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Personal exposure assessment of pesticides in residents: The association between hand wipes and urinary biomarkers

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ABSTRACT

Background: Residential exposure to pesticides may occur via inhalation of airborne pesticides, direct skin contacts with pesticide-contaminated surfaces, and consumption of food containing pesticide residues. The aim was to study the association of dermal exposure to pesticides between the use and non-use periods, between farmer and non-farmer families and between dermal exposure and the excretion of metabolites from urine in residents living close to treated agricultural fields.

Methods: In total, 112 hand wipes and 206 spot urine samples were collected from 16 farmer and 38 non-farmer participants living within 50 m from an agricultural field in the Netherlands. The study took place from May 2016 to December 2017 during the use as well as the non-use periods of pesticides. Hand wipes were analysed for the parent compound and urines samples for the corresponding urinary metabolite of five applied pesticides: asulam, carbendazim (applied as thiophanate-methyl), chlorpropham, prochloraz and tebuconazole. Questionnaire data was used to study potential determinants of occurrence and levels of pesticides in hand wipes according to univariate and multivariate analysis.

Results: Carbendazim and tebuconazole concentrations in hand wipes were statistically significantly higher in the pesticide-use period compared to the non-use period. In addition, especially during the use periods, concentrations were statistically significantly higher in farmer families compared to non-farmer families. For asulam, chlorpropham and prochloraz, the frequency of non-detects was too high (57–85%) to be included in this analysis. The carbendazim contents in urine samples and hand wipes were correlated on the first and second day after taking the hand wipe, whereas chlorpropham was only observed to be related on the second day following the spray event.

Conclusions: Concentrations in hand wipes were overall higher in pesticide use periods compared to non-use periods and higher in farmer families compared to non-farmer families. Only for carbendazim a strong correlation between concentrations in hand wipes and its main metabolite in urine was observed, indicating dermal exposure via contaminated indoor surfaces. We expect this to be related to the lower vapour pressure and longer environmental lifetime of carbendazim compared to the other pesticides studies.

1. Introduction

People living in close vicinity of agricultural activities can become exposed to pesticides through non-occupational pathways. Residential exposure can occur via different routes and from different sources, for example by consumption of food containing pesticide residues, inhalation of airborne pesticides originating from volatilization or spray drift, and skin contact to surfaces contaminated with drift droplets, soil particles or indoor dust (Bradman et al., 1997; Quiros-Alcala et al., 2011; Hogenkamp et al., 2004; Health Council of the Netherlands, 2014).

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The potential for adverse health effects associated with the application of pesticides has been an important issue for almost 100 years in Europe, the concerns about residential exposure to pesticides originate from the early '80s in the Netherlands (Mulder et al., 1993). A literature review by The European Food and Safety Authority (EFSA) showed statistically significant associations between pesticide exposure in children and childhood leukaemia and the development of Parkinson's disease at old age (Ntzani et al., 2013). A more recent systematic review by Van Maele-Fabry et al. supported the association between residential exposure to pesticides and childhood brain tumors (Van Maele-Fabry, Gamet-Payrastre, and Lison, 2017). More specifically, Brouwer et al. suggested an association between working in flower bulb cultivation and an increased risk of Parkinson's disease in the Netherlands (Brouwer et al., 2017). Overall, many studies suggest that pesticide exposure could be associated with adverse birth outcomes, neurological diseases, several types of cancer, immune disorders, renal diseases, and endocrine disruption (Gonzalez-Alzaga et al., 2015; Chen et al., 2015; VoPham et al., 2015; Thrasher et al., 1993; Cosselman et al., 2015; Lebov et al., 2015; Piccoli et al., 2016; Larsen et al., 2017; Brouwers et al., 2011).

A few studies focused on the difference in residential exposure between the use and the non-use periods of pesticides. It is suggested that seasonal peak pesticide exposure can decrease acetylcholinesterase activity of people who are living near plantations resulting from post-harvesting exposures (Suarez-Lopez et al., 2018; Ramirez-Santana et al., 2020). Furthermore, people living in agricultural areas have a higher risk of exposure during spray periods. This was indicated by increased levels of urinary biomarkers of pesticide exposure (Galea et al., 2015; Crane et al., 2013).

We did not find studies reporting on differences in hand wipes concentrations or dermal exposure in residents between use and non-use periods. A previous study regarding six commonly applied pesticides in Iowa, US, reported that the majority of the hand wipe samples were non-detectable (Curwin et al., 2005). Nevertheless, it can be important to collect hand wipe samples as they might reflect a stronger relation to internal exposure compared to dust measures and to reveal the importance of different routes of exposure (e.g. hand-mouth contact and dermal absorption) (Hoffman, EHS, 2015). Moreover, hand wipe samples are non-invasive and easy to collect. For the determination of external personal exposure, pesticide levels in hand wipe samples can give an indication of dermal exposure, in addition to urinary metabolite levels (Curwin et al., 2005).

Biological monitoring of pesticides and their metabolites in urine is a preferred method for assessing integrated exposure, as pesticides are usually excreted rapidly. Therefore, biological monitoring usually reflects short-term exposures. Urine provides an integrated dose estimate reflecting recent exposure from all sources across different routes of exposure due to the relatively short biological half-lives of most pesticides (Budnik and Baur, 2009; Armon and Hänninen, 2015). Furthermore, collection of urine samples is often preferred over blood samples because it is less invasive, easy to collect at home by study participants, and available in sufficient quantity, while ethics permission for blood sampling is less accessible for young children (e.g. more difficult to collect blood samples of sufficient volume) (Barr et al., 2006).

In this study, hand wipe samples were collected and analysed for five commonly used pesticides in the Netherlands, i.e. asulam, thiophanate-methyl (degrades into carbendazim), chlorpropham, prochloraz, and tebuconazole. In addition, urine samples from the participants were collected in the same time-frame as the hand wipes and were analysed for the corresponding primary urinary metabolites. The aims were to study the association between hand exposure (as a proxy of dermal exposure) to pesticides and the excretion of metabolites from urine collected by residents living close to treated agricultural fields. Additionally, hand wipes and urine biomarker levels were compared between participants from farmer and non-farmer families and between the use and non-use periods of the corresponding pesticide.

2. Material and methods

2.1. Study design

The current study was nested within the study population of the research project on residential exposure to pesticides in the Netherlands named 'Onderzoek Bestrijdingsmiddelen en Omwonenden' (OBO), which aimed to assess the exposure to pesticides among residents living close to agricultural land. Selection of agricultural fields, pesticides chosen, and participant recruitment were described previously (Vermeulen RCH, 2019). The study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht (no. NL54727.041.15).

Asulam, carbendazim, chlorpropham, prochloraz, and tebuconazole were the pesticides selected based on authorised use in tulip and lily cultivation in The Netherlands, frequency of application, dose per hectare, physicochemical properties, estimated dermal absorption rate, and contribution from non-agricultural sources, like diet. Carbendazim itself was not sprayed but was measured as an environmental metabolite from applications of thiophanate-methyl used in postharvest treatment and storage.

Non-farmer families (NF) and farmer families (FF) living within 50 m from the perimeter of an agricultural field from a farmer participating in the study were recruited by door-by-door distribution of information leaflets and was the only inclusion criteria. A farmer family was defined as a participating residence were at least one person reported to work in agriculture. We chose a distance of 50 m from the residence to the agricultural field since there is the highest pesticide burden expected due to spray drift and volatilization. Potential participants expressed their interest in participation by returning a response card. In total, 54 participants were included of which 16 were members of a FF. All participants collected at least one urine and hand wipe sample during the study period from May 2016 to December 2017. However, not all urine samples from all participants were analysed.

Participants were asked to collect urine samples and hand wipes during two measurement series. The first series of spot urine sample collection was during a pesticide use period between March and September of a selected pesticide and the second series of sample collection during a pesticide non-use period between October and December.

2.2. Sample and data collection

Sample and data collection took place in the horticulture regions in the North-West part of the Netherlands. Each participant was visited on two occasions per measurement campaign. During each visit, the urine samples and hand wipes samples were picked up within three days after completion. The start of the spraying event was announced by a text message (on day 0) and the first morning void was collected on the following day (day 1). The first-morning urine was also collected by the participant on each of six subsequent days (days 2–7). Each urine void was collected in a 500 mL polyethene container and immediately stored in a refrigerator until shipment to the laboratory, where it was aliquoted in portions of 15 mL and kept at −18 °C until analysis. This measurement series was repeated for the collection of samples in the non-use period. In total, 206 first-morning voids of urine were analysed. Certain urine samples were analysed for multiple pesticide biomarkers as participants could be residents among different agricultural fields, therefore 458 urine results were available.

Participants collected a hand wipe sample by themselves (according to a detailed written instruction) in the evening of day 0 (i.e. after a spray application was announced and executed). For 11 participants hand wipes were collected a second time during the use period given the occurrence of another spray application and possibility of collecting a second hand wipe. The same procedure was followed in a non-use period. An overview of the total number of analysed urine samples

and hand wipes for NF and FF, as well as for each pesticide and for the use and non-use period are presented in supplemental material A. The summarizing numbers are given in Table 1.

The protocol of the self-performed hand wipe by the participants was based on the procedures described previously (Gorce and Roff, 2015). Participants were provided with written instructions and a labelled 175 mL Nalgene container with the wipe consisting of a 10 × 10 cm paper tissue (Kimtech Science, Irving, Texas, USA) moistened with 3 mL of 50% of Milli-Q water and 50% of ethanol. Participants were instructed to not wash or rinse their hands in the 2 h before sampling and to avoid contact with wet surfaces (e.g. laundry, hand cloths or kitchen towels). The procedure involved wiping both sides of both hands and fingers, starting at the wrist and wiping in the distal direction. The tissue was placed in the labelled Nalgene container, closed with the cap and stored in a refrigerator until shipment to the laboratory where it was kept at −18 °C until analysis. In total, 112 hand wipes were collected and analysed for the assessment of dermal exposure.

A questionnaire was provided to all participants for each occasion of sample collection. The questions included concerned age, sex, body weight, lifestyle, exact times of urine and hand wipe collection, occupation, pesticide use, and home characteristics.

2.3. Sample analysis

Urinary biomarkers were only analysed for the pesticide that was allocated to one or multiple selected fields, whereas hand wipes were analysed for all five pesticides in one multi-method (Vermeulen RCH, 2019). The total number of urine samples analysed per biomarker ranged from 48 to 133 and can be found in supplemental material B.

The urinary metabolites were analysed separately as the method of analysis was optimized to achieve the highest sensitivity for each metabolite. Therefore, sample preparation and LC-MS/MS conditions were different for all metabolites. The methods are explained briefly below and a more detailed description of the procedure of sample analysis can be found in supplemental material C.

As asulam is mainly excreted unmetabolized, no deconjugation is required (Vermeulen RCH, 2019). An aliquot of thawed urine was spiked with the isotope-labelled analogue as internal standard (ILIS) and homogenized. The extraction was performed by using the Quick Easy Cheap Effective Rugged Safe (QuEChERS) method through the addition of acetonitrile and acetic acid. After shaking, it was followed by the addition of magnesium sulphate and sodium acetate to induce phase separation (Lehotay, 2007). The upper acetonitrile layer was subsequently analysed by LC-MS/MS.

The metabolite of carbendazim, methyl 5-hydroxy-2-benzimidazole carbamate (5-HBC), is partly excreted in urine as conjugates (Leenheers et al., 1993). An aliquot of thawed and homogenized urine was spiked with ILIS, acetate buffer, and 15 μL of β-glucuronidase/arylsulfatase. After overnight incubation at 37 °C, the extraction was carried out by addition of acetonitrile and acetic acid and shaking. Magnesium sulphate and sodium acetate were added for phase separation. An aliquot of the upper acetonitrile layer was transferred to a clean test tube and evaporated under a gentle flow of nitrogen. The residue was reconstituted in Milli-Q water and subsequently analysed by LC-MS/MS.

The metabolite of chlorpropham, 4-hydroxychlorpropham-O-sulphonic acid (4-HSA), is excreted in urine as a sulphate conjugate. Deconjugation was not needed as the reference standards for 4-HSA were available. An aliquot of the pre-treated sample was thawed, spiked with ILIS and homogenized. Thereafter, the aliquot was filtered using a 30 kDa ultracentrifuge cartridge. The filtrate was transferred into a vial for LS-MS/MS analysis.

The metabolite of prochloraz, 2,4,6-trichlorophenoxyacetic acid (2,4,6-TCP), is excreted in urine as a conjugate (Vermeulen RCH, 2019). An aliquot of thawed and homogenized urine was spiked with ILIS, acetate buffer, and β-glucuronidase/arylsulfatase. After overnight incubation at 37 °C a solid-phase extraction (SPE) was performed. The SPE column was loaded with 5 mL of deconjugated urine, washed with 10% of acetonitrile in aqua pure, and eluted with 50% acetonitrile in aqua pure. The eluate was dried under nitrogen and reconstituted in acetonitrile and Milli-Q water and subsequently analysed by LC-MS/MS.

As the human metabolite of tebuconazole, 1-hydroxytebuconazole (TEB-OH), is excreted in urine as a conjugate, deconjugation during sample treatment is required (Oerlemans et al., 2018). An aliquot of thawed and homogenized urine was spiked with ILIS, acetate buffer, and β-glucuronidase/arylsulfatase. After overnight incubation at 37 °C, a sub-zero temperature liquid-liquid extraction was performed to induce phase separation (Yoshida and Akane, 1999). According to the method of Yoshida and Akane, 3 mL of acetonitrile was added to an aliquot of 1 mL of deconjugated urine and placed at −20 °C for 20 min. The upper acetonitrile layer was subsequently analysed by LC-MS/MS.

All methods included matrix-matched calibration standards, quality control samples, and blanks and were analysed simultaneously with the samples. In-house method validation was done according to SANTE/11945/2015 (European Commission, 2015). All methods included normalisation to the response of the ILIS. Limits of quantification (LOQ) for hand wipe and urine sample analysis are given in Table 2. Summaries for the method performances are presented in supplemental material C (Table S3) and D (Table S4). Creatinine was analysed after centrifuging the sample but prior to further pre-treatment of the samples by the laboratory for clinical chemistry of the Radboud university medical center according to the modified Jaffe method (Slot, 1965). Relative metabolite concentrations were expressed in μg/g creatinine.

The hand wipes were analysed in a multi-method for the five pesticides using liquid chromatography tandem mass spectrometry (LC-MS/MS). Sample extraction was performed in the Nalgene container in which the wipe was stored to reduce extraction losses. The wipe material was cut in small pieces and desorbed with methanol, after which the container was placed in an ultrasonic bath and on a mechanical shaker. An aliquot of methanol was transferred into a test tube and dried under a gentle flow of nitrogen. The residue was reconstituted and was centrifuged to remove remaining solids. The supernatant was transferred to a vial for LC-MS/MS analysis. A more detailed description of the procedure for the hand wipe analysis can be found in supplemental material D.

Table 1
Number of analysed urine samples and hand wipes.

	Non-farmer families		Farmer families	
	Use period	Non-use period	Use period	Non-use period
Asulam	18	8	21	2
Carbendazim	74	39	14	6
Chlorpropham	52	25	16	8
Prochloraz	16	8	15	8
Tebuconazole	64	28	28	8
Hand wipes	49	31	16	16

Table 2
Limits of quantification (LOQ) for hand wipe and urine sample analysis using LC-MS/MS.

Pesticide (urinary metabolite)	LOQ hand wipe (ng/wipe)	LOQ urine (ng/mL)
Asulam (asulam)	0.50	0.1
Carbendazim (5-HBC)	0.50	0.05
Chlorpropham (4-HSA)	2.5	0.1
Prochloraz (2,4,6-TCP)	1.0	0.3
Tebuconazole (TEB-OH)	0.25	0.05

5-HBC: methyl 5-hydroxy-2-benzimidazole carbamate, 4-HSA: 4-hydroxychlorpropham-O-sulphonic acid, 2,4,6-TCP: 2,4,6-trichlorophenoxyacetic acid, TEB-OH: 1-hydroxytebuconazole.

2.4. Data analysis

The LC-MS/MS technique has pesticide-specific LOQs as shown in Table 2. In this study, the LOQ was set as cut-off level for detection. For levels below the LOQ, imputation was done when the analyte was quantified (>LOQ) in at least 60% of the samples measured for that analyte (Succop et al., 2004). The values below LOQ were imputed based on the maximum likelihood estimation, while accounting for the distribution of the data and correlation between different compounds across the same medium (Lubin et al., 2004). For urine, imputations were only possible for the biomarkers of tebuconazole and chlorpropham. For hand wipes, imputation was only possible for tebuconazole and carbendazim. We compared FF vs NF to study any significant change between farmers and the remaining rural population (NF).

We used the Wilcoxon non-parametric test to determine if the concentration mean ranks in the use and confirmed non-use period were different from each other, as well as FF vs NF. Spearman's Rho correlation coefficients were calculated between concentrations of three different pesticides in hand wipes on day 0 compared to the levels of excreted metabolites in morning urine samples on day 1 and 2. As the Spearman method gives a rank correlation coefficient, imputed data can be included in the analysis. Here, the requirement of a minimum of 10 paired samples was used, given that applying this correlation with a smaller sample size reduces statistical power and increases the likelihood for type I or type II errors (see Knudson and Lindsey, 2014 for details).

2.5. Univariate and multivariate models

A univariable analysis was applied for the evaluation of the questionnaire data with the aim of understanding the relationship between each different variable and the concentration of pesticides in hand wipes. We also performed a multivariable analysis taking into account all variables in a forward stepwise regression to identify which were associated with the hand wipe concentrations. The inclusion of all variables was based on i) many of these variables have been indicated as possible determinants of pesticide levels in the home environment, such as pets (González-Alzaga et al., 2020), flooring type (Harley et al., 2019), shoes and clothes (Coronado et al., 2011), ii) expert decision. In these analysis we constructed separate models for each pesticide, with the different questionnaire data as independent variables (see supplementary material I for the complete list) and the concentrations in handwipe as dependent variable. The house identification number was added as fixed parameter in every model to account for participants living in the same residence.

FF were excluded from the univariate and multivariate analysis since these homes are known to have different determinants of pesticide burden than the general population compared to NF homes (Curl et al., 2002). The participants aged ≤17 (N = 13) were also excluded, since a different questionnaire was given to this age group and various questions in this questionnaire were not answered.

All statistical analyses were performed using R version 3.5.3 (R Development Core Team, 2010).

3. Results

3.1. Study population

In total, 54 residents enrolled in the hand wipe collection, 28 (52%) of them were males and the median (SD) age of the population was 40.2 ± 24.4 y. The median (SD) BMI was 22.8 ± 4.5 kg/m^2. Moreover, the median (±SD) reported time spent at home (i.e. indoors) was 15.6 ± 2.6 h per day. Eighteen (34%) residents reported application of a pesticide at least once within 6 months before the start of the study.

3.2. Urine biomarkers

For the different analysed biomarkers in urine, imputation was only possible for chlorpropham (Fig. 1A) and tebuconazole (Fig. 1B). Supplemental material B summarizes the measured concentrations and the percentage of detects for all five analysed biomarkers. For chlorpropham (4-HSA) in FF, the median concentrations were 0.79 and 0.26 μg/g creatinine for the use and non-use period, respectively. While for NF, the median concentrations were 0.62 and 0.26 μg/g creatinine for the use period and non-use period, respectively. The median concentrations where significantly higher for FF in the use period compared to the non-use period ($p = 0.03$). The same being true when comparing NF groups ($p = 0.03$). Additionally, when comparing FF with NF for both periods, the median concentrations were close to each other, thus no statistical difference ($p > 0.05$).

In FF the median concentrations of the urinary metabolite of tebuconazole (TEB-OH) were 0.47 and 0.03 μg/g creatinine for the use and non-use period, respectively. In NF, the median concentrations were 0.16 and 0.07 μg/g creatinine for the use and non-use period, respectively. The median concentrations in FF were significantly higher in the use period when compared with the non-use period ($p = 0.006$). The same conclusion is valid for NF ($p = 0.031$). Moreover, concentrations were significantly higher for FF in the use period when comparing with NF ($p = 0.026$). There were no statistically significant differences in urine concentrations between FF vs NF in the non-use period.

3.3. Hand wipes

A total of 65 hand wipe samples were collected from different residents during the use period of a pesticide; 47 samples were collected in the non-use period. Supplemental material E summarizes the concentrations measured and the percentage of detects for the five pesticides analysed. Imputation of the data was only applicable to two of these, i.e. tebuconazole (Fig. 2A) and carbendazim (Fig. 2B), the percentages of non-detects (<LOQ) were too high to perform imputation for the other compounds.

Regarding residential exposure in FF, the median concentrations of tebuconazole were 1.82 and 0.02 ng/hand wipe for the use and non-use period, respectively. Whereas for NF, the median concentrations were 0.17and 0.03 ng/hand wipe, for the use and non-use period, respectively. For carbendazim, the median concentrations were 1190 and 295 ng/hand wipe, for the use and non-use period in FF, respectively. Whereas for NF, the mean concentrations were 64.2 and 1.04 ng/hand wipe, for the use and non-use period, respectively.

When comparing the hand wipe results of the FF between periods, the concentrations were significantly higher for tebuconazole in the use period ($p = 0.007$). However, this result is not statistically significant when comparing NF groups ($p = 0.103$). In addition, during the use period, concentrations where significantly higher in FF when comparing with NF ($p < 0.001$).

For carbendazim, we can see that concentrations in FF are significantly higher than NF ($p < 0.001$), for both use and non-use periods. The concentrations were also significantly higher in the use period vs. non-use period within the NF group ($p = 0.001$).

3.4. Association between hand wipes and urine samples

A study into the association between hand wipe concentrations and corresponding urine concentrations was possible for three of the five studied pesticides (i.e. tebuconazole, chlorpropham and carbendazim). The hand wipes from day 0 and the morning urine samples of day 1 and 2 were included based on the time the metabolites are expected to be excreted from the body (Oerlemans et al., 2019). Comparisons were performed, including the imputed data (NF + FF) and solely for the use period. For asulam and prochloraz this was not possible since nearly all results were below the LOQ in both the hand wipe and in the urine

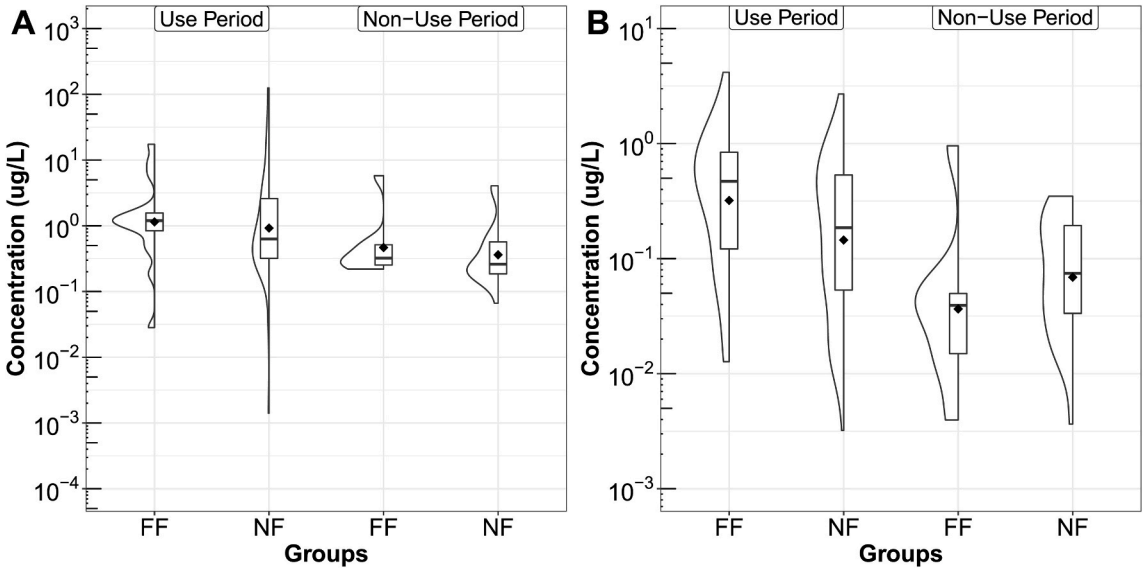

Fig. 1. Concentrations of chlorpropham (A) and tebuconazole (B) in the morning urine of residents during the use and non-use period. The x-axis represents the groups (FF = farmer families, NF = Non-farmer families). The y-axis represents the concentration in urine in μg/g creatinine on a[10]log scale. The box plots are representing summary statistics (min, max, 1st and 3rd quartile and median) and on the left side the distribution is presented. The black diamond represents the arithmetic mean.

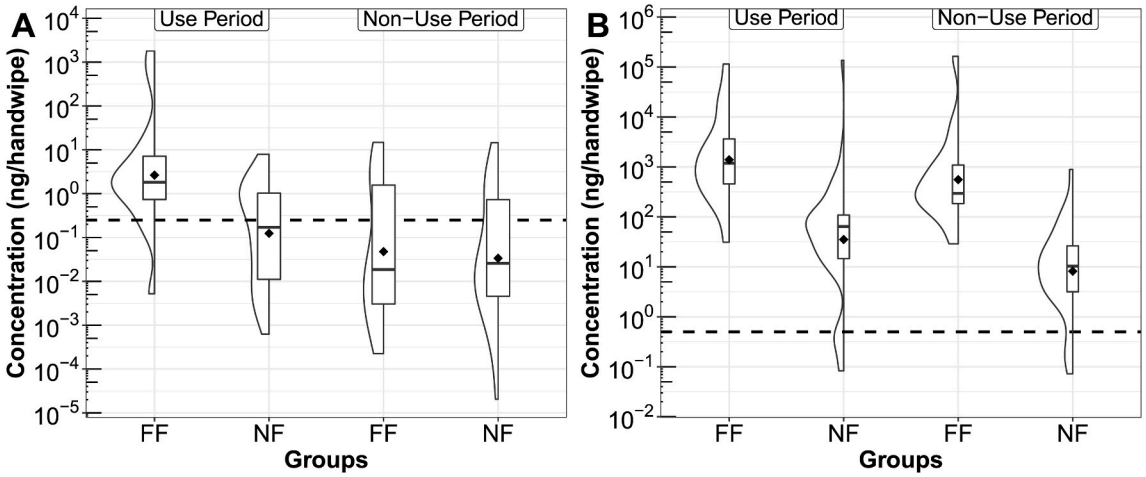

Fig. 2. Concentrations of tebuconazole (A) and carbendazim (B) in hand wipes collected from residents during the use and non-use period. The x-axis represents the groups (FF = farmer families, NF = Non-farmer families). The y-axis represents the concentration in ng/hand wipe on a[10]log scale. The box plots are representing summary statistics (min, max, 1st and 3rd quartile and median) and on the left side the distribution is presented. The black diamond represents the arithmetic mean of the logged data. The dotted line indicates the LOQ.

sample.

N-paired: Number of paired samples between hand wipe and measured urine biomarker.

A strong Spearman correlation was observed between the concentrations of carbendazim in hand wipes and carbendazim metabolite in urine on day 1 and 2, with a higher correlation on day 1. For chlorpropham results of pesticides found in hand wipes and urine samples correlated on day 2, but not on day 1. For tebuconazole no statistically significant correlations were found, see Table 3.

In addition, a sensitivity analysis was performed with only the paired observations that were > LOQ (i.e. not imputed). These results can be found in supplemental material F. Overall, similar associations were obtained, except for chlorpropham since there were not enough paired observations to perform a rank correlation.

Table 3
Spearman correlations between concentrations of three different pesticides in hand wipes on day 0 compared to the levels of excreted metabolites in morning urine samples of day 1 and 2.

Pesticides	Day	Spearman Rho	P-value	N-paired
Carbendazim	1	0.909	**< 0.001**	11
	2	0.731	**0.006**	13
Chlorpropham	1	0.309	0.387	10
	2	0.627	**0.043**	11
Tebuconazole	1	0.340	0.060	32
	2	−0.133	0.480	30

3.5. Univariable and multivariable analysis

To test the relationship between different variables and the concentrations found in hand wipes, but also to study changes in coefficient

(β) direction when adding multiple variables, both univariable and multivariable analyses were performed. In Table 4, the signs of β for each paired independent variable and outcome, as well as the regression p-values are presented. The results of the multivariable analysis were added in Table 4, indicating the selected variables.

Three reported characteristics of the residence appeared to be significantly associated with changes of carbendazim concentrations in hand wipes. Associated with an increase in concentration (positive β) were: use of pesticides at least once in the home, presence of pets in the home, and specifically, owning a cat. However, in the forward multivariable stepwise regression, only owning a cat remained in the model and continued to be statistically significant without a change in β direction. Additionally, four variables that were not statistically significant in the univariable analysis were selected in the model: two variables with a p-value < 0.05 and two with a p-value < 0.001. The latter two are 'the age of the floor' and 'the ventilation rate (expressed as exchanges per hour)'. The first variable indicates that the older the floor in the residence, the higher the hand wipe concentration. The second variable indicated that an increase in the number of air changes per hour calculated using the gComis model (Vermeulen RCH, 2019), is associated with a decrease in the hand wipe concentrations.

When hand wipes were collected during the tebuconazole use period the concentrations of tebuconazole were significantly increased. In the multivariable stepwise regression, this variable continued to be statistically significant, the β sign did not change and was maintained in the model. Moreover, many other variables were also selected in this step (all $p < 0.05$). Associated with an increase in β were: the average time a person spent indoors, the age of the floor, owning a pet and drying laundry outdoors. In contrast, the reported conditions associated with a decrease in β were: the use of pesticides at least once, average ventilation rate, owning a dog, and reporting the smell of pesticides.

Table 4
Regression coefficients and p-values: results from a univariable and multivariable analysis for carbendazim and tebuconazole concentrations in hand wipes.

Independent variables	Carbendazim				Tebuconazole			
	Univariable		Multivariable		Univariable		Multivariable	
	β	α	β	α	β	α	β	α
Use period = Yes	−	>0.05			+	< **0.001**	+	< **0.001**
Age (y)	−	>0.05	+	>0.05	−	>0.05		
Gender = Male	+	>0.05	+	>0.05	+	>0.05	+	>0.05
BMI (kg/m^2)	−	>0.05			−	>0.05	+	>0.05
Average time spent indoors (h)	−	>0.05			+	>0.05	+	<0.05
Use pesticides at least once	+	< **0.001**			+	>0.05	−	<0.05
Number of persons in home	−	>0.05			−	>0.05	+	>0.05
Living room size (m^2)	−	>0.05			+	>0.05	−	>0.05
Air changes per hour of the home (1/h)	−	<0.05	−	< **0.001**	−	>0.05	−	<0.05
Type of floor = Smooth	+	>0.05	+	>0.05	−	>0.05	+	>0.05
Age of the flooring (y)	−	>0.05	+	< **0.001**	−	>0.05	+	<0.05
Distance from a field (m)	+	>0.05	+	>0.05	+	>0.05		
Owning pets	+	< **0.001**			+	>0.05	+	<0.05
Own a dog	+	>0.05			−	>0.05	−	<0.05
Own a cat	+	< **0.001**	+	< **0.001**	+	>0.05		
Eat vegetables from garden	+	>0.05			+	>0.05	+	>0.05
Bring shoes inside	−	>0.05			−	>0.05	+	>0.05
Dry clothes outside	+	>0.05	+	<0.05	−	>0.05	+	<0.05
Can smell pesticides	+	>0.05	−	<0.05	−	>0.05	−	<0.05

β – Effect estimate signal. For a numerical independent variable: an increase in 1 unit will lead to an increase (+) or decrease (−) in concentrations. For a categorical variable: It is a binary option (e.g. Yes/No leads to an increase (+) or decrease (−) in concentrations).
α - Significance level.

4. Discussion

In this study, we found higher concentrations of tebuconazole in hand wipes in the pesticide use period compared to the non-use period. This was expected since tebuconazole is commonly applied in vegetables, cereals, seeds, and ornamentals, including flower bulbs. Furthermore, this substance is not highly persistent resulting in relatively low environmental background (Dong et al., 2018). For carbendazim the differences of hand wipe concentrations between the use and the non-use period were only significant for NF, with higher concentrations in the use period of its precursor thiophanate-methyl. It is most likely that higher hand wipe concentrations reflect increased background concentrations, which are expected to be higher in the use period both for FF and NF. Carbendazim occurs in the Netherlands mainly as environmental degradation product of benomyl and thiophanate-methyl and it can persist for more than one year in the environment (Leistra and Matser, 2004). These results agree with previous findings that populations are usually exposed to multiple pesticide mixtures, and also to higher concentrations in the use period compared to the non-use period (Smith et al., 2017).

Regarding the measurement results in the morning urine samples and period of sample collection, a similar pattern could be observed for pesticide residues retrieved from hand wipes. However, we only observed statistically significant differences in concentrations between the use and non-use periods for chlorpropham, with higher concentrations in the use period. This outcome matches the results described in a recent review on biomonitoring studies, in which all studies demonstrated higher chlorpropham concentrations for residents in the use period (Dereumeaux et al., 2020). We hypothesize that diet plays a major role in pesticide intake, making it more difficult to detect a contribution from environmental exposure on the total amount of pesticide excreted in urine as a metabolite. Another challenge is the contribution from local or non-professional use of pesticides to the total amount of pesticide exposure. A recent systematic review concluded that there is a lack of consensus regarding of differences in urinary metabolite concentrations between exposed and control groups (Lopez-Galvez et al., 2019). In addition, the contribution of pesticide exposure from environmental background levels and indoor sources to the excretion of corresponding metabolites is unknown.

Previous studies described that FF are exposed to higher concentrations of pesticides than residents (Curwin et al., 2007). In our study, we observed a similar pattern, especially for hand wipes. Both tebuconazole and carbendazim concentrations are, overall, higher in FF. However, when looking at urinary biomarkers, the difference between FF and NF is less pronounced, indicating that other routes, such as dietary intake, might play a bigger role in exposure than the dermal pathway.

A statistically significant correlation was found between hand wipe and urine concentrations for carbendazim on both day 1 and 2. This could be an indication of a contribution from the dermal exposure pathway because skin absorption is a comparatively slow absorption process (Atabila et al., 2017). The small decrease in Spearman Rho from day 1 to day 2 could reflect variability in excretion rate after dermal exposure, since it is expected that the concentrations will decrease continuously over time, depending mainly on the toxicokinetics of the pesticide (Oerlemans et al., 2019). We also observed a moderate correlation between urine from day 2 and hand wipe concentrations for

chlorpropham, but we cannot draw any conclusions from this, given that many of the paired values were below LOD. These results are similar to a previous study that showed moderate to strong correlations between exposure via hands and excreted concentrations in urine among pesticide applicators (Tuomainen et al., 2002). Therefore, the hand wipe could likely be used as a proxy for environmental exposure.

To the best of our knowledge no previous studies have described possible determinants that drive the concentrations of pesticides in hand wipes from residents. The univariable analysis in this study indicated that for tebuconazole primarily the time of sample collection explains observed variability. However, when adding multiple variables together, other variables become also potential determinants ($p < 0.05$), such as average time spent indoors, indoor applications of pesticides, ventilation rate, owning a dog and reported smells attributed to pesticides.

Regarding carbendazim, our results showed that ventilation rate, age of the flooring and owning a cat are associated with the hand wipe concentrations. A higher airflow rate in a house is associated with lower pesticide concentrations in the indoor environment. We suggest that the age of the floor reflects the function of the floor as a depot for contaminants potentially leading to secondary exposure, which is especially relevant to carbendazim because of the long environmental half-life. And finally, owning a cat increases the hand wipe concentration, which corresponds to previous findings were it was described that pets could carry pesticides from outdoor to the indoor environment as part of the take-home pathway (Deziel et al., 2015). This might be an important indirect exposure route especially for pesticides with a longer environmental half-life.

Prochloraz and asulam were detected in relatively low number of hand wipe and urine samples. Therefore, no statistical analysis were possible for these two pesticides. A probable reason for the low number of detects for asulam is that this pesticide had only a temporary authorization for use in 2016. Background concentrations in homes might therefore be low as well as the contribution from diet. Additionally, in indoor and outdoor air samples collected in the OBO study, asulam was also detected in low number of samples (up to 10%). For prochloraz, we were not able to find an explanation for the low number of detects in urines and hand wipes. In the environmental samples it was detected in high numbers (up to 89% in the use period). In combination with the physicochemical properties, market share and applied amounts on the field it was expected to find sufficient urine and hand wipe samples above the LOQ. Regarding human environmental exposure, prochloraz remains an important fungicide to monitor as it was detected in high numbers, both in indoor and outdoor air, as well as in house dust (Vermeulen RCH, 2019).

Although the sample size was small, the composition of the population studied reflects the situation of many rural residents in the Netherlands, i.e. residents of FF and NF with a comparable gender distribution. Furthermore, the methodology used for sample analysis allowed us to detect very low concentrations, which is usually not the case in population studies (Huen et al., 2012). In addition, the data collected via questionnaires proved to be a valuable tool to identify the determinants of dermal exposure. Finally, only few studies combined urine sampling and hand wipe collection in a non-occupational setting. This study adds value to the body of evidence that the dermal pathway is an important route of exposure in a residential setting, since we detected pesticides in many of the hand wipes and we observed, a strong correlation for carbendazim between the observed pesticide concentrations in hand wipes and urine samples.

One of the limitations of the study is that babies and toddlers were not included. This group is considered more susceptible to pesticides and the relative exposure per kg body weight is expected to be higher due to their behaviour (e.g. crawling and playing on the ground, frequent hand-mouth contact) they have more frequent contact with contaminated surfaces. Therefore, the exposure levels found in the studied population cannot be used to make a judgment on the exposure situation for young children.

Although the self-assessment wipe test had a better sampling efficacy compared to wiping by a scientist, the protocol was previously tested for metals and not for pesticides. Additionally, the self-assessment wipe test was previously done by employees and not by residents. The sampling efficacy of the described wipe-method was estimated to be between 70 and 88% (Gorce and Roff, 2015). The efficacy of the sampling procedure used in this study was not tested which is a limitation.

The relation between hand wipe concentrations and urine levels would have been better studied if also hand wipes were available on follow-up days. Now the hand wipes were only available on the day of spraying (day 0). Finally, a relatively high number of residents reported that they applied at least one pesticide within 6 months prior to the start of the study, and although this variable was included in the multivariable analysis for both compounds, it only was statistically significant as a determinant of the tebuconazole concentration in hand wipes. This is likely due to the fact that the environmental half-life of tebuconazole (49–610 days) is much longer compared to chlorpropham (35–65 days) and tebuconazole occurs mainly in the particle-phase, whereas chlorpropham is mainly present in the vapour phase due to its higher vapour pressure (Wang et al., 2017; Rokbani et al., 2019).

5. Conclusion

In this study we observed that the levels of carbendazim and tebuconazole in hand wipes from residents, as surrogate for dermal exposure, were statistically different between the use and the non-use period of these pesticides in the Netherlands. The concentrations in urine of tebuconazole were also significantly higher in the use periods compared to the non-use periods. We observed large differences in concentrations in hand wipes between FF and NF, but not for urine. Correlations in pesticide concentrations between hand wipes and urine samples were found for carbendazim on the first two days after taking the hand wipe, whereas for chlorpropham a correlation was only detected on day 2. Ventilation rate of the home, age of flooring and owning a cat were observed to be associated with higher carbendazim concentrations in hand wipes and the time of sample collection was the main variable determining dermal tebuconazole concentrations.

Author contribution

A. Oerlemans, Conceptualization, Methodology, Writing – original draft, D.M. Figueiredo, Formal analysis, Methodology, Writing – original draft, Data curation, J.G.J. Mol, Validation, Writing – review & editing, R. Nijssen, Validation, Resources, Investigation, R.B.M. Anzion, Resources, Investigation, M.F.P. van Dael, Resources, Investigation, J. Duyzer, Supervision, Methodology, N. Roeleveld, Supervision, Writing – review & editing, F.G.M. Russel, Supervision, Writing – review & editing, R.C.H. Vermeulen, Supervision, Methodology, Writing – review & editing, Project administration, P.T.J. Scheepers, Supervision, Methodology, Writing – review & editing, Project administration

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111282.

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Ethical approval

The study protocol with one amendment was approved by the Medical Ethical Committee of the University Medical Center Utrecht in accordance with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

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