

Identification of biomarkers of pesticides exposure for residents living close to vineyards in France - Supplementary materials

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Analytical methods:

Reagents and chemicals

Acetone and dichloromethane (DCM) (PESTIPUR - For pesticide analysis) were purchased from CARLO ERBA Reagents S.A.S (Val de Reuil, France). Acetonitrile and methanol (MeOH) absolute (ULC/MS - CC/SFC) was purchased from Biosolve Chimie SARL (Dieuze, France). Acetic acid (For LC-MS) was purchased from CARLO ERBA Reagents S.A.S (Val de Reuil, France). Ammonium hydroxide (28-30% solution in water) was purchased from Acros Organics (NJ, USA). Ammonium chloride (For analysis) was purchased from Merck KGaA (Darmstadt, Germany). Nitric acid (67-69% - For trace metal analysis) was purchased from VWR International (Radnor, PA, USA). Potassium fluoride (≥99%), anhydrous sodium acetate (For molecular biology, ≥99%), N-methyl-N-(trimethylsilyl)trifluoroacetamide) (MSTFA) for GC derivatization (≥98.5%), β-glucuronidase from limpets (*Patella vulgata*) and sulfatase from *Helix pomatia* were purchased from Sigma Aldrich (Saint Louis, MO, USA). Strata-X cartridges (6 mL, 200 mg) were purchased from Phenomenex France (Le Pecq, France). Chem Elut cartridges (3 mL, unbuffered) were purchased from Agilent Technologies (Folsom, CA, USA).

Certified standards of boscalid, ethylene thiourea (ETU), ethylene urea (EU), folpel, phthalic acid, phthalimide and tebuconazole were purchased from LGC Labor GmbH (Augsburg, Germany). Certified standard of hydroxytebuconazole (TEB-OH) was purchased from Sigma Aldrich (Saint Louis, MO, USA). Certified standard of tetrahydrophthalimide (THPI) was purchased from Toronto Research

Chemicals (Toronto, ON, Canada). Standards of phthalic acid D₄ and THPI D₆ were purchased from Chiron AS (Trondheim, Norway). Standards of boscalid D₄ and phthalimide D₄ were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Standard of TEB-OH D₆ was purchased from Alsachim (Illkirch, France). Standard of ETU D₄ was purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). Standard of EU D₄ was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Standard of folpel D₄ was purchased from LGC Labor GmbH (Augsburg, Germany). The purity of native standards was above 98% and that of labelled standards above 95%. Individual standard stock solutions (1 g/L) were prepared in acetone or MeOH by accurately weighing 10 mg (\pm 0.1 mg) of standards using a Sartorius Cubis MSE 225P semi-micro balance (Sartorius AG, Göttingen, Germany) into 10-mL volumetric flasks, and stored at -18 °C. Acetone solution (100 mg/L) of tebuconazole D₆ was purchased from LGC Labor GmbH (Augsburg, Germany). Intermediate and spiking solutions were prepared in acetone by appropriate dilution of individual standard stock solutions and commercial solutions.

Pooled urine and hair samples for quality controls were obtained by donation. Surine™ Negative Urine Control was purchased from Sigma Aldrich (Saint Louis, MO, USA).

Determination of ETU and EU in urine samples

Preparation of urine samples

After adding 10 μ L of nitric acid to 2 mL of urine in a 10-mL tube, the sample was agitated and transferred in a 8-mL tube containing 70 mg of ammonium chloride and 1 g of potassium fluoride that were used to adjust pH and ion strength, respectively. The tube was then vortexed before adding the labelled ISTDs.

Extraction of ETU and EU from urine samples

Supported liquid extractions (SLE) were performed using a Supelco Visiprep DL (disposable liner) vacuum manifold and a GAST vacuum pump. The pre-treated urine sample (2 mL), previously

vortexed to ensure the dissolution of salts, was loaded on a Chem Elut cartridge (3 mL). After sample percolation (5 to 10 min), analytes were then extracted by passing through the column 10 x 2 mL of DCM. Organic extracts collected in 20-mL gauged glass tubes were then concentrated to 0.5 mL at 30°C under a nitrogen stream using a N-EVAP 111 Organomation Nitrogen Evaporator and adjusted to 500 µL of DCM prior to be transferred into 2 mL amber glass vials. After adding 40 µL of the derivatization agent (MSTFA), concentrated extracts were incubated at 40°C overnight (at least 16 hours) prior to analysis by GC/MS/MS.

GC/MS/MS analysis

Analyses were performed using a 7890A GC system coupled to a 7000C GC/MS Triple Quad (Agilent Technologies, Santa Clara, California, United States) operated in electron impact ionization (EI) mode (70 eV). The GC system was equipped with a Gerstel MPS (MultiPurpose Sampler) robotic autosampler (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) and a multimode inlet (MMI) fitted with a single taper ultra-inert glass liner with glass wool. Calibration solutions and SLE extracts were injected (2 µL) in the splitless mode (purge flow to split vent: 60 mL/min at 1.8 min) with the following injector temperature program: 37 °C (hold 0.1 min), ramp at 500 °C/min to 325 °C (hold 10 min). Helium was used as the column carrier gas at a constant flow rate of 1.0 mL/min. Chromatographic separation was performed on a Rtx[®]-PCB capillary column (30 m length × 0.25 mm I.D., 0.25 µm film thickness) supplied by Restek Corporation (Bellefonte, Pennsylvania, United States) with the following oven temperature program: 37 °C (hold 2 min), first ramp at 37 °C/min to 250 °C (hold 0 min), second ramp at 15 °C/min to 325 °C (hold 1 min to reach an analysis time of 13.8 min). The MSD transfer line, ion source and quadrupole temperatures were fixed at 325, 280 and 180 °C, respectively. The mass spectrometer (triple quadrupole) was operated in multiple reaction monitoring (MRM) mode. The two most intense and specific MRM transitions of each compound (quantifier and qualifier transitions) were monitored for identification, confirmation and quantification. They were selected using the pesticides and environmental pollutants MRM database

provided by Agilent Technologies for the compounds present in the database or following the usual procedure for others. Analytical characteristics of measured compounds are reported in Table SM1. MassHunter software (10.0) was used for instrument control, data acquisition and quantification.

Quality assurance and quality control (QA/QC)

The limit of detection (LOD) was defined as the lowest concentration of a substance that can be distinguished from the absence of that substance. LODs were estimated from the replicate analysis of a blank sample. The limit of quantification (LOQ) was defined as the lowest concentration of a substance for which the relative standard deviation (RSD) of the raw signal ($n = 5$) was lower than or equal to 20%, the signal-to-noise ratio (S/N) was greater than or equal to 10, and the raw signal was greater than or equal to 5 times the signal of the blank sample.

Several labeled substances were selected to best cover the physical and chemical properties of the targeted analytes. ETU D₄ and EU D₄ were added prior to the extraction step and used as internal standards (ISTDs). All compounds were quantified using the appropriate ISTD (Table SM1) to compensate for the variability associated with the analytical procedure, from calibration curves generated for each compound by analyzing at least four different calibration samples. A quadratic fit (origin ignored, no weighting) was used to compensate for the nonlinearity of the instrument response over a wide working range.

Each batch included: i) up to 32 urine samples (2 mL), ii) one procedural calibration blank sample and six procedural calibration samples prepared from Surine™ and analyzed as regular samples to assess whether contamination may have occurred during analysis and to generate quadratic calibration curves intended for quantification, respectively, and iii) at least three matrix procedural QC samples (2-mL pooled urine sample non-spiked and spiked at the LOQ level and at an intermediate level) analyzed as regular samples to check for method accuracy.

Positive values for each substance were confirmed by comparing retention times and MRM transitions ratios ($\pm 30\%$) between calibration and urine samples. The data validation protocol

included several conditions: i) the determination coefficient of the calibration curve had to be greater than 0.99, ii) the concentration of a substance measured in the procedural calibration samples had to be within $\pm 50\%$ of its theoretical concentration value at the LOD and LOQ levels and $\pm 25\%$ at all other levels, iii) the response of a substance (ISTD response ratio) in the procedural blank samples had to be lower than 50% of that in the procedural calibration sample at the LOQ level, iv) the concentration of a substance measured in the matrix procedural QC samples prepared from pooled urine samples had to be within $\pm 50\%$ of its theoretical concentration value at the LOQ level and $\pm 30\%$ at the intermediate level, and v) the concentration of a substance measured in the urine samples had to be within the method working range without exceeding 110% of the concentration of the most concentrated calibration samples. If all these conditions were not met, results were not validated and samples were reanalyzed if possible.

Determination of the other targeted substances in urine samples

Preparation of urine sample (enzymatic hydrolysis)

After adding 2 mL of a 0.2 M sodium acetate buffer at pH 4.8 to 2 mL of urine, the labelled internal standards (ISTDs) were added and the pH of the urine sample adjusted to pH 5 with the commercial solution of ammonium hydroxide or a solution of nitric acid diluted to one tenth. Then, 200 μL of a solution of β -glucuronidase from limpets (*Patella vulgata*) (25 g/L / 25 units/ μL) and sulfatase from *Helix pomatia* (2.5 g/L / 0.025 units/ μL) prepared in a 0.2 M sodium acetate buffer at pH 4.8 were added and the sample was subsequently incubated and agitated for 2 hours at 50 °C. After return to room temperature, 16 mL of ultra-pure water and 40 μL of nitric acid were added to reach a final volume of approximately 20 mL at pH 2.

Extraction of the target substances from urine samples

Solid phase extractions (SPE) were performed using a Gilson GX-274 ASPEC automatic extraction system (Gilson, Middleton, WI, USA). The Strata-X cartridge was conditioned with successively 10 mL of DCM, 10 mL of MeOH mixture and 10 mL of ultra-pure water. Then, the pre-treated urine sample

(20 mL) was loaded. The cartridge was washed with 10 mL of acidified ultra-pure water (pH 2) and subsequently completely dried for 15 minutes. Analytes were eluted with 4 x 2.5 mL of a 90% DCM / 10% MeOH mixture. Organic extracts were then evaporated to dryness at 30°C under a nitrogen stream using a N-EVAP 111 Organomation Nitrogen Evaporator and reconstituted in 500 µL of DCM prior to be transferred into 2 mL amber glass vials and stored at -18°C. 40 µL of the derivatization agent (MSTFA) were added prior to analysis by GC/MS/MS.

GC/MS/MS analysis

Analyses were performed using a 7890A GC system coupled to a 7000C GC/MS Triple Quad (Agilent Technologies, Santa Clara, California, United States) operated in electron impact ionization (EI) mode (70 eV). The GC system was equipped with a Gerstel MPS (MultiPurpose Sampler) robotic autosampler (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) and a multimode inlet (MMI) fitted with a single taper ultra-inert glass liner with glass wool. Calibration solutions and SPE extracts were injected (2 µL) in the splitless mode (purge flow to split vent: 60 mL/min at 1.8 min) with the following injector temperature program: 37 °C (hold 0.1 min), ramp at 500 °C/min to 325 °C (hold 10 min). Helium was used as the column carrier gas at a constant flow rate of 1.0 mL/min. Chromatographic separation was performed on a Rtx[®]-PCB capillary column (30 m length × 0.25 mm I.D., 0.25 µm film thickness) supplied by Restek Corporation (Bellefonte, Pennsylvania, United States) with the following oven temperature program: 37 °C (hold 2 min), first ramp at 37 °C/min to 250 °C (hold 0 min), second ramp at 15 °C/min to 330 °C (hold 3 min to reach an analysis time of 16.1 min). The MSD transfer line, ion source and quadrupole temperatures were fixed at 325, 280 and 180 °C, respectively. The mass spectrometer (triple quadrupole) was operated in multiple reaction monitoring (MRM) mode. The two most intense and specific MRM transitions of each compound (quantifier and qualifier transitions) were monitored for identification, confirmation and quantification. They were selected using the pesticides and environmental pollutants MRM database provided by Agilent Technologies for the compounds present in the database or following the usual

procedure for others. Analytical characteristics of measured compounds are reported in Table SM1. MassHunter software (10.0) was used for instrument control, data acquisition and quantification.

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Several labeled substances were selected to best cover the physical and chemical properties of the targeted analytes. Boscalid D₄, phthalic acid D₄, phthalimide D₄, TEB-OH D₆, tebuconazole D₆ and THPI D₆ were added prior to the extraction step and used as internal standards (ISTDs). All compounds were quantified using the appropriate ISTD (Table SM1) to compensate for the variability associated with the analytical procedure, from calibration curves generated for each compound by analyzing at least four different calibration samples. A quadratic fit (origin ignored, no weighting) was used to compensate for the nonlinearity of the instrument response over a wide working range.

Each batch included: i) up to 18 urine samples (2 mL), ii) one procedural calibration blank sample and seven procedural calibration samples prepared from Surine™ and analyzed as regular samples to assess whether contamination may have occurred during analysis and to generate quadratic calibration curves intended for quantification, respectively, and iii) two matrix procedural QC samples (2-mL pooled urine sample non-spiked and spiked at an intermediate level) analyzed as regular samples to check for method accuracy.

Positive values for each substance were confirmed by comparing retention times and MRM transitions ratios ($\pm 30\%$) between calibration and urine samples. The data validation protocol included several conditions: i) the determination coefficient of the calibration curve had to be

greater than 0.99, ii) the concentration of a substance measured in the procedural calibration samples had to be within $\pm 50\%$ of its theoretical concentration value at the LOD and LOQ levels and $\pm 25\%$ at all other levels, iii) the response of a substance (ISTD response ratio) in the procedural blank samples had to be lower than 50% of that in the procedural calibration sample at the LOQ level, iv) the concentration of a substance measured in the matrix procedural QC samples prepared from pooled urine samples had to be within $\pm 30\%$ of its theoretical concentration value, and v) the concentration of a substance measured in the urine samples had to be within the method working range without exceeding 110% of the concentration of the most concentrated calibration samples. If all these conditions were not met, results were not validated and samples were reanalyzed if possible.

Determination of the targeted substances in hair samples

Preparation of hair sample (pulverization)

Approximately 20 to 100 mg of hair sample were accurately weighed (± 0.1 mg) on a piece of paper and transferred to a 5-mL stainless steel grinding jar with one 10 mm ball per jar. Grinding was performed in the mixer mill MM400 (Retsch GmbH, Haan, Germany) for 10 min at 25 Hz. The hair powder, after recovery on a piece of paper, was transferred into an amber glass vial that was then sealed and stored at -18 °C until analysis.

Extraction of the targeted substances from hair samples

Solvent extractions were performed using a mixer mill MM400 (Retsch GmbH, Haan, Germany). 25 mg of hair powder, the labelled internal standards (ISTDs), three 3 mm stainless steel micro balls previously rinsed with dichloromethane and 400 μ L of acetonitrile were successively added in a 1.5-mL Eppendorf tube then placed on the mixer mill in a 24-position rack. Agitation/grinding was performed for 1 min at 25 Hz. The organic extract was then centrifuged at 3500 rpm for 20 min at 20°C using a micro centrifuge accuSpin™ Micro 17R. The supernatant was transferred to a second Eppendorf tube. The procedure was repeated twice from the addition of acetonitrile and the three

supernatants were combined in the second Eppendorf tube before a final centrifugation (3500 rpm for 20 min at 20°C). The final supernatant was transferred to a third Eppendorf tube and stored at -18°C prior to the evaporation step. Acetonitrile extracts were then evaporated to dryness at 30°C under a nitrogen stream using a N-EVAP 111 Organomation Nitrogen Evaporator and reconstituted in 500 µL of DCM prior to be transferred into 2-mL amber glass vials. After adding 40 µL of the derivatization agent (MSTFA), concentrated extracts were incubated at 40°C overnight (at least 16 hours) prior to analysis by GC/MS/MS.

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Analyses were performed using a 7890A GC system coupled to a 7000C GC/MS Triple Quad (Agilent Technologies, Santa Clara, California, United States) operated in electron impact ionization (EI) mode (70 eV). The GC system was equipped with a Gerstel MPS (MultiPurpose Sampler) robotic autosampler (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) and a multimode inlet (MMI) fitted with a single taper ultra-inert glass liner with glass wool. Calibration solutions and organic extracts were injected (5 µL) in the solvent vent mode (vent flow: 100 mL/min; vent pressure: 0 psi until 0.1 min; purge flow to split vent: 60 mL/min at 1.5 min) with the following injector temperature program: 37 °C (hold 0.1 min), ramp at 500 °C/min to 325 °C (hold 10 min). Helium was used as the column carrier gas at a constant flow rate of 1.0 mL/min. Chromatographic separation was performed on a Rtx[®]-PCB capillary column (30 m length × 0.25 mm I.D., 0.25 µm film thickness) supplied by Restek Corporation (Bellefonte, Pennsylvania, United States) with the following oven temperature program: 37 °C (hold 2 min), first ramp at 37 °C/min to 250 °C (hold 0 min), second ramp at 15 °C/min to 330 °C (hold 3 min to reach an analysis time of 16.1 min). The MSD transfer line, ion source and quadrupole temperatures were fixed at 325, 280 and 180 °C, respectively. The mass spectrometer (triple quadrupole) was operated in multiple reaction monitoring (MRM) mode. The two most intense and specific MRM transitions of each compound (quantifier and qualifier transitions) were monitored for identification, confirmation and quantification. They were selected

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Several labeled substances were selected to best cover the physical and chemical properties of the targeted analytes. Boscalid D_4 , ETU D_4 , EU D_4 , folpel D_4 , phthalimide D_4 , TEB-OH D_6 , tebuconazole D_6 and THPI D_6 were added prior to the extraction step and used as internal standards (ISTDs). All compounds were quantified using the appropriate ISTD (Table SM1) to compensate for the variability associated with the analytical procedure, from calibration curves generated for each compound by analyzing at least four different calibration samples. A quadratic fit (origin ignored, no weighting) was used to compensate for the nonlinearity of the instrument response over a wide working range.

Each batch included: i) up to 15 hair samples (25 mg), ii) one procedural calibration blank sample and six procedural calibration samples analyzed as regular samples to assess whether contamination may have occurred during analysis and to generate quadratic calibration curves intended for quantification, respectively, and iii) four matrix procedural QC samples (25-mg real hair sample non-spiked and spiked at the LOQ level, at an intermediate level and at a high level) analyzed as regular samples to check for method accuracy.

Positive values for each substance were confirmed by comparing retention times and MRM transitions ratios ($\pm 30\%$) between calibration and hair samples. The data validation protocol included several conditions: i) the determination coefficient of the calibration curve had to be greater than 0.99, ii) the concentration of a substance measured in the procedural calibration samples had to be within $\pm 50\%$ of its theoretical concentration value at the LOD and LOQ levels and $\pm 25\%$ at all other levels, iii) the response of a substance (ISTD response ratio) in the procedural blank samples had to be lower than 50% of that in the procedural calibration sample at the LOQ level, iv) the concentration of a substance measured in the matrix procedural QC samples prepared from real hair samples had to be within $\pm 30\%$ of its theoretical concentration value, and v) the concentration of a substance measured in the hair samples had to be within the method working range without exceeding 110% of the concentration of the most concentrated calibration samples. If all these conditions were not met, results were not validated and samples were reanalyzed if possible.

1 Table SM1

2 Analytical characteristics of measured compounds

Compound ^a	CAS number	Chemical family	ISTD	Time segment	t _R (min)	Quantifier MRM transition Precursor > Product (CE (V))	Qualifier MRM transition Precursor > Product (CE (V))	LOD urine ^b (µg/L)	LOQ urine ^b (µg/L)	LOD hair ^c (ng/g)	LOQ hair ^c (ng/g)	
Target compounds												
1	EU	120-93-4	Carbamates	EU D ₄	1	6.5	214.8 > 133.0 (15)	214.8 > 147.0 (15)	0.2	0.5	40	80
2	THPI	85-40-5	Phthalimides	THPI D ₆	2	7.4	222.8 > 192.0 (10)	222.8 > 207.0 (5)	1.3	2.5	20	40
3	ETU	96-45-7	Carbamates	ETU D ₄	2	7.6	230.7 > 159.7 (5)	245.7 > 231.1 (10)	0.4	1.0	20	40
4	Phthalimide	85-41-6	Phthalimides	Phthalimide D ₄	2	7.6	203.8 > 102.0 (35)	203.8 > 130.0 (25)	0.5	1.0	10	20
5	Phthalic acid	88-99-3	Phthalimides	Phthalic acid D ₄	3	8.1	294.9 > 147.1 (5)	294.9 > 73.0 (40)	2.5	5.0	-	-
6	Folpel	133-07-3	Phthalimides	Folpel D ₄	4	9.8	259.7 > 130.0 (30)	259.7 > 102.0 (30)	-	-	40	80
7	Tebuconazole	107534-96-3	Triazoles	Tebuconazole D ₆	5	11.1	249.7 > 125.0 (25)	249.7 > 153.0 (10)	1.3	2.5	10	20
8	TEB-OH	212267-64-6	Triazoles	TEB-OH D ₆	5	11.8	187.9 > 157.1 (5)	187.9 > 98.1 (5)	1.3	2.5	20	40
9	Boscalid	188425-85-6	Carboxamides	Boscalid D ₄	5	13.2	341.6 > 203.9 (10)	166.7 > 140.1 (15)	1.3	2.5	40	80
Labeled ISTDs												
a	EU D ₄	n/a	Carbamates		1	6.5	218.6 > 134.0 (10)	-				
b	THPI D ₆	203578-24-9	Phthalimides		2	7.4	228.8 > 214.1 (10)	-				
c	ETU D ₄	352431-28-8	Carbamates		2	7.6	234.8 > 163.1 (35)	-				
d	Phthalimide D ₄	60161-31-1	Phthalimides		2	7.6	133.9 > 106.0 (15)	-				
e	Phthalic acid D ₄	87976-26-9	Phthalimides		3	8.1	224.5 > 222.1 (10)	-				
f	Folpel D ₄	1327204-12-5	Phthalimides		4	9.8	265.7 > 134.1 (20)	-				
g	Tebuconazole D ₆	n/a	Triazoles		5	11.0	254.6 > 156.0 (10)	-				
h	TEB-OH D ₆	n/a	Triazoles		5	11.8	193.8 > 160.0 (10)	-				
i	Boscalid D ₄	n/a	Carboxamides		5	13.2	170.8 > 142.1 (30)	-				

^a Compounds listed in order of retention times; ^b for a 2-mL sample of urine; ^c for a 25-mg sample of hair

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7 **Table SM2: LOD and LOQ of pesticides and their metabolites analyzed in urine**

Pesticide/metabolite	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)
Phthalic acid	2.5	5.0
Phthalimide	0.5	1.0
Tebuconazole	1.3	2.5
TEB-OH	1.3	2.5
THPI	1.3	2.5
ETU	0.4	1.0
EU	0.2	0.5
Copper	0.07	0.24
AMPA	0.02	0.05

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Table SM3: LOD and LOQ of pesticides and their metabolites analyzed in hair

Pesticide/metabolite	LOD (ng/g)	LOQ (ng/g)
Folpet	40	80
Phthalimide	10	20
Tebuconazole	10	20
TEB-OH	20	40
THPI	20	40
ETU	20	40
EU	40	80

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15 **Table SM4: Normality tests**

	Adults		Children	
	Shapiro-Wilk	p-value	Shapiro-Wilk	p-value
Phthalimide (urine)				
$\mu\text{g/L}$	0.97	0.1797	0.93	0.0014
$\mu\text{g/g creat}$	0.98	0.3981	0.97	0.2115
Phthalic acid (urine)				
$\mu\text{g/L}$	0.97	0.1640	0.96	0.0295
$\mu\text{g/g creat}$	0.92	0.0007	0.93	0.0020
ETU (urine)				
$\mu\text{g/L}$	0.98	0.5316	0.98	0.3074
$\mu\text{g/g creat}$	0.99	0.7765	0.98	0.3057
EU (urine)				
$\mu\text{g/L}$	0.99	0.6949	0.97	0.0991
$\mu\text{g/g creat}$	0.98	0.6040	0.99	0.9931
TEB-OH (urine)				
$\mu\text{g/L}$	0.96	0.0427	-	-
$\mu\text{g/g creat}$	0.98	0.6666	-	-
Copper (urine)				
$\mu\text{g/L}$	0.99	0.9893	0.95	0.0137
$\mu\text{g/g creat}$	0.97	0.1816	0.98	0.4773
AMPA (urine)				
$\mu\text{g/L}$	0.97	0.1407	0.99	0.7841
$\mu\text{g/g creat}$	0.98	0.4223	0.97	0.2424
Phthalimide (hair)	0.86	<0.0001	-	-

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18 **Table SM5: variables used to characterize pesticide exposure**

Variables	Terms and conditions
Adjustment variables	
Age of the participant	Continuous (years)
Sex	Man / Woman
Marital status	Alone (single, divorced, widowed) / In couple (married, unmarried couple)
Level of education	None, Primary / Middle School, High School / College
Smoking status	Smoking / Non-smoking
Creatinine concentration (log-transformed)	Continuous ($\mu\text{g/L}$)
Explanatory variables	
Residential area	Downtown / Village / Scattered housing
Residential environment	Residential, Commercial / Industrial / Agricultural
Season	Spring-Summer / Fall-Winter
Professional activity related to the use of pesticides	Yes / No
Domestic use of pesticides in the last 12 months	Never or less than twice / More than twice
Pesticide use in the last 7 days	Yes / No
Presence of pets	Yes / No
Consumption of self-produced food	Once a month or more / Never or less than once a month
Consumption of organically grown food	Once a week or more / Between 1 and 4 times a month / Never or less than 1 time / month
Practice of gardening or forestry composition	Yes / No
Ventilation of the dwelling	Once a day or more / Less than once a day
Cleaning the floor of the house	1 to 2 times a week / More than 2 times a week

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21 **Table SM6: Description of socio-demographic characteristics of the adult participants,**
 22 **and comparison between residents LCTV and controls**

		All	Residents	Controls	p
Sex					
Men	n (%)	25 (42%)	11 (36%)	14 (48%)	0.3152 ^a
Women	n (%)	35 (58%)	20 (64%)	15 (52%)	
Age (year)					
	Mean	55.1	56.8	53.2	0.2507 ^b
	[CI 95%]	[52.0-58.2]	[52.1-61.6]	[49.1-57.4]	
BMI (kg/m²)					
	Mean	24.5	23.8	25.2	0.2258 ^b
	[CI 95%]	[23.4-25.6]	[22.4-25.2]	[23.4-27.0]	
Marital status					
Single / divorced / widow	n (%)	10 (17%)	8 (26%)	2 (7%)	0.0495^a
Married / unmarried couple	n (%)	50 (83%)	23 (74%)	27 (93%)	
Number of family members					
	Mean	2.5	2.1	2.9	0.0102^b
	[CI 95%]	[2.2-2.8]	[1.7-2.5]	[2.4-3.3]	
Household income (€/per/year)					
<15,000	n (%)	27 (45%)	15 (48%)	12 (41%)	0.5856 ^a
>15,000	n (%)	33 (55%)	16 (52%)	17 (59%)	
Education level					
None/primary education	n (%)	17 (28%)	10 (32%)	7 (24%)	0.1109 ^a
Secondary education	n (%)	20 (33%)	13 (42%)	7 (24%)	
Higher education	n (%)	23 (38%)	8 (26%)	15 (52%)	
Occupational category					
Farmer	n (%)	1 (2%)	0 (0%)	1 (3.5%)	0.6439 ^a
Artisan/independent retailer	n (%)	5 (8%)	3 (10%)	2 (7%)	
Executive	n (%)	13 (23%)	6 (19%)	7 (24%)	
Intermediate professional	n (%)	16 (27%)	7 (23%)	9 (31%)	
Employee	n (%)	15 (25%)	10 (32%)	5 (17%)	
Factory worker	n (%)	9 (15%)	5 (16%)	4 (14%)	
Other	n (%)	1 (2%)	0 (0%)	1 (3.5%)	
Occupational activity related with the use of pesticides					
No	n (%)	54 (90%)	27 (87%)	27 (93%)	0.4383 ^a
Yes	n (%)	6 (10%)	4 (13%)	2 (7%)	
Smoking status					
Smoker	n (%)	15 (25%)	8 (26%)	7 (26%)	0.8814 ^a
Non-smoker	n (%)	45 (75%)	23 (74%)	22 (74%)	
Vegetable garden intake					
Once a month or more	n (%)	14 (24%)	7 (23%)	7 (26%)	0.7665 ^a
Never or less than once a month	n (%)	44 (76%)	24 (77%)	20 (74%)	
Organic food product intake					
Once a week or more	n (%)	21 (36%)	10 (32%)	11 (39%)	0.8467 ^a
Between 1 and 4 portions per month	n (%)	13 (22%)	7 (23%)	6 (22%)	
Never or less than once a month	n (%)	25 (42%)	13 (45%)	11 (39%)	
Use of pesticides at home during the last 12 months					
Never or less than twice a year	n (%)	15 (25%)	8 (26%)	7 (24%)	0.9039 ^a
Between 3 times a year and once a month	n (%)	32 (53%)	17 (55%)	15 (52%)	
More than once a month	n (%)	13 (22%)	6 (19%)	7 (24%)	
Practice of gardening or floral composition					
No	n (%)	27 (45%)	13 (42%)	14 (48%)	0.6218 ^a
Yes	n (%)	33 (55%)	18 (58%)	15 (52%)	
Use of pesticides during the last 7 days					
No	n (%)	49 (82%)	25 (81%)	24 (83%)	0.8326 ^a
Yes	n (%)	11 (18%)	6 (19%)	5 (17%)	
Floor cleaning at home					
Once or twice a week	n (%)	34 (57%)	16 (52%)	18 (62%)	0.4141 ^a
More than twice a week	n (%)	26 (43%)	15 (48%)	11 (38%)	
Home ventilation					
Once a day or more	n (%)	45 (75%)	24 (77%)	21 (72%)	0.6545 ^a
Less than once a day	n (%)	15 (25%)	7 (23%)	8 (28%)	
Presence of pets at home					
Yes	n (%)	39 (65%)	21 (68%)	18 (62%)	0.6452 ^a
No	n (%)	21 (35%)	10 (32%)	12 (38%)	
Housing estate					
Town center	n (%)	17 (28%)	8 (26%)	9 (31%)	0.5974 ^a

<i>Village</i>	<i>n (%)</i>	27 (45%)	13 (42%)	14 (48%)	
<i>Dispersed habitat</i>	<i>n (%)</i>	16 (27%)	10 (32%)	6 (21%)	
Housing area					
<i>Residential, commercial, industrial</i>	<i>n (%)</i>	40 (67%)	21 (68%)	19 (66%)	0.8551 ^a
<i>Agricultural</i>	<i>n (%)</i>	20 (33%)	10 (32%)	10 (34%)	
Population of the place of residence (number of habitants)	<i>Mean</i>	5,043	4,652	5,462	0.4587 ^b
Surface of agricultural land in the place of residence (100 square meters)	<i>[CI 95%]</i>	[3,961-6,126]	[2,994-6,310]	[4,002-6,922]	0.0051^b
Surface of vineyards in the place of residence (100 square meters)	<i>Mean</i>	74,217	101,591	44,955	
	<i>[CI 95%]</i>	[53,556-94,878]	[71,061-132,121]	[19,897-70,014]	
Distance with the closest vineyards (meters)	<i>Mean</i>	25,609	49,555	11	<.0001^b
	<i>[CI 95%]</i>	[14,170-37,048]	[30,743-68,367]	[0-24]	
Season of the biological samples collection					
<i>Autumn /Winter</i>	<i>Mean</i>	2,990	148	6,030	<.0001^b
<i>Spring / Sumer</i>	<i>[CI 95%]</i>	[2,010-3,971]	[100-195]	[4,724-7,335]	
	<i>n (%)</i>	37 (62%)	18 (58%)	19 (66%)	0.5530 ^a
	<i>n (%)</i>	23 (38%)	13 (42%)	10 (34%)	

23 ^a chi-square test

24 ^b two sample t-test

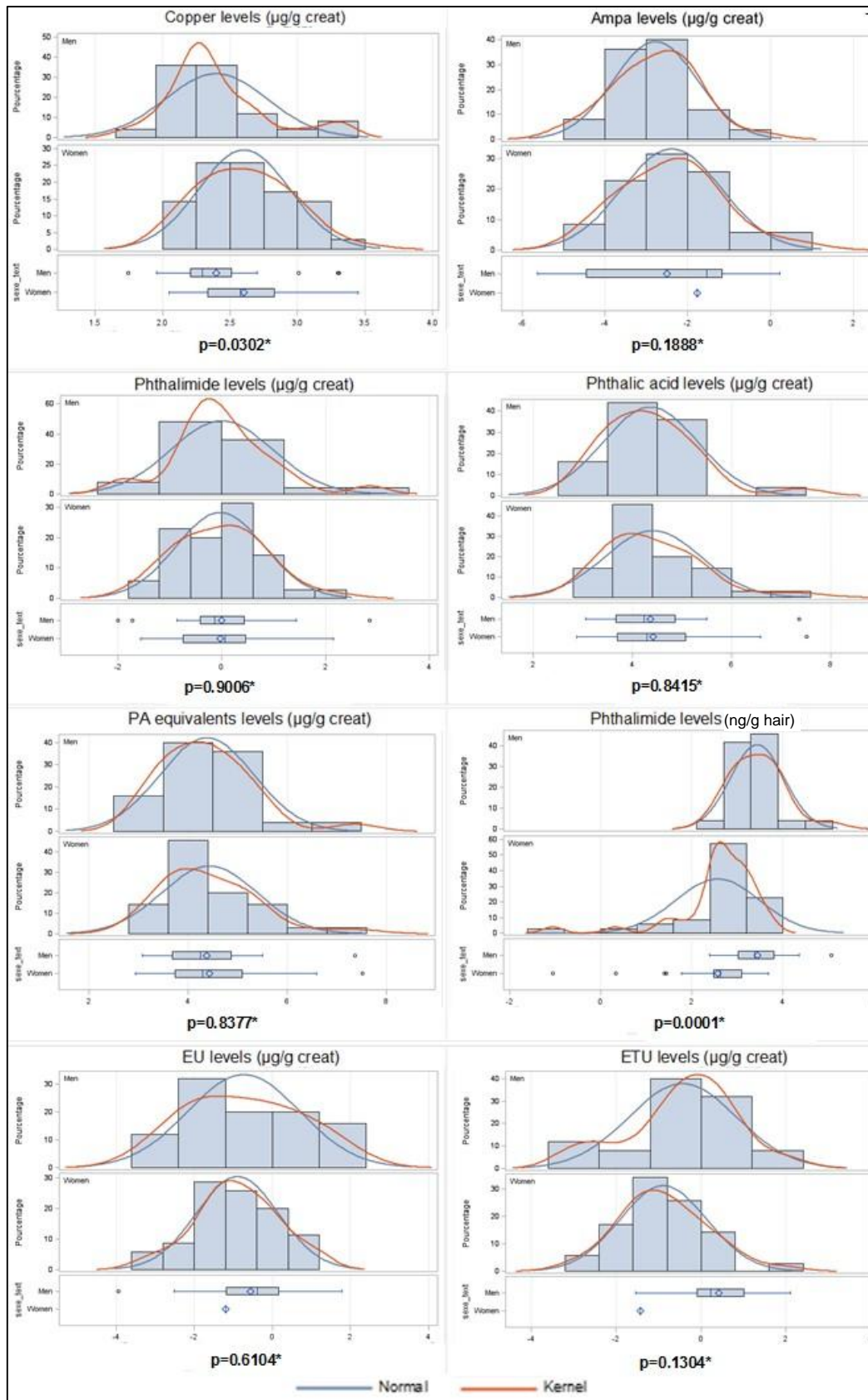
25

26 **Table SM7: Crude distribution of urinary concentrations for folpel metabolites, mancozeb**
 27 **metabolites, tebuconazole, copper and AMPA in the adult population (in µg/L)**

Pesticide/metabolite	N	%>LOD	GM [CI 95%]	P25	P50	P75	P90	P95	Max
Phthalimide	60	48%	0.7 [0.6 – 0.9]	<LOD	<LOQ	1.2	1.7	2.4	7.4
Phthalic acid	60	100%	60.0 [46.2 – 77.8]	27.5	55.0	129.5	201.0	288.2	1,281.8
PA equivalents	60	-	61.0 [47.1 – 79.0]	28.0	56.0	130.6	202.1	291.2	1,283.4
EU	60	63 %	0.3 [0.2 – 0.5]	<LOD	<LOQ	0.7	1.9	3.1	5.6
ETU	60	47 %	0.4 [0.3 – 0.5]	<LOD	<LOQ	<LOQ	1.2	2.3	5.9
TEB-OH	60	37%	NC	<LOD	<LOD	<LOQ	5.7	7.2	18.7
Tebuconazole	60	0 %	NC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Copper	60	100%	9.2 [8.0 – 10.6]	6.2	9.5	12.9	19.5	23.0	36.3
AMPA	60	83%	0.06 [0.04-0.08]	<LOQ	0.06	0.11	0.20	0.33	2.7

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29 **Figure SM1: Comparisons of folpel metabolites, mancozeb metabolites, copper and AMPA**
 30 **levels between men and women in the adult population (log-transformed data)**



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* t-test – equal variance – using log-transformed concentrations

33 **Table SM8: Folpel metabolites, mancozeb metabolites, tebuconazole, copper and AMPA**
 34 **urinary concentrations in adult residents LCTV and controls (µg/g of creatinine)**

	Residents				Controls			
	N	%>LOD	GM [CI 95%]	P95	N	%>LOD	GM [CI 95%]	P95
Phthalimide	31	48%	1.1 [0.8 – 1.5]	4.3	29	48%	0.9 [0.6 – 1.3]	4.2
Phthalic acid	31	100%	85.1 [56.9-127.0]	1,580.2	29	100%	76.2 [56.2-103.2]	244.2
PA equivalent	31	-	86.6 [58.1–128.9]	1,597.5	29	-	77.4 [57.2-104.7]	246.4
EU	31	68%	0.4 [0.3-0.6]	2.7	29	59%	0.5 [0.3-0.8]	3.5
ETU	31	42%	0.4 [0.3 – 0.6]	2.1	29	52%	0.6 [0.4 – 0.9]	4.0
TEB-OH	31	29%	1.8 [1.3-2.6]	19.1	29	45%	1.5 [1.0-2.2]	7.9
Copper	31	100%	12.9 [11.2 – 14.9]	27.1	29	100%	11.9 [10.5 – 13.5]	22.1
AMPA	31	81%	0.07 [0.04 – 0.11]	0.71	29	86%	0.09 [0.06 – 0.13]	0.54

35 **Table SM9: Folpel metabolites, mancozeb metabolites, tebuconazole, copper and AMPA**
 36 **urinary concentrations in adult residents LCTV and controls (µg/L)**

	Residents				Controls			
	N	%>LOD	GM [CI 95%]	P95	N	%>LOD	GM [CI 95%]	P95
Phthalimide	31	47%	0.8 [0.6 – 1.0]	2.2	29	48%	0.7 [0.5 – 1.0]	2.7
Phthalic acid	31	100%	60.9 [40.2-92.3]	679.5	29	100%	59.0 [42.3-82.3]	233.0
PA equivalent	31	-	62.0 [41.1 – 93.6]	686.9	29	-	59.9 [43.0 – 83.4]	235.7
EU	31	67%	0.3 [0.2-0.4]	2.2	29	52%	0.4 [0.2-0.6]	4.9
ETU	31	40%	0.3 [0.2 – 0.4]	1.1	29	45%	0.5 [0.3 – 0.7]	3.3
Copper	31	100%	9.2 [7.3 – 11.6]	24.3	29	100%	9.2 [7.7 – 11.0]	20.8
AMPA	31	81%	0.05 [0.03 – 0.08]	0.30	29	86%	0.07 [0.05 – 0.10]	0.37

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38 **Table SM10: Phthalimide concentrations in hair in adulte residents LCTV and controls in the**
 39 **adult population (ng/g of hair)**

	Residents				Controls			
	N	%>LOD	GM [CI 95%]	P95	N	%>LOD	GM [CI 95%]	P95
Phthalimide	31	93%	19.5 [15.0 – 25.5]	48.0	29	83%	17.6 [11.7 – 26.6]	48.0

40 **Table SM11: Description of socio-demographic characteristics of the children and comparison**
 41 **between residents LCTV and controls**

	All	Residents	Controls	p	
Sex					
Boy	<i>n</i> (%)	36 (59%)	17 (55%)	19 (63%)	0.5 ^a
Girl	<i>n</i> (%)	25 (41%)	14 (45%)	11 (37%)	
Age (year)	<i>Mean</i>	11.1	10.9	11.4	0.4802 ^b
	<i>[CI 95%]</i>	[10.4-11.9]	[9.8-11.9]	[10.3-12.5]	
BMI (kg/m²)	<i>Mean</i>	17.8	17.5	18.1	0.5423 ^b
	<i>[CI 95%]</i>	[16.9-18.8]	[16.3-18.8]	[16.6-19.7]	
Parents marital status					
Single / divorced / widow	<i>n</i> (%)	5 (8%)	3 (10%)	2 (7%)	0.6683 ^a
Married / unmarried couple	<i>n</i> (%)	56 (92%)	28 (90%)	28 (93%)	
Number of family members	<i>Mean</i>	4.2	4.2	4.3	0.6436 ^b
	<i>[CI 95%]</i>	[4.0-4.5]	[3.9-4.5]	[4.0-4.6]	
Household income (€/per/year)					
<15,000	<i>n</i> (%)	25 (43%)	18 (60%)	7 (25%)	0.0072 ^a
>15,000	<i>n</i> (%)	33 (57%)	12 (40%)	21 (75%)	
Education level (responsible adult)					
None/primary education	<i>n</i> (%)	16 (26%)	7 (23%)	9 (30%)	0.4677 ^a
Secondary education	<i>n</i> (%)	7 (11%)	5 (16%)	2 (7%)	
Higher education	<i>n</i> (%)	38 (62%)	19 (61%)	19 (63%)	

Occupational category (responsible adult)					
Farmer	<i>n</i> (%)	2 (3%)	2 (7%)	0 (0%)	0.5242 ^a
Artisan/independent retailer	<i>n</i> (%)	3 (5%)	2 (7%)	1 (3%)	
Executive	<i>n</i> (%)	9 (15%)	6 (19%)	3 (10%)	
Intermediate professional	<i>n</i> (%)	24 (39%)	11 (35%)	13 (43%)	
Employee	<i>n</i> (%)	13 (21%)	5 (16%)	8 (27%)	
Factory worker	<i>n</i> (%)	10 (16%)	5 (16%)	5 (17%)	
Other	<i>n</i> (%)	0 (0%)	0 (0%)	0 (0%)	
Occupational activity related with the use of pesticides (responsible adult)					
No	<i>n</i> (%)	11 (18%)	24 (77%)	26 (87%)	0.3476 ^a
Yes	<i>n</i> (%)	50 (82%)	7 (23%)	4 (13%)	
Smoking status of the child					
Non-smoker exposed to passive smoking	<i>n</i> (%)	14 (23%)	8 (26%)	6 (20%)	0.5898 ^a
Non-smoker non exposed to passive smoking	<i>n</i> (%)	47 (77%)	23 (74%)	24 (80%)	
Vegetable garden intake					
Once a month or more	<i>n</i> (%)	32 (55%)	14 (48%)	18 (62%)	0.2909 ^a
Never or less than once a month	<i>n</i> (%)	26 (45%)	15 (52%)	11 (38%)	
Organic food product intake					
Once a week or more	<i>n</i> (%)	21 (36%)	10 (35%)	11 (38%)	0.6955 ^a
Between 1 and 4 portions per month	<i>n</i> (%)	21 (36%)	12 (41%)	9 (31%)	
Never or less than once a month	<i>n</i> (%)	16 (28%)	7 (24%)	9 (31%)	
Use of pesticides at home during the last 12 months					
Never or less than twice a year	<i>n</i> (%)	20 (37%)	11 (35%)	9 (30%)	0.6483 ^a
More than three times per year	<i>n</i> (%)	41 (67%)	20 (65%)	21 (70%)	
Practice of gardening or floral composition					
No	<i>n</i> (%)	50 (82%)	23 (74%)	27 (90%)	0.1084 ^a
Yes	<i>n</i> (%)	11 (18%)	8 (26%)	3 (10%)	
Floor cleaning at home					
Twice a week or less	<i>n</i> (%)	33 (57%)	17 (59%)	16 (55%)	0.7909 ^a
More than twice a week	<i>n</i> (%)	25 (43%)	12 (41%)	13 (45%)	
Home ventilation					
Once a day or more	<i>n</i> (%)	47 (77%)	22 (71%)	25 (83%)	0.2509 ^a
Less than once a day	<i>n</i> (%)	14 (23%)	9 (29%)	5 (17%)	
Presence of pets					
Yes	<i>n</i> (%)	40 (69%)	21 (72%)	19 (66%)	0.5703 ^a
No	<i>n</i> (%)	18 (31%)	9 (28%)	9 (34%)	
Housing estate					
Town center	<i>n</i> (%)	13 (21%)	5 (16%)	8 (27%)	0.3204 ^a
Village	<i>n</i> (%)	38 (62%)	19 (61%)	19 (63%)	
Dispersed habitat	<i>n</i> (%)	10 (16%)	7 (23%)	3 (10%)	
Housing area					
Residential, commercial, industrial	<i>n</i> (%)	37 (61%)	21 (68%)	16 (53%)	0.2495 ^a
Agricultural	<i>n</i> (%)	24 (39%)	10 (32%)	14 (47%)	
Time spent outdoor (garden, park,...) (hour/day)					
	Mean	2.4	2.3	2.6	0.1679 ^b
	[CI 95%]	[2.2-2.6]	[1.9-2.6]	[2.3-2.9]	
Population of the place of residence					
	Mean	4,451	3,858	5,063	0,2717 ^b
	[CI 95%]	[3,363-5,539]	[2,295-5,420]	[3,487-6,639]	
Surface of agricultural land in the place of residence (100 square meters)					
	Mean	105,992	137,277	73,664	0,1028 ^b
	[CI 95%]	[67,053-144,931]	[63,321-211,232]	[51,121-96,207]	
Surface of vineyards in the place of residence (100 square meters)					
	Mean	22,308	43,721	181	0.0001 ^b
	[CI 95%]	[10,520-34,097]	[22,735-64,706]	[0-541]	
Distance with the closest vineyards (meters)					
	Mean	3,856	202	7,626	<.0001 ^b
	[CI 95%]	[1,916-5,790]	[145-259]	[4,096-11,156]	
Season of the biological samples collection					
Autumn /Winter	<i>n</i> (%)	44 (72%)	20 (65%)	24 (80%)	0.1775 ^a
Spring / Sumer	<i>n</i> (%)	17 (28%)	11 (35%)	6 (20%)	

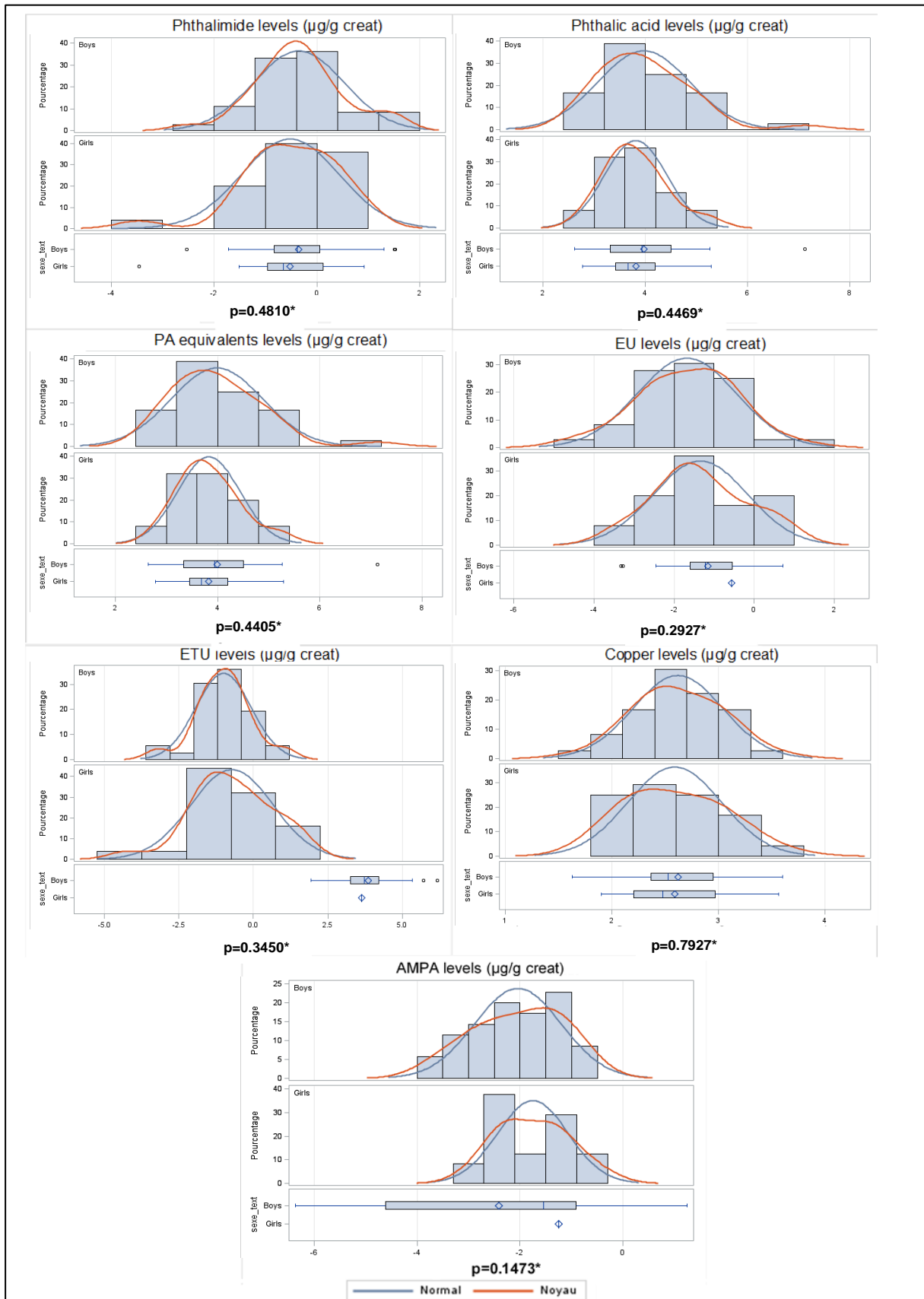
42 ^a chi-square test
 43 ^b two sample t-test
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45 **Table SM12: Crude distribution of urinary concentrations for folpel metabolites, mancozeb**
 46 **metabolites, tebuconazole, copper and AMPA in the children population (in µg/L)**

	N	%>LOD	GM [CI 95%]	P25	P50	P75	P90	P95	Max
Phthalimide	61	48%	0.6 [0.5 – 0.7]	<LOD	<LOQ	<LOQ	1.1	1.3	2.0
Phthalic acid	61	100%	43.7 [35.7 – 53.5]	27.8	38.6	62.4	113.0	211.5	479.3
PA equivalent	61	-	44.6 [36.5 – 54.4]	28.1	39.1	63.2	113.2	212.4	480.3
EU	61	53%	0.2 [0.1 – 0.3]	<LOD	<LOQ	<LOQ	0.6	0.7	2.6
ETU	61	47%	0.4 [0.3 – 0.5]	<LOD	<LOD	<LOQ	1.2	2.3	4.3
TEB-OH	61	7%	NC	<LOD	<LOD	<LOD	<LOQ	<LOQ	3.9
Tebuconazole	61	0%	NC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Copper	60	100%	11.9 [10.5 - 13.6]	9.4	12.0	17.3	20.5	23.7	34.6
AMPA	60	88%	0.13 [0.11 – 0.16]	0.07	0.14	0.22	0.46	0.57	1.53

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48 **Figure SM2: Comparisons of folpel metabolites, mancozeb metabolites, copper and AMPA levels between boys and girls in the children population**
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* t-test – equal variance – using log-transformed concentrations

52 **Table SM13: Folpel metabolites, mancozeb metabolites, copper and AMPA urinary**
 53 **concentrations in children residents LCTV and controls (µg/g of creatinine)**

	Residents				Controls			
	N	%>LOD	Mean [CI 95%]	P95	N	%>LOD	Mean [CI 95%]	P95
Phthalimide	31	50%	0,9 [0,7 – 1,2]	4,5	30	47 %	0,5 [0,3 – 0,6]	1,3
Phthalic acid	31	100%	56,3 [41,5-76,6]	164,6	30	100 %	43,5 [33,1-57,1]	192,1
PA equivalent	31	-	57,6 [42,4-78,1]	169,2	30	-	44,2 [33,7-57,9]	194,0
EU	31	50%	0,2 [0,1 – 0,4]	2,5	30	57 %	0,2 [0,1 – 0,3]	1,0
ETU	31	47%	0,5 [0,3 – 0,8]	3,9	30	47 %	0,3 [0,2 – 0,5]	2,0
TEB-OH	31	10%	NC	2,8	30	3%	NC	<LOD
Copper	30	100%	14,3 [12,1 – 16,9]	35,3	30	100 %	12,9 [11,1 – 15,0]	23,7
AMPA	30	90%	0,2 [0,1 – 0,2]	0,5	30	87 %	0,1 [0,1 – 0,2]	0,4

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56 **Table SM14: Folpel metabolites, mancozeb metabolites, copper and AMPA urinary**
 57 **concentrations in children residents LCTV and controls (µg/L)**

	Residents				Controls			
	N	%>LOD	Mean [CI 95%]	P95	N	%>LOD	Mean [CI 95%]	P95
Phthalimide	31	50%	0,7 [0,6 – 0,8]	1,4	30	47 %	0,5 [0,4 – 0,6]	1,3
Phthalic acid	31	100%	43,9 [32,6-59,1]	303,5	30	100 %	43,6 [32,6-58,4]	204,6
PA equivalent	31	-	44,8 [33,5-60,1]	304,7	30	-	44,3 [33,2-59,1]	205,9
EU	31	50%	0,2 [0,1 – 0,3]	0,7	30	57%	0,2 [0,1 – 0,3]	2,0
ETU	31	47%	0,4 [0,3 – 0,6]	2,3	30	47 %	0,3 [0,2 – 0,5]	1,2
TEB-OH	31	10%	NC	3,2	30	3%	NC	<LOD
Copper	30	100%	11,1 [9,2 – 13,3]	24,3	30	100 %	12,9 [10,7 – 15,6]	20,7
AMPA	30	90%	0,1 [0,1 – 0,2]	0,5	30	87 %	0,1 [0,1 – 0,2]	0,6

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