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Relationship between dietary pesticide intake and urinary excretion: a pilot study using duplicate portion analysis

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ABSTRACT

Dietary uptake is estimated using residue data from food commodities and predicting the effect of food processing, such as peeling. The aim of this study was to explore the value of the analysis of duplicate portions supplemented by the analysis of urinary metabolites. Forty-three participants, consuming organic and non-organic diets collected a duplicate portion of food and beverages for 24 h. Urine was collected up to 36 h to account for metabolism and prolonged excretion of metabolites. Pesticide residues were analysed in food portions and urine samples using LC-MS/MS and GC-MS/MS. Multiple pesticide residues were detected per food portion. Out of 183 pesticides, 86 were detected in the diet. Glyphosate and AMPA were detected in 42 % and 30 % of all urine samples, respectively, while detection in the diet was low. A positive relationship between dietary intake and urinary excretion was found for 2,4-D and MCPA. No diet-urine relationship was observed for glyphosate and AMPA, indicating contribution from external routes of exposure. Hazard index (HI) indicated no exposure above the acceptable daily intake (ADI) for all participants. This study demonstrates how DPA combined with urine analysis gives insight into contribution of diet to total pesticide exposure compared to standard monitoring.

1. Introduction

Pesticides are used during the production of crops in agriculture, resulting in chemical residues of these compounds ending up in the diet. Especially pesticides that are applied close to harvest or post-harvest are expected to leave residues in or on food (Markantonis et al., 2018). Maximum residue levels (MRLs) are defined as the highest level of a pesticide residue legally tolerated in or on food or feed when pesticides are applied according to Good Agricultural Practice (European Food Safety Authority (EFSA), 2022).

The three main exposure routes to pesticides for humans are dietary intake, dermal absorption and inhalation. Dermal and inhalation routes are considered relevant for applicators of pesticides, and for residents of

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Abbreviations

2,4-D = 2,4-dichlorophenoxyacetic acid
3-PBA = 3-phenoxybenzoic acid
3-PBA = 3-Phenoxybenzoic acid
4F-3-PBA = 4-Fluoro-3-phenoxybenzoic acid
ADI = Acceptable Daily Intake
AMPA = Aminomethylphosphonic acid
ARfD = Acute Reference Dose
CE = Collision Energies
CIF3CA = cis-3-(2-Chloro-3,3,3-trifluoroprop-1-en-1-yl)
CSS = Case study site
DBCA = (1R-cis)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid
DBCA = (1R-cis)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid
DCCA-CIS = 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
DCCA-cis = 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
DCCA-trans = 2,2-dimethylcyclopropane carboxylic acid
DCCA-trans = trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
DEAMPY = 2-Diethylamino-6-hydroxy-4-methylpyrimidine
DEAMPY = 2-Diethylamino-6-hydroxy-4-methylpyrimidine
DF = Detection Frequency
DPA = Duplicate portion analysis
EDI = Estimated Daily Intake
EFSA = European Food Safety Authority
FMOC chloride = 9-Fluorenylmethoxycarbonyl chloride
GC-MS/MS = Gas chromatography–mass spectrometry
HI = Hazard Index
HQ = Hazard Quotient
LC-MS/MS = Liquid chromatography–mass spectrometry
LOD = Limit of Detection
LOQ = Limit oOf Quantification
LOQm = Method LOQ
MCPA = 2-Methyl-4-ChloorPhenoxyAcetic acid
MCRA = Monte Carlo Risk Assessment
MeOH = Methanol
$MgSO_4$ = Magnesium Sulphate
MRLs = Maximum residue levels
MRM = Multi Reaction Monitoring
$Na_2C_6H_6O_7$ = Disodium Citrate Sesquihydrate
NaCitrate = Sodium Citrate
NaCl = Sodium Chloride
NH_4COOH = Ammonium Formate
PSA = Primary Secondary Amine
QuEChERS = Quick, Easy, Cheap, Effective, Rugged and Safe
RT = Retention times
SD = Standard deviation
TCPy = 3,5,6-trichloro-2-pyridinol
TCPy = 3,5,6-trichloro-2-pyridinol
Tebuconazole-OH = Hydroxy-tebuconazole
UHPLC = Ultra high-performance liquid chromatography

agricultural areas who live in close proximity to fields where pesticides are applied (Figueiredo et al., 2021). Secondary exposure to pesticides through dust or spray drift might also be important for rural inhabitants (Figueiredo et al., 2022). For the general population, dietary intake is considered the most relevant route of exposure (Nougadère et al., 2020; Oates et al., 2014; Rempelos et al., 2022). Changes in the diet are expected to be reflected in overall pesticide exposure. Prior human biomonitoring studies have reported a significant decrease in the urinary levels of organophosphorus or pyrethroid pesticide metabolites after switching to an organic diet (Curl et al., 2019; Hyland et al., 2023; Lu et al., 2006).

Since diet is considered the main source of pesticide exposure, information about human exposure through dietary intake is necessary to accurately assess the human health risks. In risk assessment, monitoring of food commodities and consumption data combined with Monte Carlo Risk Assessment (MCRA) can be applied to estimate the risk of dietary exposure (Vasco et al., 2025). Another approach to assess pesticide intake through food is by duplicate portion analysis (DPA) (Rempelos et al., 2022; Nijssen et al., 2023). In the DPA approach, a study participant is asked to collect a duplicate portion of all of their prepared and consumed food plus beverages and keep a food diary with a detailed log of the food commodities complemented with information on the presence of home-grown or locally purchased food items. The collection of duplicate portions can be done over any specified period of time as long as the normal dietary pattern is reflected (WHO). These studies are useful for small surveys; however, as food is collected all together in food containers, analysis of individual food items is not possible. Combining DPA with measurements that reflect internal exposure such as urine or blood, can provide additional information on the extent of uptake of residues from food and beverages (WHO). The novelty of the approach presented in this study lies in the combination of screening for a large number of pesticides across multiple countries using a harmonized approach.

The aim of this study was to analyse the dietary pesticide residues in duplicate portions and compare these results to the excretion of urinary metabolites. With this we present a proof-of-concept of the DPA approach to study dietary pesticide exposure in multiple countries. The methods presented in this paper are expected to improve interpretation of biomonitoring data and integrating food processing and portioning into pesticide exposure- and risk assessment.

2. Methods

2.1. Recruitment

This study was carried out as part of the SPRINT project (https://sprint-h2020.eu/). Participants were recruited from seven countries (Spain, Portugal, Croatia, Slovenia, the Netherlands, Denmark and Argentina). Most participants were recruited from the biomonitoring study of the SPRINT project (Silva et al., 2021). They were grouped in three categories: (a) farmers, (b) neighbours, the residents of rural areas living close to pesticide applications, and (c) consumers, participants not classified in the first or second category. The exact criteria for this classification have been described previously (Silva et al., 2021). The farmer and neighbour groups consisted of participants with higher potential to additional non-dietary exposure to pesticides, including environmental exposure to highly persistent pesticides and pesticide applications in private homes.

2.2. Sample collection

2.2.1. Questionnaires and diaries

Participants were re-recruited from the SPRINT biomonitoring campaign (Silva et al., 2021). During the prior campaign, participants were classified as ‘organic’ or ‘conventional’ based on their farming system (for farmers or neighbours) or self-reported following a mostly organic diet (consumers). Participants were informed that a diet consisting of at least 70 % organic products containing the SKAL EU-organic

produce logo would be labelled as organic. After re-recruitment, it was confirmed with the participants whether their diet composition had changed or remained the same compared to a year earlier. For participants which were recruited outside of the SPRINT biomonitoring study, the CSS-leaders confirmed with the participants what percentage of their diet consisted of organic products.

For the Dutch participants, organic products were labelled in a food diary kept by the participants. The percentage of organic products in the diets of these participants was later calculated, and participants who had more than 70 % organic products in their diet were labelled as 'organic' participants.

2.2.2. Diet and urine sampling

Sampling was carried out between November 2021 and May 2022. Participants were asked to collect an exact equal (prepared) portion of their food and beverages in a container for 24 h (one full day). Foods consumed the next morning were not included. Inedible parts of the food (e.g. popsicle sticks, gum or orange peels) were discarded. Food portions were stored in a cool box. Full sampling instructions can be found in **Supplementary S1.** In every country, local researchers homogenized the duplicate portion with a laboratory blender and a 500g aliquot of the slurry was sent frozen to the Technological Center of Catalonia (Eurecat), Tarragona, Spain for analysis. Participants noted the consumed food and beverage items in a diary.

For urine sampling, two different batches were collected. Batch-A consisted of all urine voids over 24 h (24-h urine) collected on the same day as the duplicate food portions, including the first void of the next day (morning urine). The first morning urine of Batch-A was discarded to account for pesticide excretion from the previous day. Batch-B included all urine voids of the second day, collected after the first morning urine until lunchtime (around 12PM) to measure pesticides with longer excretion times. Batch-A and Batch-B were aliquoted and analysed separately. Urine containers were gently homogenized at room temperature. From each batch (A and B), five aliquots of 10 mL each were transferred to 12 mL propylene tubes. Urine samples were shipped in frozen condition for analysis to Wageningen Food and Safety Research (WFSR) in Wageningen, The Netherlands.

2.3. Analysis of duplicate portions

2.3.1. Reagents and chemicals

Methanol (MeOH) for LC-MS and acetonitrile was purchased from Merck (Darmstadt, Germany). Water for LC-MS was purchased from Scharlab (Barcelona, Spain). Formic acid OptimaTM LC/MS Grade and ammonium formate (NH_4COOH) 10M HPLC-MS were purchased from Fisher Scientific (New Hampshire, USA). Medronic acid was obtained from Agilent Technologies (California, USA). In turn, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction salt packets, EN 15662 method, containing 4g magnesium sulphate ($MgSO_4$), 1g sodium chloride (NaCl),1g sodium citrate (NaCitrate), 0.5g disodium citrate sesquihydrate ($Na_2C_6H_6O_7$), QuEChERS Dispersive Kit, Universal, AOAC method, containing 50 mg primary secondary amine (PSA), 50 mg C18EC, 150 mg $MgSO_4$ and QuEChERS Dispersive Kit, Universal, AOAC method, containing 50 mg C18EC, 150 mg $MgSO_4$ were obtained from Agilent Technologies (California, USA), while Oasis® HLB 1 cc (30 mg) Extraction Cartridges were purchased from Waters (Massachusetts, USA).

Hexachlorobenzene-13C6, glyphosate pestanal and aminomethyl phosphonic acid (AMPA) were obtained from Sigma-Aldrich (Missouri, USA). Lindane-d6, fenvalerate-d5, fenbuconazole-d5 and isoproturon-d3 were purchased from TRC Canada (Ontario, Canada). Metalaxyl-M d3, 2,4′-DDT-d8, acetamiprid-d3 (N-methyl-d3), propyzamide-d3 and methiocarb-d3 were obtained from Dr. Ehrenstorfer (Nordrhein-Westfalen Germany), while linuron-(methyl-d3, methoxy-d3), oxyfluorfen-(ethoxy-d5), pyraclostrobin-(N-methoxy-d3), metribuzin-(S-methyl-d3), fipronil (pyrazole-13C3, cyano 13C) and MCPA-(methyl-d3) were purhcased from Merck (Darmstadt, Germany). Finally, AMPA 13C-15N and glyphosate 1,2–13C2-15N were obtained from LGC Standards (Barcelona, Spain).

Native standards for PPPs were obtained from Wageningen University (Wageningen, Netherlands) in six different mixtures: 1) GC compounds, 2) P LC POS-1 compounds, 3) P LC-POS-2 compounds, 4) special compounds, 5) P/M LC-NEG compounds, and 6) M LC-POS compounds. All the mixtures were prepared at a concentration of 1 μg/mL in acetonitrile (0.1 % of formic acid).

2.3.2. Multiresidue extraction and analysis

Extraction of pesticides for the multi-residue analysis in food samples has been described previously (González et al., 2023). In short, 10g of sample was weighed into a 50 mL centrifuge tube and 20 μL of internal standard (IS) solution was added. 10 mL of acetonitrile was added before samples were vortexed for 30s. A mixture of salts (4 mg $MgSO_4$, 1g NaCl, 1g sodium citrate, 0.5g of disodium citrate) was added to the samples, and subsequently vortexed for 1 min. Next, samples were centrifuged for 5 min at 4700 rpm. 1 mL of supernatant was transferred to a tube that contained 150 mg of $MgSO_4$ and 25 mg of C_{18} while another mL was transferred to 50 mg of PSA, 50 mg of C_{18} and 150 mg of $MgSO_4$. Finally, samples were vortexed for 30s and rapidly centrifuged for 5 min at 13500 rpm. Supernatant was carefully transferred to a clean 2 mL vial and injected for analysis.

Multiresidue analysis was carried out through LC-MS/MS (positive and negative modes) and GC-MS/MS methods. For the LC-MS/MS analysis, an UHPLC 1290 Infinity II Series coupled to a triple quadrupole QQQ/MS 6490 Series was used, both from Agilent Technologies (Santa Clara, CA, USA). The chromatographic column used was a Zorbax Eclipse Plus C_{18} 1.8 μm (150 × 2.1 mm) (Agilent Technologies). Chromatographic separation was performed using water with 5 mM NH_4COOH (mobile phase A) and MeOH with 5 mM NH_4COOH (mobile phase B), both complemented with 0.1 % formic acid, in the gradient described in the **Supplementary materials**. Temperature of the column was set at 40 °C and the injection volume was 2 μL. Source parameters of the mass spectrometer in positive and negative ionization mode are listed in **Supplementary S2.3 and S2.4**. Multi Reaction Monitoring (MRM) transitions and collision energies (CE), as well as retention times (RT) are summarized in **Supplementary S2**.

For the GC-MS/MS analysis, a HP 5MSUI chromatographic column (30 m × 0.25 mm x 0.25 μm) was used. The oven temperature was programmed as follows: initial temperature 60 °C, linearly raised by 40 °C/min to 170 °C (0 min), (iii) then linearly raised at 10 °C/min to 310 °C (3 min). The column flow was set at 1 mL/min using He as carrier gas. The injector was set at 280 °C, and the extracts were injected in solvent mode. The ionization was carried out by electronic impact (70eV) and the mass analyser operated on MRM. The *m/z* ions are listed in Supplementary Table S2.4.

For glyphosate and AMPA, a separate extraction and analysis method was used. Extraction of glyphosate and AMPA was performed following the protocol of an earlier study (González et al., 2023). Briefly, 2g of homogenized sample was weighted, to which 10 mL of extraction solvent (MeOH:H_2O 1:1 0.1 % Formic acid) was added. After shaking in an automatic shaker for 15 min, it was centrifuged at 4600 rpm, 4 °C for 1.5 min. After this, 300 μL of the supernatant was passed through an Oasis HLB cartridge (30 mg), previously conditioned with 200 μL MeOH and 2 mL of the extracting solvent. Then, 200 μL of the extract was collected into an Eppendorf tube. Next, 100 μL of the collected sample was transferred to an autosampler vial and 100 μL water containing the internal standard (IS) at a concentration of 200 ng/mL was added. A final 2 μL volume of sample was injected into the LC-MS/MS system.

For quantification of glyphosate and AMPA, an HPLC 1260 Infinity Series coupled to a triple quadrupole QQQ/MS 6490 A Series was used, both from Agilent Technologies (Santa Clara, CA, USA). The chromatographic column used was a InfinityLab Poroshell 120 HILIC-Z (100 × 2.1 mm), PEEK Lined (Agilent Technologies). Chromatographic

separation was performed using water (mobile phase A) and acetonitrile (mobile phase B), both complemented with 0.04 % formic acid and 5 μM medronic acid, in a gradient shown in Table S5. The temperature of the column was set at 35 °C with an injection volume of 2 μL. Source parameters of the mass spectrometer in positive ionization mode are listed in **Supplementary S2**. MRM transitions and collision energies (CE), as well as retention times (RT) are also summarized in **Supplementary S2**.

2.3.3. Quality assurance and quality control

A reference homogenized sample was used for method validation, according to the EU SANTE document (SANTE/2020/12830) (SANTE/2020/12830, 2021). To validate, spiked samples of reference homogenized samples were used. Samples were spiked with all pesticides included in the method. Method validation performance criteria were evaluated by assessing the linearity of the calibration curve, trueness (as % recovery), precision (as repeatability % RSD) and limit of quantification (LOQ), following the EU SANTE Guideline on analytical quality control and validation procedures (SANTE/2020/12830, 2021). Trueness and intra-day precision in the matrix were determined via injecting spiked reference homogenized sample at three concentration levels: either 0.2 μg/kg, 1 μg/kg and 10 μg/kg, and 1 μg/kg, 10 μg/kg and 50 μg/kg, based on their respective MRLs and the method LOQ (LOQm) obtained for each pesticide. The trueness (as % recovery, $n = 5$) and inter-day precision (as % RSD, $n = 5$) of the recovery experiments were determined. Recoveries were calculated using the isotope-labelled internal standards (deuterated carbon-13 and nitrogen-15) for quantification. Reproducibility (inter-day precision) data was obtained via on-going validation of the method by collection of recovery and RSD data from the QC-samples in routine analysis series, as stated in the SANTE document (SANTE/2020/12830). The LOQm has been defined as the lowest spike level of the recovery study that can be quantified with acceptable trueness (recovery, 70–120 %) and precision (RSD <20 %), and limit of detection (LOD) estimated as LOQ/3.33. Quality control data for the analysis of both the duplicate portions and urine can be found in the **Supplementary S3**.

2.4. Analysis of urine samples

Each participant collected their full urine samples for 24 h (batch A) and until 12:00am the next day (batch B) and placed it in a cool box (2–10 °C). Within 24 h after collection of the samples, the urine was aliquoted into different tubes/portions. Next, all tubes were placed into a sorbent pouch to absorb excess liquid during transportation and put into a safety bag with SPRINT participant ID and date for storage in the freezer (−18 °C or lower) until transport to the analytical laboratory (WFSR). Before handling, the samples were allowed to reach room temperature. The samples were vortexed during 30 s and centrifuged at 3000 rpm during 5 min. Quantification of pesticide residues was performed according to the protocol of Nijssen et al. (2023).

To extract glyphosate, 10 μL of the isotopically labelled internal standard at 100 ng/mL was added to 1 mL of urine. After homogenization, the samples were loaded to the SPE cartridges (Strata SAX, 200 mg) previously conditioned with 1 mL of acetonitrile followed by 1 mL of water. After that, the cartridges were washed with 2 mL of water followed by 2 mL of acetonitrile. Then, the cartridges were dried under vacuum. The samples were eluted with 1 mL of NaCl 200 mM in HCl 0.1M into a polypropylene reaction tube and 20 μL of NaOH at 5M was added. The derivatization procedure was performed by addition of 500 μL of borate buffer 5 % followed by 500 μL of FMOC chloride at 6.5 mM. The tubes were gently homogenized and allowed to stand for 30 min for derivatization. After that, the derivatization reaction was stopped by addition of 50 μL of formic acid. The samples were shaken and placed in autosampler vials for analysis. For extraction of AMPA, a separate method was used. To a 200 μL sample portion, 10 μL of the isotopically labelled internal standard at 20 ng/mL was added. Then, 100 μL of borate buffer 5 % was added followed by 100 μL of 9-fluorenylmethoxycarbonyl chloride (FMOC chloride) at 6.5 mM. The tubes were gently homogenized and allowed to stand for 30 min for derivatization at room temperature. After that, the derivatization reaction was stopped by addition of 10 μL of formic acid. The samples were shaken and placed in autosampler vials for analysis.

2.5. Data analysis

2.5.1. Imputation of left-censored data and statistical analysis

Both urine and dietary datasets contained high numbers of non-detects (i.e. left-censored data) for many pesticides of interest. Data imputation was used to improve reliability of calculated summary statistics. In the dietary dataset, 97 (45 %) of the pesticides included in the multi-method analysis were not detected above LOD. In the urine samples, 29 (66 %) and 30 (68 %) were not detected in batch A and batch B urine, respectively. Data > LOD but < LOQ were reported by the analytical laboratories for urine, but not diet.

For imputation, we used the *survreg* function from the *survival* package in Rstudio v.4.4.3. Here, the data < LOQ was fitted to a lognormal distribution. In the survival function, the lower boundary was defined as 10e-8, allowing for flexibility in the imputation and the upper-boundary as equal to LOQ, meaning the imputed values can vary between LOQ and lower boundary. Imputation was only applied to pesticides which had a minimum positive (>LOQ) rate of 40 %. This threshold was defined based on results from previous studies (Figueiredo et al., 2022; Fuhrimann et al., 2022). For the calculation of the hazard quotient values < LOQ were set on the LOQ and values < LOD were not taken into account.

For the analysis of the occurrence of pesticide residues in urine, the time-weighted-average was calculated between the results from the analysis of batch A and batch B. In case of a single detection in either A or B, the concentration measured was kept unchanged. Median concentrations were calculated after imputation < LOQ. Correlations between diet and urine were calculated using Spearman's rank correlation.

A risk characterization was applied using the Hazard Index (HI) approach (More et al., 2019). The total amount of pesticide present in each duplicate portion was calculated using the measured concentrations and duplicate portion weight. In case of missing data on portion weight, the average weight of all portions was used (2730 g). The total amount of pesticide was then divided by body weight of the participant, or a standard bodyweight of 70 kg to calculate the Estimated Daily Intake (EDI, mg/kg bw/day) for every pesticide separately. A Hazard Quotient (HQ) was calculated by dividing the EDI with the Acceptable Daily Intake (ADI) obtained from the online European Food Safety Authority (EFSA) Pesticide Database (accessed 2021, Supplementary Table S6) (More et al., 2019). For pesticides without an ADI set by the EU, such as the co-formulant piperonyl butoxide, or some of the metabolites of active substances, the ADI from ECHA (2024) or the parent compound was applied. The sum of all HQs for a single participant resulted in the Hazard Index (HI). Significance was calculated using the Wilcoxon Mann-Whitney *U* test.

3. Results

3.1. Demographics

At each case study site (CSS), six participants were recruited, except for Denmark (n = 5) and the Netherlands (n = 8). Participants were recruited from the SPRINT biomonitoring campaign, or through informal channels (Netherlands and Croatia). In total 43 participants (21 women and 22 men) were recruited across all countries (see Table 1). Of these, 25 were consumers (14 female, 11 male), 9 worked as farmers (5 female, 4 male) and 9 were residents in agricultural areas but did not work as farmers (2 female, 7 male).

Table 1
Summary of participant demographics including age, weight, sex and country of origin. Participants are mutually exclusive across subgroups. Values are presented as mean ± standard deviation or counts.

	Total	Organic			Conventional		
		Consumer	Farmer	Neighbour	Consumer	Farmer	Neighbour
n	43	9	6	4	16	3	5
Sex, n							
Male	22	3	2	3	8	2	4
Female	21	6	4	1	8	1	1
Age in years							
Mean (SD)		55 (10)	57 (5)	43 (20)	52 (10)	53 (4)	47 (19)
Weight in kilograms							
Mean (SD)		67 (12)	80 (12)	82 (12)	75 (11)	75 (5)	77 (14)
Country, n							
Argentina	6	0	2	0	2	0	2
Croatia	6	2	0	0	4	0	0
Denmark	5	0	1	1	3	0	0
Netherland	8	4	0	0	4	0	0
Portugal	6	1	1	1	1	1	1
Slovenia	6	1	1	1	1	1	1
Spain	6	1	1	1	1	1	1

3.2. Occurrence and concentrations of pesticides in diet and urine

The 24-h duplicate food portions were analysed for 183 pesticides. Table 2 summarizes the data of 20 pesticides with the highest detection frequency. In total, 115 pesticides or metabolites were identified in food portions of the 43 participants. In the top 20, most pesticides were detected in parent form, except forthe metabolite spirotetramat-enol-glucoside. Pesticides with the highest detection frequency were metalaxyl (100 %), tebuconazole (86 %) and pirimiphos-methyl (77 %). Piperonyl butoxide, a co-formulant in pyrethroid-based products was detected in all food portion samples. The number of pesticides per dietary portion ranged from 7 to 43. The highest concentration measured was 102 μg/kg for a pyrimethanil metabolite (M605F002), followed by 94.8 μg/kg for fludioxonil and 62.3 μg/kg for imazalil. The median concentration observed in the food portion was below 1 μg/kg food for most of the pesticides. Fungicides were the most frequently detected functional class of pesticides in the top 20 (n = 13), followed by insecticides (including the synergist piperonyl butoxide) (n = 6) and herbicides (n = 1), respectively.

In urine most pesticides were detected as their metabolites (Table 3). Some metabolites are not specific because they can be derived from multiple parent compounds. 3,5,6-trichloro-2-pyridinol (TCPy) was found in 41 samples (95 %) and DEAMPY in 35 (81 %). Common metabolites for pyrethroids were 3-phenoxybenzoic acid (3-PBA), trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA-trans), (1R-cis)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA) and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA-CIS), detected in 28 (65 %), 18 (42 %), 11 (26 %) and 8 (19 %) of the urine samples, respectively. 5-Hydroxy-tebuconazole (Tebuconazole-OH), the main metabolite of tebuconazole, was detected in 23 (53 %) of the samples. Parent compounds were detected at a lower rate, of which 2,4-D was most often detected (16 %). Overall, most measured (median) concentrations were below 1 μg/L urine. The highest concentration was measured for pyrimethanil-OH, a metabolite of pyrimethanil, but this compound was only measured in the urine of one participant and can thus be considered an outlier resulting from exceptionally high exposure. Among the most often detected metabolites, 2-Diethylamino-6-hydroxy-4-methylpyrimidine (DEAMPY) had a relative high concentration (1.96 ng/mL ± 3.34). Metabolites of insecticide pesticides were the most common in the urine data. All concentrations can be found in Supplementary Table S3.1 and S3.2.

Table 2
Pesticide residues detected (>LOD) and quantified (>LOQ) in the duplicate portions of 43 participants. The top 20 pesticides in terms of detection frequency are shown.

	Name	Type	P/M	N	DF (%)	Median concentration (μg/kg)	Range (μg/kg)
1	Metalaxyl	Fungicide	P	43	100	0.17	0.11–16.6
2	Piperonyl butoxide	Synergist/Biocide	P	43	100	0.81	0.02–20
3	Tebuconazole	Fungicide/Biocide	P	37	86	0.28	0.03–4.49
4	Pirimiphos-methyl	Insecticide	P	33	77	0.23	0.02–27
5	Trifloxystrobin	Fungicide	P	33	77	0.01	0–0.59
6	Propamocarb (hydrochloride)	Fungicide	P	32	74	0.06	0.01–23.4
7	Pyraclostrobin	Fungicide	P	32	73	0.03	0.01–0.81
8	Fludioxonil	Fungicide	P	31	72	0.12	0.11–94
9	Azoxystrobin	Fungicide	P	31	72	0.11	0.11–31
10	Fluopyram	Fungicide	P	31	72	0.10	0.11–24
11	Boscalid	Fungicide	P	28	65	0.19	0.21–45
12	Chlorantraniliprole	Fungicide	P	28	65	0.12	0.01–7.79
13	Difenoconazole	Fungicide	P	28	65	0.15	0.01–0.98
14	Acetamiprid	Insecticide/Biocide	P	27	63	0.1	0.02–2.51
15	Metamitron	Herbicide	P	26	60	0.16	0.01–3.63
16	Prothioconazole	Fungicide	P	24	55	0.39	0.05–39
17	Imidacloprid	Insecticide/Biocide	P	23	53	0.11	0.03–7.94
18	Thiacloprid	Insecticide/Biocide	P	22	51	0.03	0–2.44
19	Dimethomorph	Fungicide	P	20	47	0.08	0.02–56
20	Spirotetramat-enol-glucoside	Insecticide	M	20	47	0.16	0.05–7.66

Median concentration and range were calculated > LOD with imputed values > LOD and <LOQ, detection frequency (DF) > LOD. Abbreviations: Metabolite (M), Parent (P), Detection frequency (DF). N corresponds to the total number of detects > LOD.

Table 3
Pesticide residues and metabolites detected (>LOD) and quantified (>LOQ) in urine of 43 participants.

	Name	Type	P/M	Parent compound	N	DF (%)	Median concentration (ng/mL)	Range (ng/mL)
1	TCPy	Insecticide	M	Chlorpyrifos	41	95	0.33	0.13–6.5
2	3-PBA	Insecticide	M	Pyrethroids	39	91	0.20	0.02–3.9
3	DEAMPY	Insecticide	M	Pirimiphos	37	86	1.71	0.21–18
4	DCCA-trans	Insecticide	M	Pyrethroids	30	70	0.19	0.02–2.9
5	Tebuconazole-OH	Fungicide	M	Tebuconazole	27	63	0.26	0.08–17
6	Glyphosate	Herbicide	P		24	56	0.37	0.03–1.6
7	AMPA	Herbicide	P/M	Glyphosate	19	44	0.18	0.04–0.43
8	DBCA	Insecticide	M	Pyrethroids	17	40	0.23	0.10–8.3
9	DCCA-cis	Insecticide	M	Pyrethroids	16	37	0.10	0.02–2.2
10	2,4-D	Herbicide	P		12	28	0.15	0.03–1.1
11	Quinmerac	Herbicide	P		12	28	1.21	0.33–3.6
12	Boscalid-OH	Fungicide	M	Boscalid	8	19	0.58	0.27–5.6
13	CIF3CA	Insecticide	M	Pyrethroids	5	12	0.18	0.03–0.56
14	Chlorpyrifos-methyl-desmethyl	Insecticide	M	Chlorpyrifos-methyl	4	9	0.34	0.14–0.91
15	Pyrimethanil-OH M605F002	Fungicide	M	Pyrimethanil	4	9	1.1	0.59–136
16	MCPA	Herbicide	P		3	7	0.92	0.28–2.8
17	Propamocarb	Insecticide	P		3	7	0.32	0.17–0.34
18	Trifloxistrobin CGA321113	Fungicide	M	Trifloxistrobin	3	7	0.11	0.11–0.14
19	Cyprodinil CGA304075	Fungicide	M	Cyprodinil	2	5	5.1	1.1–9.1
20	4-F-3-PBA	Insecticide	M	Pyrethroids	1	2	*NA*	*NA*

Abbreviations: Metabolite (M), Parent (P), Detection frequency (DF), 3,5,6-trichloro-2-pyridinol (TCPy), 3-Phenoxybenzoic acid (PBA), 2-Diethylamino-6-hydroxy-4-methylpyrimidine (DEAMPY), 2,2-dimethylcyclopropane carboxylic acid (DCCA-trans), Aminomethylphosphonic acid (AMPA), (1R-cis)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA-cis), 2,4-dichlorophenoxyacetic acid (2,4-D), cis-3-(2-Chloro-3,3,3-trifluoroprop-1-en-1-yl) (CIF3CA), 2-Methyl-4-ChloorPhenoxyAcetic acid (MCPA), 4-Fluoro-3-phenoxybenzoic acid (4F-3-PBA). No measurements > LOQ available for calculation of statistics (NA), Standard deviation (SD). N corresponds to the total number of detections > LOD; DF is the detection frequency expressed as % of results at LOD or higher compared to all analysed samples.

3.3. Mixture exposure from individual food products

In the food diaries of the participants, the exact composition of each duplicate food portions were reported. Self-reported components of the duplicate food portions were classified according to the EFSA FoodEx2 system (Authority (EFSA) EFS, 2015) (Supplementary Table S5). Composite dishes and water and water-based beverages were among the most often reported components. In Argentina, grains and grain-based products were most commonly included (17.9 %) in the diet of the participants compared to other countries. In Spain, milk and milk-based products (dairy) were included more often compared to other countries (12.8 %). Notably, the food diaries from Argentina did not report vegetables separately as participants only noted the full dish (e.g. 'salad' instead of ingredients). Denmark's food diaries did not report any alcoholic beverages where diaries from other countries did. Food portions from the Netherlands contained the highest reported number of garden vegetables (11.9 %) and Spain contained the highest number of fruits (10.6 %). A positive relationship between detection in diet and urine was found for 2,4-D and MCPA ($r^2 = 0.49$ and 0.37, $p = 0.001$ and 0.019 resp.) while this was slightly negative for glyphosate and AMPA ($r^2 = -0.55$ and -0.22, $p = 0.119$ and 0.165 resp.)

3.4. Effect of organic diet and occupation on detection frequency in food and urine

For all countries, stratification by type (consumer, rural resident or farmer) did not significantly affect pesticide detection in the diet (Fig. 1). Overall, there were no significant effects by country, diet or weight of the DPA portion on the number of pesticides detected ($p < 0.05$). In total, 20 participants reported following an organic diet and 23 a conventional diet. According to the participants the availability of organic products differed per country, thus the opportunities for participants to follow a strict organic diet varied. Overall, the strongest effect of an organic diet on the number of pesticides per diet sample was seen in the Netherlands, where we observed a clear division in the number of pesticides per conventional diet sample (13 or more) compared to samples from participants adhering to an organic diet (9 or less). The lowest number of pesticides in the diet was found for one organic Dutch consumer whose diet portion contained only 7 pesticide residues. It should be noted that only consumers participated in the Netherlands and Croatia. For two residents of agricultural areas (i.e., neighbours) in Slovenia and Spain, relatively high numbers of pesticides were detected in their food samples, i.e., 43 and 42, respectively, despite their self-reported consumption of organic food. In this pilot study, the most frequently detected pesticides in organic diets were metalaxyl and the synergist piperonyl butoxide (100 %), followed by pirimiphos-methyl, pyraclostrobin, tebuconazole and trifloxystrobin (84 %). In conventional diets, metalaxyl and piperonyl butoxide were also found in all samples. Fluopyram, tebuconazole and fludioxonil were found in 87 %, 87 % and 83 % of the diets, respectively.

Occurrence of pesticides in both matrices was evaluated by counting which participants contained a trace of the same chemical in urine and diet. Piperonyl butoxide, which indicates the presence of pyrethroids, was found together with 3-PBA, DCCA-trans, DCCA-cis, DBCA, and 4-F-3-PBA in urine in 38 participants. Tebuconazole and tebuconazole-oh were detected together in 24 participants. Boscalid was detected in 28 diets, but only 7 participants also contained boscalid metabolite in their urine. Notably, there are several other pesticides with a high detection in the diet but low detection in urine, such as trifloxystrobin, pyraclostrobin, azoxystrobin, and imidacloprid. Similarly, some compounds with a high detection in urine had a low detection in diets, e.g. chlorpyrifos and glyphosate.

3.5. Mixture risk assessment based on estimated daily intake (EDI)

The results of the HI calculations are presented in Fig. 2. No statistically significant differences were found by sex or country. There was no statistically significant difference between the HI of organic and conventional diets. Daily intake, expressed as the EDI, was calculated per pesticide. The highest EDI was calculated for fludioxonil, having a median exposure of 3.86 μg/kg bw/day, followed by pyrimethanil metabolite M605F002 with an EDI of 3.44 μg/kg bw/day. The HI did not exceed 1, which indicates that there is no risk resulting from exposure to multiple chemicals from the diet in this dataset. Two participants with the highest reported hazard index were from Argentina and Slovenia. Tebuconazole was detected in the diet and urine of multiple

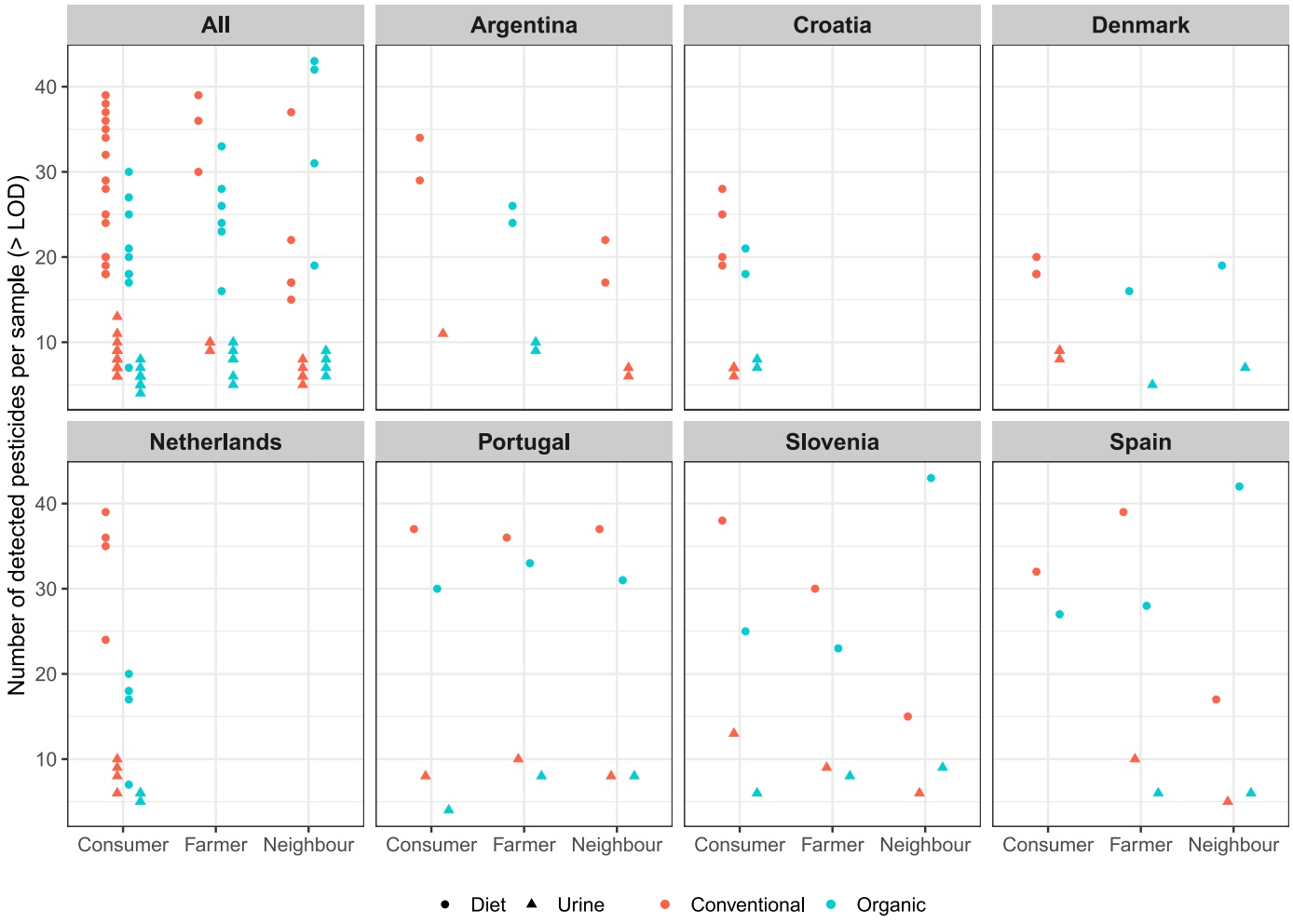


Fig. 1. Total number of pesticides detected (>LOD) per sample stratified by country, diet and matrix (urine or diet). In the Netherlands and Croatia all participants were consumers.

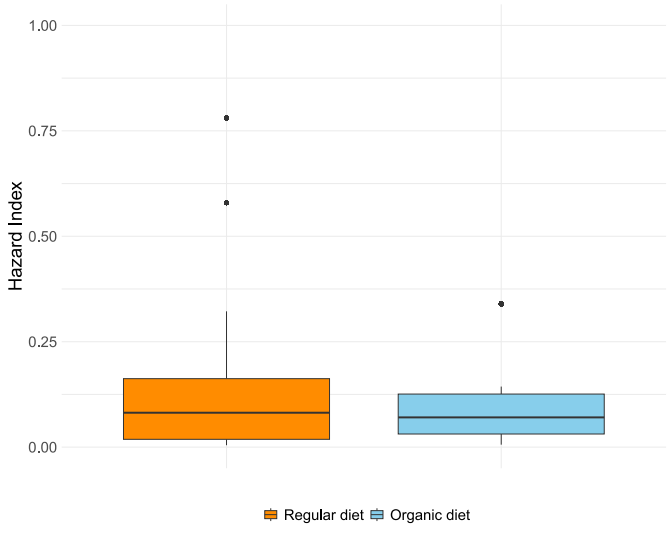


Fig. 2. Hazard index (HI), calculated as the sum of all hazard quotients (HQ) per participant, stratified per farm (for farmers and neighbours) or diet type (for consumers). No significant difference between organic and conventional participants was found (Wilcoxon rank sum exact test, p = 0.598).

participants, where low concentrations in the diet often reflected low concentrations of the metabolite as well. Other parent compounds which were measured in both diet and urine were cyprodinil, glyphosate, MCPA and pyrimethanil, although overall numbers were low. For pyrimethanil, a very high concentration in the diet (35 ng/kg food) was also reflected by a relatively high concentration of 136 μg/L in urine. The quantitative relationship is shown in Fig. 3.

4. Discussion and conclusions

This study aimed to investigate a new method for assessing pesticide residues in duplicate portions and compare them with urinary metabolite excretion, demonstrating the DPA approach as a proof-of-concept for studying dietary pesticide exposure across multiple countries. The analytical methodology used it was possible to identify different classes of pesticides from complex matrices. In addition, data was collected from multiple countries to investigate inter-country differences. The data presented here can be compared with EFSA food residue reports to provide additional information on the specificity of current EFSA methods. In this study, the strength of using a real-life sample from the diet to estimate exposure is shown. In addition to demonstrating the importance of diet in the exposure to pesticides, the most frequent pesticides in 24-h portions were identified.

DPA allows a comprehensive assessment of the dietary intake because it includes home food processing and portioning, and their effects on the uptake of pesticide residues reflecting real-life dietary

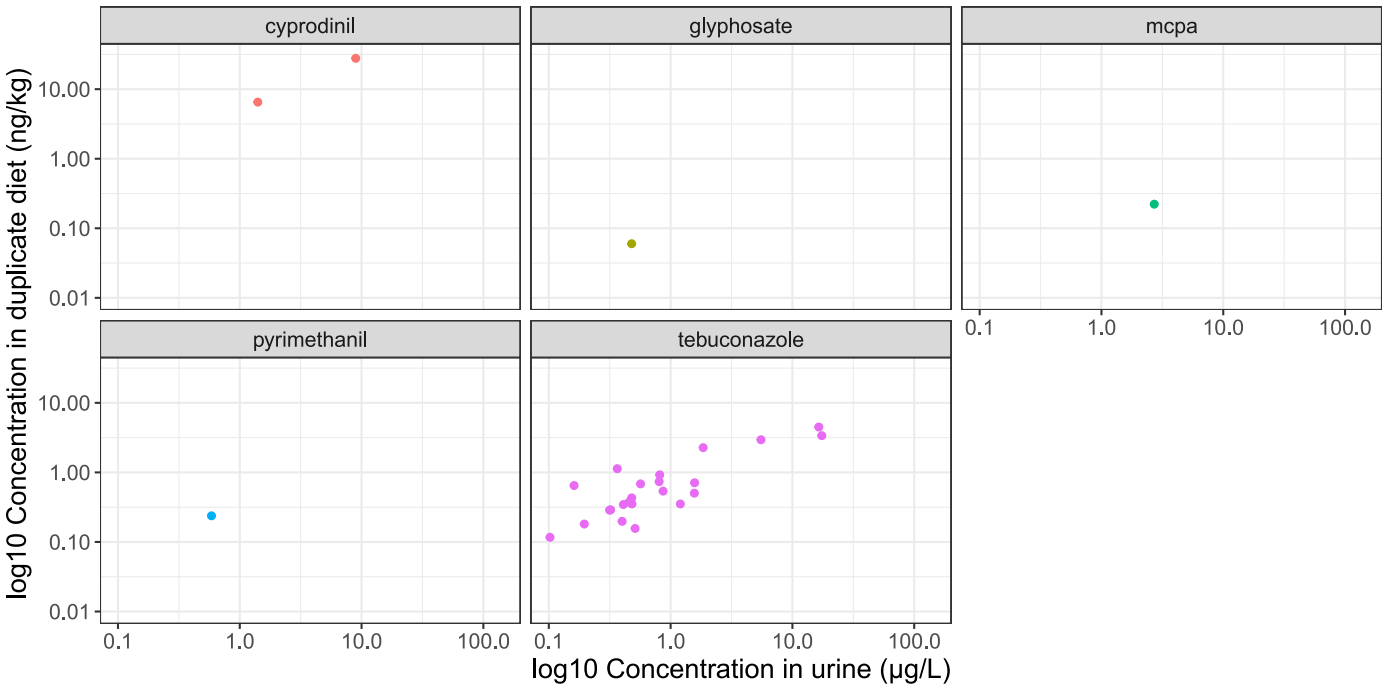


Fig. 3. Quantitative relationship between pesticides tebuconazole, cyprodinil, pyrimethanil in diets (log10, ng/kg)and their respective metabolites in urine (log10, µg/L).

exposure from realistic scenarios. For some pesticides, we were able to establish the diet as driver of exposure as increasing concentrations in the diet also showed increasing concentrations in urine, such as the pyrethroids and tebuconazole. The participant with the highest reported concentration of pesticides in the study (pyrimethanil-OH, 102.32 ng/kg) also contained the highest concentration of this metabolite in their urine (140 µg/L). Similarly, some pesticides were detected with a high frequency in urine, but not in the diets of the same participant, indicating that other routes and sources of exposure may contribute. An example of this is chlorpyrifos, which is also used as an acaricide and nematicide next to being a plant-protection product. By combining data from the diet with urinary exposure data, we were able to exclude contribution of the dietary route to the total exposure for some while establishing the diet as a route for other pesticides. Acetamiprid was found above 100 % of the Acute Reference Dose (ARfD) in the EFSA reference report in samples of pears, grapes and lettuce among others. In this study, acetamiprid was present in more than half the diet samples, indicating that this compound is present often in the diet but might be (partially) removed during processing activities. Thus, this pilot study shows that combining the DPA approach with (urinary) biomonitoring provides a detailed and informative assessment of dietary pesticide exposure.

4.1. Occurrence and concentrations

In a previous study in the Netherlands 5–21 pesticide residues per duplicate portion sample were reported (Nijssen et al., 2023). Pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin, chlorpropham and boscalid were among the most detected pesticides in the this study (Nijssen et al., 2023). In the current study we observed a higher number of pesticides per diet (n =7–43). Metalaxyl was the most frequently detected pesticide with occurrence in all the duplicate portions. Metalaxyl is a fungicide applied to three main crop groups, namely as a foliar application on tomatoes and lettuce and as a seed treatment to cereals. In addition, it is used on primary crops, grapes, tomatoes, spinach and sunflowers. Metalaxyl is stable to pasteurization, baking, boiling, brewing and sterilization, and <3 % of the ADI can be attributed to uptake from the groundwater (European Food Safety Authority (EFSA), 2013). This resistance to thermal degradation could explain its omnipresence in the diet of this study population. Another fungicide that was frequently detected was tebuconazole, mostly used as a foliar spray agent on grapes and cereals and as a seed treatment for barley. Tebuconazole is a dual use compound also authorized for wood preservation. It controls a variety of fungal diseases and is resistant to hydrolysis after sterilization, pasteurization and baking, brewing or boiling, similar to metalaxyl (European Food Safety Authority (EFSA), 2007). A third prevalent residue in this study was pirimiphos-methyl, which is often detected in flour or flour-based products since its applied as an insecticide in stored grains (European Food Safety Authority (EFSA), 2005). Processing of flour by baking does not have a significant impact on the composition of residues in matrices of plant origin. Pirimiphos-methyl is hydrolytically unstable, resulting in 50 % of the parent compound to be transformed into two major degradation products: O-2-diethyl-amino-6-methylpyrimidin-4-yl-O-methyl-phosphorothioate and 2-diethylamino-6-methylpyrimidin-4-ol (European Food Safety Authority (EFSA), 2005). Across all countries, 8–18 % of the diets consisted of grains or grain-based products, which could explain the high detection rate of this pesticide.

Approximately 91 % of all diet samples contained piperonyl butoxide, a co-formulant in pyrethroid-based insecticides used as a plant-protection product. Pyrethroids are also dual-use compounds used in biocide applications to control flies in stables, mosquito repellents for home applications and flea and tick repellent in dogs and cats. The high occurrence of piperonyl butoxide is explained by its common use as co-formulant in pyrethroid-based plant-protection products. Pyrethroid insecticides have common metabolites in humans, such as 3-PBA and DCCA cis/trans, which were frequently detected in the urine samples of the study participants. In a 2021 EFSA residue report, a detection frequency of 2.01 % was presented for piperonyl butoxide, while in our study this was 100 %. Piperonyl butoxide was not included in the EFSA residue report but its occurrence can also be explained by its use in pyrethroid-based products that contain lambda-cyhalothrin, cypermethrin and deltamethrin as active ingredients, detected in 1–3 % of the diet samples. The difference between reported frequencies by EFSA and

this study may also be attributed to a higher LOQ used in the analysis.

4.2. Effect of diet

No significant differences regarding exposure were observed between organic and conventional diets. However, the ability for study participants to follow an organic diet was highly variable between countries: the Netherlands generally showed a better discrimination between diets reported as 'conventional' or 'organic'. These groups also show a more prominent effect of an organic diet on lower urinary pesticide concentrations and metabolite detection frequencies. Inferences based on stratification by participant subgroup or self-reported dietary label need to be made with caution, since the sample size was small (N =43). No MRLs exist specifically for organic products, but restrictions on the use of pesticides in organic products are in place as stated in Article 5 of Regulation (EC) No 889/2008. Often a tolerance of 0.01–0.02 mg/kg is used for certifying products as 'organic'. A high consumption of organic products would be expected to be reflected in a lower frequency of detects of pesticides and their metabolites in urine. While some consumers may have followed an organic diet, exposure to pesticides from sources other than the diet may have contributed to urinary concentrations in this study. For example, occupational exposure by inhalation or hand-to-mouth (Oerlemans et al., 2021; Khemiri et al., 2018). Stratification by diet was based on farm type or self-reported consumption of organic products, except for participants from the Netherlands who were retrospectively categorized based on the percentage of consumed organic products from their diaries. Since these participants were also the ones where the largest difference between diet types was observed, future studies should consider the stratification and definition of an organic diet. We suggest making a distinction between participants based on a pre-defined cut-off, e.g. a contribution of 70 % organic products bought with a focus on products that contribute most based on expected pesticide residues such as vegetables, fruits, and grains.

Some pesticides were detected in the diet but not in urine, such as piperonyl butoxide and the non-specific pyrethroid metabolite 3-PBA, whereas others were found in both diet and urine, such as tebuconazole(-OH). An explanation for this can be that urine as a matrix is not sufficient as biomonitoring matrix for some pesticides when other routes of excretion are more important, such as fecal elimination after oral ingestion. Additionally, after absorption by the gastro-intestinal tract pesticides are metabolized. For analysis of this pesticide in urine, the most abundant human urinary metabolite needs to be known. Information about the most relevant human urinary metabolite is not always available, or if known, the analytical reference standard is sometimes not available. Without this, the metabolite cannot be determined as no identification and quantification is possible. For this reason, the scope of urinary metabolites included in this study was much smaller compared to the scope of the parent compounds in food.

Often, pesticides have rapid elimination kinetics and are excreted in urine within 24 h after ingestion but this does not always apply to the metabolites that are formed, as is the case for acetamiprid-N-desmethyl that was still detected at an elevated concentration in urine 48h after oral dosing in a human volunteer study (Wrobel et al., 2023). This suggests an added value of extending the period of urine collection beyond 24 h.

For pesticides detected in urine, but not in the duplicate portions, other sources than the diet are probable. Peels and other inedible parts of the fruits and vegetables were not included in the analysis but could have been a route of exposure for our participants. Pesticides may remain on the peels and cause exposure via dermal absorption or by hand-to-mouth-contact.

4.3. Residue compliance

None of the participants had HIs exceeding the threshold of 1, suggesting safe dietary exposure. However, this study does not allow for conclusions regarding individual risk. The estimates are not sufficiently precise to characterize risk at the individual level, as the current study aimed as a proof-of-concept for population-based approached with larger sample sizes in the future. The approach used in this study followed that proposed by EFSA, using an component-based approach assuming dose addition (EFSA (European Food Safety Authority) et al., 2020). Currently, two cumulative assessment groups (CAGs) have been established by EFSA; one for pesticides with acetylcholine esterase altering properties (CAG-NAN) and one for pesticides causing disruptions in neuromuscular control of movement (NAM) (Crivellente et al., 2019). Risk assessments performed within these CAGs provide better estimates of toxicity for these specific endpoints compared to the lower-tier approach presented in this study. However, even when summing the HQ of all individual compounds, the threshold of 1 was not exceeded. As such, there was no need to perform another assessment on the CAG level, which would exclude many compounds and result in an even lower HI.

In the 2020 EFSA residue report, copper compounds, chlorates and chlorpyrifos had a high frequency of detection (European Food Safety Authority (EFSA), 2022b). This can be partly explained by environmental formation of copper or non-agricultural use that sometimes produces chlorates. Compared to non-organic food, MRL exceedance and median concentrations are generally lower in organic food. Rempelos et al. found that switching from a conventional to an organic (mediterranean) diet resulted in a reduced exposure to all types and classes of pesticides (Rempelos et al., 2022).

Current evaluation of pesticide residues in food by EFSA includes the monitoring of food commodities, including baby food and other processed foods for infants and young children and processed products such as wine (grapes) or dried herbs (European Food Safety Authority (EFSA), 2022; European Food Safety Authority (EFSA), 2023). Currently, compliance of processed products with the MRL of a given pesticide is assessed by applying processing factors to the raw agricultural commodity in each product, separately. For example, the raw material of dried chilli spices is a chili pepper, and the processing factor for drying and grinding the pepper is added to verify compliance with the MRL of a given pesticide. The DPA approach presented in the current study offers the advantage of using the food portion as consumed, providing a measurement closer to real life dietary exposure based on the composition and portion of consumed food and beverage. Analytic methods used in the current study had lower LODs and LOQs than generally used, e.g. for data reported by EFSA (European Food Safety Authority (EFSA), 2022; European Food Safety Authority (EFSA) et al., 2023).

4.4. Limitations and recommendations for future research

There are some differences between our approach and that of earlier studies. Not all pesticides reported in previous studies were included in our multimethod analysis, such as TCPy (chlorpyrifos/-methyl metabolite) and pirimiphos-methyl-desmethyl in the duplicate diet and pyrimethanil-OH M605F003 in the urine. Since the compounds included in the dietary and urinary dataset do not fully overlap, it is unknown whether some pesticides are metabolized and excreted in the urine.

Pesticide use may differ per country, but the diet is made up of imported and locally grown products which doesn't always reflect a pattern based on country of residence. From the current study set-up, no conclusions on the effects of country of residence could be drawn, due to the small sample size per country. In addition, there are limitations with the use of self-reported data. For example, the self-classification into organic or conventional consumption might be difficult to estimate based on the 70 % limit that was established in this study.

Another notable difference is that none of the commonly found pyrethroids reported by Nijssen et al. were detected in the diets of the participants in the current study (Nijssen et al., 2023). This could be related to an additional freeze-drying step and addition of acetonitrile in

their approach, which may have resulted in a more efficient extraction and recovery of (specifically) pyrethroid pesticides after the QuECheRs. In addition, acetamiprid and acetamiprid-n-desmethyl could not successfully be measured in urine using the multi-method, even though other studies have shown extensive uptake and metabolism of acetamiprid (Wrobel et al., 2022). As acetamiprid was detected in 63 % of all diet portions, this is expected to be reflected in urine, but due to exclusion from the analysis this relationship could not be established. Future research should pay special attention to extraction methods of multiple compounds with varying physicochemical properties from complex matrices.

It is possible that there is a prolonged excretion of urinary metabolites, causing detection in urine samples of day 1 due to exposure the day before. This is known to be the case for acetamiprid-N-desmethyl (Wrobel et al., 2022, 2023). In this study, this was accounted for by discarding the first urine of day 1 (Batch-A), but for some pesticides it is possible that metabolites are still detected 24 h after exposure. Adding another day to the dietary sampling could help account for this in follow up studies.

Since this study included left-censored data, due to limits of detection and quantification, concentrations below the limit of quantification were imputed. Imputation based on distribution instead of common LOQ/2 imputation improves reliability of calculated summary statistics, but can over or underestimate values < LOD if the amount of detects is low in sparse datasets such as the one in the current study. Moreover, self-reporting or self-collecting data relies heavily on compliance of participants and close monitoring by the principal investigator.

In future research, adding faecal samples to investigate the amount of pesticide excreted unchanged or via biliary excretion can further support the interpretation of the urinary metabolites that are sometimes not specific to the parent. In the current set-up, when a pesticide or its metabolite(s) is not detected in urine it is assumed to be excreted via the faeces. The addition of the analysis of faecal samples may provide the supporting data for a correct interpretation in such cases in future studies. Additionally, other methods of assessing non-dietary exposure via silicone wristbands or measuring house dust for pesticide residues could be incorporated (Aerts et al., 2018; Navarro et al., 2023).

5. Conclusions

In conclusion, this pilot study provides a feasible set-up to perform duplicate-portion analysis combined with urinary metabolite analysis to assess dietary intake of pesticides in the general population. This has resulted in real life dietary pesticide uptake data from multiple countries. The approach can be used to study the contribution of the diet to pesticides exposure and indicate which proportion of the pesticide exposure may be attributed to other routes than ingestion and other sources than the diet.

CRediT authorship contribution statement

Nina C. Wieland: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Daniel M. Figueiredo:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Hans Mol:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Neus González:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Conceptualization. **Nelson Abrantes:** Writing – review & editing, Project administration, Investigation. **Virginia Aparicio:** Writing – review & editing, Project administration, Investigation. **Isabel Campos:** Investigation. **Josefina Contreras:** Writing – review & editing, Project administration, Investigation. **Alcon Francisco:** Writing – review & editing, Project administration, Investigation. **Matjaž Glavan:** Writing – review & editing, Project administration, Investigation. **Tanja Blagus:** Investigation. **Vita Dolžan:** Writing – review & editing, Project administration, Investigation. **Paula Harkes:** Writing – review & editing, Project administration, Investigation. **Trine Nørgaard:** Writing – review & editing, Project administration, Investigation. **Vivi Schlünssen:** Writing – review & editing, Project administration, Investigation. **Igor Pasković:** Writing – review & editing, Project administration, Investigation. **Marija Polić Pasković:** Writing – review & editing, Project administration, Investigation. **Martien Graumans:** Writing – review & editing. **Ad M.J. Ragas:** Writing – review & editing, Supervision. **Frans G.M. Russel:** Writing – review & editing, Supervision. **Paul T.J. Scheepers:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization.

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.yrtph.2025.105972.

Data availability

The data of this study are available on **10.5281/zenodo.16920027**.

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