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Assessment of children's exposure to currently used pesticides in wallonia, Belgium

Catherine Pirard[a,*], Suzanne Remy[b], Arnaud Giusti[b], Léa Champon[b], Corinne Charlier[a]

[a] Laboratory of Clinical, Forensic and Environmental Toxicology, CHU of Liege, B35, 4000 Liege, Belgium
[b] Direction of Chronic Risks, Environment and Health Team, Scientific Institute of Public Service (ISSeP), Rue du Chera 200, 4000 Liege, Belgium

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ABSTRACT

In spring 2016, a study was carried out to characterize currently used pesticide (CUP) exposure among children living in Wallonia (Belgium). Pesticides were measured in both first morning urine voids of 258 children aged from 9 to 12 years and in ambient air collected close to the children's schools. Out of the 46 pesticides measured in the air, 19 were detected with frequencies varying between 11 % and 100 %, and mean levels ranging from < 0.04 to 2.37 ng/m^3. Only 3 parent pesticides were found in 1–10% of the urine samples, while all the metabolites analyzed were positively detected at least once. The captan metabolite (THPI) was quantified in 23.5 % of the samples, while 3,5,6-trichloro-2-pyridinol (chlopryrifos metabolite) was detected in all urines with levels ranging from 0.36–38.96 µg/l. 3-phenoxybenzoic acid (3-PBA), trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (t-DCCA) and diethylphosphate were the most abundant pyrethroid metabolites and dialkylphosphate measured. The air inhalation was demonstrated to be a minor route of exposure for the selected CUPs. Statistical regressions highlighted predictors of exposure for some pesticides such like consumption of grey bread, presence of carpets at home or indoor use of pesticides, although no clear source was identified for most of them.

1. Introduction

Beside China, South and Central America, Europe ranked this past decade as the third area in the world where the use of pesticides per area of cropland is the highest, and despite the different regulations and directives implemented to reduce pesticide adverse effects on human health and environment, the average use per area continued to increase between 2010 and 2016 (FAOSTAT, 2019). Within Europe, Belgium was the second pesticide user per area of cropland just behind the Netherlands with 6.89 kg of pesticides used per ha in 2016 while the European average was evaluated at 1.67 kg/ha, demonstrating an intensive use of such potentially harmful substances in Belgium.

The assessment of the human exposure to currently used pesticides (CUPs) used to be challenging. Different measurement strategies can be used for the exposure characterization. The mains used to include questionnaires, environmental monitoring, or biomonitoring. Briefly, the questionnaires try to collect information affecting the individual exposure such like demographic characteristics, lifestyle activities, diet habits or recent food consumption; the environmental monitoring involves the measurement of multiple environmental media for instance food, drinking water, air, soil, or dust; and the human biomonitoring (HBM) consists in the determination of the chemical's human body burden through the analysis of biomarkers in human fluids or tissues (Bradman and Whyatt, 2005; Needham et al., 2005). If all these strategies have their own advantages and disadvantages as reviewed by Needham et al. (2005), and are often complementary, HBM has been nowadays commonly considered as the major tool to efficiently and cost effectively assess the human exposure to environmental pollutants including pesticides, mainly because integrating all routes of exposure, taking into account inter-individual variations, providing information

Abbreviations: CUP, currently used pesticide; HBM, human biomonitoring; OP, organophosphorous; LC, Liquid Chromatography; GC, Gas Chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; LOQ, limit of quantification; c- and t-DCCA, cis- and trans-3-22-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid; 4F-3-PBA, 4-fluoro-3-phenoxybenzoic acid; DAP, dialkylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DEDTP, diethyldithiophosphate; THPI, tetrahydrophthalimide; TCPY, 35,6-trichloro-2-pyridinol; 24-DB, 24-dichlorophenoxybutyric acid; DI_{inh}, inhalation daily intake; $Conc_{air}$, concentration in air; IR, inhalation rate; BW, body weight; DI, daily intake; ADI, acceptable daily intake

* Corresponding author at: Laboratory of Clinical, Forensic and Environmental Toxicology, BC + 3, Porte 166, CHU (B35), 4000 Liege, Belgium.
E-mail address: c.pirard@chuliege.be (C. Pirard).

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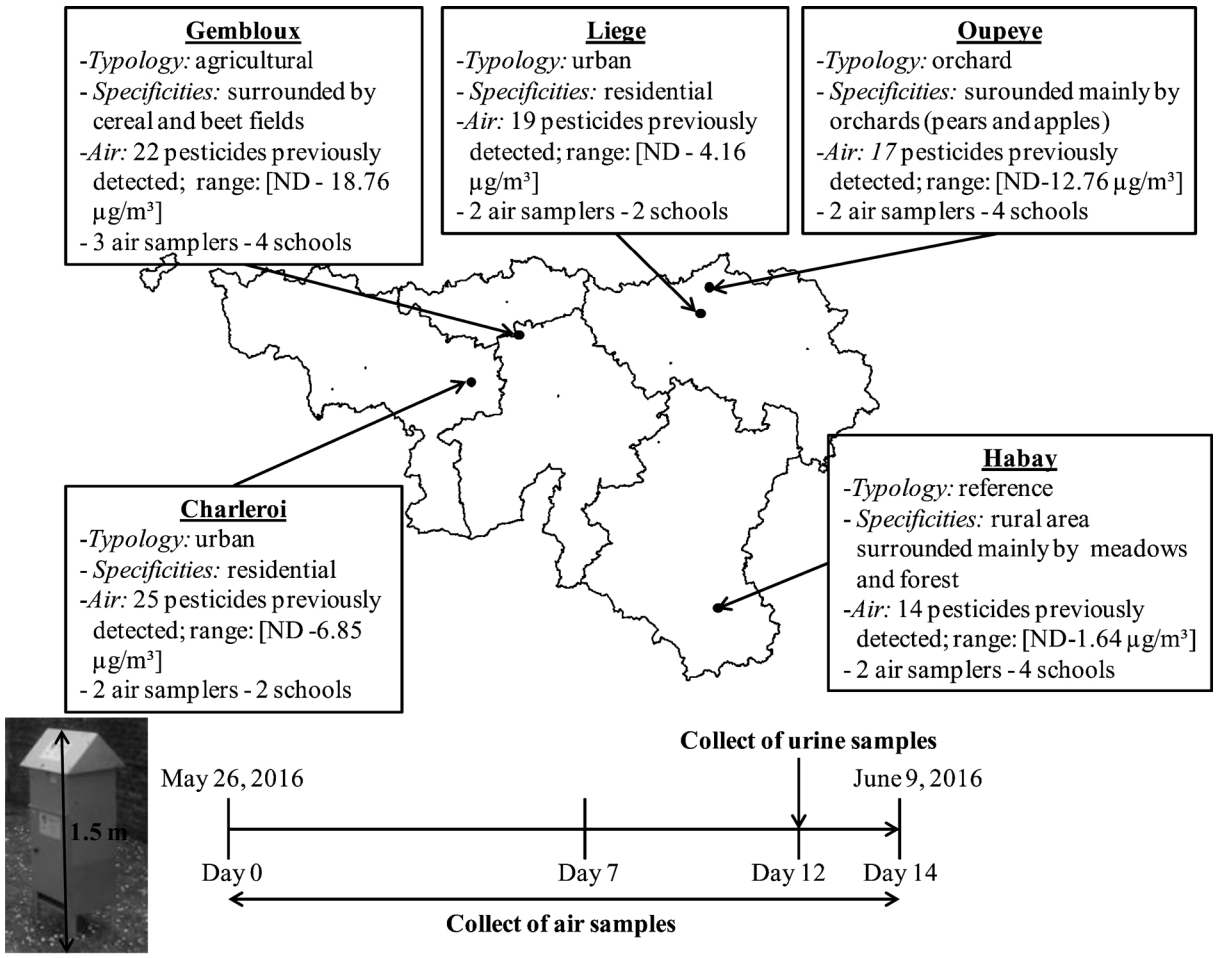

Fig. 1. Characteristics of the selected sites and timing of the sampling.

on spatial and temporal trends, reporting levels more directly related to potential adverse health outcomes, etc (Angerer et al., 2007; Egeghy et al., 2011; World Health Organization (WHO), 2019). However, a thorough knowledge of the human pharmacokinetic of the target chemicals is required to monitor in the appropriate media the relevant biomarkers reflecting the exposure (Barr, 2008).

Although the exposure to some pesticides such like pyrethroids or organophosphorous (OP) has already been studied worldwide through HBM studies, either in the general or in highly exposed populations including children (Kavvalakis and Tsatsakis, 2012; Morgan, 2012; Saillenfait et al., 2015; Yusa et al., 2015), only few recent results have been reported from children living in Western Europe. More specifically in Belgium, to our knowledge, the only available data were produced several years ago, focused on some OP metabolites and concerned Flemish children (Schoeters et al., 2011) whereas in Wallonia, HBM surveys are only under development and up to now have been not focused on CUPs.

On the other hand, if one of its strengths is to integrate all routes of exposure, HBM does not provide direct information about the identification of sources. For pesticides, even if the human exposure for the general population is thought to mainly occur through food ingestion (Sheldon, 2006), the importance of the diet contribution in the overall exposure could vary according to the extent of other sources such like the vicinity of agricultural fields, the occupational status of the family members, the domestic use of pesticides, etc (Curl et al., 2002; Holme et al., 2016; Trunnelle et al., 2014). Moreover, if some intervention studies substituting conventional to organic diet highlighted a drastic decrease of the levels of pesticide biomarkers in the urine of participants, a residual contamination was observed (Bradman et al., 2015; Göen et al., 2017; Lu et al., 2006a), suggesting other minor but non negligible routes of exposure. Because pesticides have been detected in ambient air either from rural, urban, or remote areas (Coscollà et al., 2010; Kurt-Karakus et al., 2011; LeNoir et al., 1999; Mai et al., 2013), the question about the importance of the inhalation contribution to the overall non occupational exposure should be raised. Few works tried to link the outdoor contamination and body burden levels of residents (Galea et al., 2015; Koch et al., 2002; Phung et al., 2012) but none of them perform ambient air measures to quantify the levels of this exposure pathway.

Therefore in order to fill the gap of HBM data focused on pesticides in Belgium and more specifically in Wallonia, and to assess the contribution of inhalation at environmental levels as exposure pathway, a study combining the measures of ambient air and urinary levels of CUPs was carried out in Wallonia. Children were selected as subpopulation because their exposure is of particular concern, experiencing higher exposure due to their specific diet, behaviors and activities, or their higher dose to weight ratio (Weiss et al., 2004). Preliminarily to the present study, a one-year measurement campaign (from 2015 to 2016) of CUPs in ambient air was carried out in 12 different locations spread over Wallonia, and characterized by different agricultural practices and pesticide uses (remote areas with no use of pesticides, urban sites, agricultural sites, livestock areas, sites surrounded by parks, gardens or railways where pesticide uses were suspected, etc). The 46 pesticides analyzed in air were selected according to the ranking method described earlier (Giusti et al., 2018) mainly based on toxicology endpoints, sales and uses at the national and regional levels, and their significant probability to be detected in air. The results of this previous study allowed to select the timing and the locations where air and urine

samples will be collected.

2. Materials and methods

2.1. Sample collection

Five sites were selected because showing contrasted pesticide profiles and concentrations in ambient air during the previous one-year measurement campaign, and the time of the sampling (May - June) corresponded to the period where the highest levels were measured in all sites. The location and characteristics of the sites are described in Fig. 1. For each site, between 2 and 3 air samplers placed in the close vicinity of the participating schools (between 2 and 4 schools per locality were involved in this study) collected samples during 14 days, from May 26th to June 7th 2016, at a 4 m^3/h flow (meaning a total volume of 1344 m^3 collected by sampler) using Total Suspended Particulate High Volume Samplers (ThermoFisher scientific, Breda, The Netherlands). These 11 samplers were 1.5 m high (see picture on Fig. 1) and laid on the ground except one in Oupeye placed on the roof of the school. They were equipped with a quartz filter (Pallflex, PallLife Science, Hoegaarden, Belgium) and a cartridge filled with ORBO 2500 precleaned large PUF/Amberlite® XAD®-2/PUF from Supelco (Bellefonte, PA, USA). Additionally, some Quality Controls and field blanks consisting in virgin cartridge (filled with PUF/XAD/PUF resin) respectively spiked and non-spiked with standard solution containing all analytes were run to ensure the reliability of the analysis and the absence of external contamination. All cartridges and filters were stored in the dark at 4 °C until extracted.

Children living in the selected localities and aged from 9 to 12 years old were invited to participate via their school on voluntary basis. The only inclusion criteria were to attend schools participating to the study (and thus located near an air sampler) and to be aged between 9 and 12 years old. Once the participation accepted, the parents signed the informed consent, and answered to a questionnaire about their home environment, family socioeconomic status, child outside activities, diet habits, domestic uses of pesticide, and to a diet questionnaire (48 h recall). They were asked to collect the first morning urine void of their children on June 7th 2016 in a 250 ml polypropylene vessel previously distributed (all urine samples were collected the same day, except in Oupeye where the collect was performed on June 14th due to some administrative delays, thus air samples were collected from June 2nd to 16th). However in each site, the air samples were collected from 12 days before to 2 days after the urine collection (Fig. 1). The urine samples were transferred to the laboratory at refrigerated temperature (4 − 6 °C), and were stored at − 20 °C before analysis. All communication documents were derived from those used during the COPHES/DEMOCOPHES study (Joas et al., 2012).

2.2. Analytical procedure

The air analyses were carried out by the Scientific Institute of Public Service (ISSeP) and the Walloon Center of Agronomical Research (CRA-W), while urine samples were analyzed by the Laboratory of Toxicology (CHU Liege).

2.2.1. Air samples

The 46 pesticides previously selected (Giusti et al., 2018) are gathered in Table 1. The extraction of pesticides from filters and cartridges was carried out for 4 h using an hexane:acetone:methanol mixture (50:40:10 v:v:v) in a Soxhlet apparatus. Solvent volume was then reduced to 10 ml using rotary evaporator, and split into 2 fractions of 5 ml dedicated to respectively Liquid Chromatography (LC) and Gas Chromatography (GC) coupled to mass spectrometry (MS) analyses. Further concentration and reconstitution of both extracts are detailed in Supplementary material as well as all LC-tandem mass spectrometry (MS/MS) and GC–MS/MS gradients and parameters. The quantification of the analytes detected by LC/MS was performed using isotope dilution (internal standards are reported in Table S1) with spiked calibration curves ranging from 0.5–40 pg/µl, while those determined by GC were quantified using the method of external standard addition (sample unspiked and spiked at 5 increasing levels from 5 to 100 ng/ml). The recoveries were determined on cartridges spiked at 3 levels each in duplicate (50, 500 and 1000 ng), and the means ranged between 72 and 128% in the dosing range, except for aclonifen, clorpyralid, diflufenican, and ethoprofos for which recoveries were slightly lower (Table 1). The limits of quantification (LOQs) were determined based on the recovery rates and the signal to noise ratio (S/N > 10). LOQs for both LC and GC methods were 0.04 or 0.1 ng/m^3 depending on the pesticide (Table 1).

Table 1
Mean recoveries obtained during the validation, and limits of quantification (LOQs) for each of the 46 pesticides measured in air.

Analyte	Mean recovery (%)	LOQ (ng/m^3)	Analyte	Mean recovery (%)	LOQ (ng/m^3)
2,4 D	85	0.04	Fenpropidin	82	0.04
2,4 DB	109	0.10	Fenpropimorph	72	0.04
Aclonifen	67	0.10	Iprodione	71	0.04
Benfluralin	76	0.04	Kresoxim-methyl	78	0.04
Boscalid	128	0.04	Linuron	126	0.04
Captan	72	0.04	MCPA	98	0.04
Chlorothalonil	88	0.10	Metazachlor	83	0.04
Chlorpyrifos-ethyl	79	0.04	S-Metolachlor	113	0.04
Clopyralid	67	0.10	Metribuzin	91	0.04
Cyhalothrin	87	0.04	Myclobutanil	74	0.04
Cymoxanil	92	0.04	Oxadiazon	90	0.04
Cypermethrin	80	0.04	Penconazole	81	0.04
Cyproconazole	102	0.04	Pendimethalin	98	0.10
Cyprodinil	86	0.04	Pirimicarb	87	0.10
Deltamethrin	70	0.04	Propiconazole	92	0.10
Difenoconazole	78	0.04	Propyzamide	73	0.10
Diflufenican	66	0.04	Prosulfocarb	77	0.10
Dimethenamid-P	87	0.04	Pyrimethanil	78	0.04
Dimethoate	78	0.04	Spiroxamine	79	0.04
Epoxiconazole	94	0.04	Terbuthylazine	94	0.04
Ethofumesate	94	0.10	Tebuconazole	80	0.04
Ethoprophos	65	0.10	Tetraconazole	92	0.04
Fenoxycarb	80	0.04	Triallate	97	0.10

2.2.2. Urinary analyses

The 43 pesticides or metabolites prioritized for the biomonitoring part of the study are gathered in Table 2. Compared to parent pesticides measured in air, chlorothalonil, cymoxanil, fenpropimorph and pyrimethanil were not included in the urinary analysis because of the non suitability of the multi-analyte method for these substances. In addition were also included 4 non specific metabolites of pyrethroid insecticides namely cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acids (c- and t-DCCA), 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4F-3-PBA), and 5 dialkylphosphates (DAPs) as non specific metabolites of OP pesticides namely dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP). The 43 biomarkers were determined using 3 different extraction procedures detailed in Supplementary material, and analyzed by LC–MS/MS, GC–MS/MS, or GC–MS/MS after a derivatization step. For all compounds, the quantification was performed using a matrix-matched calibration curve built in real urine from anonymous donors (previously checked for initial contamination) fortified at different levels, excepted for DAP and pyrethroid metabolite analyses for which synthetic urine (Ricca Chemical, Arlington, TX, USA) was used. The 3 analytical methods were validated according to the total error approach (Dubois et al., 2012; Hubert et al., 2007) using E-noval software V4.0 (Arlenda, Liege, Belgium), and based on the standard

Table 2
Trueness and limits of quantification (LOQs) obtained during the validation for each of the 43 pesticides or metabolites measured in urine.

Analyte	Trueness (%)	LOQ (µg/l)	Analyte	Trueness (%)	LOQ (µg/l)
Parent pesticides					
2,4-DB	105.6	0.15	Propiconazole	104.2	0.14
Aclonifen	114.4	0.18	Propyzamide	113.5	0.05
Benfluralin	116.4	0.45	Prosulfocarb	106.4	0.14
Boscalid	106.3	0.05	Spiroxamine	92.5	0.06
Captan	107.4	0.50	Tebuconazole	103.6	0.05
Chlorpyrifos ethyl	107.2	0.29	Terbuthylazine	101.3	0.05
Cyhalothrin	98.4	0.92	Tetraconazole	101.0	0.07
Cyproconazole	99.8	0.08	Triallate	103.3	0.24
Cyprodinil	103.4	0.21	***Specific metabolites***		
Difenoconazole	104.7	0.17	Desethylterbuthylazine	105.6	0.05
Dimethenamid	105.3	0.20	TCPY	114.2	0.08
Dimethoate	104.4	0.06	Tetrahydrophthalimide (THPI)	85.6	0.20
Epoxiconazole	104.4	0.17	***Dialkylphosphates (DAPs)***		
Ethoprophos	102.8	0.20	Diethylphosphate	94.2	0.50
Fenoxycarb	99.8	0.18	Diethylthiophosphate	95.0	0.50
Fenpropidin	107.6	0.05	Diethyldithiophosphate	98.9	0.50
Kresoxy-methyl	101.0	0.72	Dimethylthiophosphate	88.8	0.50
Linuron	109.4	0.05	Dimethyldithiophosphate	89.7	0.50
Metolachlor	95.8	0.19	***Pyrethroid metabolites***		
Metribuzin	103.9	0.20	c-DCCA	105.9	0.50
Oxadiazon	99.3	0.23	t-DCCA	109.1	0.15
Penconazole	94.6	0.40	4-F-3-PBA	106.7	0.11
Pendimethalin	100.4	0.12	3-PBA	97.5	0.09

addition method. The LOQs gathered in Table 2 were the smallest concentrations determined in fortified urine (or synthetic urine) samples measurable with a total error not exceeding 30 %. Trueness assessed on urine samples (or synthetic urine) fortified at 8 different levels, each level analyzed in triplicate, was also reported in Table 2. All analysis sequences consisted in the calibration curve, 30 unknown samples, 1 procedural blank, and 2 home-made Quality Controls (urine spiked at low and high levels). For DAP, TCPY and pyrethroid metabolite analyses, 2 additional materials from previous German External Quality Assessment Scheme (G-EQUAS) programs were added to the sequence. The complete analytical information is detailed in Supplementary material.

2.3. Calculation of the daily intake doses through inhalation

The inhalation daily intake doses were estimated for the pesticides detected in air in each location with the following relation:

DI_{inh} (ng/kg/d) = Conc $_{air}$ (ng/m^3) x IR (m^3/d)/BW (kg) where DI_{inh} was the inhalation daily intake, Conc $_{air}$ the mean concentration measured according to the location, IR the inhalation rate and BW the body weight, both set respectively at 12 m^3/d and 31.8 kg as suggested for children aged from 6 to 11 years old (US EPA, 2015).

2.4. Statistical analyses

The statistical analyses were performed using SAS 9.4 for Windows (SAS Institute, Cary, NC, USA) and R 3.3.1. software (R Foundation for Statistical Computing, Vienna, Austria). All urinary biomarkers showing quantification frequency (N > LOQ) lower than 50 % were used as dichotomized variables (detected vs non-detected) whereas they were considered as quantitative variables when their quantification frequencies were higher or equal to 50 %, with the measurements below the LOQ replaced by LOQ/2 (Hornung and Reed, 1990). For each urinary biomarker, the normality of the distribution was numerically evaluated by comparing medians and arithmetic means, graphically with the shape of the histogram and the Quantile Plot, and using the Shapiro-Wilk test. Due to their skewed distribution, the biomarker levels were log-transformed before being used in further statistical models to approximate a normal distribution. Univariate logistic and linear regressions were firstly carried out to compare respectively qualitative and quantitative biomarker concentrations according to the home characteristics, the socio-demographic characteristics, the children's activities and the diet characteristics, these information being collected through the questionnaire. The covariates significantly associated (statistical significance was set at $p < 0.05$) were then included in a binary logistic regression analysis or a linear multivariate model depending on the binary or the continuous character of the pesticide measure variable.

3. Results

Among the 258 children recruited, 29 and 12 did not answer respectively to the general and the 48 h recall questionnaire, while the residence area for 3 of them was not identified, and the collect of the urine of one of them was missed. Some of their characteristics are detailed in Table 3. Their mean age was 10.8 +/- 1.05 years old whereas their median body mass index (BMI) was 16.7 kg/m^2. Most of them lived in a house (95.2 %), without carpet (93.9 %) but with at least one rug for half of them (52.4 %). A large majority of parents were originated from Belgium (88.2 % for mothers, and 82.4 % for fathers), with a fair socio-economical status (both in terms of educational levels and household income). Within the 2 days before the urine collection, 91.3 % of the children had outdoor activities, while 29.3 % reported to bite their nails.

The detection rates (% > LOQ), the mean levels (average of 2 or 3 samples depending on the site) of pesticides measured in ambient air, and the estimated inhalation daily intake doses were gathered in Table 4 according to the sampling location. All other pesticides not reported in this table were never detected. 19 pesticides out of the 46 initially measured in air were detected with rates varying between 11 and 100 %.

The pesticides and metabolites positively detected in the urine of children are gathered in Table 5. While the parent pesticides and some metabolites were analyzed in all urine samples, DAP, pyrethroid metabolites, tetrahydrophthalimide (THPI) and TCPY were measured in urine with sufficient remaining volume (240 for DAP, 229 for the other metabolites). Some interferences (ion ratio between Multiple Reaction Monitoring transitions out of range) resulting in non-exploitable results were observed in 9 samples for t-DCCA and THPI, 23 and 24 samples for c-DCCA and DMTP respectively. From the 32 parent pesticides

Table 3
Characteristics of the population of children studied.

	N children (%)		N children (%)
Total	258 (100 %)	Type of dwelling	
Gender		House	218 (95.2 %)
Girls	121 (48 %)	Appartment	11 (4.8 %)
Boys	131 (52 %)	Number of pets	
Locations		0	106 (46.3 %)
Habay (Reference)	29 (11.2 %)	1	57 (24.9 %)
Charleroi (Urban 1)	26 (10.1 %)	2	27 (11.8 %)
Liege (Urban 2)	105 (40.7 %)	> 2	39 (17.0 %)
Gembloux (Agricultural)	54 (20.9 %)	Use of pet antiparasitics	
Oupeye (Orchard)	40 (15.5 %)	Yes	75 (32.8 %)
Undefined	3 (1.2 %)	No	154 (67.2 %)
Educational level of the mother		Usual proportion of organic vegetables in the diet	
Primary or secondary	56 (24.5 %)	< 50 %	199 (86.9 %)
higher short cycle	66 (28.8 %)	> 50 %	30 (13.1 %)
higher long cycle or university	107 (46.7 %)	Vegetables washed and/or peeled before consumption	
Educational level of the father		Never / rarely	1 (0.4 %)
Primary or secondary	69 (30.1 %)	Often / always	228 (99.6 %)
higher short cycle	45 (19.7 %)	Usual proportion of organic fruits in the diet	
higher long cycle or university	115 (50.2 %)	< 50 %	195 (85.2 %)
Net monthly income (household)		> 50 %	34 (14.8 %)
< 1999€	29 (12.7 %)	Fruits washed and/or peeled before consumption	
2000-3999€	97 (42.5 %)	Never / rarely	21 (9.2 %)
> 4000€	102 (44.7 %)	Often / always	208 (90.8 %)
Outdoor use of pesticides		Consumption of fruit juice	
Yes	57 (24.9 %)	Yes	141 (62.1 %)
No	172 (75.1 %)	No	86 (37.9 %)
Indoor use of pesticides		Drinking of water	
Yes	80 (34.9 %)	tap water	115 (50.2 %)
No	149 (65.1 %)	bottled water	114 (49.8 %)
Recent outdoor activities (within 2 last days)		Biting nails	
Yes	209 (91.3 %)	Yes	67 (29.3 %)
No	20 (8.7 %)	No	162 (70.7 %)

monitored in urine, only metribuzin was detected in 10 % of the samples, with concentrations ranging up to 1.68 µg/l (P95 = 0.34 µg/l), propyzamide and 2,4-DB being found in very few samples (respectively 6 and 3 out of 257). Among metabolites, desethylterbutylazine, 4F-3-PBA and DEDTP were also positively measured in very few samples (3.5 %, 2.2 % and 6.3 % repectively); while other non specific pyrethroid and OP metabolites were detected in a large majority of samples (from 28 to 100 %). TCPY, DEP, 3-PBA and t-DCCA were the most frequently detected and with the higher levels measured, ranging between < LOQ- 662 µg/l.

The binary logistic regressions are detailed in Table 6 for metribuzin, THPI, DETP, DMDTP and c-DCCA according to the detection or non-detection rate, while the multivariate linear regression results are gathered in Table 7 for TCPY, DEP, DMTP, t-DCCA, and 3-PBA. The other pesticides or biomarkers showed very low detection frequencies (below 10 %) and were thus not included in the statistical analyses. The appellation “mixed origin” of vegetables included vegetables coming as well from supermarket, local producer, local market or private garden. The outdoor pesticide uses, the number of pets, the use of pet antiparasitics, and the proportion of organic fruits and vegetables in the diet (> or < than 50 %) did not impact significantly the pesticide urinary levels whatever the biomarkers considered.

4. Discussion

4.1. Children’s exposure assessment through ambient air

Out of the 46 pesticides measured, 19 were detected in at least 10 % of the air samples. The mean levels ranging from < 0.04 to 2.37 ng/m^3 seemed to be in the same magnitude order than those reported in France or in North America (ATMO-Poitou-Charentes, 2017; Coscollà et al., 2010, Kurt-Karakus et al., 2011; Majewski et al., 2014; Yao et al., 2006). As expected, the reference site (Habay) showed the lower number of pesticides detected and the lower levels measured in the air, while the highest air levels were measured in both agricultural and orchard sites (Gembloux and Oupeye respectively), with pesticide patterns consistent with the pesticide related activities. For instance, higher levels of captan usually intended for peer and apple tree protection were measured near orchards (in Oupeye), whereas herbicides dedicated among others to cereal and potato crop protection (i.e. pendimethalin, prosulfocarb and triallate) showed higher levels in Gembloux surrounded by cereal and beet fields.

Table 4
The detection rates (% > LOQ) and the mean levels of pesticides measured at least once in ambient air. N corresponded to the number of samples collected per site. All other pesticides not reported in this table were never detected.

Pesticides	LOQ	N > LOQ	Habay -reference site (N = 2)	Charleroi - urban site (N = 2)	Liege - urban site (N = 2)	Gembloux - agricultural site (N = 3)	Oupeye - agricultural site (N = 2)	Daily intake (range)
	(ng/m^3)	(%)	(ng/m^3)	(ng/m^3)	(ng/m^3)	(ng/m^3)	(ng/m^3)	(ng/kg/day)
2,4 DB	0.04	22.2	< LOQ	< LOQ	< LOQ	0.25	0.56	0.09-0.21
Benfluralin	0.04	87.5	< LOQ	0.30	0.08	0.81	1.97	0.03-0.74
Captan	0.04	100.0	0.06	0.15	0.26	0.16	1.97	0.02-0.74
Chlorothalonil	0.10	100.0	0.26	1.58	0.27	0.95	1.03	0.10-0.60
Difenoconazole	0.04	11.1	< LOQ	< LOQ	< LOQ	< LOQ	0.06	NA-0.02
Dimethenamid-P	0.04	77.8	< LOQ	0.38	0.12	0.78	1.36	0.05-0.51
Epoxyconazole	0.04	44.4	< LOQ	0.07	< LOQ	0.06	0.04	0.01-0.03
Ethofumesate	0.10	66.7	< LOQ	0.32	0.12	0.53	0.25	0.04-0.20
Kresoxim-methyl	0.04	11.1	< LOQ	< LOQ	< LOQ	< LOQ	0.08	NA - 0.03
Linuron	0.04	44.4	< LOQ	0.12	< LOQ	0.19	< LOQ	0.05-0.07
Pendimethalin	0.10	88.9	0.12	0.86	0.34	1.70	0.39	0.04-0.64
Propiconazole	0.04	11.1	< LOQ	0.06	< LOQ	< LOQ	< LOQ	NA - 0.02
Propyzamide	0.10	22.2	< LOQ	< LOQ	< LOQ	0.15	< LOQ	NA-0.06
Prosulfocarb	0.10	77.8	< LOQ	0.59	0.28	1.45	0.11	0.04-0.55
S-metolachlor	0.04	88.9	0.05	0.62	0.22	1.67	2.37	0.02-0.89
Spiroxamine	0.04	11.1	< LOQ	< LOQ	< LOQ	< LOQ	0.05	NA - 0.02
Tebuconazole	0.04	33.3	< LOQ	0.09	< LOQ	0.06	< LOQ	0.02-0.03
Terbuthylazine	0.04	77.8	< LOQ	0.24	0.07	0.23	0.25	0.02-0.09
Triallate	0.10	66.7	< LOQ	0.68	0.19	1.15	0.34	0.07-0.43

Table 5
Number of samples (N), limit of quantification (LOQ), detection frequency (N > LOQ), percentiles, and range of concentrations for biomarkers positively detected in urine.

	N	LOQ (µg/l)	N > LOQ (%)	P25 (µg/l)	P50 (µg/l)	P75 (µg/l)	P95 (µg/l)	Min-max (µg/l)
Parent pesticides or specific metabolites								
Metribuzin	257	0.20	10.1	< LOQ	< LOQ	< LOQ	0.34	< LOQ-1.68
Propyzamide	257	0.05	2.3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ-0.05
2,4-DB	257	0.15	1.2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ-1.69
Desethylterbutylazine	257	0.05	3.5	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ-0.94
THPI	220	0.20	23.5	< LOQ	< LOQ	< LOQ	0.65	< LOQ-4.13
TCPY	229	0.08	100.0	2.33	3.87	6.09	12.12	0.36-38.96
Pyrethroid metabolites								
3-PBA	229	0.09	99.6	0.51	0.98	1.99	5.33	< LOQ-311.1
c-DCCA	206	0.50	40.3	< LOQ	< LOQ	0.75	2.01	< LOQ-55.6
t-DCCA	220	0.15	93.2	0.27	0.66	1.31	4.29	< LOQ-501.3
4-F-3-PBA	229	0.11	2.2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ-1.74
Organophosphorous metabolites								
DMTP	216	0.50	64.8	< LOQ	1.01	3.55	13.72	< LOQ-231.3
DMDTP	240	0.50	27.9	< LOQ	< LOQ	0.91	1.31	< LOQ-9.91
DEP	240	0.50	73.3	< LOQ	1.80	4.92	29.97	< LOQ-661.8
DETP	240	0.50	49.2	< LOQ	< LOQ	1.11	3.50	< LOQ-4.64
DEDTP	240	0.50	6.3	< LOQ	< LOQ	< LOQ	0.59	< LOQ-2.10

The exposure of the children through inhalation was assessed by estimating the inhalation daily intake doses (DI). The DI_{inh} ranged from 0.02 to 0.89 ng/kg/d, according to the pesticide and the location. These values are far below the acceptable daily intake (ADI) set by the European Commission varying between 0.005 and 0.1 mg/kg/d according to the pesticide, but also well below the daily intake estimated for OP pesticides based on HBM data, ranging from 0.04–30 µg/kg/day for children depending on the OP pesticide considered (Katsikantami et al., 2019), or below the DI estimated through diet studies for instance between 0.001–2.5 µg/kg/d for Spanish adult women, Moldovan or Hong Kong adults for a wide range of different pesticides (Iñigo-Nuñez et al., 2010; Sircu et al., 2015; Wong et al., 2014).

4.2. Children's exposure assessment through internal dose

Metribuzin was the only parent pesticide positively detected in the urine samples (10 %). To our knowledge, this is the first time that its presence was reported in the urine of individuals either from the general population or from occupationally exposed individuals. Data on the toxicokinetic of metribuzin are inexistent in human and scarce on animals, with mercapturic acid derivatives reported as main metabolites eliminated in the urine of treated rats and mice (Bleeke and Casida, 1984). For other triazines such like atrazine, the unmodified parent pesticide was demonstrated to represent only 2 % of the excreted doses in urine of mammals (Maroni et al., 2000). Therefore the results of the present study could be worrying because could suggest sufficiently high exposure to induce detectable urinary levels of the parent compound. On the other hand, THPI, which was usually considered as biomarker for the monitoring of captan exposure (Berthet et al., 2012; Heredia-Ortiz and Bouchard, 2012) was detected in 23.5 % of the urine samples with levels up to 4.13 µg/l, similarly to levels reported in UK adults and children living near orchards (Galea et al., 2015).

3-PBA which was a common metabolite to numerous pyrethroids was found in 228 out of the 229 urine samples analyzed, while t-DCCA was detected in 93 % of the samples. Moreover, at least one OP metabolite was detected in all urine samples, highlighting the ubiquitous exposure of children to OP and pyrethroid pesticides. Compared to previous studied carried out worldwide (Table 8), the levels of pyrethroid metabolites measured in the urine of our Belgian children seemed higher than those reported for children from Germany (Becker et al., 2006), Spain (Roca et al., 2014), France (Glorennec et al., 2017), Canada (Oulhote and Bouchard, 2013) or US (CDC, 2019). The TCPY levels measured were similar to those reported for Spanish children (Roca et al., 2014), but roughly twice higher than in US children from the 2009–2010 NHANES cycle (CDC, 2019). Conversely, the levels of DAP were lower than those reported in the urine of children living in neighboring countries such like in Germany, Spain or France, and even lower than those reported in Canadian or US children (Table 8) although higher OP exposure was usually observed in Europe compared to America (Kavvalakis and Tsatsakis, 2012). Even more surprisingly, DAP levels seemed substantially lower in children living in Wallonia than toddlers residing in Flanders (Schoeters et al., 2011) suggesting different exposure levels to pesticides between the North and South of the country. Because the environmental policy is a regional competence in Belgium, the implementation of pesticide regulations was different in the 3 regions (Brussels, Flanders and Wallonia) potentially resulting in regional specific exposure to pesticides.

4.3. Contribution of inhalation to the global exposure

To study the contribution of inhalation to the global exposure, the pesticides frequently detected in air was firstly compared to the pesticides (unchanged or urinary biomarkers) positively measured in urine. The most abundant and frequently detected pesticides in the air such like benfluralin, dimethenamid-P, ethofumesate, pendimethalin, prosulfocarb, s-metolachlor or triallate (positively detected in 66.7%–88.9% of air samples), were not detected in the urine of children as parent compound. On the other hand, desethylterbuthylazine, a commonly accepted metabolite of terbuthylazine (Mercadante et al., 2013) was sporadically detected in urine (in 3.5 % of urine samples) although terbuthylazine was found in all air samples except those from the reference site. Furthermore, metribuzin which was detected in 10 % of the urine samples was never detected in air. These observations would argue that the contribution of air inhalation to the global exposure to pesticides was too low to induce measurable levels of urinary biomarkers, even if some parent pesticides were likely not the best biomarkers for the monitoring of the environmental exposure. Moreover, the specific and unspecific urinary metabolites of OP and pyrethroids were frequently detected at sometimes high concentrations whereas the corresponding parents monitored in air were never or sparsely detected (chlorpyrifos-ethyl was found in 22 % of air samples, while dimethoate, cyhalothrin, cypermethrin or deltamethrin were never detected). And finally, levels and detection frequencies of THPI were significantly lower in the urine of children living in Oupeye where the highest captan concentrations were measured in the air. Thus for captan, pyrethroids and OP, inhalation would poorly contribute to the

Table 6
Results of the binary logistic regressions using the dichotomized variables detected or non detected for biomarkers showing detected detected at least in 10 % and up to 50 % of samples.

	Metribuzin (detected vs non detected)		THPI (detected vs non detected)		DETP (detected vs non detected)		DMDTP (detected vs non detected)		c-DCCA (detected vs non detected)	
Variable	OR (95 % CI)	p-value	OR (95 % CI)	p-value	OR (95 % CI)	p-value	OR (95 % CI)	p-value	OR (95 % CI)	p-value
Home characteristics										
Residence site										
Charleroi vs Oupeye	–	–	**7.14 (0.68–71.4)**	**< 0.001**	**0.81 (0.19–3.33)**	**0.008**	**7.14 (0.30–166.7)**	**< 0.001**	–	–
Gembloux vs Oupeye	–	–	**11.24 (1.30–100)**		**0.27 (0.07-0.98)**		**1.64 (0.08–33.3)**		–	–
Habay vs Oupeye	–	–	**52.63 (5.88–500)**		**0.29 (0.08–1.09)**		**2.13 (0.08–58.8)**		–	–
Liege vs Oupeye	–	–	**8.33 (0.98–71.4)**		**1.33 (0.37–4.76)**		**71.4 (3.85- > 1000)**		–	–
Agricultural surface (ha) 2.5 km around the school	1.00 (1.00-1.00)	0.018	–	–	1.00 (1.00–1.01)	0.790	1.00 (1.00–1.01)	0.360	–	–
Agricultural surface (ha) 500 m around home	–	–	–	–	–	–	**1.09 (1.02–1.16)**	**0.014**	–	–
Agricultural surface (ha) 2500 m around home	–	–	–	–	1.00 (1.00–1.01)	0.360	**1.00 (0.99–1.00)**	**0.031**	–	–
Sociodemographic characteritics										
Gender (girl vs boy)	–	–	–	–	–	–	1.54 (0.67–3.57)	0.310	–	–
Mother's nationality (Belgian vs foreign)	0.42 (0.11–1.54)	0.190	–	–	–	–	0.43 (0.11–1.72)	0.240	–	–
Mother's educational level										
Primary/secondary vs long cycle or university level	–	–	–	–	–	–	0.50 (0.09–2.94)	0.620	**0.38 (0.17-0.85)**	**0.024**
Short cycle higher education vs long cycle or university level	–	–	–	–	–	–	1.15 (0.09–2.94)		**0.47 (0.23-0.95)**	
Father's educational level		**0.009**	–	–	–	–	–	–	–	–
Primary/secondary vs long cycle or university level	**0.54 (0.12–2.33)**		–	–	–	–	–	–	–	–
Short cycle higher education vs long cycle or university level	**4.17 (1.08–16.67)**		–	–	–	–	–	–	–	–
Mother's job										
Office workers vs unemployed	–	–	–	–	–	–	0.55 (0.11–2.70)	0.770	–	–
Manual workers vs unemployed	–	–	–	–	–	–	0.66 (0.12–3.70)		–	–
Household income										
< 1999€ vs > 4000€	**2.00 (0.30–13.3)**	**0.038**	–	–	–	–	2.70 (0.52–13.89)	0.220	–	–
2000-3999€ vs > 4000€	**5.88 (1.35–26.3)**		–	–	–	–	2.17 (0.85–5.56)		–	–
Children activities										
Outdoor sport activities in the last 48 h (yes vs no)	0.53 (0.17–1.61)	0.260	–	–	–	–	–	–	–	–
Outdoor activities in the last 48 h (yes vs no)	–	–	–	–	–	–	0.34 (0.08–1.45)	0.150		
Nail biting (yes vs no)	**5.88 (1.88–20.0)**	**0.024**	–	–	–	–	–	–	–	–
Diet characteritics										
Fruits washed and/or peeled before consumption (never/rarely vs often/always)	–	–	**3.85 (1.32–10.99)**	**0.014**	–	–	–	–	–	–
Consumption of juices in the last 48 h (yes vs no)	–	–	–	–	–	–	1.88 (0.78–4.55)	0.160	–	–
Consumption of grey bread or similar in the last 48 h (no vs yes)	–	–	0.44 (0.17–1.15)	0.092	–	–	–	–	**0.32 (0.13-0.78)**	**0.013**
Consumption of tomatoes in the last 48 h (no vs yes)	–	–	–	–	1.56 (0.89–2.70)	0.120	–	–	–	–
Consumption of vegetables excluded tomatoes and salad in the last 48 h (yes vs no)	–	–	–	–	1.51 (0.84–2.78)	0.160	–	–	–	–
Consumption of cooked apples or peers in the last 48 h	–	–	–	–	–	–	–	–	0.42 (0.18–1.16)	0.095

Table 7
Results of the multivariate linear regression using the log-transformed concentration of biomarkers.

	TCPY (R^2 of model: 0.09)		DEP (R^2 of model: 0.27)		DMTP (R^2 of model: 0.17)		t-DCCA (R^2 of model: 0.23)		3-PBA (R^2 of model: 0.21)	
Variable	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value	β ± SE	p-value
Intercept	**2.26 ± 0.45**	**< 0.001**	**3.00 ± 0.96**	**0.002**	**1.37 ± 0.60**	**0.023**	**-1.22 ± 0.32**	**< 0.001**	−0.27 ± 0.36	0.462
Home characteristics										
Residence site										
Charleroi vs Oupeye			**-1.67 ± 0.54**	**0.002**	−0.06 ± 0.52	0.911				
Gembloux vs Oupeye			**-1.69 ± 0.46**	**< 0.001**	0.58 ± 0.47	0.225				
Habay vs Oupeye			**-1.28 ± 0.50**	**0.011**	−0.19 ± 0.47	0.680				
Liege vs Oupeye			−0.70 ± 0.49	0.154	0.22 ± 0.45	0.629				
Agricultural surface (ha) 2.5 km around the school			−0.00 ± 0.00	0.776	**-0.00 ± 0.00**	**0.034**				
Agricultural surface (ha) 100 m around home	0.19 ± 0.12	0.109	−0.00 ± 0.00	0.180			**0.56 ± 0.14**	**< 0.001**	**0.48 ± 0.12**	**< 0.001**
Agricultural surface (ha) 500 m around home	0.01 ± 0.01	0.38								
Agricultural surface (ha) 2500 m around home					0.00 ± 0.00	0.322				
Type of dwelling (appartment vs house)			0.36 ± 0.51	0.472						
Rooms with carpets (yes vs no)			**0.88 ± 0.41**	**0.033**						
Use of pesticides inside (yes vs no)							**0.54 ± 0.17**	**0.002**	**0.26 ± 0.14**	**0.073**
Sociodemographic characteritics										
BMI (kg/m²)	−0.03 ± 0.02	0.149								
Father's job										
Office workers vs unemployed	−0.25 ± 0.31	0.431								
Manual workers vs unemployed	−0.29 ± 0.32	0.365								
Father's nationality (Belgian vs foreign)					−0.24 ± 0.28	0.377				
Children activities										
Outdoor activities in the last 48 h (yes vs no)					−0.52 ± 0.36	0.144				
Outdoor sport activities in the last 48 h (yes vs no)							0.23 ± 0.16	0.170	0.21 ± 0.14	0.126
Diet characteritics										
Origin of vegetables										
Supermarket vs mixed origin			**-1.27 ± 0.53**	**0.018**						
Organic store/local producer/private garden vs mixed origin			**-1.76 ± 0.59**	**0.003**						
Usual proportion of organic vegetables in the diet (< 50 % vs > 50 %)									0.40 ± 0.28	0.149
Usual proportion of organic fruits in the diet (< 50 % vs > 50 %)									0.13 ± 0.26	0.618
Drinking of water (tap vs bottled)			**-0.45 ± 0.20**	**0.028**	**-0.40 ± 0.20**	**0.044**				
Consumption of grey bread or similar in the last 48 h (no vs yes)	**-0.29 ± 0.14**	**0.040**					**-0.56 ± 0.24**	**0.018**	**-0.49 ± 0.20**	**0.014**
Consumption of stone fruits in the last 48 h (no vs yes)			**0.48 ± 0.23**	**0.040**			**0.60 ± 0.18**	**0.001**	**0.39 ± 0.15**	**0.012**
Consumption of exotic fruits in the last 48 h (no vs yes)			**0.46 ± 0.22**	**0.041**						
Consumption of vegetables excluded tomatoes and salad in the last 48 h (yes vs no)					0.25 ± 0.21	0.249				
Consumption of raw apples or peers in the last 48 h (no vs yes)					−0.31 ± 0.23	0.177				
Consumption of cooked apples or peers in the last 48 h (no vs yes)									**-0.51 ± 0.23**	**0.027**
Consumption of cereals in the last 48 h (no vs yes)							**0.46 ± 0.16**	**0.005**		

Table 8
P50 (in µg/L) reported from worldwide HBM studies focused on children.

Years of collection	Belgium 2016	Belgium 2008-2009	Germany 2001-2002	Spain 2009–2010	France 2009-2012	Norway 2012	Canada 2007-2009	US 2007-2008	US 2009–2010
N	206-240	210	369	125	245	54	981-1036	385	386
Age (yrs)	9-12 yrs	14-15 yrs	0-17 yrs	6-11 yrs	6 yrs	6-12 yrs	6-11 yrs	6-11 yrs	6-11 yrs
Cohort	Present study	FLEHS II	GeRES IV	from Valencia	PELAGIE	from Oslo	CHMS	NHANES	NHANES
References	Present study	Schoeters[a]	Becker[b]	Roca[c]	Glorennec[d]	Cequier[e]	Oulhote[f]	CDC[g]	CDC[g]
Organophosphorus metabolites (µg/l)									
DMTP	1.0	5.8	7.9	< 0.4	–	5.3	2.5	3.5	–
DMDTP	< 0.5	< 1	< 0.1	< 0.4	–	< 0.6	< 0.3	< 0.5	–
DEP	1.8	2.5	3.3	2.3	–	3.8	3.3	< 0.4	–
DETP	< 0.5	< 1	1.1	< 0.4	–	0.4	< 0.6	< 0.6	–
DEDTP	< 0.5	< 2	< 0.1	< 0.4	–	< 0.1	< 0.3	< 0.4	–
TCPY	3.9	–	–	3.4	–	–	–	–	1.5
Pyrethroid metabolites (µg/l)									
3-PBA	1.0	–	0.3	< 0.8	0.02	–	0.2	–	0.5
c-DCCA	< 0.5	–	0.1	< 0.8	0.09	–	0.1	–	–
t-DCCA	0.7	–	0.2	< 0.4	0.22	–	< 0.15	–	< 0.6
4-F-3-PBA	< 0.1	–	< 0.1	< 0.2	< 0.003	–	0.2	–	< 0.1

[a] Schoeters et al., 2011.
[b] Becker et al., 2006.
[c] Roca et al., 2014.
[d] Glorennec et al., 2017.
[e] Cequier et al., 2017.
[f] Oulhote and Bouchard, 2013.
[g] CDC, 2019.

global exposure. Similar conclusion was reported by Galea et al. (2015) who did not observe, during some captan spray events, increased levels of THPI in the urine of UK adults and children.

4.4. Predictors of exposure

Biting nails, household income, and father's educational level were highlighted by the statistical regression as predictors of exposure for metribuzin, while living in Oupeye and often washing or peeling fruits before consumption seemed to statistically decrease the detection frequencies of THPI in the children's urine. Since metribuzin was not detected in the air whatever the site considered, other sources of exposure than outdoor air should occur. Because metribuzin was not found in any of the 3873 food samples collected during the Belgian's pesticide monitoring program in 2014 (FASFC, 2019), and very rarely measured above 2 ng/l in drinking water during the yearly internal controls recorded by the Walloon Region (unpublished data), neither food intake nor drinking water seemed to be a significant pathway of exposure. On the other hand, metribuzin was statistically more often detected in the urine of children who used to bite their nails. This could suggest an intake through hand-to-mouth behavior, and thus a contamination of either domestic dust or outdoor soil particles. Note that the number of positive samples was quite low thus questioning the power of the statistical model.

For OP and pyrethroid metabolites, the main predictors of exposure brought out by the statistical analyses were the consumption of grey bread, the indoor use of pesticides (for pyrethroids), and the presence of carpets at home (for DEP only), confirming for this latter that indoor dust contamination could also represent a non negligible pathway of OP pesticide exposure (Shalat et al., 2003). Unexpectedly, all food items recently eaten other than grey bread were not associated with increasing levels of OP or pyrethroid metabolites although some parents, like chlorpryrifos, have been positively detected in some Belgian food, such as in infusions, fruits or vegetables (FASFC, 2019). Moreover, the proportion of organic fruits and vegetables usually consumed seemed to not affect the levels of urinary pesticide biomarkers, but one should keep in mind that a large majority of children (85–87 %) reported to usually eat less than 50 % of organic food thus leveled the potential impact of such diet in the present study. The main finding is probably the very low R^2 obtained for all statistical regressions meaning that the main sources of exposure were missed and/or too many covariates were included in each model. Nevertheless, the increased urinary levels of t-DCCA and 3-PBA with the indoor use of pesticides are consistent with other studies carried out in France (Glorennec et al., 2017), Canada (Oulhote and Bouchard, 2013), United States (Bradman et al., 2015; Lu et al., 2006b) or China (Wang et al., 2016), questioning the importance of the diet contribution to pyrethroid exposure compared to indoor residential exposure for children (Trunnelle et al., 2014).

Some strange associations between urinary biomarkers and the consumption of food were quite difficult to interpret, such like the negative associations with the consumption of exotic or stone fruits, of cereals or juice, or the drinking of tap water. Similarly, the different detection frequencies of DETP and DMDTP respectively for children living in Gembloux and in Liege compared to Oupeye remained unexplained. All these statistically significances would likely due to confounding factors or to hazardous.

4.5. Strengths and limitations of the study

This study was the first assessing the Belgian children's exposure to a wide range of currently used pesticides through simultaneous human biomonitoring and ambient air analysis. Its strengths come from the concomitant analyses of environmental and biological samples, the fair number of participants, their representativeness in terms of gender and age, the high quality and reliability of the produced analytical data, and the use of statistical models using multivariate regressions incorporating among others answers to a 48 h recall questionnaire. Nevertheless this study also suffers from some limitations. Firstly, urinary levels of pesticide metabolites such like DAP or pyrethroid metabolites have been demonstrated to be subject to high within-individual variability resulting in poor reliability of a single spot urine sample to characterize individual child exposure (Attfield et al., 2014; Cequier et al., 2017; Egeghy et al., 2011; Griffith et al., 2011; Morgan, 2012). Moreover, we tried to study the potential impact of pesticide in air on urinary biomarkers although the levels in air were averaged over 2 weeks while the urinary biomarkers used to reflect the exposure from few hours to one day. Secondly, the recruitment strategy could induce a bias in the representativeness of the studied population because

voluntary based. The results of the statistical analysis should also be cautiously interpreted because of the high amount of information gathered and introduced in the regression models likely resulting in some spurious associations (Riederer et al., 2008), because the collection of these information was driven by a priori assumptions on sources of exposure and thus could lead to the miss of some confounding factors yet unknown, and finally because a wide variety of answers were collected from the 48 h recall questionnaire compelling us to arbitrarily group some food items into more general categories. Thirdly the metabolite levels measured would not only reflect the exposure to the parent pesticides but also the exposure to environmental degradation products generally less toxic (Barr, 2008; Chen et al., 2012; Sudakin and Stone, 2011), thus could result in an overestimation of the exposure risk assessment. And finally, the most relevant limitation would likely be the attempt to assess pesticide exposure by measuring some unmetabolised compounds which would probably not be the best biomarker.

5. Conclusion

This study provides the first data on the exposure to several currently used pesticides of Belgian children living in Wallonia. Concomitant measures of a wide range of pesticides in ambient air and urine were carried out to assess the internal dose of children aged from 9 to 12 years old, to study the contribution of inhalation to the overall exposure, and to identify some predictors of exposure for these children. Despite several limitations related to HBM studies, the nature of the samples and the biomarkers monitored, the obtained results allowed to highlight a ubiquitous exposure to pyrethroid and OP pesticides for Belgian children, a probable negligible contribution of inhalation compared to other routes of exposure, the effect of consuming grey bread, having carpets at home and using pesticides inside the house on the children's internal dose of some pesticides. However, the coefficients of determination of the statistical regressions were small, suggesting that the main sources of exposure were not taken into account and should therefore be still investigated.

Ethical approval

This protocol was approved by the Hospital Faculty Ethics Committee of the University of Liege (B707201627617).

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2020.04.020.

Details on materials and methods for air and urine analyses, including extraction, chromatographic conditions and mass spec parameters.

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