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2 Glossary

2,4 D	2,4-Dichlorophenoxyacetic acid
DCCA	cis-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DBCA	3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylic acid
ClF3CA	cis-3-(2-chloro-3,3,3trifluoroprop-1-enyl)-2,2dimethylcyclopropane-carboxylic acid
EBIC	Extended Bayesian Information Criterion
ESI+/ESI-	positive and negative electron spray ionisation
HCD	Higher-energy C-trap dissociation
LC-HRMS	Liquid chromatography coupled to High Resolution Mass Spectrometry
MS2	MS/MS or tandem mass spectrometry
NCE	Normalised Collision Energy
RT	Retention Time
SPECIMEn	Survey on PEstiCIde Mixtures in Europe
SS	Suspect Screening
TCPy	3,5,6-trichloro-2-pyridinol
QA/QC	quality assurance/quality control

3 Abstract/Summary

This deliverable describes the results of the joint pesticide survey 'SPECIMEn' in five partner countries: Czech Republic, Hungary, Latvia, Spain and the Netherlands, as part of task 15.2 and task 8.3. It also contains a report on the implementation of a study conducted in Switzerland, which has a different design, but data was harmonised as much as possible. Under the supervision of task lead IRAS, the local partners implemented the fieldwork as described previously in the Fieldwork Protocol (AD15.7). In all participating partner countries, samples have been collected in two seasons, i.e., winter 2019/2020 and summer 2020. Per country at least 100 locations have been included, of which about half are located in so-called hotspot areas. At each location urine samples from one adult and one child were collected, one per season. In total, at least 400 urine samples have been collected per country, summing up to 2,088 urine samples for the total SPECIMEn survey.

The urine samples have been transferred to the partner laboratories from WP16, i.e., WFSR, FISABIO, MU, UFZ and INRAE. On the collected urine samples, the WP16 laboratories applied a harmonised suspect screening method to detect multiple pesticide-related markers (parent compounds and/or metabolites) a semi-quantitative way.

At the time of this report 41 pesticide-related compounds (parent compounds and metabolites) were identified at high levels of confidence in samples across all countries.

At the time of writing of this report, and partly due to COVID related delays in the data delivery, data analysis and interpretation of the collected data is still ongoing. Here we provide a preliminary descriptive overview of the data that has been collected, focusing primarily on detection rates of the various compounds across strata within the study.

Further statistical analysis is envisaged and will be included in a peer-reviewed publication. For many of the included pesticide-related compounds we observed differences in detection rates between countries and when comparing hotspot areas versus control areas, samples collected in summer versus winter, and between children and adults.

Further interpretation of the observed patterns requires in depth evaluation of questionnaire data collected within SPECIMEn as well as scrutiny of the use characteristics of the included pesticide related compounds, such as typical applications and exposure routes.

4 Introduction

The HBM4EU-SPECIMEn (Survey on PEstiCIde Mixtures in Europe) study is designed to assess concomitant/combined exposure to multiple pesticides in hotspot and control areas using human biomonitoring. The SPECIMEn study is part of the activities of WP15 (task 15.2), WP8 (task 8.3) and WP16 (task 16.2).

The initial approach to the joint Pan-European pesticide study was to first identify 5-10 commonly applied pesticides in orchards for targeted analysis in different countries across Europe. An initial survey amongst partner countries to identify these common pesticides demonstrated that none of the countries was able to produce quantitative information about which active substances were actually applied on specific crops, i.e., in orchards.

This finding then triggered the broader approach of suspect screening (in collaboration with WP16), which is capable of screening for a large number of pesticide-related metabolites in a semi-quantitative way. The application of suspect screening gives insight into the patterns of multiple pesticides, as a prime example of mixture exposure.

The main aim of the SPECIMEn study is to generate new exposure data across Europe on a broad combination of pesticides and to assess possible local contributions (i.e. hotspot areas) and within-person variation. The approach used is a so-called ‘hotspot’ design, focusing on residential areas close to fields where pesticides are applied.

The main research questions for this survey are:

1. Which combinations of pesticide-related compounds are most commonly detected?
2. Do patterns in pesticide-related compounds detected with suspect screening differ between age groups and study populations in different countries?
3. Do patterns in pesticide-related compounds detected with suspect screening differ between people living close to pesticide application sites and the general population?
4. Do patterns in pesticide-related compounds detected with suspect screening differ between seasons (spraying and non-spraying season)?

It is hypothesised to detect higher exposure levels within the population living close to the agricultural fields (hotspots), as well as within the spraying season (summer). Difference in detection frequencies between adults and children can be due to various reasons: e.g., differences in food consumption, product use, differences in activities, such as more hand-mouth contact (children) or occupational exposure (adults). Potential differences in the detected patterns will likely be influenced by among others, food consumption and usage of pesticide containing products, these covariates will be covered by the application of a questionnaire.

In order to collect data geographically spread across Europe, countries from the regions as defined by HBMEU (North, South, East, West) were included: Hungary, Czech Republic, Spain, Latvia, and the Netherlands. Switzerland participated as well, with a slightly different study design. Data for this study started in winter 2019/2020 and finished in summer 2020.

This report describes the results of the SPECIMEn survey in the five partner countries and Switzerland. It involves a description of the study population in each participating country and a description of the total number of pesticide-related markers detected by the suspect screening methodology.

A comparison of the patterns between markers across countries is made based on detects *versus* non-detects patterns, and within each country differences across age groups, locations, and seasons are described. This report gives some first insights into the data under the current time restrictions. More elaborated analyses are foreseen in future work.

5 Methods

The SPECIMEn survey was conducted in five partners countries and Switzerland (Figure 5.1). Involved partner institutes in WP15 are MU, RSU, NPHC, RIVM, IRAS, ISCIII, and FISABIO. Switzerland conducted a similar survey with a slightly different study design, this survey was conducted by Swiss TPH. They are included in this report since they adhere to the same sample collection procedure, coding, and processing steps.

Together with five laboratories involved in WP16 (WFSR, FISABIO, MU, UFZ, INRAE), a suspect screening method was developed, harmonised, and applied on these samples, capable of detecting multiple pesticide related markers (parent compounds and metabolites) in a single assay in a semi-quantitative way (determination of detection frequencies, patterns of exposure, and variability). Each participating laboratory analysed samples originated from one country (Figure 5.1) while WFSR also included samples from Switzerland.

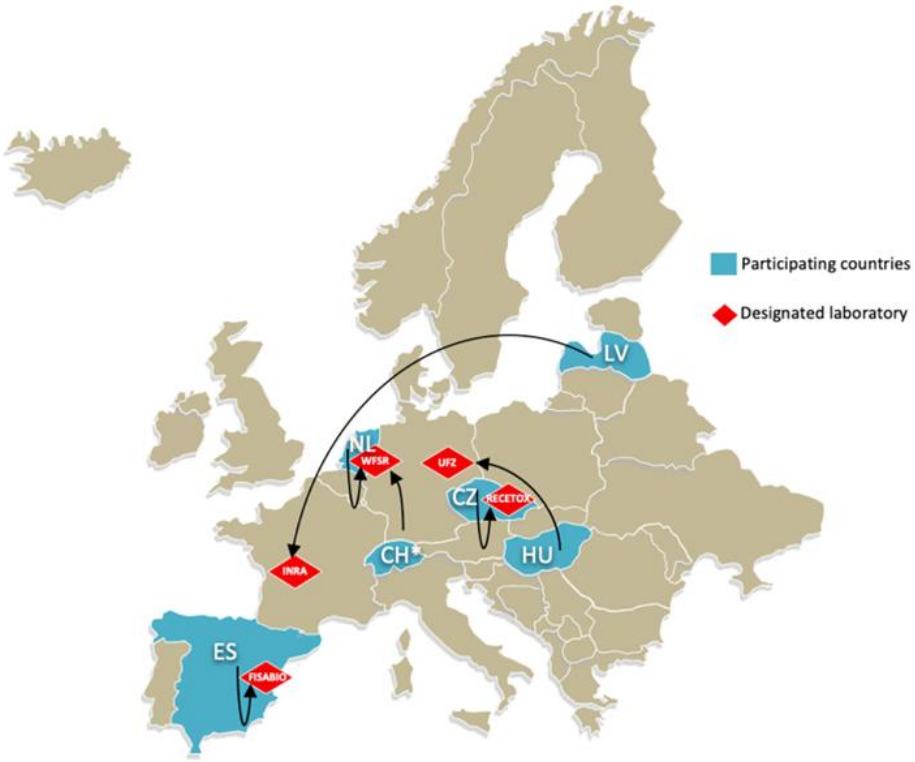

Figure 5.1: The SPECIMEn study set-up in terms of participating country (WP15) and designated analytical laboratories in charge of the large-scale pesticide suspect screening task (WP16). * Switzerland employed a slightly different study design.

5.1 Sampling protocol

5.1.1 Sampling strategy SPECIMEn

A detailed description of the general study protocol used for the SPECIMEn can be found in AD15.7, entitled “Joint survey on pesticides: details of approach and contributions”. A graphical summary of the protocol is shown in Figure 5.2. Briefly, within each country samples were collected in two different seasons (non-spraying and spraying season) and two locations (hotspot and control areas). Non-spraying season is defined as winter, spraying season as summer. Hotspot areas are defined here as residential areas within 250 meters of agricultural areas where pesticides are actively applied, focusing on orchards, olive groves, citrus groves and vineyards. Suburban areas at least 500 meters away from any agricultural fields, are defined as control areas. The control samples were a subgroup of the aligned studies from task 8.1 when it was possible. At both locations, 50 adult-child pairs (50 households) were asked to provide a morning void urine sample and to complete a questionnaire. An add-on to the general study protocol was the collection of house dust. Children of 6-11 years old (at inclusion) accompanied by one of their parent/caretakers living in the same household were included, equal to the age group defined in the Aligned studies. Adults who worked in the agricultural sector (i.e. farmers) were excluded. For each participating country a total of 200 urine samples in the hotspot area and 200 urine samples in the control area were collected.

The sampling strategy was harmonised between all countries in relation to the questionnaire, the timing of sampling, the inclusion and exclusion criteria, and the sampling materials (cups, tubes etc). Local differences were among others the recruitment strategy and the collection of house dust. The Hungarian partner involved the local public health officers to get in touch with participants, while others sent out letters (the Czech Republic and the Netherlands), contacted colleagues (Spain and the Netherlands), conducted an online campaign (the Czech Republic and the Netherlands), and/or contacted schools (Spain and Latvia).

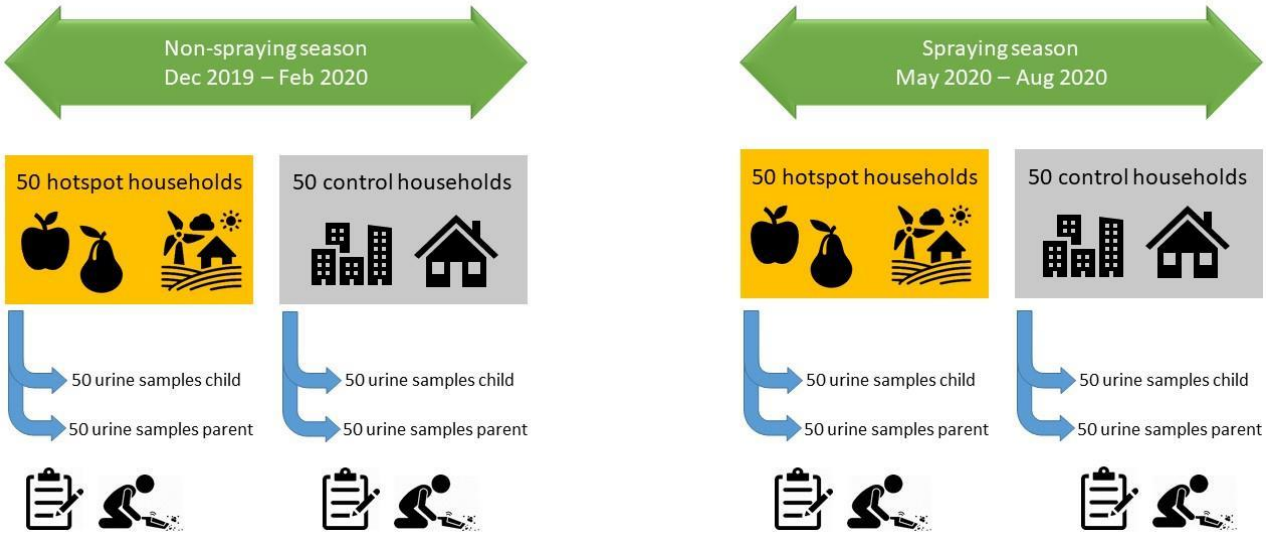

Figure 5.2. Synopsis of the SPECIMEn study set-up in within each country: two different seasons, two locations, adult-child pairs

A dedicated questionnaire was developed by the coordination team (IRAS/RIVM), based on the questionnaire developed by WP7 specified for pesticides. First, an English version was developed (Annex 1), which was then translated to the local languages for each participating country. During the first visit, the adult was asked to complete the whole questionnaire, which included among others questions related to personal characteristics, potential pesticide exposure scenarios (occupational, application of products containing pesticides) and the food consumption pattern of the day prior to sampling. During the second visit, only a subset of questions was administered (questions marked in blue in Annex 1).

The timing of sampling within each country was related to the spraying and non-spraying seasons (winter and summer). The actual dates, which differed slightly between the countries, are listed in Table 5.1. Sample collection started in November 2019, during the winter period. The second sample collection was in the summer of 2020, when the COVID-pandemic had reached Europe. Due to local lockdowns and uncertainties, the protocol was slightly altered or postponed. Some sampling periods were slightly later (end of summer) than initially planned. Local protocols were adjusted to ensure minimal physical contact with the study participants. Due to these alterations, it was not possible to collect house dust samples during the second season. Therefore, collected house dust samples of the first season (the Czech Republic and the Netherlands) were not analysed and not reported. The urine sample collection for all countries was completed in October 2020.

Table 5.1: Actual sampling dates for the SPECIMEn countries

Country	Season 1		Season 2	
Area	**Start**	**End**	**Start**	**End**
Spain				
Hotspot	07/11/2019	20/12/2019	01/09/2020	02/10/2020
Control	05/11/2019	19/12/2019	01/09/2020	05/10/2020
Latvia				
Hotspot	18/02/2020	31/03/2020	02/06/2020	18/06/2020
Control	18/02/2020	31/03/2020	02/06/2020	18/06/2020
Hungary				
Hotspot	29/01/2020	10/02/2020	07/09/2020	16/09/2020
Control	11/02/2020	18/02/2020	16/09/2020	17/09/2020
Czech Republic				
Hotspot	14/1/2020	13/3/2020	26/5/2020	30/7/2020
Control	14/1/2020	13/3/2020	26/5/2020	30/7/2020
The Netherlands				
Hotspot	22/01/2020	06/03/2020	02/06/2020	24/06/2020
Control	22/01/2020	06/03/2020	02/06/2020	24/06/2020

5.1.2 Sampling strategy Switzerland

A total of 6,000 subjects between 20 and 39 years old and living in the canton of Basel-Stadt (urban control area) were invited to participate in the Swiss study. The sampling procedure was sex-stratified (50% women and 50% men) and recruitment of the study population was performed via postal mail. The final Swiss sample-set consisted of 383 residents aged 20 to 39 years. Data of 300 participants were sent to HBM4EU and included in the analysis. An electronic pre (sample) questionnaire and a post (sample) questionnaire were distributed via the data collection tool Redcap ® and self-administered by the participants.

The collection of the biological sample was done at the participants' home. Due to the onset of the COVID-2 pandemic, the research protocol was modified and the health measurements at the Swiss TPH study center were dropped. A morning urine sample was collected at the participant's home, transported to and processed in the study center respecting the cold chain throughout until bio banking of urine aliquots. Sample collection was performed between January 8th 2020 and October 10th 2020.

5.2 Suspect screening approach

On all collected urine samples, a suspect screening approach was applied. Several consolidated quality assurance/quality control (QA/QC) dispositions, parameters and criteria were first implemented to ensure the consistency of the results obtained across the different participating laboratories as well as to document the applied method performances. The first data generation and analysis steps resulted in a large list of mass spectrometric features to be further structurally identified. These features are typically characterised by accurate mass (m/z) and retention time (RT). All unique features were componentised, leading to a calculation of all related potential elemental composition. Further evaluation of isotopic patterns was performed to increase the confidence level associated to the annotation.

This annotation process was based on a curated suspect list of compounds of interest, which included information on parent compounds and known or predicted human metabolites. The list may include multiple metabolites originating from the same parent compound, resulting in a final datafile with potentially several metabolites that reflect exposure of the same parent compound. In the case of SPECIMEn, this list was focussed on pesticides and was aggregated between the laboratories.

Due to the high number of potential annotations that could not be assessed in the given timeframe, elemental compositions containing F, Cl, Br or PO3 were prioritised for further confirmatory investigations. This confirmation procedure included re-measurements of a subset of samples to generate tandem mass spectrometric information. For metabolites where a reference standard was missing, human liver S9 experiments were performed on the pesticides to in-vitro synthesise the metabolite and gain the reference information to compare with. Fully identified features have the highest level of confidence: Schymanski level 1 if a reference standard material is commercially available or Schymanski 2b by diagnostic evidence. Features which are identified at a lower tier will end up in lower confidence levels, reflecting the level of uncertainty about the identity of that feature.

5.2.1 Harmonised sample preparation and instrumental analysis

Each of the five WP16 participating laboratories performed the sample preparation and instrumental analysis for samples originating from one country under harmonised conditions. Briefly, sample preparation consisted of buffering followed 96-well plate solid phase extraction by 5-fold pre-concentration. Internal standard mixtures were added to the samples prior to the extraction and during reconstitution. Instrumental analysis was performed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), all involved laboratories using an Orbitrap instrument. Two data acquisitions were operated respectively using positive and negative electron spray ionisation (ESI+/ESI-) with adapted chromatographic conditions for each ionisation mode. For positive mode analysis, 0.1% formic acid and 2 mM of ammonium formate were added, while for negative mode analysis 10 mM of ammonium carbonate was added to the eluents. A reversed phase column was used (1.7 µm, 2.1 mm x 100 mm, BEH C18, Waters) at 50°C and a flow rate of 0.3 mL/min. We applied a water/methanol gradient with 100/0 at 0 min, 0/100 at 15 min, 0/100 at 21 min, 100/0 at 22 min, and 100/0 at 30 min. Full scan MS analysis was performed at resolutions around or above R=100,000. Further details on the sample preparation, instrumental analysis and harmonisation procedure have been described in deliverable HBM4EU-SOP-WP16-001 "SPECIMEn study - suspect screening analytical workflow – Part 1 sample preparation and data acquisition".

Importantly, a number of quality control samples, quality control external standard mixes and internal standard mixes were distributed among and used by all contributing partners to ensure data consistency and quality across the different laboratories. Sequence orders for sample injections were also in a pre-defined format. Further details on the QA/QC provisions established are described in deliverable HBM4EU-SOP-WP16-002 " SPECIMEn study - suspect screening analytical workflow - Part 2 - Harmonised QA/QC provisions and criteria".

5.2.2 Annotation and confirmation procedures

The dataset received over all laboratories consisted of 12 sequences for each laboratory and resulted in over 5,200 full-scan data files. The first data processing comprised the following steps: a batch-wise mass correction (lock-mass correction), file size reduction, inter-laboratory correction of retention time and mass error using the measured signals of the quality control samples. For these pre-calibrated files, the retention time was then corrected in a batch-wise manner for sequence drifts by using the measured signals of the internal standards and known urine compounds. This data was then used for elemental composition analysis, annotating adducts and isotope patterns. The received list of elemental compositions was then annotated with a suspect list containing an aggregation of pesticide and pesticide metabolites.

Since the list of revealed possible annotations was too extensive for further procedures, further prioritisation strategies were needed to focus on annotations with a higher likelihood of validation in the confirmation procedures. Due to time limitations for data evaluation, we further limited the dataset to annotations with elemental compositions containing the elements of F, Cl, Br or PO3. To exclude interfering annotations of possible drug consumption, all elemental compositions were screened for high abundances of signals related to common drugs. These samples were flagged as "likely including Cl or Br- containing drug metabolites". Final annotations which were only occurring in these flagged samples were not further prioritised. As a final step, all annotations were aligned through the overall dataset and the consistency of retention time and presence of isotope

patterns was reviewed. This led to a final list of 498 tentative annotations (level 4-5 on the Schymanski scale), which was used for further confirmatory steps to increase the confidence level.

Confirmatory procedures aiming at consolidating the identity of some detected exposure markers were accomplished in the laboratories of UFZ and WFSR. Hereby, for each prioritised annotation a representative sample with high signal intensity was chosen. This resulted in the reinjection of 60 samples in ESI- and 64 samples in ESI+ originating from the sample-set of the countries Hungary and the Netherlands.

To generate reference information for phase I pesticide metabolites, human liver S9 incubation experiments were performed for 69 pesticides, measured with the same method as for the urine samples and retention time and MS2 spectra at two different collision energies (HCD 35 and 50 NCE) were extracted. Reference standards of pesticides and pesticide metabolites available at both laboratories were injected and the reference information was extracted. Further details on the data analysis strategy are described in deliverable HBM4EU-SOP-WP16-003 "SPECIMEn study - suspect screening analytical workflow - Part 3 – Data Processing".

The final annotations received a confidence level based on five levels, from the most confident scenarios (level 1, confirmed structure by a reference standard or level 2 by diagnostic evidence) to lower confidence (level 5, exact mass of interest) proposed by Schymanski et al. An overview of the different levels is presented in Figure 5.3 (from Schymanski et al., 2014). The final reported datafile consisted of a matrix of the final annotations *versus* sample codes, with a signal intensity score reported when the compound was detected.

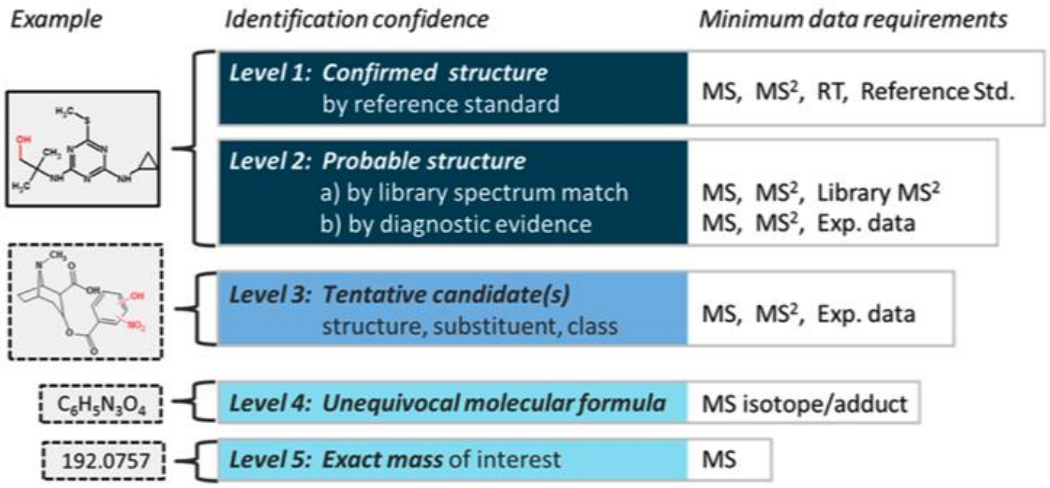

Figure 5.3: Different confidence levels established in the identification of a compound applying HRMS target, suspect and non-target screening workflows based on the levels provided by Schymanski et al. MS refers to accurate mass of the precursor ion, MS^2 to accurate mass of the fragment ions, RT is the retention time.

5.3 Statistical analysis

Descriptive statistics and network modelling approaches were applied to describe and summarise the data collected in the SPECIMEn study. The suspect screening data available in SPECIMEn is 'semi-quantitative', i.e. quantitative signal intensities for one representative mass spectrometric feature are reported per sample, yet these intensities do not directly correlate to urine

concentrations and are not standardised across laboratories. We analysed the data in two modalities: using the quantitative feature intensities (allowing calculation of correlations, etc. within studies) and by dichotomising the feature intensities into 'detect' versus 'non-detect'. The latter strategy allows comparisons across countries as well as inclusion of features with low detection rates in the statistical analysis.

The detection rate was calculated as the number of samples in which a particular feature was detected over the total number of samples collected. To assess differences in detection rates between strata (i.e. countries, season, location, age groups) in the SPECIMEn study (not applicable for Switzerland), we used a Chi-square test with a Yates' correction to accommodate strata in which the detection rate was zero. The percentage of change between categories is indicated by a shade of yellow, **<25%**, **25-50%**, **50-75%**, and **>75%**. This difference is calculated when hotspots had a higher detection frequency compared to controls and when season 2 had higher detection frequencies compared to season 1 (following the directions of our hypotheses).

Using the dichotomised data, a weighted correlation network was estimated using the *IsingFit* R package in R (Borkulo et al. 2016). This package estimates the network based on the Ising model: combining L1-regularised logistic regression with EBIC model selection (gamma 0.25). On this network a clustering algorithm was applied (*walktrap)*, to detect communities of closely related features indicated by different colours in the network. Binary networks were estimated for the total dataset, as well as subsets for season, location, and age group. Networks were not estimated on the Swiss dataset due to time restrictions.

Features with a detection rate >40% were brought forward to semi-quantitative analyses. Within each country, missing data was imputed using a Maximum Likelihood approach, where values were imputed below the lowest detected intensity score (Lubin et al. 2004). Covariates for age, gender, BMI, education, and income were used as predictors. Semi-quantitative feature levels were log-transformed to improve the distribution of this data. Weighted correlation networks were generated for each country (when applicable) using the EGAnet R package (Golino 2021), following the method described by Ottenbros et al 2020.

All statistical analyses were performed in R version 4.1.1.

6 Results

6.1 Population characteristics

A detailed description of the study population for the SPECIMEn countries and Switzerland is given in Table 6.1. The Swiss population included only adult samples from an urban control area, without a hotspot area.

Between the SPECIMEn countries the number of samples was equally spread across the countries and locations. The drop-out rates between the seasons were low, varying from 0.9% to 2.9%. Reasons for dropouts were loss of contact, divorce and/or move to another location. The adult samples mainly originated from the mothers, while gender was equally divided across the children's samples.

The mean age of the adults was comparable across the SPECIMEn countries, varying from 37.8 to 43.8 years. The included adult participants from Switzerland were slightly younger, with a mean age of 30.8 years.

The mean BMI of the adults indicated that participants from Latvia and Hungary were slightly overweight compared to the other countries. Most of the participants did not smoke, although in the hotspot areas of Spain and Hungary there was a substantial group of current smokers (resp. 34.6 and 45.1%). In all countries except Hungary, higher education levels (University) were overrepresented. This may be explained by the different recruitment strategies.

Based on the total household income categories, participants of hotspot areas mostly earned less money than those living in control areas. In the Swiss sample, the lower two income categories were most represented.

Table: 6.1: Population characteristics SPECIMEn

Country	Spain		Latvia		Hungary		Czech Republic		Netherlands		Switzerland
Area	Hotspot	Control	Hotspot	Control	Hotspot	Control	Hotspot	Control	Hotspot	Control	Control
Samples, n	206	212	200	202	201	208	204	238	219	198	300
Season 1	104	106	100	102	102	104	102	120	110	100	
Season 2	102	106	100	100	99	104	102	118	109	98	
Gender Female[1], % Adults	50.0	86.8	90.0	82.4	94.1	84.6	70.6	60.0	70.9	66.0	46.0
Children	53.8	49.1	58.0	47.1	49.0	51.9	43.1	43.3	52.7	46.0	NA
Mean age[1], years Adults	43.8	43.7	39.6	39.2	37.8	40.4	41.1	41.5	42.4	41.7	30.8
Children	8.2	8.7	8.9	8.4	9.7	9.2	8.8	9.1	8.6	8.6	NA
Mean BMI[1] Adults	24.5	24.0	26.4	25.7	26.1	26.2	24.2	24.1	23.9	23.3	23.6
Children	16.5	17.0	17.3	16.7	17.8	18.8	16.1	15.9	15.7	16.0	NA
Smoking status adult[1], % No-current smoker	65.4	73.6	88.0	82.4	54.9	78.8	84.3	91.7	94.5	100.0	77.3
Education level adult[1], % No or only primary education	0	0	2.0	0	4.1	5.8	0	1.7	1.8	0	0.7
Secondary education	7.7	17.0	30.0	11.8	25.5	19.2	2.0	3.3	5.5	2.0	0.3
Tertiary education (post-secondary)	25.0	17.0	8.0	7.8	25.5	28.8	25.5	10.0	18.2	18.4	23.0
University (BSc, MSc, PhD)	67.3	66.0	60.0	76.5	7.8	46.2	70.6	83.3	70.9	75.5	74.7
Don't Know/ NA	0	0	0	3.9	0	0	2.0	1.7	3.6	4.1	1.3

[1] Calculated based on base-line (Season 1)

Country	Spain		Latvia		Hungary		Czech Republic		Netherlands		Switzerland
Area	Hotspot	Con-trol	Hotspot	Con-trol	Hotspot	Con-trol	Hotspot	Con-trol	Hotspot	Con-trol	Control
Household income[1] [2], % of country average											
< 25%	7.7	0	14.0	9.8	27.4	17.3	15.7	16.7	1.8	0	20.3
25-50%	5.8	0	0	0	39.2	19.2	39.2	26.7	5.5	6.0	37.7
50-75%	17.3	3.8	0	0	13.7	7.7	35.3	33.3	49.1	44.0	15.7
>75%	57.7	75.5	74.0	70.6	5.9	44.4	9.8	21.7	20.0	44.0	18.0
Don't Know/NA	11.5	20.8	12.0	19.6	13.7	9.3	0	1.7	23.6	6.0	8.3

[2] Income categories from the Swiss questionnaire (1: CHF <3'000; 2: CHF 3'000 – 4'5000; 3: 4'500 – 6'000; 4: 6'000 – 9'000; 5: CHF 9'000-11'000; 6: CHF > 11'000; 7: I prefer not to answer) were assigned to the <25th, 25th - 50th, 50th – 75th and >75th percentile categories based on the publication by the Federal Department of Finance (2014): https://biblio.parlament.ch/e-docs/377581.pdf

6.2 Questionnaire results

The administered questionnaire in the SPECIMEn study can be found in Annex 1. The complete set of questions was administered during the first season, while a subset of questions was administered during the second season (marked in blue in Annex 1). Information collected via the questionnaire, could be used to explain potential high exposures and/or outliers in the data. The responses to questions related to different pesticide exposure scenarios or pesticide application activities are summarised in Table 6.2.

Table 6.2 indicates that of the participating individuals, the majority of the participants did not work professionally with pesticides (one of the selection criteria). In relation to the other household member with professional contact with pesticides, there were two locations (i.e., hotspot locations of Latvia and Hungary) where 16 and 9 household members, respectively, worked with pesticides. In Hungary, 17 of the households from the hotspot area did use some products for the treatment of the plants indoors prior to sampling. This number was substantially higher compared to the other countries and locations. Within the control area of Hungary, 11 households used some antiparasitic products for their pets prior to the sampling period. From the Spanish hotspot area, about half (25 households) of the participants used insect repellent or antiparasitic products for human use prior to the sampling period.

The SPECIMEn questionnaire also yielded information on the dietary habits of the participants. A food frequency questionnaire was administered based on the diet the day prior to sampling. Information was also collected on organic food consumption, and homegrown food consumption. The percentage of vegetables, fruits and/or herbs consumed originating from the participants' own garden is summarised in Table 6.3. Overall, the households in hotspot areas consumed more homegrown foods, and mostly during summer and autumn.

In addition to the results presented in Tables 6.2 and 6.3, the questionnaire data contains a wealth of information on additional topics such as medication use, physical activity, and time spent at different locations. Analysis and interpretation of these results is outside the scope of the current deliverable.

Table 6.2: Potential pesticide exposure scenarios based on SPECIMEn questionnaire data

Country	Spain		Latvia		Hungary		Czech Republic		Netherlands	
Area	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**
Professional contact with pesticides in the past month, n adults										
Season 1	0	1	2	0	0	0	1	0	0	0
Season 2	2	0	4	1	2	1	3	1	0	0
Having other adult household member(s) who had professional contact with pesticides, n adults	1	0	16	2	9	1	0	0	1	0
Usage of any type of products for treating the plants in the garden in 3 days prior to sample collection, n adults										
Season 1	0	2	1	1	0	2	1	0	1	0
Season 2	4	2	4	2	1	2	6	4	4	2
Usage of any type of products for treating the plants inside the house in the 3 days prior to sample collection, n adults										
Season 1	2	0	2	4	0	2	0	1	1	3
Season 2	2	0	3	3	17	4	2	0	2	2
Usage of external antiparasitic treatments for pets in the 3 days prior to sample collection, n adults										
Season 1	2	2	0	1	2	11	1	1	1	0
Season 2	1	2	4	1	4	5	0	1	6	1

Country	Spain		Latvia		Hungary		Czech Republic		Netherlands	
Area	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**
Usage of insect repellent or antiparasitic human products in the 3 days prior to sample collection, n adults										
Season 1	6	1	0	3	2	2	2	1	0	2
Season 2	25	6	5	4	4	2	6	8	5	0

Table 6.3: Homegrown food consumption by season, based on SPECIMEn questionnaire data

Country	Spain		Latvia		Hungary		Czech Republic		Netherlands	
Area	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**	**Hotspot**	**Control**
Usual homegrown vegetables, fruit and/or herbs consumption, % of total consumption										
Winter	6.7	1.1	30.4	22.1	23.4	4.5	13.4	10.2	2.0	0.1
Spring	10.0	3.4	28.1	19.0	21.2	9.4	22.0	11.5	4.8	2.2
Summer	12.4	8.0	62.5	43.6	40.9	24.5	63.9	51.4	14.6	7.8
Autumn	8.9	6.0	63.4	45.1	39.1	16.9	45.3	40.2	8.3	4.5

6.3 Annotated pesticides and metabolites

Using the suspect screening approach, we identified 41 features with confirmed structure (Schymanski level 1) or probable structure (Schymanski level 2b). These features relate to 30 parent compounds. In addition to these, 54 other features were detected for which the confidence level was lower (Schymanski levels 3-5). In Table 6.4, we present all pesticide and metabolites achieving a confidence level 1 or 2b. Results of all confidence levels (1-5) with RT confirmation can be found in Annex 2.

On all 2,088 urine samples, the suspect screening approach as described in section 5.2 was applied. The obtained tentative annotations which were prioritised for confirmatory purposes revealed a total number of 498 features (163 in ESI+ and 336 in ESI-). Limitations on reference standard availability (also for the human liver S9 incubation experiment) and the available two datasets of two laboratories led to 377 annotations (103 in ESI+ and 274 in ESI-).

The confirmation procedure was accomplished by acquiring representative data-dependent tandem mass spectra with two collision energies (MS2) and verified by spectral comparison to reference spectra and retention times generated from reference standards commercially available or produced through human liver S9 in vitro incubation experiments. Matching with the reference standard resulted in maximal identification confidence level (Schymanski level 1), see also figure 5.3. Matching with data acquired by the human liver S9 incubation experiment resulted in Schymanski confidence level 2b. Further evidence could be collected by comparing the retention time information (level 4/5 + RT) or by evaluating the acquired spectra of the tentative annotation with in silico fragmentation prediction software (level 3).

6.3.1 Annotations and detection ratio per country

Table 6.4: Pesticide (metabolite) annotations (N= 41), and their overall detection frequency (%) per country; in grey when < 10%

ID	Pesti-cide type[3]	Parent pesticide	Metabolite found in urine	Precur-sor ion	Exact m/z	RT[4] urine [min]	ID[5] level	Overall Detection Frequency[6]					
								ES	LV	HU	CZ	NL	CH
P1	H	2,4-D	Parent	[M-H]-	218.9623	9.93	1	4.07	0	2.20	2.71	0	0.33
P2_a	I	Acetamiprid	-CH2	[M-H]-	207.0443	8.71	1	98.56	32.84	94.13	98.19	93.29	87.33
P3_a	F	Ametoctradin	-C2H6 +2O	[M+H]+	278.1612	9.47	1	5.02	2.74	1.22	4.75	2.88	1.00
P5_a	F	Boscalid	+O +SO3	[M-H]-	436.9771	10.26	2b	35.65	18.41	3.91	22.85	32.85	12.67
P5_b			+O +SO3	[M+H]+	438.9917	10.49	2b	7.18	0	0	0.45	0.24	0
P6	I	Chlorantraniliprole	+O	[M-H]-	497.9564	12.67	2b	3.83	0.25	0.24	0	0.24	0
P8_a	H, GR	Chlorpropham	+O +SO3 (4-HSA)	[M-H]-	308.0003	9.5	1	55.74	31.59	31.05	34.16	75.06	40.00
P9_a	I	Chlorpyrifos (/methyl)	TCPy	[M-H]-	195.9129	10.1	1	1.67	0	0.24	0.23	0.24	0.67
P9_b			-CH2	[M-H]-	305.8723	10.72	1	36.12	0	6.85	21.72	6.47	2.00
P10	H	Clopyralid	Parent	[M-H]-	189.9465	3.5	1	0.96	0	0	1.36	0.72	0.33
P11_a	I	Clothianidin (can come from thiamethoxam)	Parent	[M-H]-	248.0015	8.09	1	34.45	1.74	21.52	24.66	19.42	13.00
P11_b			-NO2 +H	[M+H]+	205.0309	5.77	1	0.48	0	0.24	0	0.24	0.33
P11_c			-CH2	[M-H]-	233.9858	7.51	2b	21.05	0.75	9.78	6.56	3.12	5.33

[3] H: Herbicide, F: Fungicide, I: Insecticide, GR: Plant Growth Regulator, Ac: Acaricide, M: molluscide, Al: Algicide, Ab: antibacterial, Af: antifungal,
[4] Retention time
[5] Schymanski confirmation level, [1-5]
[6] <10% are shown in grey. ES= Spain, LV = Latvia, HU = Hungary, CZ = Czech Republic, NL = Netherlands, CH = Switzerland.

ID	Pesti-cide type[3]	Parent pesticide	Metabolite found in urine	Precur-sor ion	Exact m/z	RT[4] urine [min]	ID[5] level	Overall Detection Frequency[6]					
								ES	LV	HU	CZ	NL	CH
P12_a	I	Cypermethrin, cyfluth-rin, permethrin, trans-fluthrin	DCCA	[M-H]-	206.9985	10.73	1	0.48	0	0	0	0	0
P13_a	F	Cyprodinil	+O +SO3	[M-H]-	320.0710	11.87	2b	14.11	7.71	2.69	10.18	26.38	11.33
P18_a	I	Flonicamid	Parent	[M-H]-	228.0397	6.9	1	1.67	0.75	1.96	2.71	5.76	0.67
P18_b			-C2HN	[M+H]+	191.0427	6.1	2b	15.07	0.25	27.38	0.23	57.31	43.67
P19_a	H	Fluazifop	Parent	[M-H]-	326.0647	11.74	1	19.86	2.49	11.00	18.33	21.10	14.33
P19_b			Parent	[M+H]+	328.079	13.57	1	8.13	1.49	4.89	5.20	8.15	5.33
P20	F	Fludioxonil	+O +C6H8O6	[M-H]-	439.0609	11.81	2b	16.27	14.68	1.96	14.48	26.86	16.33
P21_a	F	Fluopyram	+O +SO3	[M-H]-	490.9908	12.68	2b	3.59	0.5	0.24	1.13	0.96	0
P21_b			+O +C6H8O6	[M+H]+	589.0807	13.08	2b	2.39	0.75	0.49	3.17	4.8	1.00
P21_c			-2H	[M+H]+	395.0385	13.07	2b	10.77	6.72	0.49	3.39	3.12	1.33
P22_a	I	Flupyradifurone	Parent	[M+H]+	289.0557	8.79	1	2.63	0.25	0.24	0.68	2.16	0.33
P25_a	I, Ac	Fluvalinate	-C14H9NO	[M-H]-	294.0514	13.94	2b	0.96	0	0.73	0.23	0	0.33
P27_a	F	Imazalil	+C6H8O6	[M+H]+	473.0869	11.52	2b	19.38	10.70	8.31	4.52	4.56	3.33
P28_a	I	Imidacloprid	-NO2 +H	[M+H]+	211.0739	6.01	1	17.46	1.74	4.16	0.68	9.35	10.33
P32_a	F	Penconazole	+O +C6H8O6	[M+H]+	476.0982	11.45	2b	6.46	1.74	2.2	2.04	2.4	0.33
P34_a	I, Ac	Pirimiphos-methyl	-CH2	[M-H]-	290.0734	10.75	1	85.17	10.20	6.60	23.98	47.72	19.00
P35_a	F	Propamocarb	Parent	[M+H]+	189.1597	6.00	1	9.57	1	11.49	4.98	23.26	13.00
P35_b			+O	[M+H]+	205.1546	6.45	2b	20.81	5.47	18.34	12.67	42.69	29.00

ID	Pesti-cide type[3]	Parent pesticide	Metabolite found in urine	Precur-sor ion	Exact m/z	RT[4] urine [min]	ID[5] level	Overall Detection Frequency[6]					
								ES	LV	HU	CZ	NL	CH
P37	H	Propyzamide	+H2O3	[M-H]-	304.0143	11.36	2b	8.61	0	0.49	0.9	0.96	2.00
P38_a	F	Pyrimethanil	+O +SO3	[M-H]-	294.0556	9.15	2b	26.79	14.43	4.89	21.95	31.89	11.33
P38_b			+O	[M+H]+	216.1133	11.69	2b	0.72	0	2.69	0	0.48	0
P40_a	F	Tebuconazole	-2H +2O	[M-H]-	336.1124	12.18	2b	71.29	5.47	25.18	52.26	35.97	6.67
P41_a	F	Thiabendazole	+O +C6H8O6	[M-H]-	392.0551	5.96	2b	0	0.75	0.24	0	0.48	0.33
P42_a	I	Thiacloprid	+O	[M-H]-	267.0107	9.19	2b	8.37	0.75	2.93	7.92	4.56	2.67
P43_a	I	Thiamethoxam	Parent	[M+H]+	292.0262	7.10	1	0.72	0	2.44	0	0.48	1.67
P43_b			-NO2 +H	[M+H]+	247.0413	6.20	1	23.44	0	15.16	0	0.24	1.00
P45_a	Af, Ab	Triclosan	+C6H8O6	[M-H]-	462.9759	13.23	1	84.69	16.17	24.45	46.15	12.71	41.67
P46_a	F	Trifloxystrobin	-CH2 -CH2	[M-H]-	379.0911	13.07	2b	0.72	0.5	0	3.62	3.84	4.00

[3] H: Herbicide, F: Fungicide, I: Insecticide, GR: Plant Growth Regulator, Ac: Acaricide, M: molluscide, Al: Algicide, Ab: antibacterial, Af: antifungal,
[4] Retention time
[5] Schymanski confirmation level, [1-5]

[6] <10% are shown in grey. ES= Spain, LV = Latvia, HU = Hungary, CZ = Czech Republic, NL = Netherlands, CH = Switzerland.

6.3.2 Detected compounds in SPECIMEn

The selection on confidence levels 1 and 2b resulted in 41 compounds (95 at levels 1 to 5), originating from 30 different parent pesticides (46 at levels 1 to 5). Table 6.4 shows these compounds with their detection rates per country. The ratios per country vary, with Latvia having generally the lowest number of detects and Spain the highest number of detects. Pesticides (or metabolites) which were detected in at least 10% of the samples across all five SPECIMEn countries include the parent compounds of acetamiprid, boscalid (not in Hungary), chlorpropham, chlorpyrifos (only in Spain and Czech Republic), clothianidin (not in Latvia), cyprodinil (not in Latvia and Hungary), flonicamid (not in Latvia and Czech Republic), fluazifop (not in Latvia), fludioxonil (not in Hungary), imazalil (only in Spain and Latvia), imidacloprid (only in Spain), pirimiphos-methyl (not in Hungary), propamocarb (not in Latvia), pyrimethanil (not in Hungary), tebuconazole (not in Latvia), thiamethoxam (only in Spain and Hungary), and triclosan. In all countries, the most frequently detected compound was acetamiprid (metabolite -CH2). A further assessment of differences between location, season, and age groups was focused on the above mentioned 17 pesticides (and metabolites). Parent pesticides that were detected at low frequencies (<10%) across countries include 2,4-D, ametoctradin, chlorantraniliprole, clopyralid, fluopyram, flupyradifurone, fluvalinate, penconazole, propyzamide, thiabendazole, thiacloprid, trifloxystrobin, as well as the metabolite DCCA (originates from parent pesticides cypermethrin, cyfluthrin, permethrin or transfluthrin).

Correlation coefficients were calculated based on the quantitative data to explore the relationships between compounds originating from the same parent pesticide (Annex 3). The compounds originating from the same parent compound with sufficient data are boscalid, fluazifop, and propamocarb, and they all showed high correlations (0.69 to 0.98). The lowest correlations were seen within the Czech dataset, however, these were calculated on a limited subset of 23 and 19 samples, respectively.

6.3.3 Detected compounds in Switzerland

Equal to the five SPECIMEn countries, 41 compounds of levels 1 and 2b were detected in Switzerland (See Table 6.4). The compounds with a detection rate of at least 10% include acetamiprid, boscalid, chlorpropham, chlothianidin, cyprodinil, flonicamid, fluazifop, fludioxonil, imidacloprid, pirimiphos-methyl, propamocarb, pyrimethanil, and triclosan. Stratifications performed in the next paragraphs by location, season, and age groups are not applicable for Switzerland.

6.3.4 Detection rates in hotspot versus control regions

The detection ratios stratified by hotspot and control location are presented in Table 6.5. Only compounds detected in at least 10% of the samples and of ID levels 1 and 2b are presented. The percentage difference between the locations is indicated by colour, only if the hotspot location had a higher detection ratio compared to the control location (following the hypothesis of our research question).

Several interesting patterns can be observed. Firstly, some compounds show a consistent higher detection frequency in hotspot versus control areas, which might suggest that living in a hotspot area contributed to a higher probability of exposure, regardless of the country of residence. This was the case for acetamiprid (metabolite -CH2), which was detected most frequently in the hotspot area in three of the five countries: Spain, Hungary, and the Netherlands (last two not significant). Similarly, chlorpropham was detected at a higher frequency in the hotspot areas than in control areas of the Netherlands, the Czech Republic, Hungary, and Latvia (last three not significant), while in Spain the opposite was seen.

P11_a and P11_c are both related to the parent pesticide clothiandin. Higher detection rates were consistently detected in hotspot areas than in controls in Hungary, Latvia, the Czech Republic and the Netherlands, but not in Spain, where P11_a was much higher in the control region than in the hotspot area.

Another interesting finding is the higher frequency of detection of both propamocarb related compounds (P35_a and P35_b) in the hotspot areas of Spain, Latvia, and the Netherlands. In contrast, in Hungary and the Czech Republic, both compounds were more frequently detected in the control areas. Finally, triclosan was detected more frequently in the hotspot areas of the Netherlands and Hungary, while for the other countries this was not observed.

Table 6.5: Detection ratios, stratified by location. Only compounds detected in >10% of the samples in at least one country, and of confidence level 1 or 2b are presented. Colours indicate the percentage of difference of hotspot compared to control, <25%, 25-50%, 50-75%, and >75%.

		Spain			Latvia			Hungary			Czech Republic			Netherlands		
ID	Parent pesticide	Hot-spot	Con-trol	*p-va-lue*[7]	Hot-spot	Con-trol	p-va-lue	Hot-spot	Con-trol	p-va-lue	Hot-spot	Con-trol	p-va-lue	Hot-spot	Con-trol	p-va-lue
P2_a	Aceta-miprid	100.00	97.17	*0.04*	32.50	33.17	*0.97*	94.53	93.75	*0.90*	97.55	98.74	*0.56*	94.98	91.41	*0.21*
P5_a	Boscalid	35.92	35.38	*0.99*	19.50	17.33	*0.66*	3.48	4.33	*0.85*	25.49	20.59	*0.27*	27.85	38.38	*0.03*
P8_a	Chlorpro-pham	52.91	58.49	*0.29*	33.50	29.70	*0.48*	33.33	28.85	*0.38*	34.31	34.03	*1.00*	80.37	69.19	*0.01*
P9_b	Chlorpy-rifos/me-thyl	33.98	38.21	*0.43*	0	0	-	2.99	10.58	*<0.01*	24.02	19.75	*0.33*	6.85	6.06	*0.90*
P11_a	Clothiani-din	27.18	41.51	*<0.01*	2.00	1.49	*0.99*	29.35	13.94	*<0.01*	26.96	22.69	*0.35*	21.00	17.68	*0.46*
P11_c		23.30	18.87	*0.32*	1.50	0	*0.24*	15.42	4.33	*<0.01*	7.35	5.88	*0.67*	3.65	2.53	*1.00*
P13_a	Cyprodinil	16.02	12.26	*0.34*	8.00	7.43	*0.98*	2.99	2.40	*0.95*	4.90	14.71	*<0.01*	21.46	31.82	*0.02*
P18_b	Floni-camid	14.08	16.04	*0.67*	0.50	0	*0.99*	22.89	31.73	*0.06*	0	0.42	*1.00*	57.08	57.58	*1.00*
P19_a	Fluazifop	19.90	19.81	*1.00*	4.00	0.99	*0.11*	11.44	10.58	*0.90*	19.12	17.65	*0.78*	23.29	18.69	*0.30*
P20	Fludi-oxonil	16.50	16.04	*1.00*	14.00	15.35	*0.81*	1.49	2.40	*0.76*	10.29	18.07	*0.03*	26.03	27.78	*0.77*
P27_a	Imazalil	19.42	19.34	*1.00*	13.00	8.42	*0.19*	6.97	9.62	*0.43*	4.41	4.62	*1.00*	5.48	3.54	*0.47*
P28_a	Imidaclop rid	19.42	15.57	*0.36*	2.00	1.49	*0.99*	3.98	4.33	*1.00*	1.47	0	*0.19*	10.05	8.59	*0.73*

[7] Chi-square test, with Yates' correction, p-values ≦ 0.05 are statistically significant and are marked in bold.

		Spain			Latvia			Hungary			Czech Republic			Netherlands		
ID	**Parent pesticide**	**Hot-spot**	**Con-trol**	***p-va-lue*[7]**	**Hot-spot**	**Con-trol**	**p-va-lue**	**Hot-spot**	**Con-trol**	**p-va-lue**	**Hot-spot**	**Con-trol**	**p-va-lue**	**Hot-spot**	**Con-trol**	**p-va-lue**
P34_a	Piri-miphos-methyl	83.50	86.79	*0.42*	10.00	10.40	*1.00*	1.49	11.54	*<0.01*	26.96	21.43	*0.21*	51.14	43.94	*0.17*
P35_a	Propamo-carb	10.19	8.96	*0.79*	2.00	0	*0.13*	7.96	14.90	*0.04*	3.43	6.30	*0.24*	26.48	19.70	*0.13*
P35_b		22.82	18.87	*0.38*	8.50	2.48	*0.02*	13.43	23.08	*0.02*	11.27	13.87	*0.50*	44.29	40.91	*0.55*
P38_a	Pyrime-thanil	25.24	28.30	*0.55*	15.50	13.37	*0.64*	3.48	6.25	*0.29*	21.57	22.27	*0.95*	26.94	37.37	*0.03*
P40_a	Tebuco-nazole	70.39	72.17	*0.77*	6.50	4.46	*0.50*	20.40	29.81	*0.04*	51.96	52.52	*0.98*	34.25	37.88	*0.50*
P43_b	Thiame-thoxam	20.39	26.42	*0.18*	0	0	-	19.40	11.06	*0.03*	0	0	-	0.46	0	*1.00*
P45_a	Triclosan	83.98	85.38	*0.79*	16.00	16.34	*1.00*	26.37	22.60	*0.44*	43.14	48.74	*0.28*	15.98	9.09	*0.05*

6.3.5 Detection rates in summer versus winter

The detection ratios stratified by season (winter or season 1, and summer or season 2) are presented in Table 6.6. Only compounds detected in at least 10% of the samples and of ID levels 1 and 2b are presented. The percentage of change is indicated by colour, only if the detection rate was higher in summer (following the hypothesis of our research question).

Comparing the detection frequencies between the seasons across countries, some compounds are consistently more often detected in summer. Specifically, most of the compounds in Hungary (except chlorpropham, imazalil, cyprodinil and boscalid) had a higher detection frequency during summer, suggesting that participants were exposed to a larger set of different pesticides in that season.

The compounds clothianidin, pirimiphos-methyl, pyrimethanil, and tebuconazole were most frequently detected during summer in Hungary and Latvia, but the other countries showed the exact opposite effect. Thiamethoxam was only detected in Spain and Hungary, with both countries having a significantly higher detection frequency during summer. Triclosan was most detected in summer in almost all countries except in the Czech Republic. Also, chlorpropham did have a higher detection frequency during summer in Latvia, the Czech Republic and the Netherlands; interestingly the opposite effect was seen in Spain and Hungary. Imazalil was more frequently detected in winter within all countries, suggesting that exposure to this pesticide more frequently occurs during winter.

Table 6.6: Detection ratios, stratified by season (winter, and summer). Only compounds detected in >10% of the samples in at least one country, and of confidence level 1 or 2b are presented. Colours indicate the percentage of change of season 2 compared to season 1, <25%, 25-50%, 50-75%, and >75%.

		Spain			Latvia			Hungary			Czech Republic			Netherlands		
ID	Parent pesticide	win-ter	sum-mer	p-va-lue[8]	win-ter	sum-mer	p-va-lue	win-ter	sum-mer	p-va-lue	winter	sum-mer	p-va-lue	winter	sum-mer	p-value
P2_a	Acetamiprid	99.05	98.08	0.67	37.13	28.50	0.08	93.69	94.58	0.86	98.65	97.73	0.71	95.24	91.30	0.16
P5_a	Boscalid	39.52	31.73	0.12	18.81	18.00	0.93	4.85	2.96	0.46	25.23	20.45	0.28	30.00	35.75	0.25
P8_a	Chlorpropham	63.81	47.60	<0.01	28.22	35.00	0.17	37.38	24.63	<0.01	27.93	40.45	0.01	67.14	83.09	<0.01
P9_b	Chlorpyrifos/me-thyl	50.48	21.63	<0.01	0	0	-	4.37	9.36	0.07	25.23	18.18	0.09	8.57	4.35	0.12
P11_a	Clothianidin	40.00	28.85	0.02	0.50	3.00	0.12	13.11	30.05	<0.01	28.38	20.91	0.09	22.86	15.94	0.10
P11_c		21.90	20.19	0.76	0.50	1.00	0.99	7.28	12.32	0.12	8.56	4.55	0.13	4.76	1.45	0.10
P13_a	Cyprodinil	13.33	14.90	0.75	8.91	6.50	0.47	3.40	1.97	0.56	10.81	9.55	0.78	28.57	24.15	0.36
P18_b	Flonicamid	21.90	8.17	<0.01	0.50	0	1.00	20.39	34.48	<0.01	0.45	0	1.00	58.10	56.52	0.82
P19_a	Fluazifop	25.24	14.42	<0.01	2.97	2.00	0.76	10.68	11.33	0.95	19.37	17.27	0.66	21.43	20.77	0.96
P20	Fludioxonil	20.00	12.50	0.05	15.84	13.50	0.60	1.46	2.46	0.71	16.67	12.27	0.23	31.90	21.74	0.03
P27_a	Imazalil	30.48	8.17	<0.01	14.36	7.00	0.03	13.11	3.45	<0.01	6.31	2.73	0.11	6.67	2.42	0.06
P28_a	Imidacloprid	20.95	13.94	0.08	2.97	0.50	0.13	4.37	3.94	1.00	0.45	0.91	0.99	9.05	9.66	0.96
P34_a	Pirimiphos-me-thyl	86.67	83.65	0.47	8.42	12.00	0.31	3.40	9.85	0.02	27.48	20.45	0.11	51.43	43.96	0.15
P35_a	Propamocarb	7.14	12.02	0.13	1.98	0	0.13	11.17	11.82	0.96	4.50	5.45	0.81	20.48	26.09	0.21

[8] Chi-square test, with Yates' correction, p-values ≦ 0.05 are statistically significant and are marked in bold.

		Spain			Latvia			Hungary			Czech Republic			Netherlands		
ID	Parent pesticide	win-ter	sum-mer	p-va-lue8	win-ter	sum-mer	p-va-lue	win-ter	sum-mer	p-va-lue	winter	sum-mer	p-va-lue	winter	sum-mer	p-value
P35_b		19.52	22.12	0.59	7.92	3.00	0.05	13.59	23.15	0.02	11.26	14.09	0.45	42.86	42.51	1.00
P38_a	Pyrimethanil	31.43	22.12	0.04	10.40	18.50	0.03	4.37	5.42	0.79	27.93	15.91	<0.01	36.67	27.05	0.05
P40_a	Tebuconazole	76.19	66.35	0.03	2.97	8.00	0.05	19.90	30.54	0.02	56.31	48.18	0.11	39.52	32.37	0.16
P43_b	Thiamethoxam	0.95	46.15	<0.01	0	0	-	0	30.54	<0.01	0	0	1.00	0.48	0	1.00
P45_a	Triclosan	83.33	86.06	0.52	12.87	19.50	0.10	20.39	28.57	0.07	98.65	97.73	0.99	10.00	15.46	0.13

6.3.6 Detection rates in children versus adults

The detection ratios stratified by age group (children and adults) are presented in Table 6.7. Only compounds detected in at least 10% of the samples and of ID levels 1 and 2b are presented. The percentage difference is indicated by colour, only if children had a higher detection ratio compared to adults.

A consistent effect of higher detection rates within children was detected, with the largest effect in Hungary, the Czech Republic, and the Netherlands. Compounds that were more often detected in children across all countries were chlorpropham, chlorpyrifos/methyl, clothianidin (P11_a), flonicamid, pirimiphos-methyl, and tebuconazole. Boscalid had the opposite effect since it was most frequently detected among adults in all countries. An interesting difference between the countries was related to the compound cyprodinil, which was most detected in Spanish adults, while it was most detected in Dutch children.

Table 6.7: Detection ratios, stratified by age (children and adults). Only compounds detected in >10% of the samples in at least one country, and of confidence level 1 or 2b are presented. Colours indicate the percentage of change of children compared to adults, <25%, 25-50%, 50-75%, and >75%.

		Spain			Latvia			Hungary			Czech Republic			Netherlands		
ID	Parent pesticide	Child	Adult	p-value[9]	Child	Adult	p-value	Child	Adult	p-value	Child	Adult	p-value	Child	Adult	p-value
P2_a	Acetamiprid	99.52	97.61	*0.22*	34.65	31.00	*0.50*	96.57	91.71	*0.06*	98.19	98.19	*1.00*	92.34	94.23	*0.57*
P5_a	Boscalid	25.84	45.45	*<0.01*	16.83	20.00	*0.50*	1.96	5.85	*0.08*	17.19	28.51	*<0.01*	30.14	35.58	*0.28*
P8_a	Chlorpropham	65.07	46.41	*<0.01*	40.59	22.50	*<0.01*	37.75	24.39	*<0.01*	42.08	26.24	*<0.01*	78.47	71.63	*0.14*
P9_b	Chlorpyrifos/methyl	43.54	28.71	*<0.01*	0	0	-	8.82	4.88	*0.17*	27.60	15.84	*<0.01*	7.18	5.77	*0.70*
P11_a	Clothianidin	39.71	29.19	*0.03*	2.97	0.50	*0.13*	25.00	18.05	*0.11*	24.89	24.43	*1.00*	20.10	18.75	*0.82*
P11_c		22.97	19.14	*0.40*	1.49	0	*0.25*	10.78	8.78	*0.61*	6.79	6.33	*1.00*	2.39	3.85	*0.57*
P13_a	Cyprodinil	8.13	20.10	*<0.01*	7.92	7.50	*1.00*	1.47	3.90	*0.22*	11.31	9.05	*0.53*	32.54	20.19	*<0.01*
P18_b	Flonicamid	17.70	12.44	*0.17*	0	0.50	*0.99*	28.43	26.34	*0.72*	0.45	0	*1.00*	61.72	52.88	*0.08*
P19_a	Fluazifop	19.62	20.10	*1.00*	2.48	2.50	*1.00*	13.24	8.78	*0.20*	20.36	16.29	*0.33*	23.44	18.75	*0.29*
P20	Fludioxonil	13.88	18.66	*0.23*	13.86	15.50	*0.75*	1.96	1.95	*1.00*	16.74	12.22	*0.22*	32.06	21.63	*0.02*
P27_a	Imazalil	18.66	20.10	*0.80*	7.43	14.00	*0.05*	10.29	6.34	*0.20*	2.26	6.79	*0.04*	3.83	5.29	*0.63*
P28_a	Imidacloprid	15.31	19.62	*0.40*	1.49	2.00	*0.99*	4.41	3.90	*0.99*	0.90	0.45	*1.00*	10.05	8.65	*0.75*
P34_a	Pirimiphos-methyl	89.95	80.38	*<0.01*	13.86	6.50	*0.02*	10.78	2.44	*<0.01*	29.41	18.55	*0.01*	57.89	37.50	*<0.01*

[9] Chi-square test, with Yates' correction, p-values ≦ 0.05 are statistically significant and are marked in bold.

		Spain			Latvia			Hungary			Czech Republic			Netherlands		
ID	**Parent pesti-cide**	**Child**	**Adult**	**p-va-lue[9]**	**Child**	**Adult**	**p-va-lue**	**Child**	**Adult**	**p-va-lue**	**Child**	**Adult**	**p-value**	**Child**	**Adult**	**p-va-lue**
P35_a	Propamocarb	9.09	10.05	*0.87*	1.98	0	*0.13*	15.20	7.80	*0.03*	6.33	3.62	*0.27*	23.44	23.08	*1.00*
P35_b		19.62	22.01	*0.63*	7.43	3.50	*0.13*	22.55	14.15	*0.04*	15.38	9.95	*0.12*	40.67	44.71	*0.46*
P38_a	Pyrimethanil	29.19	24.40	*0.32*	14.36	14.50	*1.00*	3.43	6.34	*0.26*	19.46	24.43	*0.25*	34.45	29.33	*0.31*
P40_a	Tebuconazole	82.30	60.29	*<0.01*	5.94	5.00	*0.85*	33.82	16.59	*<0.01*	68.78	35.75	*<0.01*	53.11	18.75	*<0.01*
P43_b	Thiamethoxam	22.97	23.92	*0.91*	0	0	-	13.24	17.07	*0.34*	0	0	-	0.48	0	*1.00*
P45_a	Triclosan	83.73	85.65	*0.68*	15.35	17.00	*0.75*	31.37	17.56	*<0.01*	48.87	43.44	*0.29*	11.00	14.42	*0.37*

6.3.7 Detection ratios stratified

To further explore the described differences by location, season, and age groups, stratification tables were constructed for each country. The tables present stratifications based on season 2 (summer), following the research question and hypothesis of higher exposures during summer/spraying season. A stratification is made based on age and location. Only compounds detected in at least 10% in one of the subcategories are shown.

Based on the Spanish samples from the summer stratified by children and adults, the majority of compounds were most frequently detected in the hotspot area (Table 6.8). Compared to the table only stratified by location (Table 6.5), some differences are seen. Within the group of children, the previously observed difference of clothianidin between hotspots and controls disappears. Other differences are only seen when the data was stratified (e.g. propamocarb in the group of children, and thiamethoxam in the group of adults), however due to the small subgroups no interpretations will be given to these differences.

Table 6.8: Detection ratios of summer samples from Spain, stratified by age (adults and children), and location (hotspot and control). Colours indicate the percentage of change of hotspots compared to control areas, <25%, 25-50%, 50-75%, and >75%.

Spain		Children			Adults		
ID	Parent pesticide	Hotspot	Control	p-value[10]	Hotspot	Control	p-value
P2_a	Acetamiprid	100.00	98.11	1.00	100.00	94.34	*0.25*
P5_a	Boscalid	29.41	18.87	0.25	41.18	37.74	*0.84*
P5_b		7.84	3.77	0.43	11.76	1.89	*0.06*
P8_a	Chlorpropham	54.90	64.15	0.42	35.29	35.85	*1.00*
P9_b	Chlorpyrifos/methyl	23.53	28.30	0.66	11.76	22.64	*0.20*
P11_a	Clothianidin	31.37	35.85	0.68	15.69	32.08	*0.07*
P11_c		23.53	22.64	1.00	15.69	18.87	*0.80*
P13_a	Cyprodinil	11.76	9.43	0.76	21.57	16.98	*0.62*
P19_a	Fluazifop	9.80	13.21	0.76	21.57	13.21	*0.31*
P19_b		5.88	5.66	1.00	11.76	5.66	*0.31*
P20	Fludioxonil	11.76	5.66	0.31	17.65	15.09	*0.79*
P21_c	Fluopyram	9.80	7.55	0.74	15.69	9.43	*0.39*
P28_a	Imidacloprid	11.76	9.43	0.76	17.65	16.98	*1.00*
P34_a	Pirimiphos-methyl	86.27	92.45	0.35	76.47	79.25	*0.82*
P35_a	Propamocarb	9.80	9.43	1.00	15.69	13.21	*0.78*
P35_b		27.45	9.43	0.02	25.49	26.42	*1.00*
P37	Propyzamide	5.88	7.55	1.00	17.65	5.66	*0.07*
P38_a	Pyrimethanil	21.57	20.75	1.00	25.49	20.75	*0.64*
P40_a	Tebuconazole	72.55	79.25	0.49	64.71	49.06	*0.12*
P42_a	Thiacloprid	11.76	5.66	0.31	5.88	9.43	*0.72*

[10] Fisher-Exact test, p-values ≦ 0.05 are statistically significant and are marked in bold.

P43_b	Thiamethoxam	43.14	47.17	0.70	37.25	56.60	*0.05*
P45_a	Triclosan	74.51	94.34	0.006	88.24	86.79	*1.00*

The Latvian data from summer stratified by children and adults showed no differences between the locations for both adults and children (Table 6.9). The non-stratified comparisons based on location (Table 6.5) showed a higher detection frequency of propamocarb (P35_b) in the hotspot area, the stratified detection frequencies became so low that no valid comparison can be made.

Table 6.9: Detection ratios of summer samples from Latvia, stratified by age (adults and children), and location (hotspot and control). Colours indicate the percentage of change of hotspots compared to control areas, <25%, 25-50%, 50-75%, and >75%.

Latvia		Children			Adults		
ID	Parent pesticide	Hot-spot	Control	p-value[11]	Hot-spot	Control	p-value
P2_a	Acetamiprid	36.00	25.49	0.29	20.00	32.65	0.18
P5_a	Boscalid	16.00	15.69	1.00	20.00	20.41	1.00
P8_a	Chlorpropham	46.00	41.18	0.69	30.00	22.45	0.49
P20	Fludioxonil	12.00	13.73	1.00	14.00	14.29	1.00
P27_a	Imazalil	6.00	3.92	0.68	12.00	6.12	0.49
P34_a	Pirimiphos-methyl	14.00	15.69	1.00	6.00	12.24	0.32
P38_a	Pyrimethanil	22.00	15.69	0.46	20.00	16.33	0.80
P40_a	Tebuconazole	6.00	9.80	0.72	10.00	6.12	0.71
P45_a	Triclosan	18.00	19.61	1.00	20.00	20.41	1.00

[11] Fisher-Exact test, p-values ≦ 0.05 are statistically significant and are marked in bold.

The Hungarian summer data stratified by children and adults are presented in Table 6.10. The effect of living in a hotspot location was more present in children's urine measured in summer. In the non-stratified comparison (Table 6.5), chlothianidin was most detected in the hotspot areas. This effect disappeared when stratified to summer season samples from adults, but remained intact with summer samples from children. For the compounds chlorpyrifos/methyl, pirimiphos-methyl, propamocarb and thiamethoxam the exact same effect was observed: only significant differences between locations in the non-stratified data and children's summer samples.

Table 6.10: Detection ratios of summer samples from Hungary, stratified by age (adults and children), and location (hotspot and control). Colours indicate the percentage of change of hotspots compared to control areas, <25%, 25-50%, 50-75%, and >75%.

Hungary		Children			Adults		
ID	Parent pesticide	Hotspot	Control	p-value[12]	Hotspot	Control	p-value
P2_a	Acetamiprid	95.92	98.08	*0.61*	94.00	90.38	*0.72*
P8_a	Chlorpropham	34.69	25.00	*0.38*	18.00	21.15	*0.80*
P9_b	Chlorpyrifos/methyl	4.08	19.23	*0.03*	4.00	9.62	*0.44*
P11_a	Clothianidin	55.10	13.46	*<0.001*	32.00	21.15	*0.26*
P11_c		26.53	0	*<0.001*	16.00	7.69	*0.23*
P18_b	Flonicamid	28.57	32.69	*0.67*	26.00	50.00	*0.02*
P19_a	Fluazifop	18.37	9.62	*0.26*	8.00	9.62	*1.00*
P19_b		14.29	1.92	*0.03*	6.00	0	*0.11*
P34_a	Pirimiphos-methyl	2.04	28.85	*<0.001*	0	7.69	*0.12*
P35_a	Propamocarb	6.12	23.08	*0.02*	12.00	5.77	*0.31*
P35_b		14.29	36.54	*0.01*	18.00	23.08	*0.63*
P38_a	Pyrimethanil	2.04	5.77	*0.62*	2.00	11.54	*0.11*
P40_a	Tebuconazole	32.65	51.92	*0.07*	12.00	25.00	*0.13*
P42_a	Thiacloprid	2.04	13.46	*0.06*	0	1.92	*1.00*
P43_b	Thiamethoxam	51.02	3.85	*<0.001*	28.00	40.38	*0.22*
P45_a	Triclosan	55.10	23.08	*0.001*	14.00	23.08	*0.31*

[12] Fisher-Exact test, p-values ≦ 0.05 are statistically significant and are marked in bold.

Czech data from summer stratified by location for children and adults is presented in Table 6.11. The observed effect of higher detection frequencies in the control areas for cyprodinil and fludioxonil, only remained for cyprodinil in children's summer samples. Another difference was a higher detection frequency of propamocarb in control areas based on children's summer samples and of chlorpyrifos-methyl in hotspot areas based on adult's summer samples, although no interpretation can be made due to small subgroups.

Table 6.11: Detection ratios of summer samples from the Czech Republic, stratified by age (adults and children), and location (hotspot and control). Colours indicate the percentage of change of hotspots compared to control areas, <25%, 25-50%, 50-75%, and >75%.

The Czech Republic		Children			Adults		
ID	Parent pesticide	Hotspot	Control	p-value[13]	Hotspot	Control	p-value
P2_a	Acetamiprid	98.04	98.31	*1.00*	98.04	96.61	*1.00*
P5_a	Boscalid	15.69	10.17	*0.41*	19.61	35.59	*0.09*
P8_a	Chlorpropham	45.10	50.85	*0.57*	27.45	37.29	*0.31*
P9_b	Chlorpyrifos/methyl	27.45	22.03	*0.66*	19.61	5.08	*0.03*
P11_a	Clothianidin	19.61	20.34	*1.00*	25.49	18.64	*0.49*
P11_c		9.80	1.69	*0.09*	3.92	3.39	*1.00*
P13_a	Cyprodinil	1.96	13.56	*0.04*	5.88	15.25	*0.14*
P19_a	Fluazifop	15.69	16.95	*1.00*	17.65	18.64	*1.00*
P20	Fludioxonil	11.76	16.95	*0.59*	7.84	11.86	*0.54*
P34_a	Pirimiphos-methyl	29.41	22.03	*0.39*	17.65	13.56	*0.60*
P35_a	Propamocarb	1.96	11.86	*0.07*	0	6.78	*0.12*
P35_b		5.88	27.12	*0.004*	7.84	13.56	*0.38*
P38_a	Pyrimethanil	11.76	10.17	*1.00*	29.41	13.56	*0.06*
P40_a	Tebuconazole	64.71	64.41	*1.00*	31.37	32.20	*1.00*
P42_a	Thiacloprid	11.76	10.17	*1.00*	5.88	3.39	*0.66*
P45_a	Triclosan	52.94	45.76	*0.57*	33.33	50.85	*0.08*

[13] Fisher-Exact test, p-values ≦ 0.05 are statistically significant and are marked in bold.

Lastly, the stratification of the Dutch data from the summer for children and adults is presented in Table 6.12. All of the detected differences based on the non-stratified analyses in Table 6.5 were not observed when stratifying data to summer season and age groups. Within the summer adult samples pirimiphos-methyl and propamocarb were more frequently detected in the hotspot areas, while this effect was not significant for non-stratified comparisons.

Table 6.12: Detection ratios of summer samples from the Netherlands, stratified by age (adults and children), and location (hotspot and control). Colours indicate the percentage of change of hotspots compared to control areas, <25%, 25-50%, 50-75%, and >75%.

The Netherlands		Children			Adults		
ID	Parent pesticide	Hotspot	Control	p-value[14]	Hotspot	Control	p-value
P2_a	Acetamiprid	92.73	91.84	*1.00*	90.74	89.80	*1.00*
P5_a	Boscalid	29.09	36.73	*0.53*	33.33	44.90	*0.31*
P8_a	Chlorpropham	89.09	83.67	*0.57*	85.19	73.47	*0.15*
P11_a	Clothianidin	5.45	10.20	*0.12*	12.96	18.37	*0.58*
P13_a	Cyprodinil	21.82	36.73	*0.13*	12.96	26.53	*0.13*
P18_a	Flonicamid	5.45	16.33	*0.11*	3.70	2.04	*1.00*
P18_b		54.55	65.31	*0.31*	53.70	53.06	*1.00*
P19_a	Fluazifop	27.27	20.41	*0.49*	14.81	20.41	*0.60*
P19_b		12.73	12.24	*1.00*	7.41	10.20	*0.73*
P20	Fludioxonil	29.09	26.53	*0.83*	12.96	18.37	*0.59*
P28_a	Imidacloprid	10.91	2.04	*0.12*	16.67	8.16	*0.24*
P34_a	Pirimiphos-methyl	63.64	51.02	*0.23*	40.74	18.37	*0.02*
P35_a	Propamocarb	25.45	22.45	*0.82*	40.74	14.29	*0.004*
P35_b		45.45	40.82	*0.69*	35.19	48.98	*0.17*
P38_a	Pyrimethanil	23.64	30.61	*0.51*	27.78	26.53	*1.00*
P40_a	Tebuconazole	43.64	51.02	*0.55*	14.81	20.41	*0.60*
P42_a	Thiacloprid	7.27	8.16	*1.00*	1.85	10.20	*0.10*
P45_a	Triclosan	16.36	8.16	*0.25*	24.07	12.24	*0.14*

[14] Fisher-Exact test, p-values ≦ 0.05 are statistically significant and are marked in bold.

6.4 Pesticide Exposure patterns

6.4.1 Binary correlation networks

To describe the co-occurrence patterns among the multiple compounds detected by the suspect screening approach in each sample, a correlation network analysis has been applied. Figure 6.1 shows the resulting weighted correlation network across the five SPECIMEn countries. The relationships between markers are indicated by a line (green = positive, red = negative). The colours indicate the different communities or groups of more closely related markers.

When assessing the relations/lines, as could be expected, the strongest positive relations are detected between metabolites of the same parent pesticide: P11_a with P11_c (chlothianidin, P11_b was however not connected, since it is rarely detected in any of the countries), P19_a with P19_b (fluazifop), P35_a with P35_b (propamocarb), P21_a/b/c (fluopyram), and P5_a, with P5_b (boscalid). Interestingly, some compounds were also correlated across parent pesticides. For example, P5_b (boscalid, a fungicide) was strongly related to P25_a (fluvalinate, an insecticide and acaricide), suggesting common exposure routes. Other strong relationships between different parent pesticides were P41_a (thiabendazole, fungicide) with P27_a (imazalil, fungicide), and P20 (fludioxonil, fungicide) with P13_a (cyprodinil, fungicide).

Within the network, 8 different communities are detected, indicated by different colours in Figure 6.1. The largest community is indicated in dark red and joins P2_a (acetamiprid, insecticide), P8_a (chlorpropham, herbicide and plant growth regulator), P9_b (chlorpyrifos, insecticide), P18_b (flonicamid, insecticide), P34_a (pirimiphos-methyl, insecticide and acaricide), P40_a (tebuconazole, fungicide), P42_a (thiacloprid, insecticide) and P45_a (triclosan, antifungal and algicide). Based on the detection frequencies, these compounds are generally most detected across the countries. Another example is the slightly lighter red community, joining P21_a, P21_b, P21_c (all three fluopyram, fungicide), P22_a (flupyradifurone, insecticide), P32_a (penconazole, fungicide) and P46_a (trifloxystrobin, fungicide).

Overall, the underlying absolute Pearson correlations are weak with values around 0.1.

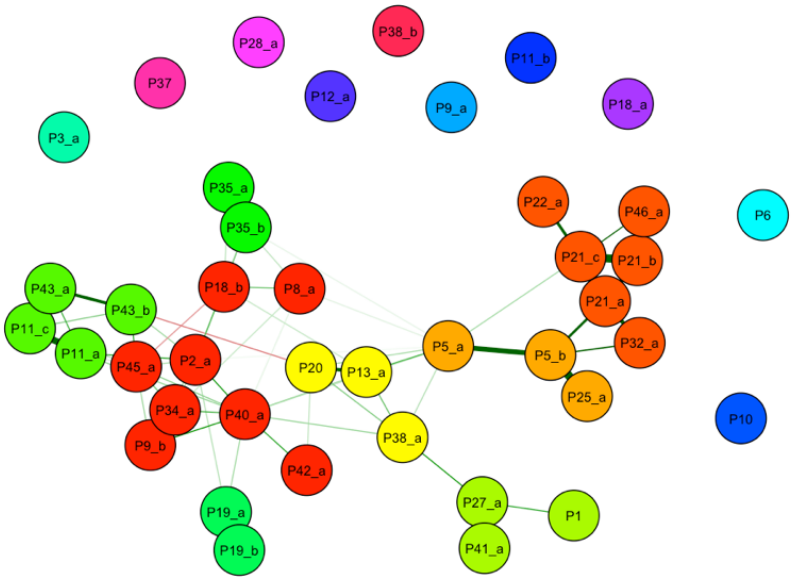

Figure 6.1: Weighted correlation network based on binary data of all five SPECIMEn countries, compounds of levels 1 and 2b are included. Colour codes in this figure reflect different communities (groups of more closely related compounds).

To explore the difference in correlation patterns, networks were estimated stratified by location (Figure 6.2), season (Figure 6.3), and age (Figure 6.4).

In figure 6.2 the network based on samples from hotspot areas consists of two separate networks (set of connected markers), while for the control area a single network with connected markers is detected. This is not as expected since we would expect to have more strongly connected compounds within the hotspot area.

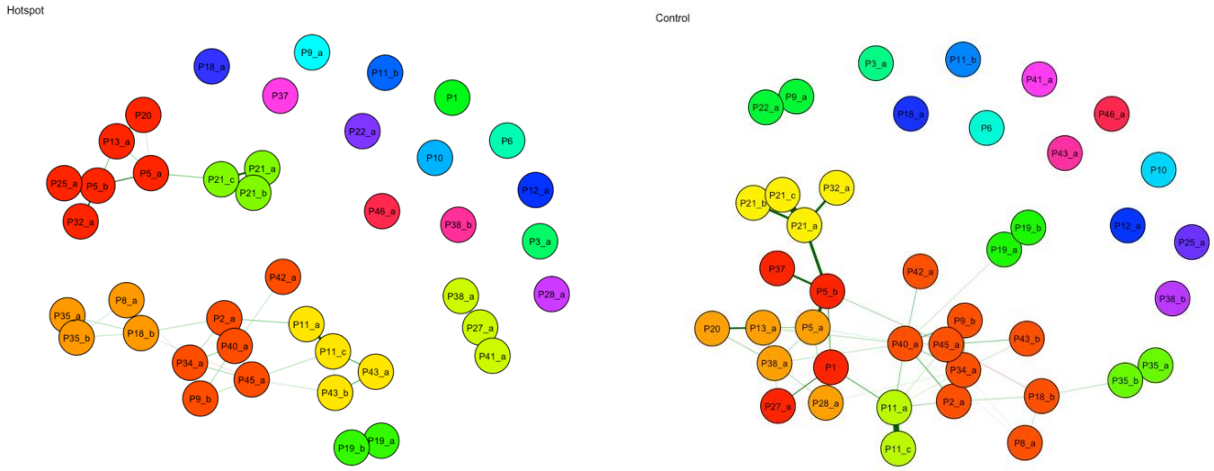

Figure 6.2: Weighted correlation network based on binary data of all five SPECIMEn countries, stratified by location (hotspot on the left, control on the right), compounds of levels 1 and 2b are included. N=41

In figure 6.3 the networks for both seasons are presented, based on 38 compounds (11_b, 12_a and 25_a were left out the networks since they were not detected in one of the two seasons). The network in summer had a higher connectivity with the largest connected set of compounds compared to winter, which confirms the expectations.

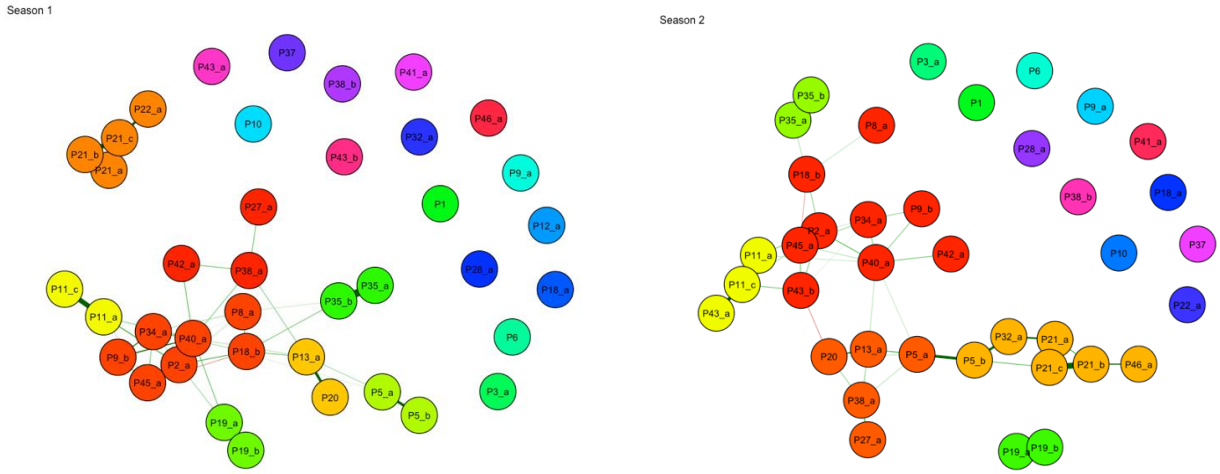

Figure 6.3: Weighted correlation network based on binary data of all five SPECIMEn countries, stratified by season (season 1 on the left, season 2 on the right), compounds of levels 1 and 2b are included. N=38

Lastly, in Figure 6.4, networks for both adults and children are presented based on 40 compounds (12_a was deleted since it was not detected within adults). The network of the children showed a higher connectivity.

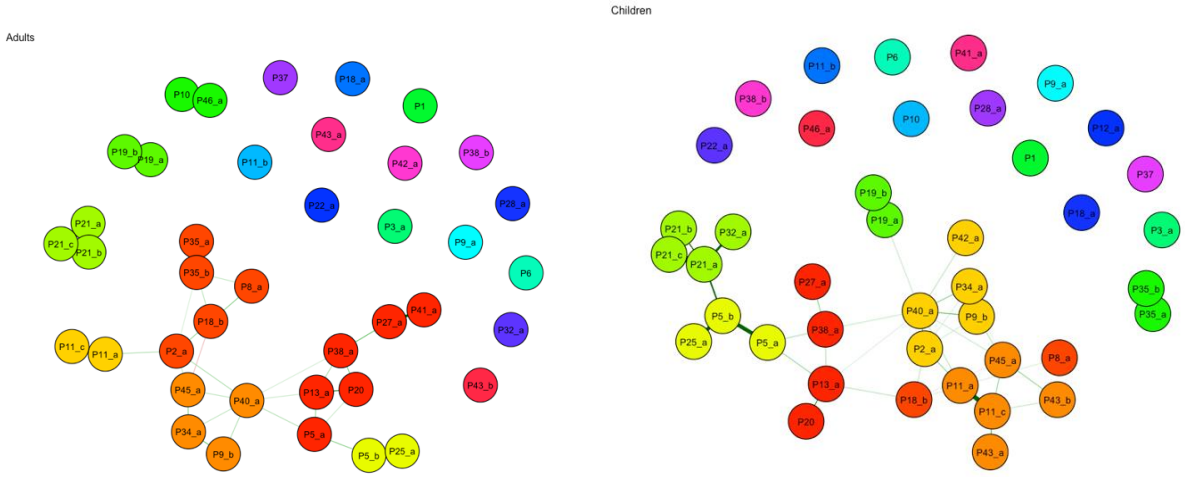

Figure 6.4: Weighted correlation network based on binary data of all five SPECIMEn countries, stratified by age (adults on the left, children on the right), compounds of ID levels 1 and 2b are included. N=40

6.4.2 Quantitative correlation networks

Based on the quantitative intensity data, weighted correlation networks have been applied within each country. Due to low detection rates, networks could only be estimated for Spain and the Netherlands.

The weighted correlation network for Spain (Annex 4) showed no clear indication of a connected network with 3 out of 5 compounds not connected (acetamiprid, chlorpropham, and triclosan). Two compounds were somewhat correlated (pirimiphos-methyl and tebuconazole), however, the Pearson correlation between these markers was rather low (0.17).

The weighted correlation network for the Netherlands (Annex 4) showed also no clear indication of a network with 3 out of 5 compounds not connected to any others (acetamiprid, pirimiphos-methyl, and propamocarb). The remaining two compounds (chlorpropham and flonicamid) were slightly correlated, but the Pearson correlation between them was low (0.21).

7 Discussion

7.1 Main findings

- A harmonised sample urine collection protocol was successfully conducted in five different countries. Within each country except Switzerland, sample collection was balanced between different locations, seasons, and adults *and* children.
- With this work we present the application of a novel suspect screening approach harmonised between different laboratories, resulting, at the time of this report, in the detection of 41 pesticide-related compounds related to 30 parent compounds.
- Across all countries, acetamiprid, chlorpropham, and triclosan were most frequently detected. Boscalid, fludioxonil, pirimiphos-methyl, and pyrimethanil were detected at frequencies above 10% in all countries except Hungary. Clothianidin, fluazifop, and propamocarb were detected at frequencies above 10% in all countries except Latvia. Finally, the markers for cyprodinil, flonicamid, and tebuconazole had detection rates > 10 % in at least three countries.
- In Switzerland a similar pattern of detection frequencies was detected compared to the SPECIMEn countries.
- Differences in detection rates are observed in hotspot *versus* control areas, in many cases not significant. The significant differences were not consistent across countries.
- In the samples collected in Hungary, generally higher detection rates were observed in summer, while in samples from Spain highest detection rates were generally found in winter. The other countries showed no consistent pattern.
- For most of the markers, the highest detection rates were observed in samples collected from children, suggesting a different exposure and/or elimination pattern between adults and children.
- The applied suspect screening methodology allows for a relatively cost-effective way of providing semi-quantitative measurements of a large number of pesticides.
- With the application of the SPECIMEn study, new data was collected in the participating countries. Some countries did not collect any prior data related to the exposure of multiple pesticides, making this study specifically useful for national governments and regulators. For example, in Latvia, where currently discussions are held in the parliament in relation to the minimum distance between agricultural areas and residential areas.

7.2 Strengths & limitations

The SPECIMEn study is a good example of the type of Pan-European studies that can be conducted utilising the infrastructure that was developed within the HBM4EU project. Through close collaboration with researchers in all five countries that participated in the SPECIMEn we were able to develop a harmonised protocol for sample collection and a harmonised questionnaire that was applied by all centres. As such the SPECIMEn has provided a first look at the possibility of pan-European application of suspect screening methods to detect pesticide exposures in various European regions.

The collection of biological material (urine) for the SPECIMEn required a minimally invasive protocol, reducing as much as possible the burden of citizens participating in the study. This opens possibilities for scaling-up of studies similar to SPECIMEn in future endeavours. The application of novel suspect screening methods allowed for the detection of a large number of pesticides and their metabolites. As such, the SPECIMEn should be seen as the first step towards a more complete assessment of the pesticide mixtures that the general population is exposed to. Using the

suspect screening data, we were able to provide clues towards the co-occurrence from potential co-exposure to some of the detected compounds. Further in-depth screening of the collected data and further methodological developments will increase the number of compounds that can be detected in the collected urine samples.

This allows an increasingly more complete coverage of all pesticides that are present in these samples as well as the detection of other compounds that might potentially interact with the pesticide-related mixture. Important steps were made towards the harmonisation of suspect screening data across the labs participating in the SPECIMEn. Such harmonisation is crucial to be able to compare suspect screening data and results coming from different laboratories and countries, a situation that is often unavoidable in large-scale European efforts. The questionnaire data collected in the SPECIMEn provides opportunities to further understand patterns observed in the collected data such as aspects that might contribute to differences in pesticide exposures, such as dietary patterns and occupational exposures. The SPECIMEn study also provides a first view of how network methods as developed within HBM4EU WP15 can yield relevant information with regards to correlation structures in the suspect screening data, providing insight into the patterns in pesticide co-exposure and potential co-exposure to other chemicals. With the development of more high-resolution quantitative methods, network methods can be applied to further scrutinise differences in correlation structures between relevant predictors of pesticide exposure.

Even though the SPECIMEn study has yielded many new insights and perspectives on approaching the difficult issue of studying and interpreting the exposure to pesticide mixtures, several limitations of our study need to be addressed. While unavoidable in a setting where one of the aims is cross-European collaboration and regardless of the significant efforts for cross-laboratory harmonisation, analysis of the SPECIMEn samples in five different labs has introduced variability in the type of information that was available for each region.

Also, most of the labs analysed the samples of each season separately, potentially introducing some variability which could affect the comparison of the two seasons. For the comparisons between countries, it is foreseeable the considerable differences that were observed in detection rates are partly due to differences in detection capabilities between the laboratories (although the same technology was used, different versions of instruments were used). Importantly, data generated by the suspect screening approach applied in the SPECIMEn can currently not be related to urine concentration levels. The semi-quantitative primary principle and objective of suspect screening, supported by the QA/QC provision established for ensuring data consistency, only allows for comparisons of data within each country. In the current state, the comparison of data between the different countries remains however limited by the lack of an appropriate data normalisation process that would require additional time and resources for being elaborated and applied. Several activities within and outside HBM4EU are working on further improvement in standardisation and harmonisation of SS data between laboratories and the interpretability of these data are therefore expected to improve in the coming years.

Annotation of the features detected in suspect screening is another remaining challenge in the interpretation of the SPECIMEn results. Full (level 1/2) identification of all features detected in the SS approach requires considerable follow-up work, which was only partially done as it was outside the scope of the current project. While features annotated with a confidence level of 3, 4, or 5 potentially reveal important information, for the current application we restricted ourselves to the features that were identified with higher confidence levels. The currently applied suspect screening approach focuses on elemental compositions with F, Cl, Br, and PO3- and a few other pesticide metabolites. Therefore, still a significant number of detected signals remains unevaluated at this stage regarding their identity, for which additional data processing and confirmatory work would be necessary. For example, the metabolites DCCA and DBCA-glucoronide are both level 4 because they only have 2Cl and 2Br isotope patterns, however they strongly correlate with free DCCA and

DBCA in quantitative measurements, suggesting a higher level of confidence than just following the Schymanski scoring. Considering the possible time frame, confirmatory MS2 acquisitions were only performed on data from two countries. This acquisition of qualitative sufficient MS2 information was also limited by the low signal intensities observed for most of the detected metabolites.

With regards to the sample collection, it must be noted that the samples from season 2 were collected during the COVID-pandemic while the samples from season 1 were not. Activity patterns or diet of participants might have been altered, and differences between seasons should be interpreted with care. Within SPECIMEn only morning void urines were collected. Due to the rapid excretion of many pesticides, the detected pesticides in the morning voids likely does not reflect the total daily exposure (for which 24h urine samples would be required). The timing of sample collection was defined in a standardised manner, by season and by location.

However, these aspects might vary between pesticides and countries. The crop types between countries vary due to differences in climate and culture, and across the countries, different pesticides could have been applied at the time of sampling. For future work, it is recommended to include local information on crop types and spraying application per pesticide (when available). Next, the definition of the spraying and non-spraying season was also somewhat arbitrary and varied between countries depending on local practice and it is not applicable for all pesticides. It could be that some pesticides were not detected due to the design of our study, e.g. when they were applied in a different season. The study was designed before we knew which pesticides could be detected by the suspect screening approach, so therefore this information could not be taken into account. Also, with the large list of parent pesticides, it would not have been possible to take the actual spraying season and location of all pesticides into account by the design of the study.

The detected differences by location and season are most likely influenced by a set of other covariates such as diet or usage of pesticide containing products. The dietary habits of participants will differ between the countries, locations within countries, seasons. Also, there will be differences in percentage of imported foods. For example, based on the questionnaire data, we saw that participants from the hotspot area more often consumed homegrown foods. The observed difference between adults and children is typically related to exposures from food consumption, since children have larger ratio of food intake per kg of bodyweight; also biological elimination mechanisms may differ between children and adults. As a more specific example, the more frequently detected metabolite of imazalil during winter could be due to differences in (imported) food consumption between the seasons. Also, based on the network analyses, a correlation between thiabendazole and imazalil was observed, both compounds are typically used to increase shelf life of citrus fruits. Next, with the current interpretation based on detection frequencies, we did not take into account legal allowance differences between countries, and it must be noted that some pesticides (chlorpropham, chlorpyrifos, chlothianidin, imidacloprid, thiamethoxam and thiacloprid) were banned since 2019 or 2020 (with grace periods). Also, triclosan is currently not a registered pesticide but a biocide and only registered for use in cosmetics.

The exploration of the results presented in this report compares regions, seasons, and age groups. The current timeframe, burdened by pandemic-related delays, did not allow for more in-depth analysis, nor for the inclusion of questionnaire data and the application of paired analysis of adult-child samples and more advanced (multivariate) statistical analysis.

7.3 Recommendations and future steps

- This Pan-European study is a first step in addressing pesticide mixture exposure under real-life conditions. It demonstrates that further development of methods and broader application and expansion of scope of similar studies is warranted to better assess pesticide mixture exposures in the European population.
- The annotation and confirmation procedures of the pesticide metabolites should be further extended to allow for the detection of more compounds, as well as for the detection at higher levels of confidence of the currently detected compounds.
- The applied suspect screening method provides knowledge on the occurrence of pesticides, which can serve as guidance for the prioritisation of substances that should be subjected to targeted methods. Further quantification of the pesticides detected through targeted analysis is recommended to allow for more detailed exposure assessments.
- The current detection rates of the urinary metabolites should be compared with detection rates in food based on data from EFSA, national pesticide monitoring programmes and the food frequency questionnaire completed by the participants. Furthermore, current pesticide modelling results should be compared to the actually observed pesticide-related markers.
- The Horizon Europe ‘Partnership for the Assessment of Risks from Chemicals’ (PARC) brings together EU Member States, the European Commission and EU agencies relevant for chemical risk assessment. This unique opportunity for close collaborations across Europe should be exploited, to strengthen and expand the work on suspect screening analyses for regulatory purposes that was initiated in HBM4EU.

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9 Annex

Annex 1: Questionnaire (questions of the second visit are marked in blue)

QUESTIONNAIRE HBM4EU-SPECIMEn study

QUESTIONNAIRE INFORMATION

ID (PARTICIPANT ADULT)	|__|__|__|__|__|__|__|
ID (PARTICIPANT CHILD)	|__|__|__|__|__|__|__|
ID (INTERVIEWER)	|__|__|__|__|__|__|__|
DATE OF THE INTERVIEW	|__|__| |__|__| |__|__|__|__|
PLACE	

This questionnaire has to be completed by the ADULT (parent) on the day you collected your urine sample.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032.

About this questionnaire

Dear participant,

Thank you for your agreement to be part of our study, for both yourself and your child. The HBM4EU Survey on PEstiCIde Mixtures in Europe (HBM4EU-SPECIMEn study) is assessing levels of multiple pesticides in urine samples of adults and children in five European countries. This questionnaire aims to collect background information together with the measurements conducted the urine samples you will collect. This questionnaire contains questions regarding your personal characteristics, living situation, lifestyle and diet.

The parent/caretaker is asked to complete this questionnaire for the child as well, no separate questionnaire has to be filled in for the child. You are asked to complete this questionnaire on the day you collected your urine sample. During the collection of the urine samples by the fieldworker, the questionnaire will be collected as well. Any remarks or questions can be addressed to the fieldworker during the visit.

This questionnaire might refer to:

- The past 3 days: These are the 72 hours before you collected your urine sample. For example if urine is collected on Wednesday, the past three days are Sunday till Tuesday.
- The past 24 hours: This is the day before you collected your urine sample.

Example questions:

1. Have you (ADULT) been in contact with pesticides and/or insecticides professionally during the past month?

Yes ☒

No ☐

Don't know ☐

2. Please indicate the portion of your diet which is homegrown?

Winter	Spring	Summer	Autumn
|_|_|0| %	|1|0| %	|2|5| %	|4|0| %
Don't know ☐	Don't know ☐	Don't know ☐	Don't know ☐

Instructions for filling in this questionnaire:

- Please complete this questionnaire with a black or blue pen; not with a marker.
- Please fill in the open questions with block letters.
- Take note of the *cursive text*, this is some explanatory text.
- If it happens that you make a mistake, please check the box with the correct answer and circle the correct box. Do not use any correction fluid or Tipp-Ex.
- If you want to clarify anything, you can write this next to the question.

In case something is unclear or if you might need help with the completion of the questionnaire, you can contact the research assistant through

XXXXXXXXXXXXXXXXXXXXXXXXXXX.

PERSONAL INFORMATION & HEALTH

ADULT

Born on: | ___ | ___ | month | ___ | ___ | ___| ___| year

Sex: Male ☐ Female ☐ Other ☐

CHILD

Born on: | ___ | ___ | month | ___ | ___ | ___| ___| year

Sex: Male ☐ Female ☐ Other ☐

What is the relation of the adult to the child?

Parent (mother/father) ☐ Caretaker ☐ Other ☐

1. Measurements ADULT	
1.1 How tall are you without shoes (in cm)?	| ___ | ___ | ___| cm
1.2. How much do you weigh (in kg)?	| ___ | ___ | ___| kg

2. Weight change ADULT	**Yes**	**No**	**Don't know**
2.1. Have you lost weight in the past year? Specify how much your weight has changed (in kg) | ___ | ___ | ___| kg	☐	☐	☐
2.2. Have you gained weight in the past year? Specify how much your weight has changed (in kg) | ___ | ___ | ___| kg	☐	☐	☐

3. Measurements CHILD	
3.1 How tall is your child without shoes (in cm)?	| ___ | ___ | ___| cm
3.2. How much does your child weigh (in kg)?	| ___ | ___ | ___| kg

SOCIODEMOGRAPHIC INFORMATION (part A)

A1. Where were you (ADULT) and your partner (only when living in the same household) born? (include name of country)

	In (Country)	If no, specify country
You (ADULT)	Yes ☐ No ☐ Don't know ☐	.....
Partner (when living in the same household)	Yes ☐ No ☐ Don't know / NA ☐	.....

A2. How long have you (ADULT) been living in…?

Please indicate the number of years (or months if less than 1 year)

This municipality	| ___ | ___ | Years | ___ | ___ | Months
Current address	| ___ | ___ | Years | ___ | ___ | Months

A3. What is the highest level of education you (ADULT) attained? (wording might need to be adapted to the national education system)

1. No education or only primary education	☐
2. Secondary education	☐
3. Tertiary education (post-secondary)	☐
4. University (BSc, MSc, PhD)	☐
5. Don't know	☐

A4. What is your (ADULT) current main labour status?

1. Employee working full-time	☐	8. Permanently disabled or/and unfit to work	☐
2. Employee working part-time	☐	9. In compulsory military community or service	☐
3. Self-employed working full-time (including family worker)	☐	10. Fulfilling domestic tasks and care responsibilities (including parental leave)	☐
4. Self-employed working part-time (including family worker)	☐	11. Other inactive person	☐
5. Unemployed	☐	12. Other status Specify……	☐
6. Pupil, student, further training, unpaid work experience	☐	13. Don't know	☐
7. In retirement or in early retirement	☐		

A5. Please describe your current professional activity/job(s) in your own words.

A6. The other member(s) of the household are persons not providing urine samples for this study.

Could you please indicate the amount of other household members by checking the boxes in the first column.

Please indicate for each other household member the age, gender, and if that person comes in contact with pesticides professionally.

Having contact with pesticides professionally means for example working at a farm (spraying activities or colleagues who spray pesticides).

Member	Age	Gender *(Female / Male/ Other)*	Professional contact with pesticides *Leave open when No/Unknown* **Yes**
No.1 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.2 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.3 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.4 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.5 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.6 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.7 ☐	| ___ | ___ |___ | Years	F / M / O	☐
No.8 ☐	| ___ | ___ |___ | Years	F / M / O	☐

A7. Could you provide the approximate range of your total household income? (It is referred to annual gross incomes from all members of your household) (Indicated by each country, will be equalized based on family size/age)

Total household income	
No.1	☐
No.2	☐
No.3	☐
No.4	☐
Don’t know	☐

RESIDENTIAL ENVIRONMENT AND HOME EXPOSURES (part B)

B1. Which of the following options best describes your home…?

Please check the box that describes your home the best.

1. Detached house	☐
2. Semi-detached house	☐
3. Townhouse	☐
4. A flat/apartment 4.1. Specify floor number……	☐
5. A farmhouse	☐
6. Other (e.g. caravan, mobile home) 6.1.Specify ……	☐
7. Don't know	☐

B2. Do you know when your home was built?

1. Before 1949	☐	4. 1998-2008	☐
2. 1950-1981	☐	5. After 2008	☐
3. 1982-1997	☐	6. Don't know	☐

B3. Approximately, what is the living surface (in m^2) of your home? | ___ | ___ | ___ |___ | **m^2** Don't know ☐

B5. Does your house have a garden and/or kitchen garden?

Please indicate if there are any vegetables, herbs or fruits for consumption in your garden, and if so, if they are consumed by your own family or sold/given to others (small scale production).

	Yes
1. No garden ***(go to question B7)***	☐
2. Garden, but without any vegetables, herbs or fruits for consumption	☐
3. Garden with vegetables, herbs or fruits for consumption for my own family	☐
4. Garden with vegetables, herbs or fruits for consumption for others *This includes small scale sale, or donations.*	☐
5. Garden with vegetables, herbs or fruit for consumption for my own family and for others	☐

B6. During the past 3 days did you use any type of products for treating the plants in your garden?

Products can be e.g. pesticides, insecticides, bio-products. Think of all sprays, liquids, tablets etc.

Yes ☐

If Yes, Please specify commercial name(s) and briefly describe for what it was used for ***(if you don't know the commercial name, please specify the purpose)***

No ☐

Don't know ☐

B7. During the past 3 days did you use any type of products for treating the plants inside your home?

Products can be e.g. pesticides, insecticides, bio-products. Think of all sprays, liquids, tablets etc.

Yes ☐

If Yes, Please specify commercial name(s) and briefly describe for what it was used for ***(if you don't know the commercial name, please specify the purpose)***

No ☐

Don't know ☐

B8. Did you have any contact with animals or pets during the past 3 days (at home [your pet] or somewhere else)?

If yes, specify the animal species and amount of animals you have been in contact with.

	Yes
1. No contact with any animal	☐
2. Dog	☐ Amount: |__|__|__|
3. Cat	☐ Amount: |__|__|__|
4. Bird	☐ Amount: |__|__|__|
5. Other animal Specify……	☐ Amount: |__|__|__|
6. Other animal Specify……	☐ Amount: |__|__|__|

B8.1. In the past 3 days, were any of the following products used for your pets, or have been in use?

Product	Yes	No	Don't know
1. Pets grooming products (e.g.shampoos, conditioners, lotions, sprays…)	☐	☐	☐
2. External antiparasitic treatments (e.g. lotions, sprays, necklace, collar…)	☐	☐	☐
4. Other pet products Specify…….....	☐	☐	☐

PERSONAL EXPOSURES (part C)

C1. Did you use insect repellents or anti-parasite products for human use, including lotions, sprays, shampoos etc. in the past 3 days?

Yes ☐

If Yes, Please specify commercial name(s) and briefly describe for what you used it *(if you don't know the commercial name, please specify the purpose)*

No ☐

Don't know ☐

C2. Did you use any medicines/drugs during the past 3 days?

Yes ☐

If Yes, Please specify name(s) of the medicines/drugs *(if you don't know the commercial name, please specify the purpose)*

No ☐

Don’t know ☐

LIFESTYLE & ACTIVITY (part D)

D1. In relation to smoking, which of the following options best describes your situation?

	Yes	No
D1. 1 I have never smoked (Go to question 2)	☐	☐
D1.2 I was a smoker but I gave up smoking	☐	☐
D1.3. I currently smoke D1.3.1 Type/Variety and average of consumption **a. Cigarretes** ☐ No. per week |__|__|__| ☐ Don't know **b. Pipes** ☐ No. per week |__|__|__| ☐ Don't know **c. Cigars** ☐ No. per week |__|__|__| ☐ Don't know **d. Electronic cigarretes** ☐ No. per week |__|__|__| ☐ Don't know **e. Others** ☐ No. per week |__|__|__| ☐ Don't know Specify……	☐	☐

D2. Does anyone else (not yourself) smoke inside the house? *Please indicate how often.*

Never	Rarely (<1/month)	Sometimes (<1/week)	Once a week	2-3 times/week	4-6 times/week	Don't know
☐	☐	☐	☐	☐	☐	☐

D3. Which of the following best describes your (ADULT) current physical exercise? Please do not take into account your physical activity at work

Please check the box that best describes your situation.

1. Never do physical activity	☐
2. Light physical exercise for relaxation fewer than three times a week	☐
3. Medium and intensive physical exercise fewer than three times a week	☐

4. Intensive physical exercise at least three times a week for 10 minutes or more	☐
5. Daily intensive exercise over 30 minutes a day	☐
6. Don't know	☐

D4. How much time on average did you (ADULT) spend in the following places (the 3 days prior to the urine sample collection)? (Day 0 is the day of urine collection)

Please indicate the approximate time, for example 8 hours at work, 10 hours at home, 1 hour 30 minutes in the car etc.

Day -3 is three days before the urine collection. Day -2 is two days before urine collection, day -1 is one day before urine collection.

Example: Urine will be collected on Wednesday. Day -3 is on Sunday (3 days before collection), Day -2 is on Monday, Day -1 is on Tuesday.

	Day -3	Day -2	Day -1
1. Inside your home	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
2. Inside other houses	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
3. In other indoor spaces(e.g. at workplace, shopping centre, sports club, cinema,restaurant...)	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
4. In your car	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
5. In other closed vehicles for daily commuting (e.g. bus, car, train...)	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
6. Outdoor traffic (on foot, bicycle, motorbike, skating, at train stations or bus stops...)	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
7. Outdoors, at home (garden, balcony...)	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know
7. Outdoors, away from home (park, garden, forest, beach, outdoor sports area...)	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know	|__|__| hours |__|__| minutes ☐ Don't know

D5. Did you (ADULT) carry out any of the following activities as DIY activities or hobbies or did you use any of the substances below in the past 3 days?

Please, do not count your professional activity/work

DIY ACTIVITIES and HOBBIES	Yes	No	Don't know
Agricultural labour (outdoors)	☐	☐	☐
Agricultural labour (greenhouses)	☐	☐	☐
Applying pesticides	☐	☐	☐
Formulation of pesticides	☐	☐	☐
Gardening (outdoors)	☐	☐	☐
Gardening (greenhouses)	☐	☐	☐
Collaborating in fruit/vegetable warehouse	☐	☐	☐
Use of compost or sewage sludge (as fertilizer)	☐	☐	☐
Use of phosphate fertilizers	☐	☐	☐
Other activities involving using or handling pesticides	☐	☐	☐

D6. Have you (ADULT) been in contact with pesticides and/or insecticides professionally during the past month?

This question is about professional usage or contact at your work.

Yes ☐

Specify product name(s) and describe the activity	
Name:	Activity:
Name:	Activity:
Name:	Activity:
Name:	Activity:

No ☐

Don’t know ☐

DIET (part E)

E1. Where do you (ADULT) usually buy vegetables and fruits?

1. Supermarkets (including large superstores)	☐
2. Local shops, convenience stores	☐
3. Local farmers	☐
4. Food markets	☐
5. Others, specify: ……..	☐
6. I do not buy any vegetables and fruits *(e.g. only consuming from your own garden)*	☐
7. Don't know	☐

E2. Did you (ADULT) consume organic food in the last 6 months?

Yes ☐

No ☐ ==> Go to question E3

Don't know ☐ ==> Go to question E3

E2.1 How often did you usually consume organic food in the last 6 months?

<1 per month	1-3 per month	1 per week	2-6 per week	Daily	Don't know
☐	☐	☐	☐	☐	☐

E2.2 Which percentage of your (ADULT) diet is based on organic food in the last 6 months? Indicate a percentage for each of the following food items *(0%= nothing organic and 100%= all the food consumed is organic)*

Vegetables and fruits	Bread	Meat	Eggs	Dairy products	Rice, pasta and other cereals	Other foods Specify……
|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|
No consumption ☐	No consumption ☐	No consumption ☐	No consumption ☐	No consumption ☐	No consumption ☐	No consumption ☐
Don't know ☐	Don't know ☐	Don't know ☐	Don't know ☐	Don't know ☐	Don't know ☐	Don't know ☐

E3. **Did you (ADULT) eat homegrown vegetables, fruit and/or herbs in the last 6 months? If yes, indicate per season the portion you have eaten homegrown products** ***(0%= nothing and 100%= all fruit/vegetable is homegrown)***

Winter	Spring	Summer	Autumn
|__|__|__|	|__|__|__|	|__|__|__|	|__|__|__|
Don't know ☐	Don't know ☐	Don't know ☐	Don't know ☐

E4. **Please indicate which of the following food items you (ADULT) have eaten during the 24 hours (1 day) <u>before urine collection.</u>** ***See the pictures for serving sizes.***

Food item	Not eaten	Eaten during the past 24h	Amount of servings *For the serving sizes, please refer to the pictures.*	Homegrown? Yes	Biological? Yes
I. DAIRY PRODUCTS AND EGGS					
Butter (1 serving = 10 g)	☐	☐	Servings	☐	☐
Milk (1 serving = 200 ml)	☐	☐	Servings	☐	☐
Fresh Cheese (1 serving = 2 units)	☐	☐	Servings	☐	☐
Aged Cheese (1 serving = 2 units)	☐	☐	Servings	☐	☐
Yoghurt (1 serving = 125g)	☐	☐	Servings	☐	☐

Food item	Not eaten	Eaten during the past 24h	Amount of servings *For the serving sizes, please refer to the pictures.*	Homegrown? Yes	Biological? Yes
Eggs (1 serving = 2 eggs)	☐	☐	Servings	☐	☐
II. CEREALS & POTATOES					
White bread (1 serving = 2 units, 100g)	☐	☐	Servings	☐	☐
Whole grain bread (1 serving = 2 units, 100g)	☐	☐	Servings	☐	☐
Cereal products (crackers, rusk…) (1 serving = 50g)	☐	☐	Servings	☐	☐
Barley (1 serving = 2 hands)	☐	☐	Servings	☐	☐
Oats (1 serving = 2 hands)	☐	☐	Servings	☐	☐
Bran (1 serving = 2 hands)	☐	☐	Servings	☐	☐
Other cereals **…….**	☐	☐	Servings	☐	☐
Pasta (all kinds) (1 serving = 75g)	☐	☐	Servings	☐	☐
Rice (all kinds) (1 serving = halve cup)	☐	☐	Servings	☐	☐
Potatoes (boiled/baked)	☐	☐	Servings	☐	☐
French fries/chips (1 serving = 100 g)	☐	☐	Servings	☐	☐
III. VEGETABLES					
Carrots (1 serving = 2 units, 200g)	☐	☐	Servings	☐	☐
Fresh tomatoes (1 serving = 2 units, 200g)	☐	☐	Servings	☐	☐
Leafy vegetables (1 serving = handful)	☐	☐	Servings	☐	☐
Eggplant, courgette	☐	☐	Servings	☐	☐
Pepper	☐	☐	Servings	☐	☐

Food item	Not eaten	Eaten during the past 24h	Amount of servings *For the serving sizes, please refer to the pictures.*	Homegrown? Yes	Biological? Yes
Asparagus	☐	☐	Servings	☐	☐
Broccoli (1 serving = 1/3 unit, 200g)	☐	☐	Servings	☐	☐
Green beans (1 serving = 10-15 units, 200g)	☐	☐	Servings	☐	☐
Other beans	☐	☐	Servings	☐	☐
Mushrooms (1 serving = 8 units, 200g)	☐	☐	Servings	☐	☐
Onions (1 serving = 1 unit, 200 g)	☐	☐	Servings	☐	☐
Garlic	☐	☐	Servings	☐	☐
Corn on the cob	☐	☐	Servings	☐	☐
Soybeans	☐	☐	Servings	☐	☐
Sunflower seeds	☐	☐	Servings	☐	☐
Basil (a hint)	☐	☐	Hints	☐	☐
Black pepper(a hint)	☐	☐	Hints	☐	☐
Tinned products (vegetables, legumes, cereals) (1 serving = 1 can)	☐	☐	Servings	☐	☐
IV. FRUITS					
Orange (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Banana (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Apple (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Pear (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Peach, apricot (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Melon, watermelon (1 serving = 1 slice)	☐	☐	Servings	☐	☐

Food item	Not eaten	Eaten during the past 24h	Amount of servings *For the serving sizes, please refer to the pictures.*	Homegrown? Yes	Biological? Yes
Grapes (1 serving = 1 hand, 200g)	☐	☐	Servings	☐	☐
Prunes	☐	☐	Servings	☐	☐
Kiwi (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Strawberries (1 serving =5-7units, 200g)	☐	☐	Servings	☐	☐
Pineapple (1 serving = 1 slice)	☐	☐	Servings	☐	☐
Dried fruits	☐	☐	Servings	☐	☐
Orange juice (fresh) (1 serving = 1 glass)	☐	☐	Servings	☐	☐
Other fresh fruit juices (1 serving = 1 glass)	☐	☐	Servings	☐	☐
V. OTHER FOODS					
Superfoods (chia seeds, quinoa etc)	☐	☐	Servings	☐	☐
Other foods you might think contain pesticides: ……	☐	☐	Servings	☐	☐
Other foods you might think contain pesticides: ……	☐	☐	Servings	☐	☐

E5. Please indicate if the diet of your CHILD differs from your own diet.

Yes ☐ ==> Go to question E6

No ☐

Don't know ☐

E6. Please indicate which of the following food items your CHILD has eaten during the 24 hours (1 day) before urine collection.
See the pictures for serving sizes.

Food item	Not eaten	Eaten during the past 24h	Amount	Homegrown? Yes	Biological? Yes
I. DAIRY PRODUCTS AND EGGS					
Butter (1 serving = 10 g)	☐	☐	Servings	☐	☐
Milk (1 serving = 200 ml)	☐	☐	Servings	☐	☐
Fresh Cheese (1 serving = 2 units)	☐	☐	Servings	☐	☐
Aged Cheese (1 serving = 2 units)	☐	☐	Servings	☐	☐
Yoghurt (1 serving = 125g)	☐	☐	Servings	☐	☐
Eggs (1 serving = 2 eggs)	☐	☐	Servings	☐	☐
II. CEREALS & POTATOES					
White bread (1 serving = 2 units, 100g)	☐	☐	Servings	☐	☐
Whole grain bread (1 serving = 2 units, 100g)	☐	☐	Servings	☐	☐
Cereal products (crackers, rusk…) (1 serving = 50g)	☐	☐	Servings	☐	☐
Barley (1 serving = 2 hands)	☐	☐	Servings	☐	☐
Oats (1 serving = 2 hands)	☐	☐	Servings	☐	☐
Bran (1 serving = 2 hands)	☐	☐	Servings	☐	☐
Other cereals ……	☐	☐	Servings	☐	☐
Pasta (all kinds) (1 serving = 75g)	☐	☐	Servings	☐	☐
Rice (all kinds) (1 serving = halve cup)	☐	☐	Servings	☐	☐
Potatoes (boiled/baked)	☐	☐	Servings	☐	☐

Food item	Not eaten	Eaten during the past 24h	Amount	Homegrown? Yes	Biological? Yes
French fries/chips (1 serving = 100 g)	☐	☐	Servings	☐	☐
III. VEGETABLES					
Carrots (1 serving = 2 units, 200g)	☐	☐	Servings	☐	☐
Fresh tomatoes (1 serving = 2 units, 200g)	☐	☐	Servings	☐	☐
Leafy vegetables (1 serving = handful)	☐	☐	Servings	☐	☐
Eggplant, courgette	☐	☐	Servings	☐	☐
Pepper	☐	☐	Servings	☐	☐
Asparagus	☐	☐	Servings	☐	☐
Broccoli (1 serving = 1/3 unit, 200g)	☐	☐	Servings	☐	☐
Green beans (1 serving = 10-15 units, 200g)	☐	☐	Servings	☐	☐
Other beans	☐	☐	Servings	☐	☐
Mushrooms (1 serving = 8 units, 200g)	☐	☐	Servings	☐	☐
Onions (1 serving = 1 unit, 200 g)	☐	☐	Servings	☐	☐
Garlic	☐	☐	Servings	☐	☐
Corn on the cob	☐	☐	Servings	☐	☐
Soybeans	☐	☐	Servings	☐	☐
Sunflower seeds	☐	☐	Servings	☐	☐
Basil (a hint)	☐	☐	Hints	☐	☐
Black pepper(a hint)	☐	☐	Hints	☐	☐
Tinned products (vegetables, legumes, cereals) (1 serving = 1 can)	☐	☐	Servings	☐	☐
IV. FRUITS					

Food item	Not eaten	Eaten during the past 24h	Amount	Homegrown? Yes	Biological? Yes
Orange (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Banana (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Apple (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Pear (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Peach, apricot (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Melon, watermelon (1 serving = 1 slice)	☐	☐	Servings	☐	☐
Grapes (1 serving = 1 hand, 200g)	☐	☐	Servings	☐	☐
Prunes	☐	☐	Servings	☐	☐
Kiwi (1 serving = 1 unit)	☐	☐	Servings	☐	☐
Strawberries (1 serving =5-7units, 200g)	☐	☐	Servings	☐	☐
Pineapple (1 serving = 1 slice)	☐	☐	Servings	☐	☐
Dried fruits	☐	☐	Servings	☐	☐
Orange juice (fresh) (1 serving = 1 glass)	☐	☐	Servings	☐	☐
Other fresh fruit juices (1 serving = 1 glass)	☐	☐	Servings	☐	☐
V. OTHER FOODS					
Superfoods (chia seeds, quinoa etc)	☐	☐	Servings	☐	☐
Other foods you might think contain pesticides: ……	☐	☐	Servings	☐	☐
Other foods you might think contain pesticides: ……	☐	☐	Servings	☐	☐

Annex 2: Full Table of all annotations, including detection frequencies

ID	Pesticide type[15]	Parent pesticide	Metabolite found in urine	precursor ion	exact m/z	RT urine [min]	ID level	Overall Detection Frequency[16]				
								ES	LV	HU	CZ	NL
P1	H	2,4-D	Parent compound	[M-H]-	218.9623	9.93	1	4.07	0	2.2	2.71	0
P2_a	I	Acetamiprid	-CH2	[M-H]-	207.0443	8.71	1	98.56	32.84	94.13	98.19	93.29
P2_b			-CH2	[M+H]+	209.0589	8.55	4	81.82	10.95	45.23	41.18	47.00
P2_c			Parent compound	[M+H]+	223.0745	8.67	4	1.44	0	0.49	0	0.72
P3_a	F	Ametoctradin	-C2H6 +2O	[M+H]+	278.1612	9.47	1	5.02	2.74	1.22	4.75	2.88
P3_b			-C2H6 +2O	[M-H]-	276.1466	8.17	5	0.72	0.5	0.49	0.45	2.16
P4	I, Ac	Bifenthrin/Cyhalothrin	F3CCA + C6H8O6	[M-H]-	417.0570	11.95	4	40.43	3.23	7.09	3.62	13.91
P5_a	F	Boscalid	+O +SO3	[M-H]-	436.9771	10.26	2b	35.65	18.41	3.91	22.85	32.85
P5_b			+O +SO3	[M+H]+	438.9917	10.49	2b	7.18	0	0	0.45	0.24
P5_c			+O (M510F01)	[M-H]-	357.0203	11.89	4	0.48	0	0	0	0
P5_d			+O (M510F01)	[M+H]+	359.0349	11.69	4	0.48	0	0	0	0
P6	I	Chlorantraniliprole	+O	[M-H]-	497.9564	12.67	2b	3.83	0.25	0.24	0	0.24
P7_a	Ac	Chloropropylate	-C3H6	[M-H]-	294.9934	12.93	4	0	0	0	0.23	0
P7_b			-C3H6 -CO2	[M-H]-	251.0036	12.93	4	0.24	0	0	0	0
P8_a	H, GR	Chlorpropham	+O +SO3 (4-HSA)	[M-H]-	308.0003	9.5	1	55.74	31.59	31.05	34.16	75.06
P8_b			-C4H6O +SO3	[M-H]-	221.9633	6.19	3	29.19	32.09	21.03	28.05	63.07

[15] H: Herbicide, F: Fungicide, I: Insecticide, GR: Plant Growth Regulator, Ac: Acaricide, M: molluscide, Al: Algicide, Ab: antibacterial, Af: antifungal,
[16] <10% are shown in grey

ID	Pesticide type[15]	Parent pesticide	Metabolite found in urine	precursor ion	exact m/z	RT urine [min]	ID level	Overall Detection Frequency[16]				
								ES	LV	HU	CZ	NL
P8_c			+2O +SO3	[M-H]-	323.9950	7.5	3	7.66	6.97	9.78	9.28	26.86
P8_d			+O +C6H8O6	[M-H]-	404.0757	8.55	4	15.55	15.42	12.96	14.03	44.84
P8_e			+O	[M-H]-	228.0433	10.97	4	1.2	0	0.73	1.36	9.35
P9_a	I	Chlorpyrifos (/methyl)	TCPy	[M-H]-	195.9129	10.1	1	1.67	0	0.24	0.23	0.24
P9_b			-CH2	[M-H]-	305.8723	10.72	1	36.12	0	6.85	21.72	6.47
P9_c			TCPy+C6H8O6	[M-H]-	371.9450	8.38	4	50.00	0	2.69	13.35	7.19
P10	H	Clopyralid	Parent compound	[M-H]-	189.9465	3.5	1	0.96	0	0	1.36	0.72
P11_a	I	Clothianidin (can come from thiamethoxam)	Parent compound	[M-H]-	248.0015	8.09	1	34.45	1.74	21.52	24.66	19.42
P11_b			-NO2 +H	[M+H]+	205.0309	5.77	1	0.48	0	0.24	0	0.24
P11_c			-CH2	[M-H]-	233.9858	7.51	2b	21.05	0.75	9.78	6.56	3.12
P11_d			Parent compound	[M+H]+	250.0160	8.08	4	1.67	0	3.18	0	2.16
P12_a	I	Cypermethrin, cyfluthrin, permethrin, transfluthrin	DCCA	[M-H]-	206.9985	10.73	1	0.48	0	0	0	0
P12_b			DCCA+C6H8O6	[M-H]-	383.0306	10.95	4	84.93	9.2	14.67	25.34	48.20
P13_a	F	Cyprodinil	+O +SO3	[M-H]-	320.0710	11.87	2b	14.11	7.71	2.69	10.18	26.38
P13_b			+2O +SO3	[M-H]-	336.0660	9.22	3	9.09	4.98	1.71	7.47	22.78
P14	I	Deltamethrin	DBCA+C6H8O6	[M-H]-	470.9296	11.43	4	76.32	0.75	7.33	9.5	21.82
P15_a		Diuron	-CH2 -CH2	[M-H]-	202.9786	12	4	5.5	1	0.24	1.13	0.48

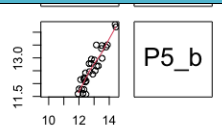
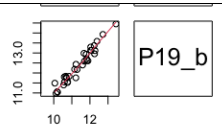
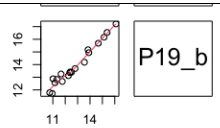
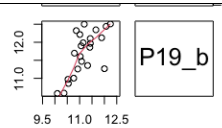
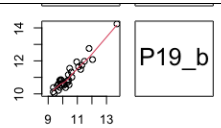
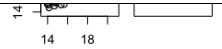
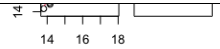
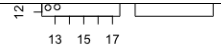
ID	Pesticide type[15]	Parent pesticide	Metabolite found in urine	precursor ion	exact m/z	RT urine [min]	ID level	Overall Detection Frequency[16]				
								ES	LV	HU	CZ	NL
P15_b	H, Al		-CH2	[M-H]-	216.9942	12.45	4	1.2	0.25	0	0	0
P15_c			-CH2	[M+H]+	219.0084	12.14	4	0.24	0	0	0	0
P16	F	Fenhexamid	+O +C6H8O6	[M+NH3]+	511.1244	9.34	3	0.96	1	1.22	2.49	6.71
P17_a	I, Ac	Fipronil	Parent compound	[M-H]-	434.9310	15.02	4	0.96	0	0	0	0
P17_b			+O	[M-H]-	450.9260	15.43	4	3.59	0.5	0	0	0
P18_a	I	Flonicamid	Parent compound	[M-H]-	228.0397	6.9	1	1.67	0.75	1.96	2.71	5.76
P18_b			-C2HN	[M+H]+	191.0427	6.1	2b	15.07	0.25	27.38	0.23	57.31
P18_c			Parent compound	[M+H]+	230.054	6.8	4	1.44	0	0.98	1.36	3.6
P19_a	H	Fluazifop	Parent compound	[M-H]-	326.0647	11.74	1	19.86	2.49	11.00	18.33	21.10
P19_b			Parent compound	[M+H]+	328.079	13.57	1	8.13	1.49	4.89	5.20	8.15
P20	F	Fludioxonil	+O +C6H8O6	[M-H]-	439.0609	11.81	2b	16.27	14.68	1.96	14.48	26.86
P21_a	F	Fluopyram	+O +SO3	[M-H]-	490.9908	12.68	2b	3.59	0.5	0.24	1.13	0.96
P21_b			+O +C6H8O6	[M+H]+	589.0807	13.08	2b	2.39	0.75	0.49	3.17	4.8
P21_c			-2H	[M+H]+	395.0385	13.07	2b	10.77	6.72	0.49	3.39	3.12
P22_a	I	Flupyradifurone	Parent compound	[M+H]+	289.0557	8.79	1	2.63	0.25	0.24	0.68	2.16
P22_b			-C2H2F2	[M+H]+	225.0425	7.54	4	1.67	0	0.24	0.23	3.12
P23	H	Fluroxypyr	Parent compound	[M+H]+	254.973	10.47	4	0.24	0	0	0	0
P24	F	Flutolanil	-C3H6 +O +SO3	[M-H]-	376.0108	8.18	3	14.11	0	4.65	0	0.24
P25_a	I, Ac	Fluvalinate	-C14H9NO	[M-H]-	294.0514	13.94	2b	0.96	0	0.73	0.23	0
P25_b			-C14H9NO +O	[M-H]-	310.0463	12.78	3	0.72	0	0.49	0	0

ID	Pesticide type[15]	Parent pesticide	Metabolite found in urine	precursor ion	exact m/z	RT urine [min]	ID level	Overall Detection Frequency[16]				
								ES	LV	HU	CZ	NL
P25_c			-C14H9NO	[M+H]+	296.066	14.35	4	0.96	0	0.49	0	0
P26	H	Haloxyfop	-CH2	[M-H]-	360.026	13.39	4	60.53	3.23	2.69	34.39	21.34
P27_a	F	Imazalil	+C6H8O6	[M+H]+	473.0869	11.52	2b	19.38	10.70	8.31	4.52	4.56
P27_b			+H2O2 +C6H8O6	[M+H]+	507.0946	9.15	3	14.35	8.21	4.16	1.81	3.6
P28_a	I	Imidacloprid	-NO2 +H	[M+H]+	211.0739	6.01	1	17.46	1.74	4.16	0.68	9.35
P28_b			Parent compound	[M+H]+	256.0596	8.04	4	5.02	0	2.44	3.85	3.84
P28_c			+O	[M+H]+	272.054	7.48	4	10.53	0.75	1.47	2.71	2.4
P28_d			-2H	[M+H]+	254.0439	7.3	4	8.37	0.25	0.98	2.94	2.88
P29	F	Iprodione	-C3H6 (RP32490)	[M-H]-	285.9786	12.93	4	5.02	0	0.24	1.58	2.64
P30_a	H	MCPA	+O	[M-H]-	215.0117	7.57	3	14.59	0.75	1.22	5.66	12.47
P30_b			Parent compound	[M-H]-	199.0167	9.95	4	0.48	0	0	0.45	0.96
P31	F	Myclobutanil	-H2 +2O	[M-H]-	317.0811	9	3	7.18	0.50	0.24	4.30	0.96
P32_a	F	Penconazole	+O +C6H8O6	[M+H]+	476.0982	11.45	2b	6.46	1.74	2.2	2.04	2.4
P32_b			-2H +2O	[M+H]+	314.0457	11.91	3	2.63	0.25	0.73	1.13	1.68
P33	F, H, I, M, GR	Pentachlorophenol	in source fragment of +SO3	[M-H]-	264.8368	13.19	4	3.11	0	2.44	3.85	0.24
P34_a	I, Ac	Pirimiphos-methyl	-CH2	[M-H]-	290.0734	10.75	1	85.17	10.20	6.60	23.98	47.72
P34_b			-CH2 -C2H4	[M-H]-	262.0422	7.47	5	16.75	0	0	0.23	4.08

ID	Pesticide type[15]	Parent pesticide	Metabolite found in urine	precursor ion	exact m/z	RT urine [min]	ID level	Overall Detection Frequency[16]				
								ES	LV	HU	CZ	NL
P34_c			-CH2 -C2H4	[M+H]+	264.0564	6.22	5	0	0.25	0	0	0.48
P35_a	F	Propamocarb	Parent compound	[M+H]+	189.1597	6.00	1	9.57	1	11.49	4.98	23.26
P35_b			+O	[M+H]+	205.1546	6.45	2b	20.81	5.47	18.34	12.67	42.69
P36_a	F	Propiconazole	-C5H10O +H2 +C6H8O6	[M-H]-	432.0371	9.00	3	2.39	0	0.98	0	1.2
P36_b			-C5H10O (CGA91304)	[M-H]-	253.9888	12.30	4	0	0	0	0	0.24
P37	H	Propyzamide	+H2O3	[M-H]-	304.0143	11.36	2b	8.61	0	0.49	0.9	0.96
P38_a	F	Pyrimethanil	+O +SO3	[M-H]-	294.0556	9.15	2b	26.79	14.43	4.89	21.95	31.89
P38_b			+O	[M+H]+	216.1133	11.69	2b	0.72	0	2.69	0	0.48
P39	G	Quinmerac	Parent compound	[M-H]-	220.0171	8.54	4	86.12	22.64	25.92	74.89	23.26
P40_a	F	Tebuconazole	-2H +2O	[M-H]-	336.1124	12.18	2b	71.29	5.47	25.18	52.26	35.97
P40_b			+O +C6H8O6	[M+H]+	500.1794	12.71	3	41.15	17.16	30.56	23.08	13.91
P41_a	F	Thiabendazole	+O +C6H8O6	[M-H]-	392.0551	5.96	2b	0	0.75	0.24	0	0.48
P41_b			+O (5-hydroxy)	[M+H]+	218.0381	6.80	5	2.15	1.49	1.47	0	3.36
P42_a	I	Thiacloprid	+O	[M-H]-	267.0107	9.19	2b	8.37	0.75	2.93	7.92	4.56
P42_b			+H2 +O	[M-H]-	269.0271	7.05	4	3.11	0.5	0.49	0.9	1.92
P43_a	I	Thiamethoxam	Parent compound	[M+H]+	292.0262	7.10	1	0.72	0	2.44	0	0.48
P43_b			-NO2 +H	[M+H]+	247.0413	6.20	1	23.44	0	15.16	0	0.24
P44	F	Tolclofos-methyl	-CH2	[M-H]-	284.9309	10.31	4	0	0.25	0	0.45	0.24

ID	Pesticide type[15]	Parent pesticide	Metabolite found in urine	precursor ion	exact m/z	RT urine [min]	ID level	Overall Detection Frequency[16]				
								ES	LV	HU	CZ	NL
P45_a	Af, Ab	Triclosan	+C6H8O6	[M-H]-	462.9759	13.23	1	84.69	16.17	24.45	46.15	12.71
P45_b			+O +C6H8O6	[M-H]-	478.9709	9.40	3	4.78	0.75	0.73	1.13	0.48
P45_c			+SO3	[M-H]-	366.9007	13.89	4	3.83	0.25	0.73	0.68	0.48
P45_d			Parent compound	[M-H]-	286.9439	16.12	4	2.15	0.5	1.22	0	0.96
P45_e			+C6H8O6	[M+NH3]+	482.0171	14.02	4	28.95	2.24	12.71	14.25	6.24
P46_a	F	Trifloxystrobin	-CH2 -CH2	[M-H]-	379.0911	13.07	2b	0.72	0.5	0	3.62	3.84
P46_b			-CH2 (CGA 321113)	[M+H]+	395.1213	14.88	5	0.24	0	0.24	2.04	0.24

Annex 3: Correlations of compounds originating from the same parent pesticide, per country

ID	Parent compound	Country				
		ES	LV	HU	CZ	NL
P5_a *P5_b*	Boscalid		NS[17]	NS	NS	NS
P19_a *P19_b*	Fluazifop		NS			
P35_a *P35_b*	Propamocarb		NS			

[17] Not Sufficient data to calculate the correlation.

Annex 4: Weighted correlation networks

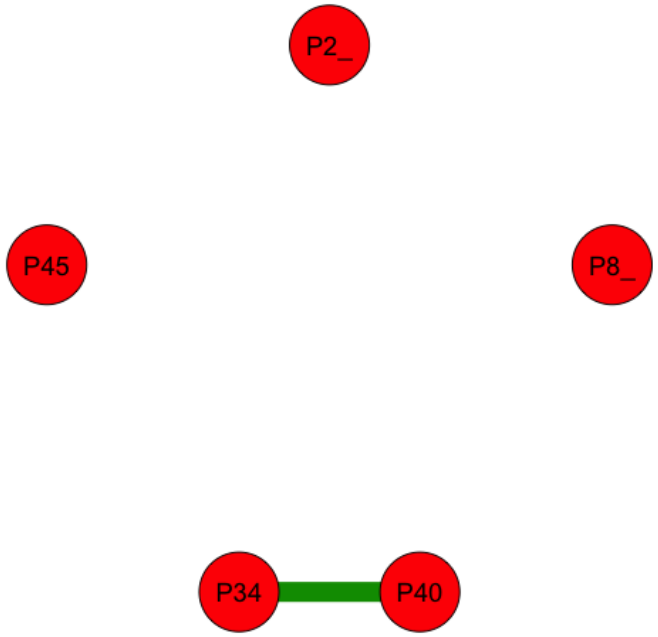


Figure S4.1: Weighted correlation network based on quantitative data from Spain

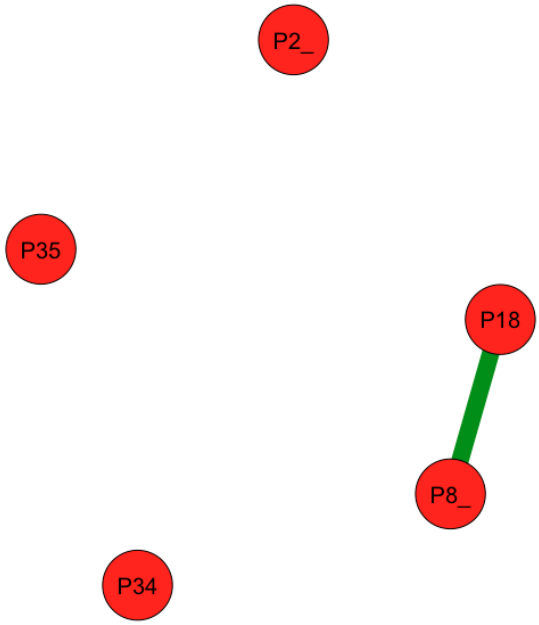
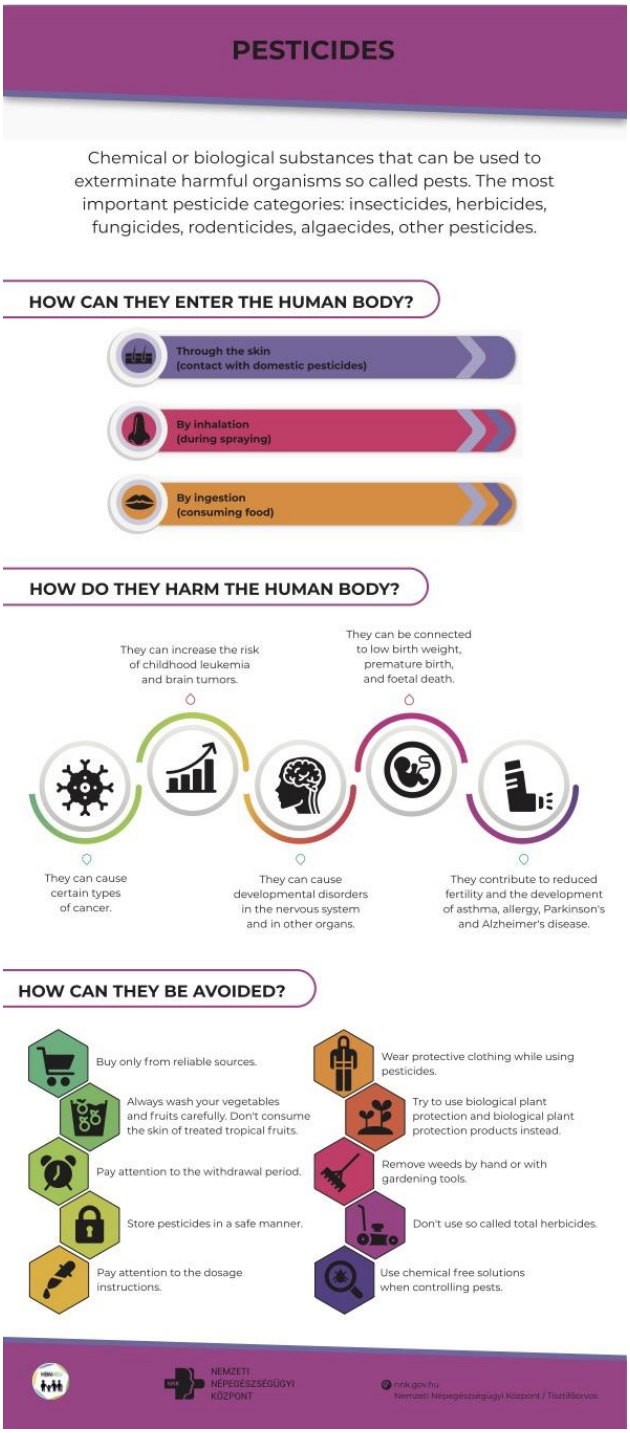
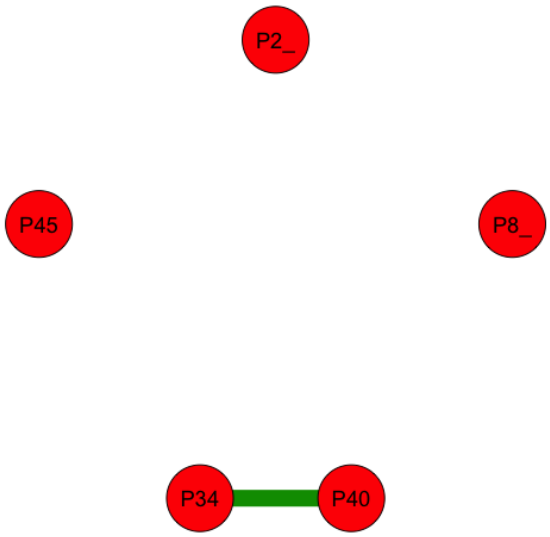


Figure S4.2: Weighted correlation network based on quantitative data from the Netherlands





Annex 5: Communication of results, poster developed by NPHC