



Exposure of Belgian children to multiple classes of currently used pesticides

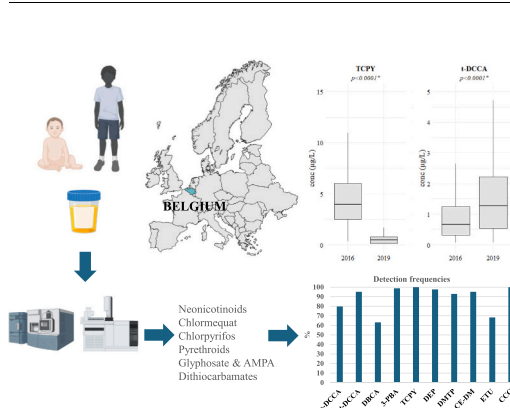
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HIGHLIGHTS

- Exposure to currently used pesticides was studied in a Belgian children population
- Chlormequat and neonicotinoids were detected in more than 95% of the samples
- Urinary levels of chlorpyrifos metabolites decreased between 2016 and 2019
- Exposure to pyrethroid insecticides seemed to slightly but significantly increase
- More than 2 out of 3 Belgian children are exposed to ethylene bis-dithiocarbamates

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Human biomonitoring
Pesticides
Children
Urine
Exposure

ABSTRACT

Pesticides have been extensively used worldwide for agricultural, public health, veterinary or domestic purposes. Because children are among the most vulnerable and exposed populations to pesticides, the monitoring of their exposure is of primary importance. However, only few currently used pesticides are usually included in biomonitoring studies, while for a large number, data are sparse or inexistant. To partly fill this gap, several classes of currently used pesticides, namely pyrethroids, organophosphorus (through dialkylphosphates and tri-chloropyridinol), neonicotinoids, dithiocarbamates (through ethylenethiourea), chlormequat chloride and glyphosate were measured in the urine of 84 Belgian children aged from 2 to 15 years and recruited in 2019. Most of the pyrethroid metabolites studied showed detection frequencies ranging between 63 and 99% with median concentrations from 0.36 to 1.54 µg/L, while trichloropyridinol and diethylphosphate (respectively specific and common metabolite to several organophosphorus including chlorpyrifos) were the most frequently measured among organophosphorus in 100% and 98% of the samples (P50 = 1.25 and 1.83 µg/L). Among neonicotinoids, acetamiprid desmethyl and imidacloprid metabolites (hydroxylated and olefin) were the most abundant confirming the relevance of their measurement compared to the corresponding parents. Ethylenethiourea and chlormequat chloride were positively detected in most samples (68% and 100% respectively) with median levels of 0.40 µg/L and 6.7 µg/L demonstrating the widespread exposure of the Belgian children, while glyphosate and aminomethylphosphonic acid were detected in 25% and 19% of the samples confirming the low levels usually found in urine. To our knowledge, this is the first study reporting biomonitoring data of neonicotinoid, ethylenethiourea and chlormequat chloride in a Belgian population.

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1. Introduction

Pesticides have been used since ancient times to struggle against pests mainly within the field of agriculture to protect crops among others from harmful insects, weeds, mold, bacteria, nematodes, but also for public health protection for instance by controlling disease vector insects, for veterinary purposes (use on domestic pets), for indoor or outdoor domestic uses, for the protection of wood building materials, etc. With the modernisation of the civilizations, natural mineral substances such as sulphur or arsenic, or plant-based substances like rotenone or aconit have been supplanted by synthetic chemicals within the 20th century and the development of organochlorine pesticides. Since then, the pesticide market has been continually evolving with regular shift to new chemicals as an adaption to new regulations implemented (Cequier et al., 2016; Donley, 2019; Shekhar et al., 2024). Although currently used pesticides are supposed to exhibit lower acute toxicity to non-targeted species including Human than their historical predecessors (e.g. organochlorines), their extensive uses have led to the ubiquitous contamination of the environment and evidence of potential chronic adverse effects on Human health and biodiversity have been growing. Long term exposure has been associated among others with neurological disorders, reproductive impairments, developmental issues, endocrine disruption, hepatotoxicity, or cancers (Cimino et al., 2017; Han et al., 2018; Saillenfait et al., 2015; Shekhar et al., 2024; Zhang and Lu, 2022). Children are of particularly susceptibility to their adverse health effects because of the different metabolic rate compared to adults, the immaturity of the blood-brain barrier, and the critical stages of organ development especially the brain (Au, 2002; Osaka et al., 2016; Oulhote and Bouchard, 2013). Moreover, they used to experience higher pesticide exposure due to their specific diet favouring fresh food more susceptible to containing pesticides (fruits and fruit juices, milk, water), the higher amount of food and water ingested compared to their body weight, and their particular behaviours (hand-to-mouth activities, time spent playing on the floor) increasing the unintentional ingestion of contaminated dust or items (Aprea et al., 2000; Au, 2002; Roberts and Karr, 2012). Therefore the assessment of children exposure to pesticides is of primary importance.

Human biomonitoring consisting the measurement of exposure markers in human biological samples has been become these past decades an essential tool for the assessment of chemical exposure, with the main advantage of integrating all sources and routes of exposure (Jones, 2020; Needham et al., 2005; Vorkamp et al., 2021). Some currently used pesticides have already been extensively studied within biomonitoring surveys such as organochlorine, organophosphorus or pyrethroid insecticides (Glorennec et al., 2017; Govarts et al., 2023; Health Canada, 2024; Katsikantami et al., 2019; Li et al., 2020; Pirard et al., 2020). However, for some others (e.g. glyphosate, neonicotinoids), the exposure has only begun to be investigated very recently and even if data are currently emerging, they remain scarcely available (Buekers et al., 2022a; Wrobel et al., 2025). Finally, the internal exposure for a numerous of pesticides is still undocumented (e.g. trazines, triazoles, carbamates, thiocarbamates, benzofurans, chloroacetamides, etc). The main difficulties encountered would include among others the lack of available toxicokinetic data ensuring the selection of the proper markers, the limited number of reference materials, or the analytical challenge for the reliable measurement of chemicals showing a wide range of different physicochemical properties.

In the present study, we quantitatively measured makers of exposure of organophosphorus, pyrethroids, glyphosate, neonicotinoids, dithiocarbamates, and chlormequat chloride in the urine of 84 Belgian children recruited in 2019. The aims were, on one hand, to increase the existing biomonitoring data on well documented pesticides and to study time and geographical trends as well as the impact of regulations recently implemented in Europe, and on the other hand, to explore the children exposure to emerging pesticides for which data are still scarce or even non-existent.

2. Materials and methods

2.1. Study population

During May 2019, 84 Belgian children aged between 2 and 15 years old were recruited through one of the biggest Belgian non-profit consumer protection organizations. Parents among members and workers of the organization were invited to participate to this study via website and internal communication supports, by collecting a random sport urine sample of their child in a previously distributed polypropylene vessels, answering to a small questionnaire about some general dietary habits and domestic uses of pesticides. The inclusion criteria were: living in Belgium at the time of the recruitment and being aged under 18 years old. The urine samples were aliquoted in glass tubes before storage at -20°C until analysis. This study and protocol were performed in compliance with relevant laws and institutional guidelines and have been approved by the Hospital Faculty Ethics Committee of the University of Liege (2019/144) in June 2019. The privacy rights of children have been observed and informed consent was obtained for experimentation with human subjects and signed by their respective parents. The individual results were not sent to the parent of the participant, but global results were publicly available on the website of the consumer protection organization.

2.2. Analytical procedures

The metabolites of pyrethroid pesticides included *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). The exposure to organophosphorus pesticides was assessed by measuring 5 dialkylphosphate metabolites (DAP), namely, dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), and trichloropyridinol (TCPY) as specific metabolite of chlorpyrifos (methyl and ethyl). Glyphosate and chlormequat chloride (CCC) were analysed as parent pesticides, while aminomethylphosphonic acid (AMPA) was measured as the degradation product of glyphosate. Ethylenethiourea (ETU) was measured to evaluate the exposure to ethylene bis-dithiocarbamates (EBDC) such as mancozeb, maneb, or zineb. Finally, the neonicotinoid pesticides and related metabolites investigated were clothianidin (CLO), clothianidin desmethyl (CLO-DM), acetamiprid (ACE), acetamiprid desmethyl (ACE-DM), imidacloprid (IMI) and two imidacloprid metabolites: 5-hydroxy-imidacloprid (5OH-IMI) and imidacloprid-olefin (IMI-OLE).

For pyrethroids, trichloropyridinol, and dialkylphosphates, the lab has successfully participated to the German External Quality Assessment Scheme (G-EQUAS) on a yearly basis (twice a year). Note that at the time of the analysis, glyphosate was not included in the G-EQUAS, and no other QA/QC scheme was organized including the other targeted pesticides (for ETU, CCC nor neonicotinoids).

2.2.1. Analysis of pyrethroids and trichloropyridinol

The measurement of pyrethroid metabolites and TCPY was previously detailed elsewhere (Pirard et al., 2020). Briefly, urine samples (3 mL) were enzymatically hydrolysed overnight, diluted in a sodium dihydrogen phosphate solution (0.2 M), and extracted using diethyl ether. After evaporation, the extract was derivatized with *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) at 70°C for 30 min, and injected into a gas chromatograph coupled to tandem mass spectrometer (GC-MS/MS) equipped with a 30 m HP5-MS column (0.25 mm $\times$ 0.25 μm , Agilent). The mass spectrometer operated in electronic impact (EI) using 2 multiple reaction monitoring (MRM) transitions for each compound to ensure specificity. Labelled isotopes of *t*-DCCA, TCPY and 3-PBA were used for quantification. Each sequence included a 7-point calibration curve (synthetic urine spiked with increasing levels from 0.1 $\mu\text{g/L}$ to 20 $\mu\text{g/L}$) extracted as real samples, 1 reagent blank, 2

quality controls (QC) consisting in materials from previous German External Quality Assessment Scheme (G-EQUAS) from Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg, and 40 unknown samples.

2.2.2. Analysis of dialkylphosphates

The DAP metabolites of organophosphorus pesticides were analysed according to the procedure fully described in Pirard et al. (2020). Briefly, after acidification, urine samples were extracted using Oasis WAX cartridge (from Waters), before being derivatized using chloriodopropane (CIP) (65 °C for 2 h). The derivatized extracts were evaporated nearly to dryness, reconstituted in nonane, and injected into GC-MS/MS operating in positive chemical ionization mode (PCI). The quantification was performed by isotope dilution using for each DAP its deuterated labelled homologue. The 6-point calibration curve was built in synthetic urine and extracted as real samples, along with 1 reagent blank, 2 QC from previous G-EQUAS, and 40 unknown samples.

2.2.3. Analysis of glyphosate

Internal standard (glyphosate $-1,2-^{13}\text{C}_2^{15}\text{N}$ and AMPA $^{13}\text{C}^{15}\text{N}_2\text{D}$) was added to 1 mL of urine, 3 mL of 9-fluorenylmethyl chloroformate (FMOC) dissolved in acetone (20 g/L), and 1 mL of disodium tetraborate solution (5%), and let derivatize at room temperature overnight in the dark. The acetone was then evaporated under a gentle stream of nitrogen, and 5 mL of ethyl acetate were added for the extraction of unwanted derivatization reaction residues (shaking for 10 min, centrifugation at 2500 rpm for 5 min) before being discarded. Then the remaining aqueous phase was acidified using 100 μL of concentrated chlorohydric acid and extracted twice using 5 mL of diethyl ether. The combined organic layers were evaporated until dryness under a gentle stream of nitrogen at 40 °C, and reconstituted in the initial mobile phases (80/20 aqueous/organic) for injection (10 μL) on a liquid chromatograph (UHPLC Acquity, Waters) coupled to a mass spectrometer (Xevo TQ-S, Waters) equipped with an Acquity BEH C18 (50 mm $\times$ 2.1 mm, 1.7 μm , Waters) kept at 40 °C, with a constant flow of 0.4 mL/min. The mobile phases consisted in a 5 mM ammonium acetate solution adjusted to pH 9 as aqueous phase (A), and acetonitrile as organic phase (B). The initial gradient started with 10% B for 1 min, then increased to 25% in 4 min, increased again to 100% in 0.5 min and maintained for 1 min before the return to initial conditions. The mass spec operated in positive electrospray (ESI+) with the capillary voltage set at 3 kV, the source and desolvation temperatures set at 150 °C and 350 °C, respectively. Nitrogen was used as desolvation gas at 800 L/min while argon was used as collision gas at a flow of 0.15 mL/min. The MRM transitions 392.0 > 87.6 and 392.0 > 213.8 were monitored for quantification and qualification of glyphosate, respectively, 334.0 > 178.8 and 334.0 > 116.0 were selected for AMPA, whereas 395.0 > 118.9 and 395.0 > 268.9, and 338.0 > 178.8 were monitored for internal standards of glyphosate and AMPA. Each sequence included a 6-point calibration curve built in synthetic urine and extracted as real samples (from 0.05 $\mu\text{g/L}$ to 10 $\mu\text{g/mL}$), a reagent blank, 2 QC from previous G-EQUAS, and 40 unknown samples.

2.2.4. Analysis of chlormequat chloride (CCC)

The measurement method of chlormequat chloride (CCC) in urine was based on an application note dedicated to diquat and paraquat determination in drinking water (Van Tran et al., 2019). After the addition of internal standard (CCC-D₄), 3 mL of urine were buffered with 3 mL of a 400 mM solution of dipotassium phosphate/monopotassium phosphate (2/1 w/w) adjusted to pH 7, and loaded to an Oasis WCX SPE (3 cc, 60 mg) previously conditioned with methanol and phosphate buffer. The cartridge was then washed with 0.7 mL of an ammonium formate solution (20 mM, adjusted to pH 8) and 0.7 mL of methanol, before being eluted by 2-fold 1 mL of a mixture of acetonitrile/isopropanol (1/1 v/v) acidified with 10% of formic acid. The extract collected was evaporated until dryness under a gentle stream of nitrogen

at 40 °C and reconstituted with 100 μL of mobile phase in the initial proportions (90% B). Ten microliters of the extract were injected on a LC-MS/MS (UHPLC Acquity coupled to Xevo TQ-S, Waters) equipped with an Acquity UPLC BEH HILIC (2.1 mm $\times$ 100 mm $\times$ 1.7 μm , Waters) maintained at 30 °C. The mobile phase consisted in an ammonium formate solution (25 mM) adjusted to pH 3 (A) and 0.1% formic acid in acetonitrile (B). The flow was set at 0.4 mL/min. The following gradient was applied: 90% B held for 1 min, then decreased to 50% B in 4 min and maintained for 1 min. The come back to the initial conditions and the equilibration were achieved in 3 min. The final determination was performed in ESI+ using the following MRM transitions: 122.0 > 58.2 and 122.0 > 62.7 as quantification and qualification traces, respectively for native CCC, and 126.0 > 58.3 and 66.8 as quantification and qualification traces for deuterated CCC. The source temperature was set at 140 °C, 13.33 mL/min of nitrogen was used as desolvation gas at 400 °C, while 0.2 mL/min of argon were introduced in the collision cell. The quantification was performed using an 8-point calibration curve consisting in synthetic urine spiked at increasing levels from 0.05 $\mu\text{g/L}$ to 50 $\mu\text{g/L}$ and extracted as real samples. In addition, 1 reagent blank and home-made QC (synthetic urine spiked at 1 $\mu\text{g/L}$) were analysed with the unknown samples.

2.2.5. Analysis of ethylenethiourea

The analytical procedure to measure ETU was based on followed by LC-MS/MS analysis and was strongly inspired from (Colosio et al., 2002). After the addition of internal standard (consisting in ETU-D₄), 5 mL of urine were loaded on a Chem Elut cartridge (diatomaceous earth) and let equilibrate for 5 min. Elution was carried out using twice 8 mL of dichloromethane. The combined dichloromethane phases were evaporated under a gentle stream of nitrogen at 40 °C and reconstituted with 100 μL of water. Ten microliters of the final extract were injected on a LC-MS/MS (UHPLC Acquity coupled to Xevo TQ-S, Waters) equipped with an Acquity UPLC HSS T3 (2.1 mm $\times$ 100 mm $\times$ 1.8 μm , Waters) heated at 40 °C and eluted at a constant flow of 0.4 mL/min. Methanol (B) and 0.1% formic acid in water (A) were used as mobile phases in the following proportions: 100% A for 1.5 min, then A decreased to 75% in 1.5 min. The column was then rinsed with 100% B for 1 min before the return to the initial conditions. The Xevo-TQS was operated in ESI+, at 150 °C and 350 °C for source and desolvation temperatures respectively, using nitrogen at 800 L/h and argon at 0.20 mL/min as desolvation and collision gas, respectively. The following MRM transitions were monitored: 102.95 > 44.1 and 102.95 > 86.0 for ETU, and 107.0 > 48.1 for ETU-D₄ for quantification and qualification respectively. The unknown samples were simultaneously analysed using a calibration curve (6 points) built in synthetic urine fortified at increasing levels from 0.1 to 50 $\mu\text{g/L}$ and extracted as real samples, 1 reagent blank, and 2 home-made QC consisting in real urine (from anonymous donors) spiked at low levels (0.5 $\mu\text{g/L}$ and 1 $\mu\text{g/L}$). At the time of the analysis, no external QC was available (i.e. G EQUAS).

2.2.6. Analysis of neonicotinoids

The internal standard solution, containing imidacloprid-D₄, clothianidin-D₄, acetamiprid-D₃, acetamiprid desmethyl- $^{13}\text{C}_4$, imidacloprid olefin- $^{13}\text{C}^{15}\text{N}_2$, and 5-hydroxy-imidaclopride- $^{13}\text{C}^{15}\text{N}_2$, was added to 3 mL of urine. Then the sample was enzymatically hydrolysed overnight at 40 °C in an ammonium acetate buffer (0.1 M, pH 5) using beta-glucuronidase and sulfatase from Helix Pomatia. An acidification of the hydrolyzate was performed using 200 μL of formic acid (2%) prior a double liquid-liquid extraction with 2 $\times$ 3 mL of ethyl acetate. The combined organic layers were then evaporated to dryness under a gentle stream of nitrogen at 40 °C and reconstituted with 100 μL of 50/50 (v/v) 0.1% formic acid in water/0.1% formic acid in methanol solution. The final determination was carried out using a LC-MS/MS (UHPLC Acquity coupled to Xevo TQ-S, Waters) equipped with a Kinetex biphenyl column (2.1 mm $\times$ 100 mm $\times$ 1.7 μm , Phenomenex) heated at 40 °C. A 0.4 mL/min flow of the mobile phase consisting in 0.1% formic acid in water

(A) and 0.1% formic acid in methanol (B) was applied using the following gradient: 80% A held for 1.5 min, then decreased linearly to 67% in 7.5 min, decreased again to 60% in 1 min, and finally decreased to 0% in 0.1 min and held for 2 min before the coming back to the initial conditions for 1.5 min. The mass spec was operated in ESI+, with the source and desolvation temperatures set at 150 °C and 350 °C, respectively. Nitrogen (800 L/h) and argon (0.20 mL/min) were used as desolvation and collision gas, respectively. The MRM transitions monitored were detailed in the supplementary material (Table S1). In addition to the unknown samples, a 6-point calibration curve built in synthetic urine fortified at increasing levels from 0.05 to 10 µg/L was extracted as well as 1 reagent blank, and 2 home-made QC consisting in real urine (from anonymous donors) spiked at low levels (0.8 µg/L and 2.5 µg/L). At the time of the analysis, no external QC were available (i.e. G EQUAS). Each metabolite was quantified using its own labelled standard except clothianidin desmethyl for which clothianidin-D₄ was used.

2.3. Statistical analyses

Statistical analyses were performed using Excel 2013 (Microsoft, Redmond, WA) and RStudio (version 2023.09.1, R Project for Statistical Computing). When a pollutant was measured below the limit of quantification (LOQ) in a sample, its concentration was replaced by the LOQ divided by two (Hornung and Reed, 1990). Pesticides with a detection frequency between 20 and 70% were dichotomised (detected versus non-detected) and treated as a binary variable. Molecules with a detection frequency below 20% were not included in statistical analysis. Due to the right-skewed distribution of urinary pesticide concentrations, these concentrations were log-transformed (in basis 10) prior to analysis. First, a univariate linear (or logistic for dichotomized pollutants) regression model was computed to assess the relationship between the outcome variable (pesticide contamination) and each of the other variables. Then, a multivariate linear (or logistic) regression model was built for each molecule. The initial model incorporated all the variables associated with the outcome in the individual model with a significance level of $p < 0.2$, and backward selection was applied. Only variables with a significance level of <0.1 were kept in the final model.

Mann-Whitney (for continuous variables) and Chi square (for dichotomized variables) tests were used to compare the pesticide concentrations measured in the urine of children recruited in 2016 in Wallonia (Pirard et al., 2020) with those obtained within the present study. The p -values were considered as significant when below 0.05.

3. Results and discussion

All the results were treated, discussed and compared with the literature based on volumetric concentrations thus independently of the hydration status, because the dilution correction is still controversial within pesticide exposure assessment (Barr et al., 2004; Mage et al., 2004; Roggeman et al., 2022), and because the biomonitoring results with or without dilution adjustment have been observed to be not drastically different (Wang et al., 2021). It has been indeed demonstrated that the intraindividual variability for such compounds with short elimination half-lives would be higher than the concentration variations due to dilution sample status and thus would be not highly improve by creatinine or specific gravity adjustment (Egghy et al., 2011; Mage et al., 2004; Roggeman et al., 2022). However, since several major HBM studies included a dilution adjustment (through creatinine or specific gravity measurement), the conclusions based on comparison with the literature should be drawn with cautious.

3.1. Study population

The characteristics of the children recruited are gathered in Table 1. The mean age was 8.9 $\pm$ 3.8 years old, with the 6–11-year-old group a

little bit more represented. The children were roughly equally distributed between girls and boys (45 and 39 respectively), with a higher proportion of residents from Flanders (53.6%), Wallonia being under-represented (17.9%). A fifth of them was reported to bite their nails, 3 children had at least one parent working with pesticides, and 52 were expected to never be exposed to pesticides at home through domestic uses (indoor nor in the garden). Most of them used to drink tap water, eat sometimes organic vegetables or fruits, while all of them used to always or often wash or peel fruits or vegetables before consumption. The consumption of some specific foods within the last 48 h are also presented in Table 1. This convenience child population was intended to provide a picture of the pesticides exposure within Belgium but couldn't be considered as representative population. Although the socio-economical status could be an important factor influencing the pesticide exposure of the children, this information was unfortunately not recorded.

Table 1
Characteristics of the studied population.

	N (%)		N (%)
<i>All</i>	84 (100%)	<i>Dietary habits</i>	
<i>Age</i>		Organic fruits/vegetables	
2–5 years	21 (25.0%)	Always	6 (7.1%)
6–11 years	38 (45.2%)	Often	25 (29.8%)
12–15 years	25 (29.8%)	Sometimes	42 (50.0%)
<i>Sex</i>		Never	11 (13.1%)
Girls	45 (53.6%)	Wash and/or peel vegetables/fruits	
Boys	39 (46.4%)	before consumption	
<i>Region</i>		Always	41 (48.9%)
Brussels	24 (28.6%)	Often	43 (51.1%)
Flanders	45 (53.6%)	Rarely or never	0 (0.0%)
Wallonia	15 (17.9%)	<i>Consumption in the last 48 h</i>	
<i>Nail biting</i>		Organic vegetables	
Yes	18 (21.4%)	Yes	32 (38.1%)
No	66 (78.6%)	No	52 (61.9%)
<i>Recent pesticide use (by parents)</i>		Organic fruits	
Occupational use	3 (3.6%)	Yes	26 (30.9%)
Outdoor domestical use	25 (29.8%)	No	58 (69.1%)
Indoor domestical use	11 (13.1%)	Grey bread or whole grains	
Never	52 (61.9%)	Yes	57 (67.9%)
<i>Pets at home</i>		No	27 (32.1%)
Yes	44 (52.4%)	Stone fruits	
No	39 (46.4%)	Yes	18 (21.4%)
No answer	1 (1.2%)	No	66 (78.6%)
<i>Drinking of water</i>		Exotic fruits (incl. Banana, kiwi, etc.)	
Tap water	45 (53.6%)	Yes	46 (54.8%)
Bottled water	17 (20.2%)	No	38 (45.2%)
Both	22 (26.2%)		

3.2. Urinary levels of pyrethroid and organophosphorus insecticides

Table 2 gathers the frequency detections ($N > \text{LOQ}$), the geometric means (GM), the percentiles 5, 25, 50, 75 and 95, and the minimum and maximum levels measured for each pesticide markers. 3-PBA and t-DCCA were the most abundant pyrethroids measured in the urine of our population with a median concentration of 1.54 and 1.40 $\mu\text{g/L}$ respectively (5.64 and 7.16 as respective P95), while 4F-3-PBA was only occasionally detected (3 positive samples out 84). It wasn't unexpected since 3-PBA and DCCA are common metabolites of several pyrethroids whereas 4F-3-PBA and DBCA are specific metabolite to respectively cyfluthrin and deltamethrin. Among organophosphorus metabolites, DMTP, DEP and TCPY were measured at higher levels, with median concentrations of 2.11, 1.83 and 1.25 $\mu\text{g/L}$, respectively. The other DAPs were detected in less than 30% of the samples, DEDTP never being measured above the LOQ (0.5 $\mu\text{g/L}$).

Last decades, an increasing number of studies have investigated pyrethroid and organophosphorus exposure through human biomonitoring, especially in Europe driven among other by projects such as COPHES/DEMOCOPHES or HBM4EU (reviewed by Andersen et al., 2022), allowing the comparison of the results and thus bringing some information about geographical and temporal variations, about specific exposure of different populations, etc. The Tables 3 and 4 gather the medians and P95 (expressed in $\mu\text{g/L}$) of respectively urinary pyrethroid and organophosphorus metabolite results reported for children in the most recent large-scale studies carried out in different parts of the world, with emphasis on Europe. Pyrethroid levels observed in European studies are rather comparable even if some differences sometimes occurred with, for instance, lower concentrations reported for 3-PBA in Slovenia, Italy, Poland or for the Spanish INMA cohorts. On the other hand, the levels measured in North American populations seemed lower than in Europe. This would likely be the result of the historical use of

different insecticide families as insect repellents between both continents, DEET being widely used for prevention of tropical mosquito and tick bites in America while in Europe pyrethroids like prallethrin, allethrin or transfluthrin would be preferred as domestic insecticides, even if DEET is not banned but regulated under the European Union's Biocidal Products Regulation. Contrariwise, urinary levels of organophosphorus pesticide metabolites varied more within the European countries. Even if pesticide regulations in terms of authorization uses or maximum residue levels allowed in food are enforced by European directives, thus similar to all European member states, some variations in the population exposure between countries could be expected depending on the specific diet (i.e. Mediterranean diet vs continental diet), the agriculture activities, the socioeconomic status, etc. Moreover, despite the harmonization efforts currently being made (Andersen et al., 2022), some differences in study design still occurred potentially impacting results (i.e., population recruitment, urine samples collected, storage, analytical method used, limits of detection as well as the way how the values below were treated, etc). From the Table 4, it's not obvious to catch any clear trend. While DAP levels in the present study seem to be lower than those from Northern population (Denmark and Norway) recruited before 2012 (Andersen and Dalsager, 2021; Cequier et al., 2017; Mørck et al., 2016), the levels reported more recently from French (Tagne-Fotso et al., 2023) or Spanish children (Pardo et al., 2020) were respectively higher and lower. For TCPY, urinary levels reported varied even more widely from very low levels in samples from Spain or Italy to high levels in samples from Belgium (FLEHS IV) and Cyprus, independently of the year of collection or of the sampling period (Bravo et al., 2019; HBM4EU dashboard; Schoeters et al., 2022). Note that our recruitment was performed exclusively in May. Although the impact of the sampling season on pesticide exposure remains unclear and reported results seem inconsistent from one study to another (Osaka et al., 2016; Šulc et al., 2022), some differences according to the sampling period could be

Table 2

Descriptive statistics: limits of quantification (LOQ), detection frequencies ($N > \text{LOQ}$), geometric means (GM), percentiles (P5 to P95), and the concentration ranges measured (min-max). All concentrations are expressed in $\mu\text{g/L}$ (except detection frequencies in %).

	LOQ ($\mu\text{g/L}$)	$N > \text{LOQ}$ (%)	GM ($\mu\text{g/L}$)	P5 ($\mu\text{g/L}$)	P25 ($\mu\text{g/L}$)	P50 ($\mu\text{g/L}$)	P75 ($\mu\text{g/L}$)	P95 ($\mu\text{g/L}$)	min-max ($\mu\text{g/L}$)
<i>Pyrethroid insecticides</i>									
c-DCCA	0.15	79.8	0.36	0.08	0.18	0.36	0.78	1.76	<0.15–4.12
t-DCCA	0.15	95.2	1.26	0.18	0.68	1.40	3.03	7.16	<0.15–10.1
DBCA	0.30	63.1	0.42	<0.30	<0.30	0.47	0.78	1.98	<0.30–3.14
4F-3-PBA	0.11	3.6	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11	<0.11–0.82
3-PBA	0.09	98.8	1.29	0.33	0.69	1.54	2.41	5.64	<0.09–7.42
<i>Organophosphorus insecticides</i>									
TCPY	0.08	100.0	1.14	0.27	0.70	1.25	1.85	2.89	0.17–9.21
DEP	0.50	97.6	2.12	0.67	1.24	1.83	2.88	11.69	<0.50–24.9
DETP	0.50	10.7	<0.50	<0.50	<0.50	<0.50	<0.50	1.10	<0.50–7.41
DEDTP	0.50	0.0	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	–
DMTP	0.50	92.9	2.12	<0.50	1.27	2.11	3.58	11.0	<0.50–12.1
DMDTP	0.50	29.8	<0.50	<0.50	<0.50	<0.50	0.50	0.60	<0.50–1.24
<i>Neonicotinoid insecticides</i>									
CLO-DM	0.05	4.8	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05–0.32
CLO	0.07	28.6	<0.07	<0.07	<0.07	<0.07	0.07	0.47	<0.07–2.86
5OH-IMI	0.10	46.4	0.13	<0.10	<0.10	<0.10	0.23	4.26	<0.10–12.4
IMI OLE	0.10	57.1	0.23	<0.10	<0.10	0.19	0.57	4.48	<0.10–15.5
IMI	0.10	10.7	<0.10	<0.10	<0.10	<0.10	<0.10	0.71	<0.10–3.47
ACE-DM	0.05	95.2	0.26	0.06	0.14	0.28	0.43	1.64	<0.05–2.83
ACE	0.10	19.8	<0.10	<0.10	<0.10	<0.10	<0.10	0.12	<0.10–0.44
<i>Dithiocarbamate fungicides</i>									
ETU	0.25	68.3	0.40	<0.25	<0.25	0.40	0.80	2.17	<0.25–12.7
<i>Plant growth regulators and herbicides</i>									
CCC	0.10	100.0	6.49	1.22	3.40	6.70	13.1	30.0	0.35–66.5
Glyphosate	0.08	25.0	<0.08	<0.08	<0.08	<0.08	<0.08	0.17	<0.08–1.13
AMPA	0.15	19.1	<0.15	<0.15	<0.15	<0.15	<0.15	0.36	<0.15–1.24

Table 3

Median concentrations and P95 in brackets (expressed in µg/L) reported for pyrethroid metabolites in several recent studies.

Country	Years	Age	N	c-DCCA	t-DCCA	DBCA	3-PBA	4-F-PBA	Ref.
Belgium (present study)	2019	2–15 yrs	84	0.36 (1.76)	1.40 (7.16)	0.47 (1.98)	1.54 (5.64)	<0.11 (<0.11)	
Belgium (Wallonia)	2016	9–12 yrs	240	<0.5 (2.01)	0.66 (4.29)	–	0.98 (5.33)	<0.11 (<0.11)	(1)
Belgium (FLEHS IV)	2017–2018	14–15 yrs	415	–	–	–	0.87 (2.77)	–	(2)
Belgium (Flanders)	2010–2015	6–8 yrs	133	0.54 (2.44)	1.11 (5.18)	0.54 (3.04)	1.72 (7.05)	–	(3)
The Netherlands (SPECIMEn)	2020	6–11 yrs	102	0.44 (1.75)	0.62 (2.72)	0.86 (3.84)	1.29 (4.81)	–	(3)
France (ESTEBAN)	2014–2016	6–17 yrs	499	0.32 (1.89)	0.18 (1.14)	1.16 (5.70)	1.03 (7.30)	<0.015 (0.09)	(4)
Slovenia (PHIME)	2016	7–8 yrs	168	–	–	–	0.40 (12.0)	–	(5)
Cyprus (ORGANIKO)	2017	10–11 yrs	177	0.61 (2.22)	0.93 (4.28)	0.60 (3.85)	1.93 (6.59)	<0.2 (<0.2)	(6)
Italy (PHIME)	2014–2015	7 yrs	199	–	–	–	0.56	<0.019	(7)
Spain (Valencia, BIOVAL)	2016	5–12 yrs	568	–	–	<1.25 (5.56)	1.63 (11.57)	<0.13 (<0.13)	(8)
Czech Republic (SPECIMEn)	2019–2020	4–12 yrs	110	Sum (c- & t-): 0.73 (3.23)	–	–	0.38 (1.57)	–	(9)
Poland (REPRO_PL)	2014–2019	7–9 yrs	399	–	–	–	0.44 (3.00)	<0.098 (0.11)	(10)
Turkey	2017–2018	3–6 yrs	186	<0.025	<0.025	–	0.07	<0.05	(11)
USA (NHANES)	2015–2016	3–5 yrs	504	–	<0.6 (7.20)	–	0.57 (7.50)	<0.10 (0.15)	(12)
USA (NHANES)	2015–2016	6–11 yrs	417	–	<0.6 (3.70)	–	0.57 (7.00)	<0.10 (0.15)	(12)
USA (NHANES)	2015–2016	12–19 yrs	402	–	<0.6 (5.60)	–	0.57 (8.00)	<0.10 (0.17)	(12)
Canada (CHMS)	2018–2019	3–5 yrs	512	0.12 (1.40)	0.24 (3.10)	0.04 (0.41)	0.39 (4.00)	<0.006 (0.53)	(13)
Canada (CHMS)	2018–2019	6–11 yrs	498	0.12 (2.70)	0.25 (5.20)	0.026 (0.23)	0.46 (5.90)	<0.006 (0.28)	(13)
Canada (CHMS)	2018–2019	12–19 yrs	499	0.14 (0.92)	0.24 (2.50)	0.024 (0.21)	0.38 (5.00)	<0.006 (0.05)	(13)
Ecuador (Espina cohort)	2016–2022	12–17 yrs	529	–	<0.60 (1.96)	–	0.37 (2.30)	–	(14)
China (SMBCS)	2016	7 yrs	454	2.19	0.16	–	3.13	–	(15)
Korea (KoNEHS)	2015–2017	3–5 yrs	570	–	–	–	0.97 (9.34)	–	(16)
Korea (KoNEHS)	2015–2017	6–11 yrs	887	–	–	–	1.29 (7.44)	–	(16)
Korea (KoNEHS)	2015–2017	12–18 yrs	904	–	–	–	1.12 (5.93)	–	(16)
New Zealand	2015–2017	5–14 yrs	501	–	1.30 (11.0)	<0.7 (<0.7)	1.60 (8.00)	<0.02 (0.38)	(17)

(1) Pirard et al., 2020; (2) Schoeters et al., 2022; (3) HBM4EU dashboard; (4) Tagne-Fotso et al., 2023; (5) Bravo et al., 2020; (6) Makris et al., 2022; (7) Bravo et al., 2019; (8) Pardo et al., 2020; (9) Sulc et al., 2022; (10) Bustamante et al., 2025; (11) Göi et al., 2023; (12) CDC (Center for Disease Control and Prevention), 2024; (13) Health Canada, 2024; (14) Parajuli et al., 2025; (15) Dai et al., 2025; (16) Jung et al., 2022; (17) Li et al., 2022

expected since some diet habits sometimes evolve with the season (i.e. eating more fresh fruits in summer) and thus could impact the pesticide exposure.

To investigate the temporal variation in the children exposure to pyrethroids and organophosphorus pesticides in Belgium, we compared the urinary levels of metabolites measured within the present study with those obtained during a previous study carried out in 2016 (Pirard et al., 2020), represented in the Fig. 1. During this previous study, 258 children aged from 9 to 12 years old and living in some Walloon localities were recruited via their school. All the details about the recruitment procedure, the selected Walloon localities, and the characteristics of the studied population were gathered elsewhere (Pirard et al., 2020).

A drastic and significant decrease was observed between 2016 and 2019 for TCPY levels and DETP frequencies of quantification (from 49.8% to 10.7%, $p < 0.0001$), both resulting from the metabolism of chlorpyrifos. This observation is consistent with the decrease in reporting frequency of chlorpyrifos in Belgian fruits and vegetables from 2014 to 2019 (AFSCA, 2022), likely related to a global decrease of chlorpyrifos levels in Belgian food, although the European decision of the non-renewal of the chlorpyrifos authorization was only implemented at the end of 2019, meaning few months after the present sample collection. A comparable decrease in TCPY urinary levels was already observed between 2010 and 2016 in Spain (F. Pardo et al., 2020). On the other hand, the levels of t-DCCA and 3-PBA seemed to slightly but significantly increase between 2016 and 2019 (borderline significance for 3-PBA), as well as DMTP levels. An increasing urinary levels of pyrethroids in children and adolescent with time were already observed earlier worldwide (Pardo et al., 2020; Lehmler et al., 2022; Norén et al., 2020), and could possibly be explained by a higher use of pyrethroids in replacement to organophosphorus and neonicotinoid insecticides which have seen the restrictions on indoor and/or outdoor uses gradually increased this last decade. However, this apparent trend should be interpreted with cautious because of the limited size of the present population, but also because both Belgian children populations were not fully similar. Indeed, the geographic distribution of the residence places was different with 100% of the children living in Wallonia within the 2016 study while most children resided in Flanders (54%) in the present

study, and lower levels of DAP (DMTP) were already previously observed in the urine of Walloon children vs Flemish adolescents (Pirard et al., 2020). Note that there are likely other differences between both populations (recruited in 2016 and 2019) such as the socio-economic status, the age, the recruitment timing and method, etc., which could influence the pesticide levels measured and thus maybe also explain partly the time-related difference.

3.3. Urinary levels of neonicotinoid insecticides

Conversely to pyrethroids and organophosphorus, data on neonicotinoid levels in urine have been poorly documented, available data mainly coming from Asia (Japan and China) which is the world's largest neonicotinoid user region, far ahead for USA or Europe (Simon-Delso et al., 2015). To the best of our knowledge, the only European countries where some data on children exposure have been produced were Poland and Ireland (Bustamante et al., 2025; Wrobel et al., 2025). Moreover, the exposure markers measured varied from one study to another, making the comparison even more difficult. Initially most studies measured the parent pesticides (imidacloprid, acetamiprid, thiacloprid, etc.) assuming that they are excreted unchanged in urine (Osaka et al., 2016; Ueyama et al., 2015; Wang et al., 2024; Zhao et al., 2022). An increasing number of studies suggested that for some neonicotinoids, such as acetamiprid or imidacloprid, metabolites are more suitable, while for some others (i.e. dinetofuran, clothianidine,) the parent substances would be recommended (Harada et al., 2016; Li et al., 2020; Suwannarin et al., 2024; Wrobel et al., 2022). Additionally, some analytical methods used in neonicotinoid biomonitoring did not include hydrolysis step while deconjugation by enzymatic hydrolysis seemed to be indicated and processed in some others (Ospina et al., 2019; Tu et al., 2023; Wrobel et al., 2025). Therefore, there are still uncertainties about the appropriate urinary markers to be measured, making any comparison difficult, and demonstrating the need of additional data to perform relevant harmonization. In the present study, the metabolites of acetamiprid and imidacloprid (ACE-DM, IMI-OLE and 5OH-IMI) were the markers the most frequently detected and the most abundant in the urine of children with a median level of 0.28 µg/L and 0.19 µg/L for

Table 4

Median concentrations and P95 in brackets (expressed in µg/L) reported for organophosphorus pesticide metabolites in several recent studies.

Country	Sampling years	Age (yrs)	N	DEP	DETP	DEDTP	DMTP	DMDTP	TCPY	Ref
Belgium (present study)	2019	2–15	84	1.83 (11.7)	<0.50 (1.10)	<0.50 (<0.50)	2.11 (11.0)	<0.50 (0.60)	1.25 (2.89)	
Belgium (Wallonia)	2016	9–12	240	1.8 (30.0)	<0.50 (3.50)	<0.50 (0.59)	1.01 (13.7)	<0.50 (1.31)	3.87 (12.1)	(1)
Belgium (FLEHS IV)	2017–2018	14–15	415						4.45 (12.3)	(2)
Belgium (3xG)	2019–2020	6–8	133						1.22 (3.24)	(3)
The Netherlands (SPECIMEn)	2020	6–11	102						1.13 (3.49)	(3)
France (ESTEBAN)	2014–2016	6–17	500	<0.6 (6.48)	<0.6 (5.64)	<0.6 (<0.6)	4.63 (26.5)	<0.6 (<0.6)	<0.05 (<0.05)	(4)
Slovenia (SLO-CRP)	2018	7–10	148						0.61 (3.08)	(5)
Cyprus (ORGANIKO)	2017	10–11	177						6.72 (13.7)	(3)
Italy (PHIME)	2014–2015	7	199						0.36	(6)
Spain (Valencia, BIOVAL)	2016	5–12	568	1.52 (5.9)	<0.5 (3.6)	<0.5 (<0.5)	<0.5 (14.7)	<0.5 (2.51)	1.13 (11.1)	(7)
Czech republic (SPECIMEn - summer)	2019–2020	4–12	110						2.01 (4.44)	(8)
Poland (REPRO_PL)	2014–2019	7–9	399						1.50 (6.30)	(9)
Denmark (Democophes)	2011	6–11	144	4.80 (19.7)	<1 (5.20)	<1 (<1)	3.20 (23.2)	<1 (<1)		(10)
Denmark (Odense)	2010–2012	10–16	143	2.99 (21.5)	<0.3 (5.66)	<0.3 (<0.3)	3.35 (23.1)	<0.3 (<0.3)	1.43 (6.05)	(11)
Denmark (Odense)	2010–2012	5	1166						1.15	(12)
Norway	2012	6–12	54	3.8 (16)	0.43 (6.43)	<0.14 (0.14)	5.3 (41)	<0.60 (6.6)		(13)
Turkey	2017–2018	3–6	186	<0.025	<0.025		<0.05		0.04	(14)
USA (NHANES)	2017–2018	3–5	418	3.55 (24.6)	0.19 (2.64)	<0.1 (<0.1)	1.04 (18.2)	0.18 (3.79)	1.2 (5.2)*	(15)
USA (NHANES)	2017–2018	6–11	331	3.41 (30.1)	0.19 (2.2)	<0.1 (<0.1)	0.92 (16.4)	0.18 (2.5)	1.5 (5.2)*	(15)
USA (NHANES)	2017–2018	12–19	353	2.2 (16.5)	0.12 (1.52)	<0.1 (<0.1)	0.62 (10.5)	0.11 (1.96)	1.5 (5.8)*	(15)
Canada (CHMS)	2018–2019	3–5	512	3.8 (23)	0.62 (4.2)	<0.067 (0.08)	2.6 (49)	0.34 (12)	1.1 (7.3)**	(16)
Canada (CHMS)	2018–2019	6–11	498	3.6 (24)	0.45 (6.0)	<0.067 (0.1)	2.3 (34)	0.26 (6.9)	1.4 (–)**	(16)
Canada (CHMS)	2018–2019	12–19	507	2.6 (14)	0.32 (5.2)	<0.067 (0.14)	1.1 (15)	0.12 (1.6)	1.2 (11)**	(16)
Ecuador (Espina cohort)	2016–2022	12–17	529						2.68 (9.5)	(17)
China (SMBCS)	2016	7	454						4.04	(18)
New Zealand	2015–2017	5–14	501	1.3 (17)	0.5 (6.4)	<0.005 (0.06)	3.7 (24)	0.044 (1.3)	13 (63)	(19)

(1) Pirard et al., 2020; (2) Schoeters et al., 2022; (3) HBM4EU dashboard; (4) Tagne-Fotso et al., 2023; (5) Bravo et al., 2020; (6) Bravo et al., 2019; (7) Pardo et al., 2020; (8) Šulc et al., 2022; (9) Bustamante et al., 2025; (10) Mørck et al., 2016 (11); Andersen and Dalsager, 2021; (12) Normann et al., 2025; (13) Cequier et al., 2017; (14) Göl et al., 2023; (15) CDC (Center for Disease Control and Prevention), 2024; (16) Health Canada, 2024; (17) Parajuli et al., 2025; (18) Dai et al., 2025; (19) Li et al., 2022

* TCPY results from the 2015–2016 cycle.

** TCPY results from the 2014–2015 cycle.

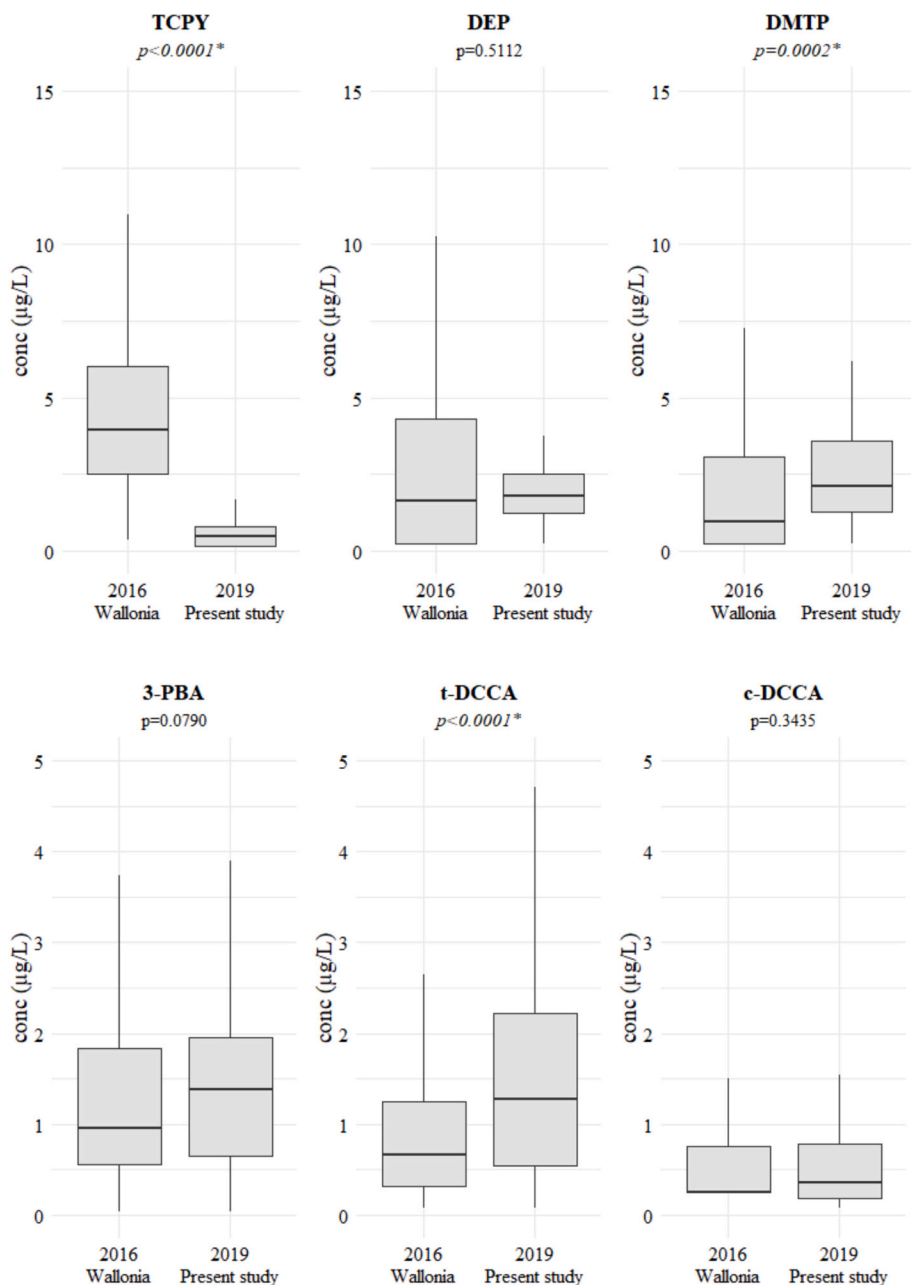


Fig. 1. Levels measured in the urine of Belgian children in 2016 (Wallonia) and in 2019 (present study) for organophosphate and pyrethroid metabolites. The boxes represent the 1st and 3rd quartiles, the middle lines are the medians, the vertical lines are the minimum and maximum levels computed without the outliers (not represented in the graph for more readability). Significant p -values were in italic.

ACE-DM and IMI-OLE respectively ($P_{95} = 1.64 \mu\text{g/L}$ and $4.26 \mu\text{g/L}$). The 4OH-IMI recently suggested as suitable marker for IMI in addition to IMI-OLE and 5OH-IMI (Wrobel et al., 2022) was not measured because of the lack of reference materials at the time of the analysis. The corresponding parents were also detected but to a much lesser extent (in 19.8% and 10.7% of the samples for ACE and IMI respectively), confirming the higher adequacy of metabolites compared to parents to assess the exposure of both acetamiprid and imidacloprid. It's noteworthy that within the European PARC project (Partnership for the Assessment of Risks from Chemicals), the 3 compulsory markers to assess neonicotinoid exposure are imidacloprid, acetamiprid and acetamiprid desmethyl (Deliverable D4.3 on <https://www.eu-parc.eu/deliverables>, last accessed in December 2025). Conversely, CLO-DM was only sparsely detected in the urine samples compared to CLO, demonstrating the higher relevance of measuring this parent pesticide, CLO

which may also result from the metabolism of thiamethoxam (Wang et al., 2020a). Globally, keeping in mind the reserve mentioned above about the difficulties to compare the different biomonitoring studies, the exposure of Belgian children to neonicotinoids seemed roughly consistent with those observed for Irish children (Wrobel et al., 2025), Polish children (Bustamante et al., 2025), and US children (Ospina et al., 2019), close to the levels reported for Japanese children (Ikenaka et al., 2019; Oya et al., 2021), and as expected, far lower than the levels usually reported for children in China or Taiwan (Tao et al., 2019; Wang et al., 2020b; Wang et al., 2024; Wu et al., 2024).

3.4. Urinary levels of ethylene bis-dithiocarbamate fungicides

Despite the ethylene bis-dithiocarbamate fungicides such as ziram, maneb, or mancozeb were widely used in agriculture, and ETU has been

suggested for many years to be a suitable marker for their exposure assessment (Aprea et al., 1997; Colosio et al., 2006), data on non-occupationally exposed populations are scarce and mostly carried out on residents living close to wine-growing activities where particularly high exposure to ethylene bis-dithiocarbamates was suspected (Stadler et al., 2022). Compared to the few studies focused on children (Table 5), the levels of ETU measured in the urine of Belgian children seem more or less comparable to those reported from New Zealand children recruited between 2015 and 2017 (Li et al., 2022) or from a set of French children from the ESTEBAN cohort (2014–2016) (Dereumeaux et al., 2022), slightly lower than the Canadian children recruited through the 2018–2019 CHMS cycle (Health Canada, 2024), but higher than US children from the NHANES 2007–2008 cycle (CDC (Center for Disease Control and Prevention), 2024), or Swedish adolescents (Norén et al., 2020). However, the median levels obtained from our population are well below those measured in the urine of children living in areas with suspected environmental pesticide contamination in Latin America (Parajuli et al., 2025; van Wendel de Joode et al., 2016) and similarly, the P95 was well below the one evaluated from an Italian children population living in a viticultural area where ethylene bis-dithiocarbamate was known to be used (Sammartano et al., 2020).

3.5. Urinary levels of chlormequat chloride

Chlormequat chloride has been one of the most used plant growth regulators applied to shorten and strengthen the stem of many cereals like wheat, oats or barley, and thus facilitate the harvesting (Temkin et al., 2024). However, literature about human exposure is even rarer than for ETU. Very few studies reported urinary CCC levels for adults or general population including adults and children, with median or geometric mean ranging between 1.4 µg/L for Floridan (US) adults recruited in 2023 (Temkin et al., 2024) and 4 µg/L for Swedes (Lindh et al., 2011), while Galea and coworkers (Galea et al., 2015) measured CCC at 12.3 to 16.5 µg/g creat in the urine of a UK population living in farms during pesticide spray events. To our knowledge, only one study published CCC levels measured in the urine of youths (Table 5), reporting a median level of 1.17 µg/L for Swedish adolescents (Norén et al., 2020) which is far lower than the levels observed for our Belgian children population (6.7 µg/L). This large difference in CCC levels could be likely explain at least by the different age ranges between both studied populations because young children have been demonstrated to experience higher exposure to most pesticides (Aprea et al., 2000; Au, 2002; Roberts and Karr, 2012). We expected even larger difference according to the age for pesticides mainly used on cereal crops because young children are more likely to consume higher proportions of breakfast and snacks based on ready-to-eat highly sugary cereals as observed for children recruited within the 2015–2018 NHANES cohort compared to adolescents (Ospina et al., 2022).

3.6. Urinary levels of glyphosate and AMPA

These last years, several reviews compiled the literature on the human exposure levels to glyphosate and AMPA, the main environmental glyphosate degradation product, all concluding that data are limited and insufficient to properly assess the related health risk, especially for vulnerable population such as children (Buekers et al., 2022a, 2022b; Connolly et al., 2020; Gillezeau et al., 2020, 2019), and this despite their classification as priority substances within HBM4EU and PARC programs (Ougier et al., 2021). The Table 5 gathers studies reporting urinary levels of glyphosate and AMPA for children, including only those using mass spectrometry as detector for comparability purposes because results produced by ELISA method have been observed to be up to 10-fold higher than those measured by LC or GC/MS (Knudsen et al., 2017) questioning the specificity of such methods for the assessment of exposure to some non-persistent substances like glyphosate and thus making comparisons tricky (Buekers et al., 2022b). As expected, the

Table 5

Median and P95 concentrations (expressed in µg/L) reported for ETU, CCC, glyphosate and AMPA in several recent studies.

Country	Sampling years	Age (yrs)	N	P50 µg/L	P95 µg/L	Ref
ETU						
Belgium (present study)	2019	2–15 yrs	84	0.40	2.17	
Sweden	2017	17–21 yrs	196	0.18	1.19	(1)
Sweden	2009	18 yrs	254	0.34	2.02	(1)
Italy (viticultural area)	2011	3–5 yrs	126	0.35	8.0	(3)
France (data set from ESTEBAN)	2014–2016	6–17 yrs	61	<1.0	2.3	(4)
France (from schools in the close vicinity of vineyards)	2011	3–10 yrs	96	winter: 0.43* summer: 0.66*		(5)
USA (NHANES)	2007–2008	6–11 yrs	382	<0.21	0.61	(6)
Canada (CHMS)	2018–2019	3–5 yrs	507	0.84	3.8	(7)
Canada (CHMS)	2018–2019	6–11 yrs	494	0.56	3.1	(7)
Canada (CHMS)	2018–2019	12–19 yrs	498	0.55	4.3	(7)
New Zealand	2015–2017	5–14 yrs	501	<0.5	1.9	(8)
Ecuador (Espina cohort)	2016–2022	12–17 yrs	529	1.45	5.84	(9)
Costa Rica	2017	6–9 yrs	140	1.2		(10)
CCC						
Belgium (present study)	2019	2–15 yrs	84	6.70	30.0	
Sweden	2017	17–21 yrs	196	1.17	16.8	(1)
Glyphosate						
Belgium (present study)	2019	2–15 yrs	84	<0.08	0.17	
Belgium (Flanders, FLEHS IV)	2017–2018	14–15 yrs	415	<0.1	0.39	(11)
Belgium (3xG)	2019–2020	6–8 yrs	133	0.12	0.41	(12)
France (ESTEBAN)	2014–2016	6–17 yrs	498	<0.05	0.57	(13)
Germany (GerES V)	2015–2017	3–17 yrs	2144	0.1	0.51	(14)
Slovenia	2018	7–15 yrs	246	<0.1	0.19	(15)
Cyprus (ORGANIKO)	2017	10–11 yrs	177	<0.1	1.01	(16)
Morocco	2019	3–12 yrs	48	0.72	2.58	(17)
Japan	2015	4–6 yrs	50	<0.03	0.57	(18)
Mexico	?	5–15 yrs	192	0.36		(19)
Ecuador (Espina cohort)	2016–2022	12–17 yrs	529	0.87	4.7	(9)
USA (NHANES)	2017–2018	3–5 yrs	325	0.32	1.43	(6)
USA (NHANES)	2017–2018	6–11 yrs	245	0.41	1.95	(6)
USA (NHANES)	2017–2018	12–19 yrs	297	0.29	1.35	(6)
Canada (CHMS)	2018–2019	3–5 yrs	393	0.45	2.1	(7)
Canada (CHMS)	2018–2019	6–11 yrs	388	0.57	3.1	(7)
Canada (CHMS)	2018–2019	12–19 yrs	389	0.45	3.3	(7)

(continued on next page)

Table 5 (continued)

Country	Sampling years	Age (yrs)	N	P50 µg/L	P95 µg/L	Ref
AMPA						
Belgium (present study)	2019	2–15 yrs	84	<0.15	0.36	
Belgium (Flanders, FLEHS IV)	2017–2018	14–15 yrs	415	0.11	0.37	(11)
Belgium (3xG)	2019–2020	6–8 yrs	133	<0.2	0.40	(12)
France (ESTEBAN)	2014–2016	6–17 yrs	498	0.17	0.45	(13)
Germany (GerES V)	2015–2017	3–17 yrs	2144	<0.1	0.48	(14)
Slovenia	2018	7–15 yrs	246	0.10	0.29	(15)
Cyprus (ORGANIKO)	2017	10–11 yrs	177	0.18	0.65	(16)
Morocco	2019	3–12 yrs	48	0.50	2.65	(17)
Canada (CHMS)	2018–2019	3–5 yrs	393	0.39	1.40	(7)
Canada (CHMS)	2018–2019	6–11 yrs	388	0.25	1.30	(7)
Canada (CHMS)	2018–2019	12–19 yrs	389	0.16	1.00	(7)

(1) Norén et al., 2020; (2) Sammartano et al., 2020; (3) Dereumeaux et al., 2022; (4) Raherison et al., 2019; (5) CDC (Center for Disease Control and Prevention), 2024; (6) Health Canada, 2024; (7) Li et al., 2022; (8) Parajuli et al., 2025; (9) van Wendel de Joode et al., 2016; (10) Schoeters et al., 2022; (11) Buekers et al., 2022b; (12) Tagne-Fotso et al., 2023; (13) Lemke et al., 2021; (14) Stajniko et al., 2020; (15) Makris et al., 2022; (16) Berni et al., 2023; (17) Nomura et al., 2022; (18) Sierra-Diaz et al., 2019.

* Results expressed in µg/g of creatinine.

detection frequencies obtained for glyphosate and AMPA in the present study were low, 25% and 19%, respectively, consistently with those usually reported in Europe (from 20 to 50% depending on the study, with similar limits of quantification), with levels measured quite similar or slightly lower than those previously measured in Belgium, France, Germany, Slovenia or Cyprus (Buekers et al., 2022b; Lemke et al., 2021; Makris et al., 2022; Schoeters et al., 2022; Stajniko et al., 2020; Tagne-Fotso et al., 2023). As expected, higher levels were reported from American populations (USA, Canada, Ecuador or Mexico) where higher amounts of glyphosate are used (USA is considered as one of the largest users of glyphosate and related products worldwide) (Benbrook, 2016) and usual agricultural practices consisted in pre-seeding and desiccation applications directly on genetically modified crops, practices not approved in Europe (Lemke et al., 2021). One should keep in mind that these low levels measured in urine samples would not necessarily mean low exposure level because of the very small fraction of glyphosate excreted in urine (likely less than 1%) (Faniband et al., 2021).

3.7. Predictors of exposure

Fig. S1 (supplemental material) shows the significant correlations (p -value <0.05) occurring between markers with detection frequencies higher than 20%. High correlations were observed between both metabolites of imidacloprid (IMI-OLE and 5OH-IMI), between both DCCA isomers (c- and t-DCCA), between DBCA and 3-PBA, between c-DCCA and 3-PBA as well as between t-DCCA and 3-PBA, as expected because coming from the metabolism of common parent pesticides. Moreover, weaker correlations were highlighted between metabolites coming from different pyrethroid parents (DBCA and c-/t-DCCA) likely resulting on the application of pesticide formulations containing mixture of active substances. TCPY was correlated with all pyrethroid metabolites, suggesting simultaneous uses of both insecticide families for domestic or agricultural uses. On the other hand, TCPY was only weakly correlated to DEP, although both being metabolites of chlorpyrifos, but other

sources of DEP exist such as organophosphate flame retardants. DETP which is another potential metabolite of chlorpyrifos was not included because of the too low detection frequency observed. Less expected, CCC levels were fairly correlated with all pyrethroid metabolites, TCPY and DEP, and weakly with neonicotinoids (CLO, 5OH-IMI, IMI-OLE). This would support the assumption of a general contamination of diet by a lot of different pesticides.

Diet used to be considered as the main source of exposure to pesticides for the general population. However, other pathways could be involved such as dermal contact or inhalation within indoor or outdoor house environment because of domestic uses at home, in the garden, or on pets, and this especially for children who could have specific behaviours increasing the risk of this exposure (i.e. hand-to-mouth contacts, play on the floor, etc.). Therefore, to investigate the impact of some potential sources on the exposure and thus on urinary pesticide concentrations, few questions were administrated to parents about children's behaviours (i.e. biting nails), domestic uses of pesticides or the presence of pets at home in addition to some items related to diet habits and food consumed in the last 48 h (Table 1). The results of the statistical regression models applied are gathered in Table 6.

The children who used to bite their nails have significantly higher urinary levels of pyrethroids (c-DCCA, t-DCCA and 3-PBA) and neonicotinoids (ACE-DM) compared to those who didn't (Fig. 2). This observation, already reported for metribuzin only (Pirard et al., 2020), demonstrates that biting nails and more generally the hand-to-mouth behaviour would increase the exposure of children to pesticides through the ingestion of household dust knowing to be contaminated by pesticides and particularly by pyrethroids (Darney et al., 2018; Heudorf and Angerer, 2001; Tang et al., 2018). This confirms the assumption previously suggested (Darney et al., 2018; Saillenfait et al., 2015; Trunelle et al., 2014) considering dust ingestion as a particularly important exposure pathway to pyrethroids for children. No association was highlighted between the urinary pesticide levels and owning pets, except for DMTP for which lower concentrations were measured for children living with pets at home, although higher pyrethroid exposure were already observed for dog ownership or pet-care product uses (Li et al., 2022). On the other hand, the recent use of pesticides in the garden (in the last 2 months) was associated with higher detection frequencies of DBCA and higher levels of CCC. Even if the information about the identification of pesticides used is missing, these observations seem to be not senseless for DBCA, the metabolite of deltamethrin which is used as active ingredient in many insecticide formulations intended to indoor and outdoor domestic fight against ants, mosquitoes, flies or cockroaches (among others). Conversely, the positive association with CCC appears more inconsistent since CCC is dedicated to professional use in agriculture only. Slight but significant differences were observed between regions, with higher levels or detection frequencies of several pesticides (t-DCCA, 3-PBA, DMTP, and ACE-DM) measured in the urine of children living in Brussels compared to those residing in Flanders, except for DMTP which was higher in the urine of Flemish children. Significance for Walloon children could be missed due to the very low number of participants in this Region. It's no obvious to attribute these differences to the different environmental policies implemented in the 3 Belgian regions or to other confounding factors such as socio-demographic status for instance.

Several studies have already highlighted higher urinary levels of some pesticides related to the consumption of fruits, vegetables, or other food items (Andersen et al., 2022; Cequier et al., 2017; Pardo et al., 2020; Harada et al., 2016; Lu et al., 2008; Rempelos et al., 2022; Šulc et al., 2022; Tagne-Fotso et al., 2023). However, some inconsistent associations were reported depending on the food items selected or the pesticide studied, but the consumption of organic food has been commonly demonstrated to decrease the pesticide exposure in most studies (Andersen et al., 2022; Bradman et al., 2015; Curl et al., 2003; Rempelos et al., 2022; Tagne-Fotso et al., 2023). In the present study, the consumption of organic fruits or vegetables in the last 48 h were only

Table 6

Results of the multivariate regression models.

Variable	c-DCCA ($R^2 = 0.2109$)		t-DCCA ($R^2 = 0.2608$)		3-PBA ($R^2 = 0.2389$)	
	Estimate (CI inf/sup)	p-value	Estimate (CI inf/sup)	p-value	Estimate (CI inf/sup)	p-value
Intercept	−0.592 (−0.771/−0.412)	<0.0001	0.167 (−0.044/0.378)	0.1190	0.126 (−0.047/0.300)	0.1510
Region Brussels vs Flanders			0.356 (0.112/0.599)	0.0048	0.257 (0.057/0.457)	0.0127
Region Wallonia vs Flanders			0.100 (−0.195/0.395)	0.5010	0.085 (−0.157/0.328)	0.4850
Nail biting (yes vs no)	0.381 (0.142/0.619)	0.0022	0.356 (0.103/0.608)	0.0065	0.337 (0.129/0.545)	0.0019
Drinking bottled vs tap water	−0.340 (−0.610/−0.071)	0.0140				
Drinking both vs tap water	0.055 (−0.179/0.290)	0.6400				
Pets at home (yes vs no)	0.180 (−0.025/0.385)	0.0851				
Consumption of grey bread or whole grains (48 h, yes vs no)			−0.420 (−0.490/−0.191)	0.0005	−0.283 (−0.471/−0.094)	0.0038

Variable	DBCA ($R^2 = 0.1518$)		TCPY ($R^2 = 0.1900$)		DEP ($R^2 = 0.0998$)	
	OR (CI inf/sup)	p-value	Estimate (CI inf/sup)	p-value	Estimate (CI inf/sup)	p-value
Intercept	1.288 (0.713/2.359)	0.4040	0.150 (−0.025/0.325)		0.389 (0.282/0.496)	<0.0001
Nail biting (yes vs no)					−0.192 (−0.930/0.008)	0.0601
Outdoor pesticide use (yes vs never)	5.789 (1.532/30.96)	0.0185				
Indoor pesticide use (yes vs never)	7.337 (0.983/163.8)	0.0988	0.217 (−0.014/0.449)	0.0656		
Pets at home (yes vs no)			−0.133 (−0.284/0.018)	0.0841		
Consumption of grey bread or whole grains (48 h, yes vs no)			−0.192 (−0.346/−0.038)	0.0152		
Consumption of stone fruits (48 h, yes vs no)	0.141 (0.026/0.561)	0.0105			−0.237 (−0.438/0.036)	0.0214
Consumption of exotic fruits (48 h, yes vs no)			0.132 (−0.017/0.282)	0.0825		

Variable	DMTP ($R^2 = 0.2207$)		DMDTP ($R^2 = 0.1144$)		CLO ($R^2 = 0.0609$)	
	Estimate (CI inf/sup)	p-value	OR (CI inf/sup)	p-value	OR (CI inf/sup)	p-value
Intercept	0.823 (0.551/1.095)	<0.0001	0.337 (0.149/0.692)	0.0049	0.082 (0.015/0.335)	0.0013
Age	−0.024 (−0.047/0.00)	0.0459			1.179 (1.026/1.377)	0.0261
Region Brussels vs Flanders	−0.327 (−0.477/−0.071)	0.0089	3.390 (1.06/11.50)	0.0421		
Region Wallonia vs Flanders	−0.152 (−0.395/0.091)	0.2160	0.330 (0.017/2.115)	0.3210		
Pets at home (yes vs no)	−0.225 (−0.400/−0.050)	0.0124				
Wash and/or peel vegetables/fruits (always vs often)	−0.167 (−0.341/0.008)	0.0606				
Consumption of stone fruits (48 h, yes vs no)			0.232 (0.032/1.042)	0.0876		

Variable	5OH-IMI ($R^2 = 0.0620$)		IMI-OLE ($R^2 = 0.0632$)		ACE-DM ($R^2 = 0.2352$)	
	OR (CI inf/sup)	p-value	OR (CI inf/sup)	p-value	Estimate (CI inf/sup)	p-value
Intercept	1.261 (0.731/2.200)	0.4060	2.286 (1.245/4.420)	0.0099	−1.154 (−1.476/−0.832)	<0.0001
Age					0.036 (0.090/0.064)	0.0104
Region Brussels vs Flanders					0.253 (0.042/0.465)	0.0197
Region Wallonia vs Flanders					−0.118 (−0.380/0.144)	0.3720
Nail biting (yes vs no)					0.244 (0.016/0.471)	0.0360
Consumption of organic fruits (48 h, yes vs no)	0.264 (0.084/0.742)	0.0152				
Consumption of organic vegetables (48 h, yes vs no)			0.292 (0.108/0.752)	0.0122		
Consumption of exotic fruits (48 h, yes vs no)					0.267 (0.066/0.468)	0.0100

Variable	ETU ($R^2 = 0.0727$)		CCC ($R^2 = 0.0492$)		GLY ($R^2 = 0.2352$)	
	OR (CI inf/sup)	p-value	Estimate (CI inf/sup)	p-value	OR (CI inf/sup)	p-value
Intercept	3.333 (1.805/6.639)	0.0003	0.870 (0.696/1.043)	<0.0001	–	–
Outdoor pesticide use (yes vs no)			0.283 (0.057/0.510)	0.0150		
Consumption of organic fruits (48 h, yes vs no)	0.254 (0.088/0.702)	0.0091				

associated with lower detection frequencies of imidacloprid metabolites (IMI-OLE and 5OH-IMI) and ETU, all other pesticides or metabolites studied showed levels or detection frequencies not significantly affected. Note that the lack of such association have been previously observed between organic food and pyrethroid and OP levels in the urine of Belgian children (Pirard et al., 2020). Also surprisingly, while eating grey bread or whole grains has been already reported to increase the risk of higher CCC, pyrethroid and OP exposure (Andersen et al., 2022; Pirard et al., 2020; Rempelos et al., 2022), the opposite trend was observed for t-DCCA, 3-PBA and TCPY. This statistical result seems inconsistent with the higher pesticide contamination expected in the

whole flour compared to refined flour because not cleared from the wheat grain envelope, thus another dietary intake closely related to the consumption of grey bread or whole grains would likely drive this negative association. Similarly, the lower DEP levels and DBCA detection frequencies associated with the recent consumption of stone fruits would be probably due to the inherent decreased consumption of other more contaminated fruits. Finally, eating exotic fruits significantly increase the urinary levels of ACE-DM, and children drinking tap water seemed to have higher c-DCCA urinary levels than those who used to drink bottled water but because this association only occurred with c-DCCA and not with the other pyrethroids, one can reasonably assume

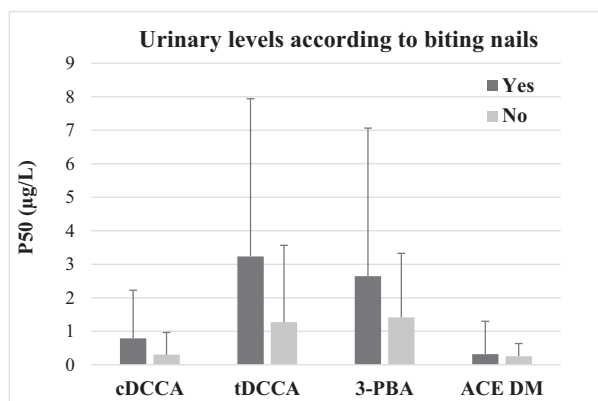


Fig. 2. Median urinary levels (and P75 as upper error bars) of some pesticides according the biting nails behaviour of the children.

that these statistically significance would likely due to confounding factors or to chance, similarly to the inconsistent relation with age (DMDTP detection frequencies decrease with increasing age while CLO and ACE-DM increased).

Note that all these statistical results should be interpreted with cautious due the low coefficients of determination obtained (from 0.05 to 0.26) meaning that the main sources of exposure were missed. Moreover, the limited size of the population studied, the use of volumetric concentration (without any correction for the potential sample dilution) and the possible inaccuracy of the parent answers to the self-administered questionnaire on their child's behaviour or food consumption could bias the results. Finally, high intraindividual variability in urinary levels has been demonstrated for most pesticides or pesticide metabolites measured within this study, questioning the reliability of spot urine samples to accurately assess the pesticide exposure of individual child (Cequier et al., 2017; Li et al., 2019; Morgan, 2012).

4. Conclusions

This small-scaled study provides human biomonitoring data on children exposure to several classes of currently used pesticides in Belgium. If some of them such as glyphosate, chloromequat chloride or dithiocarbamates have been so far poorly studied or not at all in children's populations, others have been already well-documented in the past, and thus present data collected also allows the assessment of the exposure time trend. Some pyrethroids, organophosphorus, and neonicotinoids were detected in more than 95% of the samples demonstrating the widespread exposure to pesticides among Belgian children. Moreover, on average 10 to 11 markers were detected by sample, knowing that few pesticides were investigated here compared to large number of substances authorized in Belgium (Giusti et al., 2018). Similarly to acetamiprid desmethyl, ethylenethiourea and chormequat chloride were detected in the urine of a large majority of children (100% for CCC, 68% for ETU) with median levels ranging from 0.40 µg/L for ETU to 6.7 µg/L for CCC. Because they have been yet sparsely included in previous biomonitoring studies, these observations highlight the need to consider these pesticides as prioritized substances for future studies to increase the current limited knowledge about their exposure. Glyphosate and AMPA showed as expected low detection frequencies and low levels likely because of the low excretion rate in urine. If the urinary levels of chlorpyrifos metabolites in Belgian children seemed to decrease between 2016 and 2019 although the non-renewal of the chlorpyrifos authorization in Europe was only implemented at the end of 2019, the exposure to pyrethroid insecticides seemed to slightly, but significantly, increase. Even if these trends should be considered with cautious due to the several limitations of the study (i.e. the limited size and the non-representativeness of the studied population), these time trends are

supported by statistically significant differences and are confirming previous observations in Europe reported in the literature. This could be additional evidence of the pesticide market shift reacting to the recent implementation of restrictions on some substance uses, for instance organophosphorus and neonicotinoid insecticides. Thus, the exposure to pyrethroids should still be monitored in the future. Finally, the present study highlighted the need of harmonization in the selection of markers to be measured for the assessment of the neonicotinoid exposure and suggested imidacloprid metabolites (hydroxy- and olefin-) and acetamiprid metabolite (acetamiprid desmethyl) instead of the respective parent compound.

CRediT authorship contribution statement

Catherine Pirard: Writing – original draft, Methodology, Investigation, Conceptualization. **Patrice Dufour:** Writing – review & editing. **Corinne Charlier:** Writing – review & editing, Supervision, Funding acquisition.

Funding sources

This work was partly supported by the Belgian non-profit consumer protection organization Test Achats. In addition to financial contribution, they performed the recruitment and sample collection. The remaining part of the work was own funded.

Declaration of competing interest

The authors declare to have no financial interests or personal relationships that could inappropriately influence or bias the work reported in this manuscript.

Acknowledgements

We would like to thank all the parents for their consent concerning their children, for their involvement and their availability. We would also like to acknowledge Carine Deschamps from Test Achats for the efficient recruitment organization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2026.181881>.

Data availability

The authors do not have permission to share data.

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