Environmental Research 214 (2022) 114002

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Pesticide exposure among Czech adults and children from the CELSPAC-SPECIMEn cohort: Urinary biomarker levels and associated health risks

Libor Šulc [a,1], Tomáš Janoš [a,1], Daniel Figueiredo [b], Ilse Ottenbros [b,c], Petr Šenk [a], Ondřej Mikeš [a], Anke Huss [b], Pavel Čupr [a,*]

[a] RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno, Czech Republic
[b] Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands
[c] Center for Sustainability, Environment and Health, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

ARTICLE INFO

Keywords:
Pyrethroids
Chlorpyrifos
Tebuconazole
Urine
HBM4EU
Cumulative risk assessment

ABSTRACT

Current-use pesticides (CUP) are extensively applied in both agricultural and urban settings. Exposure occurs mainly via the dietary pathway; however, other pathways such as inhalation or skin contact are also important. In this study, urinary levels of 12 CUP metabolites were investigated among 110 parent-child pairs during two seasons of 2020. Metabolites of pyrethroids (3-PBA, t/c-DCCA), chlorpyrifos (TCPY), and tebuconazole (TEB-OH) were detected in more than 60% of the samples. Chlorpyrifos metabolite was found at the highest concentration and tebuconazole was detected in almost all samples. CUP urinary metabolite levels were significantly higher in children in comparison to adults, except for tebuconazole, which was similar in both groups. In children, winter samples had significantly higher concentrations of pyrethroid and chlorpyrifos metabolites in comparison to the summer samples, but in adults, only chlorpyrifos metabolite concentrations were higher in the winter. No association between CUP urinary metabolite levels and proximity/surface of agricultural areas around residences was observed. Based on our findings, we suspect that CUP exposure is mainly driven by diet and that the effect of environmental exposure is less significant. Daily Intakes were estimated with three possible scenarios considering the amount of the metabolite excreted in urine and were compared to Acceptable Daily Intake values. Using a realistic scenario, exposure to chlorpyrifos exhibited the highest health risk, but still within a safe level. The Acceptable Daily Intake was exceeded only in one child in the case of cypermethrin. The cumulative risk assessment of pesticide mixtures having an effect on the nervous system, based on the total margin of exposure calculations, did not indicate any risk. The overall risk associated with pesticide exposure in the observed population was low. However, the risk observed using the worst-case scenario suggests the need for continuous evaluation of human exposure to such compounds, especially in children.

1. Introduction

Pesticides are agrochemicals used worldwide for the protection of crops from various types of pests. The application of pesticides boomed after the Second World War, although the utilization of measures to reduce the impact of pests goes back much further into the history of mankind (Damalas, 2009). So-called legacy pesticides, heavily used in the second half of the 20th century, are a group of now phased-out pesticides. Many of these legacy pesticides are characterized by their environmental persistence, ease of long-range transport, low polarity, and wide range of adverse effects on non-target species (Rasmussen et al., 2015).

In comparison to legacy pesticides, current-use pesticides (CUP) are more polar, have a shorter half-life in the environment, are less toxic to non-target species, and are less prone to long-range transport (Balmer et al., 2019; Climent et al., 2019; Gao et al., 2019). CUPs include, for example, organophosphates (chlorpyrifos, malathion, glyphosate), pyrethroids (cypermethrin, cyfluthrin), and triazoles (tebuconazole,

* Corresponding author. RECETOX Centre, Faculty of Science, Masaryk University, Kamenice 753/5, pavilion A29, 625 00, Brno, Czech Republic.
E-mail address: pavel.cupr@recetox.muni.cz (P. Čupr).
[1] Both first authors Libor Šulc and Tomáš Janoš contributed equally.

https://doi.org/10.1016/j.envres.2022.114002
Received 13 May 2022; Received in revised form 4 July 2022; Accepted 25 July 2022
Available online 5 August 2022

propiconazole). Although CUPs are a better alternative to legacy pesticides, there are still issues and potential risks associated with them. CUPs were found in environmental samples from arctic areas, indicating to some extent their long-range transport (Balmer et al., 2019). The bioaccumulation of pyrethroid pesticides in fish was reported in a recent study (Xie et al., 2022) and there is also evidence of biomagnification in arctic fauna (Morris et al., 2016).

In humans, exposure to CUP mixtures occurs mainly via diet, due to the consumption of fruits and vegetables with CUP residues (Nougadère et al., 2012; Demur et al., 2013; Hertz-Picciotto et al., 2018). Occupational exposure is another important source of pesticide exposure, especially in farming and pesticide application, pesticide manufacture, and other associated activities (Mamane et al., 2015). CUP mixing, handling, and application, as well as crop harvesting are the general activities where exposure to CUPs occurs (Mehrpour et al., 2014). Environmental exposure may occur due to residency near sprayed areas (e.g., fields, orchards). Drift and volatilizations of applied CUPs and subsequent inhalation and contact with contaminated matter are the main exposure routes (Felsot et al., 2010; Lichiheb et al., 2016; Torrent et al., 2017).

There is increasing concern about exposure of the general population to CUPs and possible adverse health effects (Angerer et al., 2007). For this reason, internal exposure to CUPs is assessed via human biomonitoring (HBM). Often used matrices are human fluids such as serum (Chang et al., 2017) or urine (Dereumeaux et al., 2018). Urine is the preferred matrix since its collection is non-invasive, it can be reliably self-collected, sample volumes are generally sufficient, and its use is more convenient when working with children. Urine can also well reflect short-term exposure, due to the rapid metabolism and excretion of CUPs (Oerlemans et al., 2021). In previously conducted studies, exposure to CUPs was related to several health outcomes, such as male reproductive disorders, neurodevelopmental problems, potential endocrine-disruption, and cancer, among others (Koureas et al., 2012; González-Alzaga et al., 2014; Saillenfait et al., 2015; Kim et al., 2017).

In this study, we collected morning void urine samples and questionnaires from residents (adults and children) of the Czech Republic. The urine samples were analysed for twelve CUP metabolites or parental compounds. The main aims of this study were i) to assess pesticide levels in the general population of the Czech Republic; ii) to compare urinary pesticide biomarkers between the winter and summer seasons in order to better understand possible exposure sources to CUPs; iii) to compare urinary pesticide biomarkers between adults and children to assess whether the susceptible group of children is more exposed to CUPs, and iv) to assess the health risks associated with exposure to the studied CUPs.

2. Methods

2.1. Study design

The SPECIMEn study (Survey on PEstiCIde Mixtures in Europe) was carried out under the HBM4EU project (The European Human Biomonitoring Initiative[2]). The overall aim of the SPECIMEn study was to assess exposure to pesticide mixtures in the general population by applying a high-resolution suspect screening approach in five countries across Europe. Detailed information on the SPECIMEn study design and sample collection is provided by Vlaanderen et al. (2019). In brief, parent-child pairs (direct kinship relationship, mother/father and daughter/son) collected urine samples and completed questionnaires in the winter season and again in the summer season. Only adults older than 20 years with school-age children were accepted into the study. Farmers and other professionals with potential occupational exposure to CUPs were excluded.

This work was focused on the Czech cohort of the SPECIMEn study: CELSPAC-SPECIMEn (Central European Longitudinal Studies of Parents and Children[3]). The CELSPAC-SPECIMEn study in the Czech Republic received ethical approval under ref. no. ELSPAC/EK/3/2019. Primarily, but not exclusively, study participants resided in the South Moravian Region of the Czech Republic (Fig. 1). Recruitment into the CELSPAC-SPECIMEn study took place from September 2019 until the beginning of January 2020. A total of 199 applicants expressed interest in the study. 88 applicants declined to participate or lost interest in the study during the recruitment phase. The remaining 111 adult applicants (with 111 children) were recruited into the study. We lost contact with one adult-child pair after sample collection in winter. This adult-child pair was subsequently excluded from further analysis, reducing the final number of participants to 110 adults and 110 children.

2.2. Sample collection

The first round of sample collection took place from mid-January 2020 to mid-March 2020 (hereinafter “winter season”), the second round of sample collection took place from the end of May 2020 to the end of July 2020 (hereinafter “summer season”). Samples were not collected on weekends and Mondays due to possible differences in the participant behavior during the weekend in comparison to working days.

Each participant received the materials needed for successful urine sample collection; these included: urine containers, plastic cups for urine collection, plastic bags for storage, a permanent marker, informed consent, and a questionnaire. Urine samples (first-morning void) were self-collected by participants using the supplied containers and then stored in plastic bags in the fridge until the arrival of the field worker. Samples were transported to the laboratory under refrigeration, aliquoted, and stored at −80 °C until analysis. The whole process from sample collection to sample storage took no longer than 24 h and typically less than 12 h.

2.3. Urine sample analysis

Collected urine samples were analysed for biomarkers of exposure to CUPs. The selection of pesticide biomarkers was based on the recommendation of HBM4EU (Prioritised substance group: Pesticides) (Ougier et al., 2021), the annual reports of Plant Protection Products in the Czech Republic (CISTA, 2022), and also on the European Food Safety Authority (EFSA) report (EFSA, 2021). The analysed biomarkers were: 3-phenoxybenzoic acid (3-PBA), 4-fluoro-3-phenoxybenzoic acid (4F3-PBA), trans/cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopro-pane carboxylic acid (t/c-DCCA), diethyl dithiophosphate (DEDTP), 3,5,6-trichloro-2- pyridinol (TCPY), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), dicarboxylic acid (MDA), pethoxamide (PM), pethoxamid metabolite-42 (PM MET), fipronil sulfone (FPS), coumaphos and hydroxy-1-tebuconazole (TEB-OH). More information per analysed compound is shown in Table 1.

Target analysis was performed by means of high-performance liquid chromatography (HPLC) in tandem with a mass spectrometer-mass spectrometer system (MS-MS). HPLC was conducted using an Agilent 1290 series instrument (Agilent Technologies, Waldbronn, Germany) consisting of a vacuum degasser, a binary pump, a thermostatted autosampler (10 °C), and a thermostatted column compartment kept at 30 °C. The column was an Acquity UPLC BEH C18 (3 μm) 100 × 2 mm i. d, equipped with an ACQUITY UPLC BEH C18 VanGuard Pre-column, 130 Å, 1.7 μm, 2.1 mm × 5 mm. The mobile phase consisted of 1 Mm ammonium fluoride in water (A) and 1 Mm ammonium fluoride in acetonitrile (B) for negative MS-MS (0.1% Formic acid in water (A) and 0.1% Formic acid in acetonitrile (B) for positive MS-MS). The binary

[2] www.HBM4EU.eu

[3] www.recetox.muni.cz/hear/projects/specimen

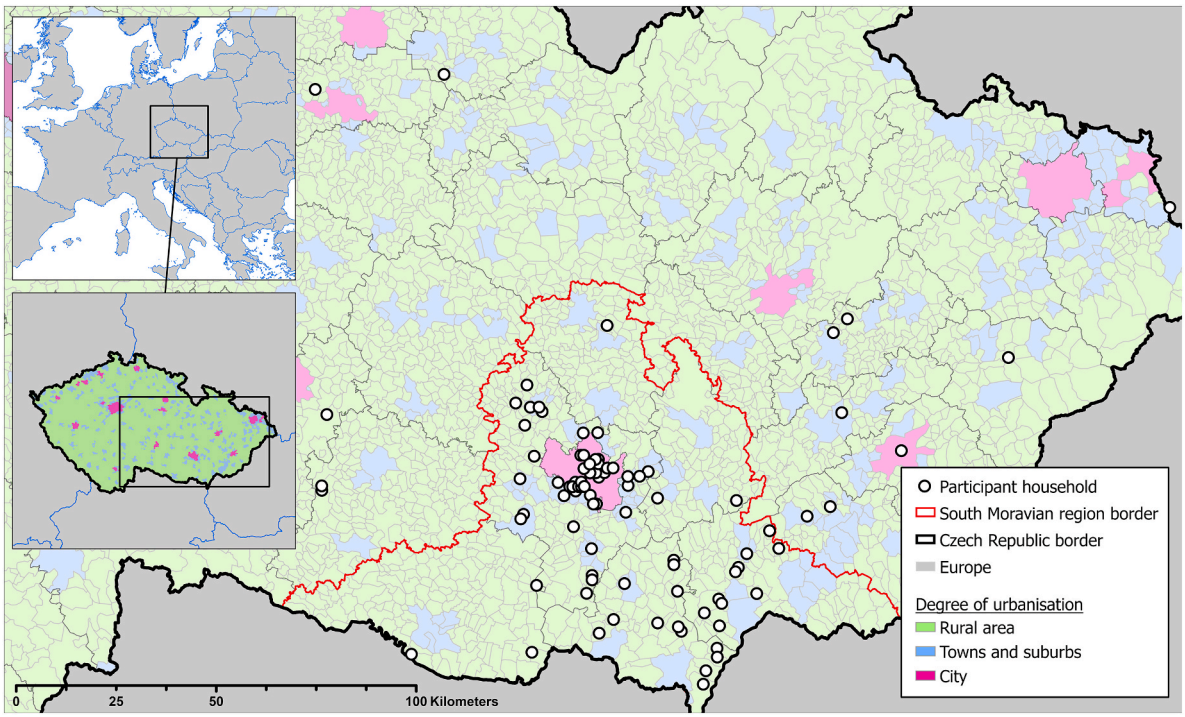

Fig. 1. Spatial distribution of participants in the CELSPAC-SPECIMEn study in the Czech Republic and the degree of urbanisation, DEGURBA (EUROSTAT, 2018).

Table 1
Analysed urinary biomarkers of exposure to CUPs.

Chemical group	CUP name	CUP use	Biomarker name	Biomarker abbr.	Biomarker CAS	Biological half-life (h)	LOD/LOQ (ng/mL)
Pyrethroid	Cypermethrin	insecticide	3-phenoxybenzoic acid	3-PBA	3739-38-6	6.4 [a]	0.04/0.14
	Cyfluthrin		4-fluoro-3-phenoxybenzoic acid	4F3-PBA	77279-89-1	6.1 [b]	0.08/0.27
	Permethrin		trans/cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopro-pane carboxylic acid	t/c-DCCA	55667-40-8	6.3 (trans) [a] 6.4 (cis) [a]	0.03/0.11
Organophosphate	Chlorpyrifos	insecticide	diethyl dithiophosphate	DEDTP	298-06-6	–	0.11/0.36
			3,5,6-trichloro-2- pyridinol	TCPY	6515-38-4	27 [c]	0.03/0.09
	Malathion		2-isopropyl-4-methyl-6-hydroxypyrimidine	IMPY	2814-20-2	–	0.08/0.28
	Coumaphos		dicarboxylic acid	MDA	1190-28-9	–	0.14/0.46
			coumaphos	–	56-72-4	–	0.02/0.06
Chloroacetamide	Pethoxamid	herbicide	pethoxamid	PM	106700-29-2	–	0.04/0.12
			pethoxamid metabolite-42	PM MET	–	–	0.04/0.14
Phenylpyrazole	Fipronil	insecticide	fipronil sulfone	FPS	120068-36-2	–	1.95/6.51
Triazole	Tebuconazole	fungicide	hydroxy-1-tebuconazole	TEB-OH	212267-64-6	7.8 [d]	0.02/0.05

[a] Related to cypermethrin, (Ratelle et al., 2015).
[b] Related to cyfluthrin, (Leng et al., 1997).
[c] Related to chlorpyrifos, (Nolan et al., 1984).
[d] Related to tebuconazole, (Oerlemans et al., 2019).

pump gradient was non-linear. The following gradient program was used for elution: 10% B (initial), 10–100% B (from 1 to 4 min for negative mode and from 1 to 3 min for positive mode). After 6.1 min, the ratio was decreased to 10% B and held for 3 min to achieve column equilibrium before the next injection. The flow rate of the mobile phase was 0.20 mL/min for negative MS-MS (0.30 mL/min positive MS-MS). 5 μL of the individual sample was injected for analysis.

The MS was an AB Sciex Qtrap 5500+ instrument (AB Sciex, Concord, ON, Canada) with electrospray ionization (ESI). Ions were detected in either negative or positive mode. The ionization parameters were as follows: capillary voltage −4.5 kV/5.5 kV; desolvation temperature 550 °C/500 °C; Curtain gas 25 psi, Ion Source Gas1 40 psi, Ion Source Gas 2 60 psi. Quantitative analysis was performed using Analyst software. IMPY, PM, and TEB-OH were analysed by positive HPLC-MS-MS. The remaining biomarkers were analysed by negative HPLC-MS-MS.

2.4. Data analysis

Data were analysed and visualized in the R programming language, version 4.1.1 (R Core Team, 2021). Measured pesticide/metabolite concentrations were adjusted to creatinine and specific gravity (SG). SG adjustment was based on the following equation:

$$c_{SG\ adj.} = B \times \left[\left(SG_{avg.} - 1 \right) / \left(SG - 1 \right) \right]$$

where $c_{SG\ adj.}$ is the SG adjusted concentration of the biomarker, B is the measured concentration of the biomarker, $SG_{avg.}$ is the average specific gravity of all adult (1.017) or child (1.021) samples, and SG is the specific gravity of the urine sample (Sauvé et al., 2015).

The limit of quantification (LOQ) and limit of detection (LOD) for each measured compound is shown in Table 1. Values below LOQ and/or LOD were imputed on the basis of maximum likelihood multiple estimation dependent on observed values, which were expected to have

a lognormal distribution (Lubin et al., 2004). The imputation was done only for compounds detected in at least 40% of all the samples.

A paired test (the Wilcoxon signed-rank test) was used to compare adult and child mean concentrations between seasons. A two sample test (the Wilcoxon rank-sum test) was used to compare adult and child mean concentrations within each season. The Spearman Rho coefficient (r) was used to determine correlations between pesticide metabolites/parent compounds, distance to agricultural areas, and agricultural area surface area in both seasons and age categories.

BMI (body mass index) calculation and categorization was based on information from the Centers for Disease Control and Prevention website (CDC, 2020). Participants were divided into urban, suburban, and rural area categories. This division was based on the degree of urbanisation provided by Eurostat (EUROSTAT, 2018) and participants' home addresses. Agricultural area and distance to the nearest field were also calculated for each household. Agricultural field area (fields, orchards, vineyards) was determined according to the Agricultural Field Area dataset, freely available from the Ministry of Agriculture of the Czech Republic (MACR) on the web portal of the Land Parcel Identification System (LPIS) (MACR, 2022). The absolute areas of fields, orchards, and vineyards were calculated in 250 m, 500 m, and 1000 m buffer zones around each participant's household and were valid for March 2020.

2.5. Daily intake estimation

To estimate the daily intake of parent pesticides and assess the possible risks of exposure to them, a model based on urinary metabolite levels was used. We performed the daily intake calculation using the following toxicokinetic model derived from an equation used in previous studies (Katsikantami et al., 2019):

$$EDI\ (\mu g\ /\ kg-bw\ /\ day) = \frac{c_{met.}(\mu g/g\ crea.) \times CE(g\ crea./day) \times \frac{MW_{parent}}{MW_{met.}}}{BW(kg) \times F_{UE}}$$

where EDI is Estimated Daily Intake, $c_{met.}$ is the concentration of the relevant metabolite in urine, CE is the anthropometry- and gender-based reference value for creatinine excretion in urine derived for children (Remer et al., 2002) and adults (Forni Ogna et al., 2015), MW_{parent} and $MW_{met.}$ are the molecular weights of the parent compound and metabolite respectively, BW is bodyweight, and F_{UE} is the ratio between the intake of parent pesticide and the amount of metabolite excreted in the urine. In the case of pyrethroid metabolites, cypermethrin was selected as a parent compound on the basis of annual data on the use of active substances contained in Plant Protection Products in the Czech Republic (CISTA, 2022). The urinary excretion factors (F_{UE}) for TCPY and TEB-OH are 0.7 and 0.38 (Nolan et al., 1984; Oerlemans et al., 2019), meaning that, on average, 70% and 38% of parent pesticide is excreted as a respective urinary metabolite. The excretion of pyrethroid metabolites is in the range 0.13–0.27 for 3-PBA and 0.28–0.64 for t/c-DCCA (Eadsforth and Baldwin, 1983; Eadsforth, 1988; Woollen et al., 1992; Ratelle et al., 2015); thus, mean excretion factors of 0.20 and 0.47 were used. Using obtained excretion factors, realistic scenario EDIs were calculated. As obtained F_{UE} are derived on the basis of healthy volunteers and are only applicable to adults, daily intakes with 0.05 and 0.95 F_{UE} were additionally estimated to affect the worst-case and the best-case scenario, when 5% or 95% of the parent compound is excreted in urine as a metabolite, as proposed by Bravo et al. (2019), 2020.

2.6. Risk assessment

To quantify the potential risk from exposure, the estimated daily intake was divided by the Acceptable Daily Intake (ADI) set by the EFSA. The ADIs used for calculation are shown in Table 4. This ratio was then multiplied by 100%, indicating the percentage of the ADI used up by a specific pesticide. If this percentage exceeds 100%, exposure could be a potential cause of concern. This % of ADI approach is similar to the Hazard Quotient (HQ) used as an indicator of possible health risk (Koch et al., 2011).

To assess the possible risk of combined exposure to a mixture of pesticides with respect to effects on the nervous system, the EFSA cumulative risk assessment approach was deployed. This approach is based on the presumption that pesticides included in the same cumulative assessment group (CAG) might produce joint, cumulative toxicity, even if they do not have similar modes of action (EFSA., 2019a). Therefore, primarily health risks in two CAGs were assessed: CAG on chronic functional effects on the motor division (CAG motor) and CAG on the brain and/or erythrocyte acetylcholinesterase inhibition (CAG AChE inhibition) as the highest risks are expected to be observed for these effects (EFSA., 2019a). Firstly, the margin of exposure (MOE) for each parent pesticide (i) and each CAG (j) was calculated:

$$MOE_{i,j} = \frac{NOAEL_{i,j}}{EDI_i}$$

where $NOAEL_{i,j}$ (No Observed Adverse Effect Level) is pesticide (i) and CAG (j) specific No Observed Adverse Effect Level set as a toxicological reference point.

The total margin of exposure (MOET) was then summed to assess the cumulative risk in every CAG as follows:

$$\frac{1}{MOET_j} = \sum \frac{1}{MOE_{i,j}}$$

An overview of CAGs and parameters used for the calculation of $MOET_j$ is shown in SI Table 1. If $MOE_{i,j}$ or $MOET_j$ are greater than 100, exposure is within safe limits (EFSA, 2008).

3. Results

3.1. Study population

The adult participants indcluded 65% of females with a median age of 41, mostly falling into the normal weight BMI category. Male adult participants had a similar median age (42) and most fell into the normal weight BMI category, although males were more frequently obese or overweight than females. The sex of child participants was more equally distributed, with girls representing 43% and boys, 57%. The median age was the same in both groups (9) and BMI was also similar, with most children falling in the normal weight category. Boys were somewhat more likely to be underweight, overweight, or obese than girls. The study population is characterized in Table 2.

Participants were mostly living in rural areas (50%) followed by suburbs (29.1%) and urban districts (20.9%) (SI Table 2). In rural areas, the median distance to fields was 66 m, with fields representing 21% (median) of the total area of 250 m buffer zones. In suburbs, the median distance to the nearest field was 198 m, with fields representing 1% (median) of the total area of 250 m buffer zones. Participants living in urban areas had a mean distance to the nearest field of 818 m and, therefore, there was a median field area of 0% in the 250 m buffer zones. Most participants' home addresses identified as urban were located within the city limits of Brno, the second-largest city in the Czech Republic.

3.2. Urinary CUP metabolite levels

Overall, 440 urine samples were analysed for 12 pesticides or their metabolites. Descriptive statistics on non-adjusted, SG-adjusted, and creatinine-adjusted concentrations and the detection frequencies for all 12 compounds across seasons and subgroups are shown in SI Tables 3–14. Creatinine-adjusted data did not differ from SG-adjusted data, thus, we used SG-adjusted data in further analyses and for the presentation of results. Furthermore, SG adjustment is the preferred method due to its achievement of more robust outputs, especially in

Table 2
Characteristics of the studied population (baseline data).

Adults	Female (n = 71), 65%			Male (n = 39), 35%		
	Median	Min.	Max.	Median	Min.	Max.
Age (years)	41	33	54	42	31	54
Height (cm)	168	158	183	180	167	197
Weight (kg)	64	45	90	82	60	114
BMI						
Underweight (%)	4.2			0		
Normal weight (%)	67.6			48.7		
Overweight (%)	26.8			38.5		
Obese (%)	1.4			12.8		
Live in						
Urban area (%)	20.9					
Suburbs (%)	29.1					
Rural area (%)	50					
Children	Girls (n = 47), 43%			Boys (n = 63), 57%		
	Median	Min.	Max.	Median	Min.	Max.
Age (years)	9	4	12	9	4	15
Height (cm)	134	109	156	133	105	172
Weight (kg)	28	17	46	28	17	73
BMI						
Underweight (%)	4.3			12.7		
Normal weight (%)	91.5			76.2		
Overweight (%)	4.3			6.3		
Obese (%)	0			4.8		

children (Pearson et al., 2009). Only 4 metabolites were detected in more than 40% of all samples and were thus used in further analyses. An overview of medians and 95th percentiles of concentrations and detection frequencies across seasons and subgroups are given in Table 3 (SG-adjusted). The most frequently detected metabolite was TEB-OH, with a detection frequency varying from 94.6% to 99.1% across seasons and subgroups, followed by 3-PBA (51.8%–88.2%), t/c-DCCA (50%-86.4), and TCPY (40.9%-87.3). MDA reached a detection frequency of 56.36%, but only in adults in the winter season. Similarly, the detection frequency of PM MET was 39.09%, this almost reaching the inclusion threshold, but again only in adult winter samples. The remaining biomarkers had detection frequencies ranging from 0% to 10.91%, well below the inclusion threshold.

We compared both adult and child levels of CUP metabolites in the winter and summer seasons (Fig. 2). TCPY levels in adults were significantly higher in the winter season (median = 3.27 ng/ml) in comparison to the summer season (median = 0.16 ng/ml). This was also the most pronounced difference among all comparisons. We found no significant differences between the winter and summer seasons for all the remaining metabolites in adult samples.

For children, there were statistically significant differences in metabolite concentrations between the winter and summer seasons. The only exception was in TEB-OH, where there was no difference between seasons. As in the case of adults, metabolite levels were higher in winter (3-PBA median = 0.56 ng/ml, t/c-DCCA median = 1.45 ng/ml, TCPY median = 4.6 ng/ml) in comparison to the summer season (3-PBA median = 0.38 ng/ml, t/c-DCCA median = 0.73 ng/ml, TCPY median = 2.01 ng/ml).

There were also significant differences in levels of CUP metabolites between adults and children (SI Fig. 1). In both seasons, CUP metabolites were significantly higher in child urine samples in comparison to adult urine samples. The only exception to this rule was TEB-OH. No differences were observed in the case of TEB-OH when comparing adult urine samples (winter median = 0.47 ng/ml, summer median = 0.44 ng/ml) and child urine samples (winter median = 0.44 ng/ml, summer median = 0.46 ng/ml). The most pronounced difference was between summer TCPY levels in adults (median = 0.16 ng/ml) and children (median = 2.01 ng/ml).

We also compared differences in levels of CUP metabolites among participants living in urban, suburban, and rural areas, both between seasons and between adults and children (SI Figs. 2–3). There were very few significant differences when comparing participants living in different areas. Winter levels of 3-PBA were significantly higher in adults living in an urban area in comparison to adults living in a rural area. However, there was no difference between adults living in urban areas and suburbs. The same was true for summer levels of t/c-DCCA. There were no differences in winter levels of TEB-OH regardless of area. However, during summer, adults living in urban areas had significantly lower levels in comparison to adults living in rural areas and suburbs.

There was also a difference in children's winter levels of 3-PBA, where children's levels in urban areas were significantly higher in comparison to children's levels in both rural and suburban areas. Summer levels of t/c-DCCA were higher in children living in suburbs in comparison to children living in rural areas, but not in comparison to children living in urban areas. The same was true for children's levels of TEB-OH.

As an alternative, we also categorized and compared distance to the nearest field in a similar way (SI Figs. 4–7). The results of these comparisons were largely similar to those for the previous categorization into rural, suburban, and urban areas.

3.3. Urinary CUP metabolite correlations

Spearman correlation coefficients (r) for child and adult samples are given in Fig. 3. Statistically significant correlations are observed between the pyrethroid metabolites 3-PBA and t/c-DCCA in both study groups and seasons, with moderate correlation coefficients in summer samples (r = 0.64 and 0.59) and weak correlations in winter samples. (r = 0.27 and 0.34). The remaining correlations are weak ($r < 0.4$) or insignificant, showing no conclusive pattern.

The statistically significant pattern in pyrethroid metabolites is observed again in crossed correlation analysis (between winter and summer samples and in adult-child pairs) (see Fig. 4). Pyrethroid metabolites are generally weakly-to-moderately intercorrelated between seasons and adult-child pairs. Correlations between adults and children in summer samples are stronger (r = 0.36–0.53) in comparison to winter samples (r = 0.1–0.38). Correlations between summer and winter samples in adults are moderately stronger (r = 0.21–0.44) in comparison to child samples (r = 0.21–0.29).

Levels of TCPY and TEB-OH are also weakly ($r < 0.4$) intercorrelated between adults and children in each season, but not between seasons. The rest of the correlations are weak ($r < 0.4$) or insignificant, showing no patterns across season or age group.

Table 3
Percentage of measurements above LOQ for metabolites with an overall detection frequency higher than 40%; median values and 95th percentiles (in brackets) of imputed and SG-adjusted concentrations (ng/mL) in urine samples.

Metabolite	Adults, winter season (n = 110)		Adults, summer season (n = 110)		Children, winter season (n = 110)		Children, summer season (n = 110)	
	% >LOQ	Median (P95)	% >LOQ	Median (P95)	% >LOQ	Median (P95)	% >LOQ	Median (P95)
3-PBA	51.8	0.16 (0.91)	51.8	0.19 (1.18)	88.2	0.56 (2.25)	82.7	0.38 (1.57)
t/c-DCCA	60.9	0.56 (3.16)	50	0.3 (1.77)	86.4	1.45 (6.66)	76.4	0.73 (3.23)
TCPY	87.3	3.27 (7.37)	40.9	0.16 (3.07)	83.6	4.6 (9.73)	83.6	2.01 (4.44)
TEB-OH	98.2	0.47 (1.74)	94.6	0.44 (4.05)	99.1	0.44 (1.77)	97.3	0.46 (9.86)

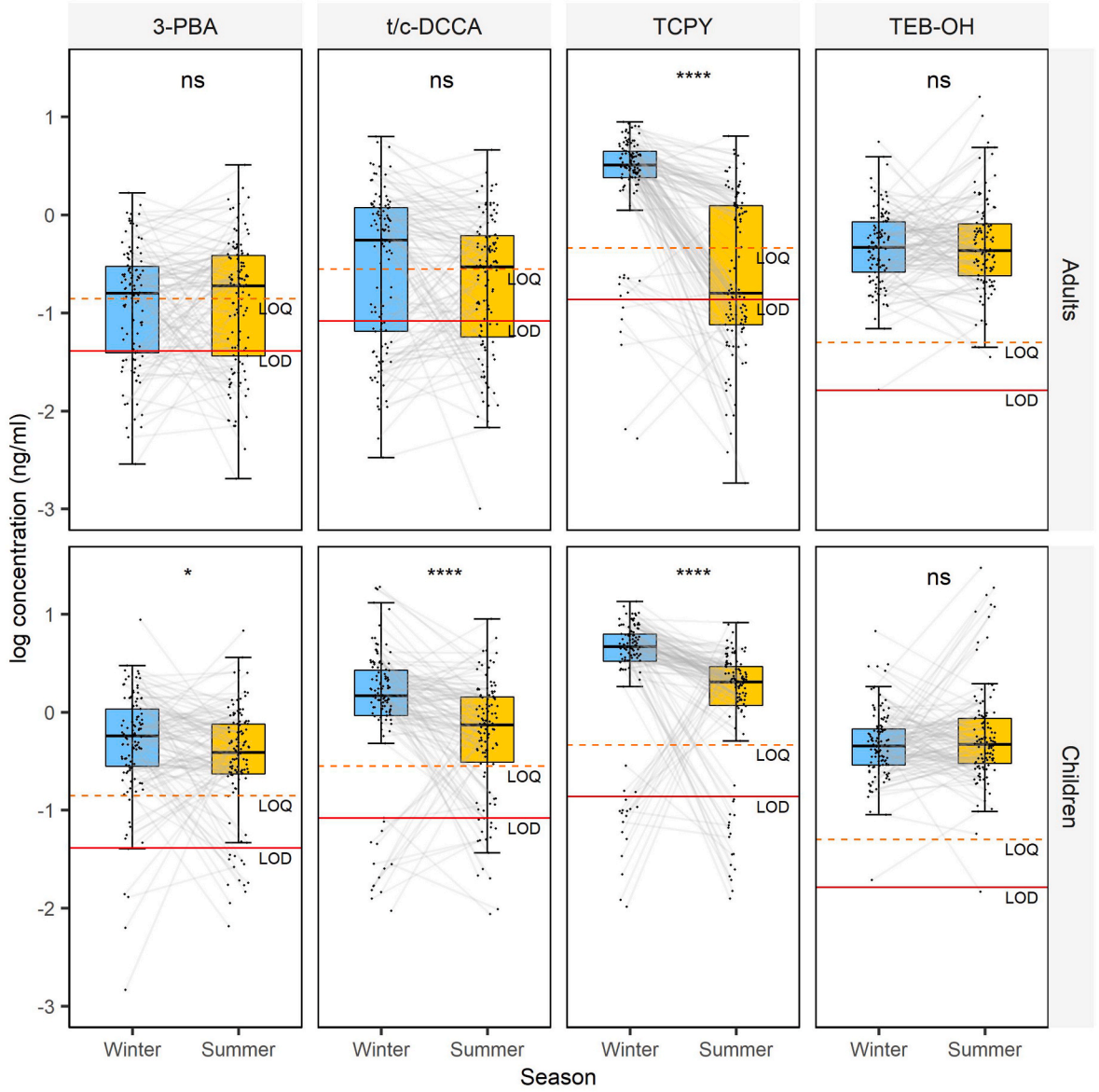

Fig. 2. Box plots comparing imputed and SG-adjusted urine concentrations of 3-PBA, t/c-DCCA, TCPY, and TEB-OH in adults and children in each season according to the Wilcoxon signed-rank test (ns statistically insignificant; *significant at $p \leq 0.05$; ****significant at $p \leq 0.0001$).

Correlation analysis between pesticide metabolites and location characteristics (distance to the field and field area in 3 buffer zones) did not show any conclusive relationships (SI Fig. 8). Most of the correlations were insignificant and those that were significant were weak ($r < 0.4$ or > -0.4) and inversed. Distance to the fields was weakly ($r < 0.4$) correlated with winter levels of 3-PBA, TCPY, and TEB-OH in children. This relation was not observed in summer or in any of the correlations among adult participants.

In addition, we performed a sensitivity analysis excluding imputed values (using only observations > LOQ). The results of the sensitivity analysis can be found in SI Figs. 9–11. No obvious differences were observed between child and adult correlations. In the crossed correlation analysis and correlations with location characteristics, a few dissimilar results can be observed, but this is mainly due to the low number of evaluated samples in the cross-correlation matrix.

3.4. Estimated daily intake

The daily intake for 3 parent compounds (cypermethrin, chlorpyrifos, and tebuconazole) was estimated. Medians and 95th percentiles (P95) for adults and children are presented in Table 4. All EDIs were significantly higher in children than in adults, except for tebuconazole (Wilcoxon paired test). The daily intake of cypermethrin was estimated twice according to the concentrations of the two most common pyrethroid metabolites, 3-PBA and t/c-DCCA. When comparing these two approaches, no statistically significant difference in cypermethrin EDI was found in either adults or children. With respect to realistic scenario EDIs for the studied pesticides, the highest daily intake in children was observed for cypermethrin, regardless of the analysed metabolite (median = 0.153 and 0.170 μg/kg-bw/day for 3-PBA and t/c-DCCA respectively), followed by chlorpyrifos (median = 0.132 μg/kg-bw/day) and tebuconazole (median = 0.019 μg/kg-bw/day). In the case of adults, the highest EDI was obtained for chlorpyrifos in all evaluated scenarios (realistic scenario median = 0.077 μg/kg-bw/day).

3.5. Risk assessment

Comparison of EDIs with their respective ADIs shows the percentage of ADI used up by specific pesticides (Table 5). In a realistic scenario, the ADI was exceeded (% of ADI >100%) only in one child, this in the case of

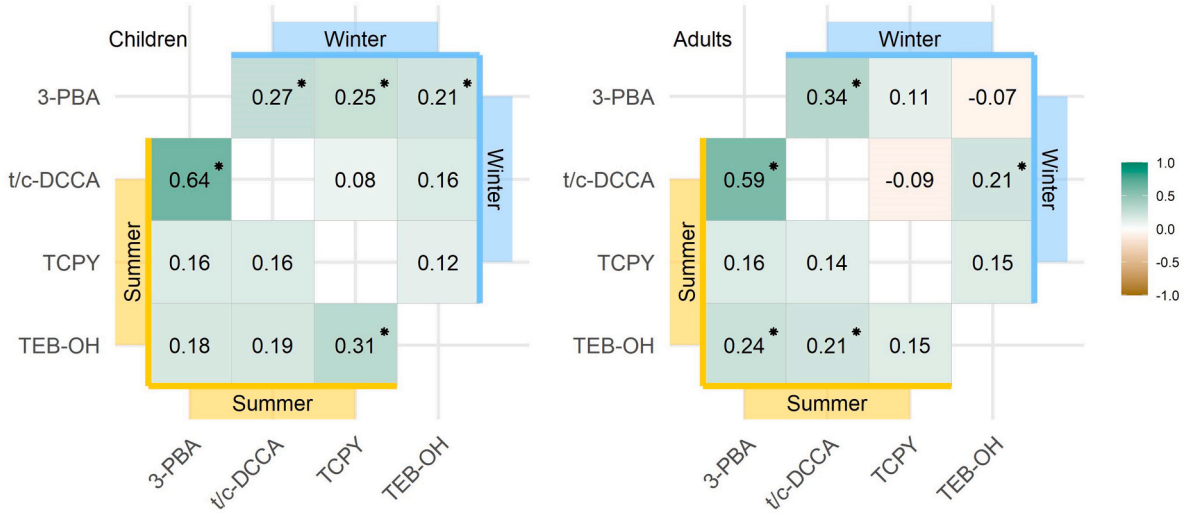

Fig. 3. Spearman correlations among SG-adjusted and imputed urine pesticide metabolites in children and adults. Bottom left (yellow) – correlation coefficients in summer samples; upper right (blue) – correlation coefficients in winter samples (*significant at $p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

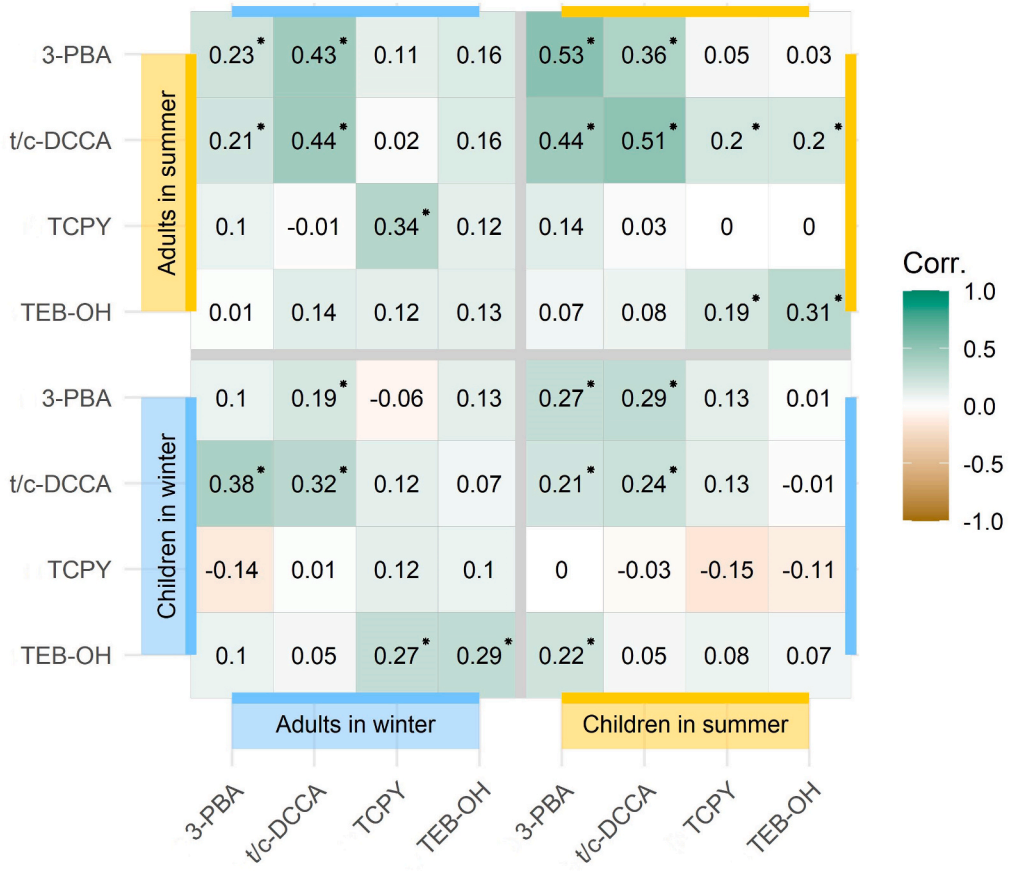

Fig. 4. Spearman correlations among SG-adjusted and imputed urine pesticide metabolites in adults (summer vs. winter) – upper left; children (summer vs. winter) – bottom right; winter (children vs. adults) – bottom left; and summer (children vs. adults) – upper right (*significant at $p \leq 0.05$).

cypermethrin. When considering the worst-case scenario, overall, 23 (10%) and 158 (72%) of 220 child samples exceeded cypermethrin and chlorpyrifos ADIs, respectively. In the worst-case scenario for adults, 117 of 220 samples (53%) exceeded the chlorpyrifos ADI. The lowest percentages of ADI were shown for tebuconazole, with a median value of only 0.07% in the realistic scenario, which is a result of the low EDI and higher ADI compared to the other assessed pesticides.

Assessment of the cumulative risk of the combined exposure of pesticides with an effect on the nervous system was primarily performed for two CAGs: CAG motor and CAG AChE inhibition. Using a realistic scenario, none of the median MOETs was below 100 (CAG motor MOET = 23,313; CAG AChE inhibition MOET = 891). Considering the worst-

Table 4
Median values and 95th percentiles of estimated daily intakes (μg/kg-bw/day) under different scenarios, respective Acceptable Daily Intakes (μg/kg-bw/day), and Wilcoxon paired test results.

Metabolite (pesticide)		Estimated Daily Intake (ug/kg-bw/day)							
		Best-case scenario		Realistic scenario		Worst-case scenario			ADI (ug/kg-bw/day)
		median	P95	median	P95	median	P95	p-value	
3-PBA (cypermethrin)*	All	0.013	0.112	0.064	0.531	0.255	2.134		
	Adults	0.004	0.030	0.020	0.140	0.081	0.564	<0.001	5[a]
	Children	0.032	0.173	0.153	0.815	0.615	3.278		
t/c-DCCA (cypermethrin)*	All	0.031	0.246	0.062	0.501	0.581	4.674		
	Adults	0.011	0.077	0.022	0.156	0.209	1.454	<0.001	5[a]
	Children	0.083	0.389	0.170	0.793	1.584	7.387		
TCPY (chlorpyrifos)	All	0.083	0.282	0.112	0.383	1.571	5.359		
	Adults	0.057	0.245	0.077	0.333	1.076	4.657	<0.001	1[b]
	Children	0.097	0.315	0.132	0.428	1.846	5.994		
TEB-OH (tebuconazole)	All	0.008	0.054	0.021	0.135	0.159	1.024		
	Adults	0.010	0.046	0.025	0.116	0.187	0.879	0.177	30[c]
	Children	0.008	0.057	0.019	0.142	0.146	1.083		

* Wilcoxon paired test shows no statistically significant difference between realistic scenario estimations based on 3-PBA and t/c-DCCA metabolites in both adults and children (p-value > 0.1).
[a] ADI, cypermethrin (EFSA, 2018)
[b] ADI, chlorpyrifos (EFSA, 2014a).
[c] ADI, tebuconazole (EFSA, 2014b).

Table 5
Median and 95th percentile of the percentage of ADI consumed by specific pesticide under different scenarios.

Metabolite (pesticide)		% of ADI					
		Best-case scenario		Realistic scenario		Worst-case scenario	
		median	P95	median	P95	median	P95
3-PBA (cypermethrin)	All	0.27	2.25	1.27	10.6	5.11	42.7
	Adults	0.09	0.59	0.40	2.81	1.62	11.3
	Children	0.65	3.45	3.06	16.3	12.3	65.7
t/c-DCCA (cypermethrin)	All	0.61	4.92	1.25	10.0	11.6	93.5
	Adults	0.22	1.53	0.45	3.12	4.19	29.1
	Children	1.67	7.78	3.40	15.9	31.7	148
TCPY (chlorpyrifos)	All	8.27	28.2	11.2	38.3	157	536
	Adults	5.67	24.5	7.69	33.3	108	466
	Children	9.71	31.6	13.2	42.8	185	599
TEB-OH (tebuconazole)	All	0.03	0.18	0.07	0.45	0.53	3.41
	Adults	0.03	0.15	0.08	0.39	0.62	2.93
	Children	0.03	0.19	0.06	0.47	0.49	3.61

case scenario, when only 5% of the parent pesticide is excreted in the urine, the MOET for CAG AChE inhibition was 64 and thus exposure could be considered a risk for humans. The results of cumulative risk assessment across all CAGs and scenarios are presented in SI Table 15.

4. Discussion

4.1. Urinary concentrations

We investigated levels of CUP metabolites in adult and child urine samples in the winter and the summer. We found little difference across seasons regarding CUP levels in adult urine. This difference was limited only to TCPY, as differences between seasons in the remaining CUP metabolites were statistically insignificant. Higher levels of TCPY metabolites were also found in the winter season in comparison to the summer season. This was also true for children's urine samples. In children's samples, the levels of all CUP metabolites were higher in the winter season in comparison to the summer season. The only exception was in children's TEB-OH levels, which were similar in both seasons. We also observed a considerable difference in CUP levels between adults and children. Children had higher levels of CUP, with the exception of TEB-OH, which was similar among both age groups and seasons. Although the exposure scenarios may be similar in both children and adults, children are not simply tiny adults, thus other factors should be considered. Children's breathing rate is higher in comparison to adults', further increasing children's susceptibility to volatile CUPs (Garry, 2004). This is also directly connected with children's hand-to-mouth behavior and possible CUP contamination of household dust (Bennett et al., 2019) or contact with pesticide-treated carpets or fabric (Becker et al., 2006). Diet, whether similar to, or different from adults, is another factor. Children have higher food intake than adults in comparison to their body weight, and dietary preferences may also play a major role (Landrigan and Goldman, 2011). Lice treatments, repellents, indoor and outdoor household pesticides are examples of other factors possibly affecting pesticide levels in adults and children (Lu et al., 2009).

The Czech population appears to be exposed to CUPs at more-less similar levels as other populations in European and North American countries (SI Table 16), although there is noticeable variability in the results among studies. Some of this variability is expected, due to different methods of CUP adjustment (creatinine/SG), but also to different exposure levels among the studied populations. These levels are dependent on the degrees of environmental, dietary, and occupational exposure to CUPs in the given population. Urinary levels of 3-PBA and t/c-DCCA are mostly in line with previous studies, although levels of t/c-DCCA in Czech children were increased in comparison to French (Glorennec et al., 2017), German (Becker et al., 2006), and Canadian (Health Canada, 2019) children. We found only a few studies reporting on urinary levels of TEB-OH, where such levels were lower in

comparison to our study (Norén et al., 2020; Oerlemans et al., 2021). The TCPY levels found in this study deviate the most from the pesticide levels reported in literature (Bravo et al., 2019, 2020; Health Canada, 2019; Fernández et al., 2020), and were also the highest among the measured CUPs.

It is reasonable to expect increased environmental exposure to CUPs in the summer season, due to processes associated with such exposure (Brouwer et al., 2018) and increased field activity (Figueiredo et al., 2021). This is supported by Osaka et al. (2016); Llop et al. (2017); Doğanlar et al. (2018); and Paglia et al. (2021), who reported increased levels of CUP in summer (use season) in comparison to winter (non-use season) in the non-occupationally exposed population. However, CUP levels between seasons in the Czech population deviate from previously reported observations, suggesting that environmental exposure to CUPs contributes less to overall exposure to CUPs than dietary exposure. There is also virtually no correlation between CUP metabolite levels and agricultural area distance or surface area.

Levels of TEB-OH indicate stable exposure to tebuconazole among seasons and age groups. Tebuconazole was recently reported in wheat flour (Tao et al., 2021) and the use of this pesticide on cereals is abundant in the Czech Republic (CISTA, 2022). These clues lead us to the assumption that tebuconazole exposure is probably driven by common food items such as pastry or bread, and that environmental exposure is secondary. It is worth pointing out through, that there is only a weak or no correlation in TEB-OH concentrations between seasons and age categories. This suggests more than one significant source of exposure to this pesticide which changes based on season and age category. Exposure to the remaining pesticides varies among seasons and age groups. Chlorpyrifos residues were previously found in vegetables such as eggplant, cabbage, cauliflower (Sinha et al., 2012), cucumber, kale, celery (Yuan et al., 2014; Hongsibsong et al., 2020), and also in apples and bananas (Ferré et al., 2018). Consumption of such food may therefore lead to exposure to chlorpyrifos. On the other hand, it is also worth pointing out that in mid-2020, chlorpyrifos was banned in the Czech Republic in response to the non-renewal of approval criteria for chlorpyrifos by the European Commission (EFSA, 2019b). This may be part of the reason for decreased levels of TCPY in the summer season, although there is still a significant ($p \leq 0.0001$) difference in TCPY levels between adults and children. Chlorpyrifos biomarker correlations indicate at least partially related sources of exposure in adults between seasons, but there seems to be no connection between adult and children's levels in the winter nor summer. This leads us to the assumption that adult's exposure is more uniform across the population in comparison to children's exposure to chlorpyrifos. Based on our findings, chlorpyrifos biomarker levels vary between age categories and seasons, but there also appear to be significantly different exposure sources in adults and children. Exposure to pyrethroids can also be driven by food as pyrethroid residues were found in leafy vegetables (Zhang et al., 2021) and various other types of vegetables (Tang et al., 2018), milk, eggs, and meat (Dallegrave et al., 2018). Similarly, as in the case of chlorpyrifos, the consumption of such products is generally not negligible in the Czech population (CZSO, 2020) and may vary across the year seasons. Concentrations of two pyrethroid metabolites (3-PBA, t/c-DCCA) were moderately correlated in children samples in the summer season. The same is true for the adults in the same season. A similar but slightly weaker correlation was also observed between adults and children in the summer samples. The similar trends in both pyrethroid metabolites are not too surprising, given that both compounds share parental pesticides. But the observed relation of these metabolites is the strongest among all studied biomarkers and points to a similar driver of pyrethroids exposure in children and adults during the summer season. Subtle, yet still significant are the correlations between adults and children in the winter season. These trends suggest that the sources of exposure vary between age categories and most predominantly in the winter season. One of the reasons for increased levels of pesticides in the winter season may be an increase in consumption of certain types of foods while overall consumption of food is increased in winter as well (Spence, 2021), further increasing the exposure. The import of foreign food with possibly increased pesticide residues consumed in winter months could be another reason for the observed winter increase (EFSA, 2021).

4.2. Estimated daily intake and risk assessment

We used the non-specific pyrethroid metabolites 3-PBA and t/c-DCCA for the estimation of parent compound daily intake. Cypermethrin was selected as a representative pyrethroid on the basis of annual data on the consumption of active substances contained in Plant Protection Products in the Czech Republic (CISTA, 2022), despite the fact that several parent pyrethroids (cypermethrin, permethrin, deltamethrin, cyfluthrin, cyhalothrin, etc.) could be metabolized into these metabolites (Ueyama et al., 2010). Two approaches to estimation allowed us to compare the use of both non-specific pyrethroid metabolites in exposure assessment. Higher concentrations in urine, combined with higher excretion factors in the case of t/c-DCCA (Eadsforth and Baldwin, 1983; Eadsforth, 1988; Woollen et al., 1992; Ratelle et al., 2015) gave us similar results using both metabolites ($p > 0.1$), indicating the possibility of the simultaneous use of both 3-PBA and t/c-DCCA in daily intake estimation. Moreover, our EDIs (regardless of metabolite) are consistent with a mother-child study conducted in Slovenia (Bravo et al., 2020), and children in Spain (using cyhalothrin as a representative pyrethroid) (Fernández et al., 2020). Higher daily intakes of pyrethroids and organophosphates in children than in adults reported in previous studies (Cequier et al., 2017; Katsikantami et al., 2019; Bravo et al., 2020) are in agreement with our results. Less is known about tebuconazole exposure assessment based on internal dose measures in both children and adults. No significant difference between adult and child EDIs presents a pattern in exposure and/or metabolism which is dissimilar to that presented by the other pesticides.

Excretion factors for pesticide metabolites in urine derived from toxicokinetic studies are usually applicable only to adults, and these studies are performed with a small number of healthy volunteers (Ratelle et al., 2015; Oerlemans et al., 2019). Due to this gap in toxicokinetic knowledge, which is a barrier to the appropriate interpretation of biomonitoring data, we estimated daily intake in two additional scenarios. The best-case and the worst-case scenarios assume that respectively 95% and 5% of a given pesticide is excreted in urine as the respective metabolite. Under the worst-case scenario conditions, comparison with ADIs for cypermethrin (EFSA, 2018), chlorpyrifos (EFSA, 2014a), and tebuconazole (EFSA, 2014b) shows us that chlorpyrifos exceeded safe levels in more than 60% of samples. Moreover, chlorpyrifos toxicity has been discussed with a view to reconsidering the recommended ADI of 1 μg/kg-bw/day (Mie et al., 2018), despite the fact that no exceedance of safe levels were reported in previous European studies (Fernández et al., 2020), even under the worst-case scenario (Cequier et al., 2017; Bravo et al., 2019). The few exceedances of the safe level for cypermethrin were mainly due to its strict selection as a representative pyrethroid, its ADI (5 μg/kg-bw/day) being lower in comparison with other pyrethroid parent compounds (permethrin ADI 50, deltamethrin ADI 10 μg/kg-bw/day) (EFSA, 2019a). In the case of tebuconazole, the first health risk assessment based on biomonitoring data using a toxicokinetic model to reconstruct the intake showed a risk of two orders of magnitude below the safety threshold. The same practically negligible risk was also observed by an OBO study in the Netherlands which compared participants' urinary levels with the urinary levels of volunteers exposed to pesticide concentrations just below the level of the ADI (OBO, 2019). In contrast, Lozowicka (2015) reported exposure to tebuconazole to carry the highest risk in comparison with all other pesticides, this based on pesticide residues in food and exceeding the safety limit in some cases.

Because of multiple exposures to pesticides as well as other environmental contaminants, single-substance health risk assessment could

underestimate any real health impact and, therefore, cumulative risk assessment is needed (Bopp et al., 2018). Considering the dissimilar modes of action (MoA) of the assessed pesticides for which ADIs are derived, we deployed a response addition approach, as introduced by the EFSA Panel on Plant Protection Products and their Residues (PPR) (EFSA, 2019a) for pesticides that are toxic to the central nervous system. In any realistic exposure scenario, MOETs for all CAGs were higher than 100, which is generally considered the protective threshold for humans. In the worst-case scenario, we estimated a median MOET value lower than 100 for CAG on brain and/or erythrocyte acetylcholinesterase inhibition. It should be noted that of all the assessed pesticides, only chlorpyrifos has an established NOAEL associated with this effect. Therefore, in this case, results from individual risk assessment are comparable with risk attributable to the respective CAG.

With respect to other studies, a toxic risk to the central nervous system was also not found in various populations. MOET associated with brain and/or erythrocyte acetylcholinesterase inhibition was slightly higher (MOET 2,177) in Spanish children using an internal exposure approach (Fernández et al., 2020), and also higher (MOET in the range 1,630–3,820) in 9 European countries, including the Czech Republic, using an external dietary exposure assessment approach (Dujardin and Bocca, 2019). In contrast, in the above-mentioned studies, lower MOET was reported in association with functional alterations of the motor division, but was still within the safe range, as in the current study.

4.3. Strengths and limitations

This study has several strengths in comparison to other studies. We collected samples from both an adult and a child in one household, which enabled us to directly compare urinary pesticide levels in both age groups while limiting variability. We were able to compare exposures during winter and summer in the same manner. We also adjusted our measurements for SG and creatinine, increasing comparability with other studies. On the other hand, our study also bears limitations. One was the inability to reliably distinguish between non-use period and use period, meaning that we cannot rule out the possibility of pesticide application during late winter and can only assume increased spraying in the summer months. In addition, CUP application itself may also have varied among specific CUPs within the season. Furthermore, although urine samples provide a good insight into short-term exposure to CUPs (Oerlemans et al., 2021), the use of such samples might also be a limiting factor, specifically for those metabolites with a short half-life. This is because we collected only one urine sample per participant in each season. Thus, the results cover a brief window in each season, which may not be fully representative of CUP levels in the given season.

5. Conclusion

In this study, we analysed urine for the presence of several CUP metabolites in adult and child samples in a repeated design. Significantly higher concentrations of CUPs in child samples indicate increased exposure in this vulnerable population group. Exposure patterns of the analysed CUPs indicate that dietary exposure is probably a more relevant pathway in comparison to environmental exposures. That said, exposure pathways should be more thoroughly investigated in future studies. The highest health risk, based on the calculation of daily intake and comparison with EFSA acceptable daily intake, was associated with chlorpyrifos. Nonetheless, risks associated with exposure to all pesticides were still within safe levels. To the best of our knowledge, we conducted here the first health risk assessment of a CUP mixture for the Czech population based on an internal dose. Despite the low risk of this mixture, it is justified to continuously evaluate human exposure to such compounds and focus on the accuracy of dose-effect relationships. Using worst-case scenario parameters, exposure to chlorpyrifos and cypermethrin indicated a possible risk, especially in children. Therefore, further research is needed to distinguish between excretion factors in children and adults in order to responsibly evaluate associated health risks. In our study, the cumulative risk assessment of effects on the nervous system yielded similar results as the single pollutant approach; nevertheless, there is a need for a methodology pertinent to other organ systems. There is also a need to extend the range of commonly analysed substances and add emerging chemicals of potential interest.

Credit author statement

Libor Šulc: Formal analysis, Investigation, Writing – original draft, Visualization; **Tomáš Janoš**; Formal analysis, Investigation, Writing – original draft, Visualization; **Daniel Figueiredo**: Conceptualization, Writing – review & editing; **Ilse Ottenbros**: Conceptualization, Writing – review & editing; **Petr Šenk**: Validation, Investigation, Writing – review & editing; **Ondřej Mikeš**; : Conceptualization, Writing – review & editing; **Anke Huss**: Conceptualization, Writing – review & editing; **Pavel Čupr**: Conceptualization, Coordination, Resources, Writing – review & editing, Supervision.

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.

Acknowledgment

This project received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 733032 and grant agreement No 857340. This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101057014. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them. The authors thank Research Infrastructure RECETOX RI (No LM2018121) financed by the Ministry of Education, Youth and Sports, and Operational Programme Research, Development and Innovation – project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) for supportive background.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114002.

References

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int. J. Hyg Environ. Health 210, 201–228. https://doi.org/10.1016/j.ijheh.2007.01.024.

Balmer, J.E., Morris, A.D., Hung, H., Jantunen, L., Vorkamp, K., Rigét, F., Evans, M., Houde, M., Muir, D.C.G., 2019. Levels and trends of current-use pesticides (CUPs) in the arctic: an updated review, 2010–2018. Emerg. Contam. 5, 70–88. https://doi.org/10.1016/j.emcon.2019.02.002.

Becker, K., Seiwert, M., Angerer, J., Kolossa-Gehring, M., Hoppe, H.W., Ball, M., Schulz, C., Thumulla, J., Seifert, B., 2006. GerES IV Pilot Study: assessment of the exposure of German children to organophosphorus and pyrethroid pesticides. Int. J. Hyg Environ. Health 209, 221–233. https://doi.org/10.1016/j.ijheh.2005.12.002.

Bennett, B., Workman, T., Smith, M.N., Griffith, W.C., Thompson, B., Faustman, E.M., 2019. Longitudinal, seasonal, and occupational trends of multiple pesticides in house dust. Environ. Health Perspect. 127, 017003 https://doi.org/10.1289/EHP3644.

Bopp, S.K., Barouki, R., Brack, W., Dalla Costa, S., Dorne, J.L.C.M., Drakvik, P.E., Faust, M., Karjalainen, T.K., Kephalopoulos, S., van Klaveren, J., Kolossa-Gehring, M., Kortenkamp, A., Lebret, E., Lettieri, T., Nørager, S., Rüegg, J., Tarazona, J.V., Trier, X., van de Water, B., van Gils, J., Bergman, A., 2018. Current EU research activities on combined exposure to multiple chemicals. Environ. Int. 120, 544–562. https://doi.org/10.1016/j.envint.2018.07.037.

Bravo, N., Grimalt, J.O., Bocca, B., Pino, A., Bin, M., Brumatti, L.V., Rosolen, V., Barbone, F., Ronfani, L., Alimonti, A., Calamandrei, G., 2019. Urinary metabolites of

organophosphate and pyrethroid pesticides in children from an Italian cohort (PHIME, Trieste). Environ. Res. 176, 108508 https://doi.org/10.1016/j.envres.2019.05.039.

Bravo, N., Grimalt, J.O., Mazej, D., Tratnik, J.S., Sarigiannis, D.A., Horvat, M., 2020. Mother/child organophosphate and pyrethroid distributions. Environ. Int. 134, 105264 https://doi.org/10.1016/j.envint.2019.105264.

Brouwer, M., Kromhout, H., Vermeulen, R., Duyzer, J., Kramer, H., Hazeu, G., de Snoo, G., Huss, A., 2018. Assessment of residential environmental exposure to pesticides from agricultural fields in The Netherlands. J. Expo. Sci. Environ. Epidemiol. 28, 173–181. https://doi.org/10.1038/jes.2017.3.

CDC, 2020. Assessing Your Weight. WWW Document]. https://www.cdc.gov/healthyweight/assessing/index.html. accessed 3.14.22.

Cequier, E., Sakhi, A.K., Haug, L.S., Thomsen, C., 2017. Exposure to organophosphorus pesticides in Norwegian mothers and their children: diurnal variability in concentrations of their biomarkers and associations with food consumption. Sci. Total Environ. 590– 591, 655–662. https://doi.org/10.1016/j.scitotenv.2017.03.017.

Chang, C., Chen, M., Gao, J., Luo, J., Wu, K., Dong, T., Zhou, K., He, X., Hu, W., Wu, W., Lu, C., Hang, B., Meeker, J.D., Wang, X., Xia, Y., 2017. Current pesticide profiles in blood serum of adults in Jiangsu Province of China and a comparison with other countries. Environ. Int. 102, 213–222. https://doi.org/10.1016/j.envint.2017.03.004.

Cista, 2022. Pesticide Use in Czech Republic [WWW Document]. Czech Repub. – Usage Act. Subst. 2020. URL. https://eagri.cz/public/web/ukzuz/portal/pripravky-na-or/ucinne-latky-v-por-statistika-spotreba/spotreba-pripravku-na-or/spotreba-v-jednotlivych-letech/. accessed 3.14.22.

Climent, M.J., Coscollà, C., López, A., Barra, R., Urrutia, R., 2019. Legacy and current-use pesticides (CUPs) in the atmosphere of a rural area in central Chile, using passive air samplers. Sci. Total Environ. 662, 646–654. https://doi.org/10.1016/j.scitotenv.2019.01.302.

Core Team, R., 2021. R: A Language and Environment for Statistical Computing.

CZSO, 2020. Food Consumption - 2020 [WWW Document]. URL. https://www.czso.cz/csu/czso/food-consumption. accessed 6.15.22.

Dallegrave, A., Pizzolato, T.M., Barreto, F., Bica, V.C., Eljarrat, E., Barceló, D., 2018. Residue of insecticides in foodstuff and dietary exposure assessment of Brazilian citizens. Food Chem. Toxicol. 115, 329–335. https://doi.org/10.1016/j.fct.2018.03.028.

Damalas, C.A., 2009. Understanding benefits and risks of pesticide use. Sci. Res. Essays 4, 945–949.

Demur, C., Métais, B., Canlet, C., Tremblay-Franco, M., Gautier, R., Blas-Y-Estrada, F., Sommer, C., Gamet-Payrastre, L., 2013. Dietary exposure to a low dose of pesticides alone or as a mixture: the biological metabolic fingerprint and impact on hematopoiesis. Toxicology 308, 74–87. https://doi.org/10.1016/j.tox.2013.03.004.

Dereumeaux, C., Saoudi, A., Goria, S., Wagner, V., De Crouy-Chanel, P., Pecheux, M., Berat, B., Zaros, C., Guldner, L., 2018. Urinary levels of pyrethroid pesticides and determinants in pregnant French women from the Elfe cohort. Environ. Int. 119, 89–99. https://doi.org/10.1016/J.ENVINT.2018.04.042.

Doğanlar, Z.B., Doğanlar, O., Tozkir, H., Gökalp, F.D., Doğan, A., Yamaç, F., Aşkın, O.O., Aktaş, Ü.E., 2018. Nonoccupational exposure of agricultural area residents to pesticides: pesticide accumulation and evaluation of genotoxicity. Arch. Environ. Contam. Toxicol. 75, 530–544. https://doi.org/10.1007/s00244-018-0545-7.

Dujardin, B., Bocca, V., 2019. Cumulative dietary exposure assessment of pesticides that have acute effects on the nervous system using SAS® software. EFSA J. 17 https://doi.org/10.2903/j.efsa.2019.5764.

Eadsforth, C.V., 1988. Human Dose-Excretion Studies with Pyrethroid Insecticides Cypermethrin and Alphacypermethrin: Relevance for Biological Monitoring, vol. 18, pp. 603–614.

Eadsforth, C.V., Baldwin, M.K., 1983. Human dose-excretion studies with the pyrethroid insecticide, cypermethrin. Xenobiotica 13, 67–72. https://doi.org/10.3109/00498258309052238.

EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005, 2008 EFSA J. 6 (4), 84. https://doi.org/10.2903/j.efsa.2008.705, 705.

EFSA (European Food Safety Authority), 2014a. Conclusion on the peer review of the pesticide human health risk assessment of the active substance chlorpyrifos, 2014 EFSA J. 12 (4), 34. https://doi.org/10.2903/j.efsa.2014.3640, 3640.

EFSA (European Food Safety Authority), 2014b. Conclusion on the peer review of the pesticide risk assessment of the active substance tebuconazole, 2014 EFSA J. 12 (1), 98. https://doi.org/10.2903/j.efsa.2014.3485, 3485.

EFSA (European Food Safety Authority), Arena, M., Auteri, D., Barmaz, S., Brancato, A., Brocca, D., Bura, L., Carrasco Cabrera, L., Chiusolo, A., Civitella, C., Court Marques, D., Crivellente, F., Ctverackova, L., De Lentdecker, C., Egsmose, M., Erdos, Z., Fait, G., Ferreira, L., Greco, L., Ippolito, A., Istace, F., Jarrah, S., Kardassi, D., Leuschner, R., Lostia, A., Lythgo, C., Magrans, J.O., Medina, P., Mineo, D., Miron, I., Molnar, T., Padovani, L., Parra Morte, J.M., Pedersen, R., Reich, H., Sacchi, A., Santos, M., Serafimova, R., Sharp, R., Stanek, A., Streissl, F., Sturma, J., Szentes, C., Tarazona, J., Terron, A., Theobald, A., Vagenende, B., Van Dijk, J., Villamar-Bouza, L., 2018. Conclusion on the peer review of the pesticide risk assessment of the active substance cypermethrin, 2018 EFSA J. 16 (8), 28. https://doi.org/10.2903/j.efsa.2018.5402, 5402.

EFSA (European Food Safety Authority), Crivellente, F, Hart, A, Hernandez-Jerez, AF, Hougaard Bennekou, S, Pedersen, R, Terron, A, Wolterink, G, Mohimont, L, 2019a. Scientific report on the establishment of cumulative assessment groups of pesticides for their effects on the nervous system. EFSA J. 17 (9), 5800 https://doi.org/10.2903/j.efsa.2019.5800, 115 pp.

EFSA (European Food Safety Authority), 2019b. Statement on the available outcomes of the human health assessment in the context of the pesticides peer review of the active substance chlorpyrifos. EFSA J. 17 (8), 5809 https://doi.org/10.2903/j.efsa.2019.5809, 23 pp.

EFSA (European Food Safety Authority), Carrasco Cabrera, L, Medina Pastor, P, 2021. 2021. The 2019 European Union report on pesticide residues in food. EFSA J. 19 (4), 6491 https://doi.org/10.2903/j.efsa.2021.6491, 89 pp.

Eurostat, 2018. Degree of urbanisation (DEGURBA) [WWW Document]. URL. https://ec.europa.eu/eurostat/web/degree-of-urbanisation/background. accessed 3.14.22.

Felsot, A.S., Unsworth, J.B., Linders, J.B.H.J., Roberts, G., Rautman, D., Harris, C., Carazo, E., 2010. Agrochemical spray drift; assessment and mitigation—a review. J. Environ. Sci. Heal. Part B 46, 1–23. https://doi.org/10.1080/03601234.2010.515161.

Fernández, S., Pardo, O., Corpas-Burgos, F., Yusà, V., 2020. Exposure and cumulative risk assessment to non-persistent pesticides in Spanish children using biomonitoring. Sci. Total Environ. 746, 140983 https://doi.org/10.1016/j.scitotenv.2020.140983.

Ferré, D.M., Quero, A.A.M., Hernández, A.F., Hynes, V., Tornello, M.J., Lüders, C., Gorla, N.B.M., 2018. Potential risks of dietary exposure to chlorpyrifos and cypermethrin from their use in fruit/vegetable crops and beef cattle productions. Environ. Monit. Assess. 190, 1–10. https://doi.org/10.1007/S10661-018-6647-X/TABLES/4.

Figueiredo, D.M., Duyzer, J., Huss, A., Krop, E.J.M., Gerritsen-Ebben, M.G., Gooijer, Y., Vermeulen, R.C.H., 2021. Spatio-temporal variation of outdoor and indoor pesticide air concentrations in homes near agricultural fields. Atmos. Environ. 262, 118612 https://doi.org/10.1016/j.atmosenv.2021.118612.

Forni Ogna, V., Ogna, A., Vuistiner, P., Pruijm, M., Ponte, B., Ackermann, D., Gabutti, L., Vakilzadeh, N., Mohaupt, M., Martin, P.Y., Guessous, I., Péchère-Bertschi, A., Paccaud, F., Bochud, M., Burnier, M., Conen, D., Hayoz, D., Erne, P., Binet, I., Muggli, F., Gallino, A., Suter, P.M., 2015. New anthropometry-based age- and sex-specific reference values for urinary 24-hour creatinine excretion based on the adult Swiss population. BMC Med. 13, 1–10. https://doi.org/10.1186/s12916-015-0275-x.

Gao, Y., Zheng, H., Xia, Y., Chen, M., Meng, X., Cai, M., 2019. Spatial distributions and seasonal changes of current-use pesticides from the North pacific to the arctic oceans. J. Geophys. Res. Atmos. 124, 9716–9729. https://doi.org/10.1029/2018JD030186.

Garry, V.F., 2004. Pesticides and children. Toxicol. Appl. Pharmacol. 198, 152–163. https://doi.org/10.1016/j.taap.2003.11.027.

Glorennec, P., Serrano, T., Fravallo, M., Warembourg, C., Monfort, C., Cordier, S., Viel, J. F., Le Gléau, F., Le Bot, B., Chevrier, C., 2017. Determinants of children's exposure to pyrethroid insecticides in western France. Environ. Int. 104, 76–82. https://doi.org/10.1016/j.envint.2017.04.007.

González-Alzaga, B., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Ballester, F., Rebagliato, M., Hernández, A.F., 2014. A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. Toxicol. Lett. 230, 104–121. https://doi.org/10.1016/j.toxlet.2013.11.019.

Health Canada, 2019. Fifth Report on Human Biomonitoring of Environmental Chemicals in Canada. Government of Canada.

Hertz-Picciotto, I., Sass, J.B., Engel, S., Bennett, D.H., Bradman, A., Eskenazi, B., Lanphear, B., Whyatt, R., 2018. Organophosphate exposures during pregnancy and child neurodevelopment: recommendations for essential policy reforms. PLoS Med. 15, e1002671 https://doi.org/10.1371/journal.pmed.1002671.

Hongsibsong, S., Prapamontol, T., Xu, T., Hammock, B.D., Wang, H., Chen, Z.-J., Xu, Z.-L., 2020. Monitoring of the organophosphate pesticide chlorpyrifos in vegetable samples from local markets in northern Thailand by developed immunoassay. Int. J. Environ. Res. Publ. Health 17, 4723. https://doi.org/10.3390/ijerph17134723.

Katsikantami, I., Colosio, C., Alegakis, A., Tzatzarakis, M.N., Vakonaki, E., Rizos, A.K., Sarigiannis, D.A., Tsatsakis, A.M., 2019. Estimation of daily intake and risk assessment of organophosphorus pesticides based on biomonitoring data – the internal exposure approach. Food Chem. Toxicol. 123, 57–71. https://doi.org/10.1016/j.fct.2018.10.047.

Kim, K.H., Kabir, E., Jahan, S.A., 2017. Exposure to pesticides and the associated human health effects. Sci. Total Environ. 575, 525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009.

Koch, H.M., Wittassek, M., Brüning, T., Angerer, J., Heudorf, U., 2011. Exposure to phthalates in 5-6 years old primary school starters in Germany-A human biomonitoring study and a cumulative risk assessment. Int. J. Hyg Environ. Health 214 (3), 188–195. https://doi.org/10.1016/j.ijheh.2011.01.009.

Koureas, M., Tsakalof, A., Tsatsakis, A., Hadjichristodoulou, C., 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol. Lett. 210, 155–168. https://doi.org/10.1016/j.toxlet.2011.10.007.

Landrigan, P.J., Goldman, L.R., 2011. Protecting children from pesticides and other toxic chemicals. J. Expo. Sci. Environ. Epidemiol. 21, 119–120. https://doi.org/10.1038/jes.2011.1.

Leng, G., Kühn, K.H., Idel, H., 1997. Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: applications and limitations. Sci. Total Environ. 199, 173–181. https://doi.org/10.1016/S0048-9697(97)05493-4.

Lichiheb, N., Personne, E., Bedos, C., Van den Berg, F., Barriuso, E., 2016. Implementation of the effects of physicochemical properties on the foliar penetration of pesticides and its potential for estimating pesticide volatilization from plants. Sci. Total Environ. 550, 1022–1031. https://doi.org/10.1016/j.scitotenv.2016.01.058.

Llop, S., Murcia, M., Iñiguez, C., Roca, M., González, L., Yusà, V., Rebagliato, M., Ballester, F., 2017. Distributions and determinants of urinary biomarkers of organophosphate pesticide exposure in a prospective Spanish birth cohort study. Environ. Heal. A Glob. Access Sci. Source 16, 1–15. https://doi.org/10.1186/S12940-017-0255-Z/TABLES/5.

Lozowicka, B., 2015. Health risk for children and adults consuming apples with pesticide residue. Sci. Total Environ. 502, 184–198. https://doi.org/10.1016/j.scitotenv.2014.09.026.

Lu, C., Barr, D.B., Pearson, M.A., Walker, L.A., Bravo, R., 2009. The attribution of urban and suburban children's exposure to synthetic pyrethroid insecticides: a longitudinal assessment. J. Expo. Sci. Environ. Epidemiol. 19 (1), 69–78. https://doi.org/10.1038/jes.2008.49.

Lubin, J.H., Colt, J.S., Camann, D., Davis, S., Cerhan, J.R., Severson, R.K., Bernstein, L., Hartge, P., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ. Health Perspect. 112, 1691–1696. https://doi.org/10.1289/ehp.7199.

MACR, 2022. Land Parcel Identification System (LPIS). WWW Document]. URL. https://eagri.cz/public/app/lpisext/lpis/verejny2/plpis/. accessed 3.14.22.

Mamane, A., Baldi, I., Tessier, J.-F., Raherison, C., Bouvier, G., 2015. Occupational exposure to pesticides and respiratory health. Eur. Respir. Rev. 24, 306–319. https://doi.org/10.1183/16000617.00006014.

Mehrpour, O., Karrari, P., Zamani, N., Tsatsakis, A.M., Abdollahi, M., 2014. Occupational exposure to pesticides and consequences on male semen and fertility: a review. Toxicol. Lett. 230, 146–156. https://doi.org/10.1016/j.toxlet.2014.01.029.

Mie, A., Rudén, C., Grandjean, P., 2018. Safety of safety evaluation of pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl. Environ. Heal. A Glob. Access Sci. Source 17, 1–5. https://doi.org/10.1186/s12940-018-0421-y.

Morris, A.D., Muir, D.C.G., Solomon, K.R., Letcher, R.J., McKinney, M.A., Fisk, A.T., McMeans, B.C., Tomy, G.T., Teixeira, C., Wang, X., Duric, M., 2016. Current-use pesticides in seawater and their bioaccumulation in polar bear-ringed seal food chains of the Canadian Arctic. Environ. Toxicol. Chem. 35, 1695–1707. https://doi.org/10.1002/etc.3427.

Nolan, R.J., Rick, D.L., Freshour, N.L., Saunders, J.H., 1984. Chlorpyrifos: in human volunteers. Toxicol. Appl. Pharmacol. 73, 8–15.

Norén, E., Lindh, C., Rylander, L., Glynn, A., Axelsson, J., Littorin, M., Faniband, M., Larsson, E., Nielsen, C., 2020. Concentrations and temporal trends in pesticide biomarkers in urine of Swedish adolescents, 2000–2017. J. Expo. Sci. Environ. Epidemiol. 30, 756–767. https://doi.org/10.1038/s41370-020-0212-8.

Nougadère, A., Sirot, V., Kadar, A., Fastier, A., Truchot, E., Vergnet, C., Hommet, F., Baylé, J., Gros, P., Leblanc, J.-C., 2012. Total diet study on pesticide residues in France: levels in food as consumed and chronic dietary risk to consumers. Environ. Int. 45, 135–150. https://doi.org/10.1016/j.envint.2012.02.001.

OBO, 2019. Research on Exposure of Residents to Pesticides in The Netherlands: OBO Flower Bulbs. http://www.rivm.nl/sites/default/files/2019-04/Onderzoeksrapport%20OBO.pdf. (Accessed 15 March 2022). Accessed.

Oerlemans, A., Verscheijden, L.F.M., Mol, J.G.J., Vermeulen, R.C.H., Westerhout, J., Roeleveld, N., Russel, F.G.M., Scheepers, P.T.J., 2019. Toxicokinetics of a urinary metabolite of tebuconazole following controlled oral and dermal administration in human volunteers. Arch. Toxicol. 93, 2545–2553. https://doi.org/10.1007/s00204-019-02523-5.

Oerlemans, A., Figueiredo, D.M., Mol, J.G.J., Nijssen, R., Anzion, R.B.M., van Dael, M.F.P., Duyzer, J., Roeleveld, N., Russel, F.G.M., Vermeulen, R.C.H., Scheepers, P.T.J., 2021. Personal exposure assessment of pesticides in residents: the association between hand wipes and urinary biomarkers. Environ. Res. 199, 111282 https://doi.org/10.1016/J.ENVRES.2021.111282.

Osaka, A., Ueyama, J., Kondo, T., Nomura, H., Sugiura, Y., Saito, I., Nakane, K., Takaishi, A., Ogi, H., Wakusawa, S., Ito, Y., Kamijima, M., 2016. Exposure characterization of three major insecticide lines in urine of young children in Japan—neonicotinoids, organophosphates, and pyrethroids. Environ. Res. 147, 89–96. https://doi.org/10.1016/J.ENVRES.2016.01.028.

Ougier, E., Ganzleben, C., Lecoq, P., Bessems, J., David, M., Schoeters, G., Lange, R., Meslin, M., Uhl, M., Kolossa-Gehring, M., Rousselle, C., Vicente, J.L., 2021. Chemical prioritisation strategy in the European human biomonitoring initiative (HBM4EU) – development and results. Int. J. Hyg Environ. Health 236, 113778. https://doi.org/10.1016/j.ijheh.2021.113778.

Paglia, G., Del Greco, F.M., Carli, C., Sigurdsson, B.B., Smarason, S., Wegher, M., Pattaro, C., Wegher, L., 2021. Longitudinal assessment of chlorpyrifos exposure in farmers and residents of an Italian alpine region. Expo. Heal. 13, 651–659. https://doi.org/10.1007/s12403-021-00409-5.

Pearson, M.A., Lu, C., Schmotzer, B.J., Waller, L.A., Riederer, A.M., 2009. Evaluation of physiological measures for correcting variation in urinary output: implications for assessing environmental chemical exposure in children. J. Expo. Sci. Environ. Epidemiol. 19, 336–342. https://doi.org/10.1038/jes.2008.48.

Rasmussen, J.J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Cedergreen, N., McKnight, U. S., Kreuger, J., Jacobsen, D., Kristensen, E.A., Friberg, N., 2015. The legacy of pesticide pollution: an overlooked factor in current risk assessments of freshwater systems. Water Res. 84, 25–32. https://doi.org/10.1016/j.watres.2015.07.021.

Ratelle, M., Coté, J., Bouchard, M., 2015. Time profiles and toxicokinetic parameters of key biomarkers of exposure to cypermethrin in orally exposed volunteers compared with previously available kinetic data following permethrin exposure. J. Appl. Toxicol. 35, 1586–1593. https://doi.org/10.1002/jat.3124.

Remer, T., Neubert, A., Maser-Gluth, C., 2002. Anthropometry-based reference values for 24-h urinary creatinine excretion during growth and their use in endocrine and nutritional research. Am. J. Clin. Nutr. 75, 561–569. https://doi.org/10.1093/ajcn/75.3.561.

Saillenfait, A.M., Ndiaye, D., Sabaté, J.P., 2015. Pyrethroids: exposure and health effects - an update. Int. J. Hyg Environ. Health 218, 281–292. https://doi.org/10.1016/j.ijheh.2015.01.002.

Sauvé, J.F., Lévesque, M., Huard, M., Drolet, D., Lavoué, J., Tardif, R., Truchon, G., 2015. Creatinine and specific gravity normalization in biological monitoring of occupational exposures. J. Occup. Environ. Hyg. 12, 123–129. https://doi.org/10.1080/15459624.2014.955179.

Sinha, S.N., Rao, M.V.V., Vasudev, K., 2012. Distribution of pesticides in different commonly used vegetables from Hyderabad, India. Food Res. Int. 45, 161–169. https://doi.org/10.1016/j.foodres.2011.09.028.

Spence, C., 2021. Explaining seasonal patterns of food consumption. International Journal of Gastronomy and Food Science 24 (March), 100332. https://doi.org/10.1016/j.ijgfs.2021.100332.

Tang, W., Wang, D., Wang, J., Wu, Z., Li, L., Huang, M., Xu, S., Yan, D., 2018. Pyrethroid pesticide residues in the global environment: an overview. Chemosphere 191 (308), 990–1007. https://doi.org/10.1016/j.chemosphere.2017.10.115.

Tao, Y., Jia, C., Jing, J., Zhang, J., Yu, P., He, M., Wu, J., Chen, L., Zhao, E., 2021. Occurrence and dietary risk assessment of 37 pesticides in wheat fields in the suburbs of Beijing, China. Food Chem. 350, 129245 https://doi.org/10.1016/j.foodchem.2021.129245.

Torrent, X., Garcerá, C., Moltó, E., Chueca, P., Abad, R., Grafulla, C., Román, C., Planas, S., 2017. Comparison between standard and drift reducing nozzles for pesticide application in citrus: Part I. Effects on wind tunnel and field spray drift. Crop Protect. 96, 130–143. https://doi.org/10.1016/j.cropro.2017.02.001.

Ueyama, J., Saito, I., Kamijima, M., 2010. Analysis and evaluation of pyrethroid exposure in human population based on biological monitoring of urinary pyrethroid metabolites. J. Pestic. Sci. 35, 87–98. https://doi.org/10.1584/jpestics.R10-01.

Vlaanderen, J., Ottenbros, I., Bogers, R., Lebret, E., Antignac, J.P., Krauss, M., Debrauwer, L., Oberacher, H., Cupr, P., Szigeti, T., Martinsone, I., Pardo, O., 2019. Joint Survey of Pesticides: Details of Approach and Contributions Additional Deliverable Report AD15.7 WP15 - Mixtures, HBM and Human Health Risks. https://www.hbm4eu.eu/work-packages/additional-deliverable-15-7-joint-survey-of-pesticides-details-of-approach-and-contributions/. (Accessed 10 March 2022). Accessed.

Woollen, B.H., Marsh, J.R., Laird, W.J.D., Lesser, J.E., 1992. The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration. Xenobiotica 22, 983–991. https://doi.org/10.3109/00498259209049904.

Xie, W., Zhao, J., Zhu, X., Chen, S., Yang, X., 2022. Pyrethroid bioaccumulation in wild fish linked to geographic distribution and feeding habit. J. Hazard Mater. 430, 128470 https://doi.org/10.1016/j.jhazmat.2022.128470.

Yuan, Y., Chen, C., Zheng, C., Wang, X., Yang, G., Wang, Q., Zhang, Z., 2014. Residue of chlorpyrifos and cypermethrin in vegetables and probabilistic exposure assessment for consumers in Zhejiang Province, China. Food Control 36, 63–68. https://doi.org/10.1016/j.foodcont.2013.08.008.

Zhang, Q., Ying, Z., Tang, T., Guo, B., Gu, S., Fu, L., Dai, W., Lin, S., 2021. Residual characteristics and potential integrated risk assessment of synthetic pyrethroids in leafy vegetables from Zhejiang in China – based on a 3-year investigation. Food Chem. 365, 130389 https://doi.org/10.1016/j.foodchem.2021.130389.