trichloro-2-pyridinol), 100%; DEP (di-ethyl-phosphate), 98%; DETP (di-ethyl-thiophosphate), 97%; and IMPy (2-isopropyl-4-methyl-6-hydroxypyrimidine), 97%), three pyrethroid compounds (3-PBA (3-phenoxybenzoic), 100%; Cl_2CA (cis-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid), 99%; and permethrin, 95%), one carbamate pesticide (carbendazim, 100%), one dinitroaniline pesticide (trifluralin, 100%), one thiocarbamate pesticide (prosulfocarb, 100%), one phenylpyrazole metabolite (fipronil sulfone, 99%), three acid herbicides (2,4-D (2,4-dichlorophenoxyacetic acid), 97%; MCPA (4-chloro-2-methylphenoxyacetic acid), 97%; and mecoprop, 93%), and one azole pesticide (thiabendazole, 90%).

In decreasing order, the chemicals found at the highest concentrations were permethrin (median: 37.9 pg/mg of hair), *p*-nitrophenol (13.2 pg/mg), pentachlorophenol (10.0 pg/mg), DEP (7.46 pg/mg), Cl_2CA (3.5 pg/mg), TCPy (2.7 pg/mg), fipronil sulfone (2.3 pg/mg), 3-PBA (1.7 pg/mg), lindane (1.6 pg/mg), and cypermethrin (1.1 pg/mg).

Overall, we observed similar detection frequencies for pesticides and metabolites in both the northeastern and southwestern areas, except for isoproturon (42% vs. 20%, respectively) and imidacloprid (32% and 57%); they were also similar across the inclusion periods (June–July, September–October, November–December), except for several specific compounds, such as imazalil (8%, 9%, 52%, respectively), isoproturon (18%, 27%, 47%), and chlortoluron (4%, 2%, 24%) (Tables S5 and S6).

3.3. Variability across hair bundle segments

Overall, 28 chemicals were detected in at least 70% of the 141 3-cm hair-segments (from 47 women). Using linear mixed models, we found that the intraindividual variability was lower than the interindividual variability for all these chemicals, with ICCs ranging from 0.59 for DMP (di-methyl-phosphate) to 0.94 for IMPy (Table 3).

Of the 20 chemicals detected in 30–70% of the 3-cm hair-segments,

Table 3
Intra- and interindividual variability of pesticide concentrations across 3-cm hair segments from the same bundles of hair (n = 47).

Compounds	Interindividual variability	Intra-individual variability (temporal and analytical variability)	Intraclass correlation coefficient (ICC)
Pesticides banned before 2011 (France)			
γ-HCH (lindane)	0.09	0.01	0.92
Hexachlorobenzene	0.06	0.03	0.69
Pentachlorophenol	0.33	0.05	0.87
α-Endosulfan	0.33	0.03	0.90
Dieldrin	0.65	0.12	0.84
Carbendazim	0.06	0.03	0.70
Trifluralin	0.10	0.02	0.82
Pesticides with on-going usage in 2011 (France)			
Permethrin	0.29	0.07	0.81
Cypermethrin	0.79	0.21	0.79
Carbofuran	0.56	0.07	0.90
Prosulfocarb	0.11	0.01	0.94
Fipronil sulfone	0.49	0.07	0.88
Fipronil	0.99	0.12	0.89
2,4D	0.20	0.04	0.84
Mecoprop	0.47	0.14	0.77
MCPA	0.13	0.04	0.77
Thiabendazole	1.00	0.09	0.92
Oxadiazon	0.18	0.05	0.79
Azoxystrobin	0.81	0.16	0.83
Pesticide metabolites			
P-nitrophenol	0.06	0.01	0.84
TCPy	0.20	0.05	0.80
DEP	0.54	0.07	0.89
DETP	0.66	0.16	0.80
IMPy	0.35	0.02	0.94
DMP	0.65	0.45	0.59
3Me4NP	0.58	0.16	0.79
3-PBA	0.22	0.04	0.83
Cl_2CA	0.28	0.03	0.90

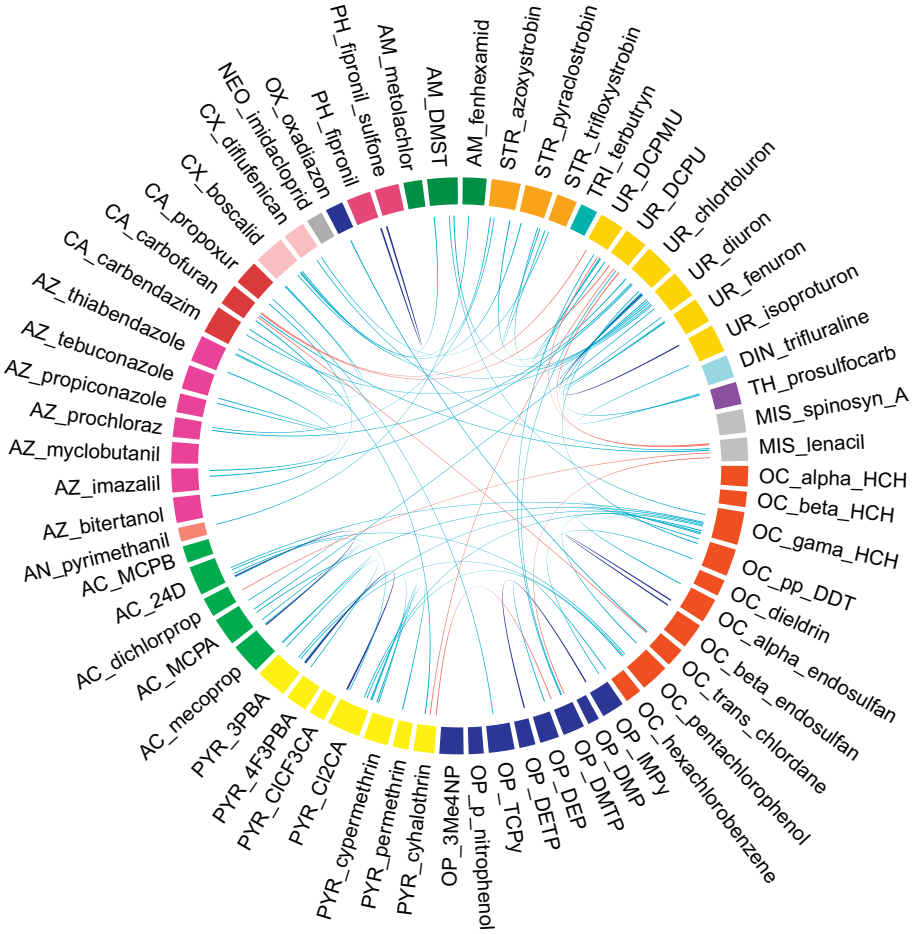

Fig. 2. Correlation between the 64 compounds detected in > 30 women's bundles of hair.
Tetrachoric/polychoric correlations between two compounds are represented by a blue line for positive correlations (light blue for coefficients between 0.4 and 0.6, dark blue above 0.6), and by a red line for negative correlations (coefficients below −0.4). The first letters correspond to the chemical family of the molecules: AC, acid herbicide; AM, amide; AN, anilo–pyrimidine; AZ, Azole; CA, carbamate; CX, carboxamide; DIN, dinitroaniline; MIS, miscellaneous; NEO, neonicotinoid; PH, phenylpyrazole; PYR, pyrethroid; OC, organochlorine; OP, organophosphate; OX, oxadiazin; STR, strobilurin; TH, thiocarbamate; UR, urea. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

17 had a Fleiss' Kappa > 0.7; this indicates good agreement in terms of detection/non-detection across these segments (Table S7). The three chemicals with the poorest Fleiss' Kappa values were metolachlor (0.68), DCPMU (1-(3,4-dichlorophenyl)-3-methylurea; 0.65), and DCPU (1-(3,4-dichlorophenyl)urea; 0.59).

3.4. Correlations

Fig. 1 presents the correlation matrix for the 28 chemicals with detection rates ≥70%. The highest correlations were observed between pesticides and their metabolites (e.g., fipronil and fipronil sulfone, rho = 0.84) or between some pesticide metabolites sharing a parent compound (IMPy and DETP, 0.67; 3-PBA and Cl_2CA, 0.82). We also observed high correlations between chemicals from the same chemical family, especially pyrethroids, and to a lesser extent, organochlorines and acid herbicides. Except for some one-off correlations (hexachlorobenzene and DEP, 0.44; pentachlorophenol and Cl_2CA, 0.42; pentachlorophenol and 2,4-D, 0.41; lindane and MCPA, 0.41; carbendazim and Cl_2CA, 0.40), all the other correlation coefficients were < 0.4. Among the few negative correlations, none reached −0.20.

Fig. 2 graphically presents the tetrachoric and polychoric correlation coefficients > 0.4 or < −0.4 between the 64 chemicals detected in the hair of > 30 women. Besides the correlations mentioned above (except for carbendazim and Cl_2CA), we observed multiple correlations within the urea family and between some of the urea compounds and most of the other chemical families. Among these, chlortoluron and diuron tended to be positively correlated with several chemicals (chlortoluron with azoxystrobin, prochloraz, imazalil, *p-p′*-DDT, prosulfocarb, and pyraclostrobin; and diuron with boscalid, carbendazim, MCPA, and DMST). DCPU and DCPMU, both metabolites of diuron, tended to be correlated with other chemicals negatively (carbofuran, lenacil, and lambda-cyhalothrin) or positively (di-methyl-thiophosphate (DMTP)). Compounds of the organochlorine, pyrethroid, and acid herbicide families (lindane, pentachlorophenol, Cl_2CA, 3-PBA, 2,4-D, and MPCA) showed multiple correlations with one another, but very few other correlations with compounds from the other families.

Interestingly, some chemical concentrations were not correlated with any others, including some chemicals from the 20 most frequently detected (i.e., *p*-nitrophenol and trifluralin). Similarly, fipronil and its metabolite, fipronil sulfone, were not related to any other family.

4. Discussion

Of the 140 pesticides and metabolites screened, 122 were detected in at least one of the 311 new mother hair samples. More than 40 pesticides and metabolites were detected in hair samples from half the women. Twenty-height of these were detected in > 70% of the samples. Several chemical groups tested here have never or only rarely been included in previous human biomonitoring studies; these include azole, oxadiazin, and carboxamide fungicides, phenylpyrazole and neonicotinoid insecticides, and amide pesticides. The good ICC values reported in our results, even for pesticides/metabolites with short half-lives, suggest a limited influence of the intraindividual variability of the exposure in our measures. To the best of our knowledge, this is the largest study yet conducted in terms of the number of pesticides simultaneously monitored in a population of pregnant women, as well as the first to explore the intraindividual variability of pesticide concentrations in hair over time.

The literature contains very little or no information on total human exposure to one third (trifluralin, carbendazim, prosulfocarb, fipronil,

MCPA, thiabendazole, and mecoprop) of the 20 chemicals with detection frequencies > 90%. Nonetheless, the International Agency for Research on Cancer reports that trifluralin is suspected to be carcinogenic (IARC, 2017), and the EU CMR classification (for chemicals that are "carcinogenic, mutagenic or toxic for reproduction") list carbendazim as a suspected mutagenic and reprotoxicant agent (EFSA, 2010; INRS, 2016). Moreover, WHO lists fipronil as a suspected endocrine disruptor (WHO, 2013), as European Commission experts did for trifluralin in 2007 (European Commission, 2016). Overall, according to WHO and the European commission, half of the 20 most detected chemicals are pesticides or metabolite of pesticides suspected to be carcinogens, mutagens, or reproductive toxicants in humans, and 14 are suspected endocrine disruptors. Most of these pesticidal active substances have been banned for agricultural uses in Europe (European Commission, 2017). Table 1 indicates their status (banned or not) in France in 2011.

Our findings are in line with past French and European studies of the organochlorine, organophosphorus, and pyrethroid pesticide families; studies have shown the presence of these families in a large majority of urine or serum samples from the general population or from populations of pregnant women (Chevrier et al., 2009; Chevrier et al., 2013; Dereumeaux et al., 2016; Fréry et al., 2013; Heudorf et al., 2006; Koureas et al., 2016; Ramos et al., 2017; Ye et al., 2008). Detailed comparisons with these studies remains difficult because of the diversity of the study populations, and the variations due to the biological matrices used and the sensitivity of the associated analytical methods. Hair, by aggregating exposures over time, is probably able to cover longer exposure periods to molecules with short half-lives than urine and serum can. We should, nonetheless, note the greatest discrepancies. Lindane, a persistent organochlorine present in 100% of the hair samples in our study, has previously been detected in 7% of serum samples in the French population (Fréry et al., 2013) and 20% of serum samples in the Spanish population (Ramos et al., 2017). Moreover, our detection rates are higher than previous findings in urine samples from women from this ELFE cohort (partially overlapping population) (Dereumeaux et al., 2016) for pentachlorophenol (100% in hair vs 4% in urine), some organophosphorus metabolites (from 1% to 90% vs. 0% to 28%), propoxur (45% vs 4%), and 4-F-3-PBA (35% vs 6%). We also detected Br_2CA and p,p′DDE at lower frequencies in this study (7% and 9%, respectively) than reported in previous findings in urine samples from the PELAGIE birth cohort (68% and 81%, respectively) (Chevrier et al., 2013; Viel et al., 2015) and from the French general population (83% and 100%) (Fréry et al., 2013). In a previous analysis of the ELFE cohort, the detection rate for Br2CA in urine was 100% (Dereumeaux et al., 2016).

Among the 10 chemicals that we detected at the highest concentrations, we found 4 pyrethroid compounds (permethrin, cypermethrin, and two metabolites). In France, pyrethroids are frequently used not only in agriculture, but also in domestic contexts, to treat flying bugs or as antiparasitics, and as a wood preservative (ANSES, 2010; Fréry et al., 2013). We note that in 2011, when these samples were collected, permethrin was restricted to domestic usage and wood preservation. We also found high concentrations of fipronil, which has not been authorized for agricultural purposes since 2005 in France (Legifrance, 2005), but is still widely used as an antiparasitic for pets. Moreover, pentachlorophenol and lindane have both been banned for at least a decade in France but were nonetheless detected in 100% of hair samples, and at much higher concentrations than the other organochlorine pesticides. Given that these persistent compounds were previously used as wood preservatives (ANSES, 2010), it is likely that release from construction materials in indoor environments is a source of exposure. The other three chemicals found at the highest concentrations were organophosphorus metabolites. TCPy and DEP are both metabolites of chlorpyrifos, still widely used as an agricultural pesticide and frequently detected in food products in France (mainly in fruits and vegetables) (Nougadère et al., 2012) and in Europe (EFSA, 2017). *P*-nitrophenol is a metabolite of parathion (banned in 2004 in France (INRS, 2007)), but it is, by itself, also widely used in the manufacture of drugs, fungicides, insecticides, and dyes and to darken leather (US-EPA, 2000); accordingly, its high level of contamination does not necessarily mean that the women were highly exposed to parathion. Overall, of these 10 compounds, only half were used in agriculture at the time of sampling, while most of them are still available for on-going domestic usage and have or had applications in wood preservation.

In selecting women living in two different agricultural environments, we expected to find different exposure profiles for the pesticides still in use. However, except for a few agricultural pesticides (e.g., boscalid, imidacloprid, isoproturon, and prosulfocarb), we observed that detection frequency and hair concentration remained fairly similar in both regions (Table S6). Boscalid and isoproturon were used in large quantities in agriculture in Champagne-Ardenne (northeast), but not in Aquitaine (southwest), which is consistent with our findings. Imidacloprid was used homogenously in agriculture in both regions, but its concomitant domestic use might explain the variation observed. We did not observe clear difference in agriculture usage for prosulfocarb. Overall, this stability throughout different agricultural areas might indicates either that the contribution of local agricultural practices to the pesticide concentrations in women's hair is probably lower than that of other sources of exposure shared nationwide (e.g., food, household practices, and construction materials), or that 9-cm hair samples are unable to capture short-term exposure variations that might be induced by local agricultural practices.

Another key finding concerned the characterization of the inter-individual variability of exposure, based on repeated measures of the 3-cm hair segments. The poor ICC observed with the standard matrices (urine and serum) is often an issue in biomonitoring chemicals with short half-lives, so that multiple repeated measures are often necessary to limit the risk of misclassification bias (Attfield et al., 2014). In our results, we observed that intraindividual variability (including variability across segments of individual bundles of hair strands and variability related to the analytical procedures) was lower than inter-individual variability, even for compounds with short half-lives. This implies that intraindividual variability should not impair our ability to discriminate subjects according to their level of exposure measured in a single hair sample. In fact, hair is assumed to archive exposure for a long period, as shown for other organic chemicals (Pragst and Balikova, 2006; Appenzeller et al., 2007). It thus enables detection over a wide time window, even when the sampling took place several months after exposure. The high ICCs we observed may be related to the ability of the hair to aggregate chemical contaminations over a definite period.

As expected, we observed some strong correlations between pesticides and their metabolites and between metabolites sharing the same parent compounds. However, among the organophosphorus metabolites, DMP and DMTP were not clearly correlated, as well as DEP, DETP, and TCPy, even though they share common parent compounds (see Fig. 1). This finding might be related to different degradation pathways among their precursors, leading to different proportions of the different metabolites. Women may also be directly exposed to some of the organophosphorus metabolites by food (Radford et al., 2016). Acid herbicides were correlated to several organochlorine and pyrethroid compounds, but not to the other families. Inversely, urea herbicides and metabolites were correlated with numerous chemicals and families, but hardly at all with organochlorines, pyrethroids, or acid herbicides. We cannot currently explain these differences in patterns. Overall, although some correlations – mainly positive – were observed between chemicals, they tended to be weak to moderate, as illustrated by the Figs. 1 and 2, with no evidence of strong correlation patterns.

Although the mechanisms by which chemicals are incorporated in hair have not been fully elucidated, Appenzeller et al. (2017) have reported good correlations between hair, urine, and serum concentrations of 27 pesticides and metabolites (including pp′DDE) in rats. They also reported good consistency between hair and serum concentrations of

organochlorine, organophosphorus, and pyrethroid compounds, with detection rates higher in hair, as observed in humans. Chata et al. (2016) have suggested that the physicochemical properties of pesticides do not influence their incorporation into hair. At the same time, several studies of both animal and humans have suggested that pigmentation has only a limited effect or none at all on the concentration of chemicals in hair (Grova et al., 2013; Appenzeller et al., 2007; Kharbouche et al., 2010); this is suggested to be the case in rats for several of the pesticides studied here (Appenzeller et al., 2017). Duca et al. (2014a) performed artificial contamination to simulate pesticide deposition on hair surface by different procedures (with silica, cellulose, and aqueous solutions). Their findings suggest efficient decontamination when hair is washed with water and acetonitrile, with limited or no influence on the concentration of chemicals biologically incorporated. In a previous study of 14 subjects, Raeppel et al. (2016) suggested positive associations between indoor air measurements and hair concentrations for some of the 27 pesticides analyzed in their study, including alpha- and beta-endosulfan, lindane, MCPA, and trifluralin. In our study, we estimated correlations between agricultural pesticide concentrations in hair and estimates of daily dietary intakes (lower-bound scenario) produced by De Gavelle et al. (2016) for 20 compounds (see Supplemental materials, Table S8). We also explored associations between the hair concentrations of pesticides frequently used in the domestic context – including fipronil, cypermethrin, and permethrin (mentioned above to be among the 10 pesticides detected at the highest concentration) – and self-reported domestic usage (Supplemental materials, Table S9). Our results suggest concordance is good between these indirect measurements and hair concentrations for various pesticides studied here. Finally, however, it is necessary to bear in mind that absence of detection does not necessarily mean absence of exposure. Beyond the limitation of analytical sensitivity, in most situations, studies of hair samples have screened only for parent compounds, and not their metabolites. Given that chemicals are incorporated continuously in the hair over long time periods, we assume that parents and metabolites might be simultaneously present in hair samples when the metabolism is endogenous and the half-life long enough. Overall, despite these supportive elements, it should be noted that the literature focusing on hair matrix remains sparse and not specifically focused on our pesticides of interest. Further studies are still needed to better understand the relations between exposure and pesticide concentrations in hair and to explore in more depth the influence of several external factors, such as high UV exposure, hair treatment, and hair properties.

It is difficult for the moment to interpret the public health implications of these findings, because we lack information linking hair contamination to health outcomes. Nonetheless, the large panel of pesticides monitored provides more comprehensive information on women's exposure and should help to prioritize future research on pesticides.

5. Conclusion

We reported measurable concentrations of a large range of pesticides and their metabolites in hair from pregnant French women, including some that may have endocrine disrupting properties. Our results enhance knowledge about the pesticide exposure of pregnant women, especially in Europe where this topic is still understudied, and for several chemical families that have been explored rarely if ever. We also reported high ICCs for various pesticides with short half-lives, based on repeated hair samples. Nevertheless, these results should be interpreted bearing in mind that our population is not representative of the French general population but rather of the studied regions. Additional work is needed to understand more clearly the incorporation of chemicals from blood to hair so that we can better interpret hair measures in exposure assessment.

Conflict of interest

The authors declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.07.023.

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