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ABSTRACT

Within the Human Biomonitoring for Europe initiative (HBM4EU), a study to determine new biomarkers of exposure to pesticides and to assess exposure patterns was conducted. Human urine samples (N = 2,088) were collected from five European regions in two different seasons. The objective of the study was to identify pesticides and their metabolites in collected urine samples with a harmonized suspect screening approach based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) applied in five laboratories. A combined data processing workflow included comprehensive data reduction, correction of mass error and retention time (RT) drifts, isotopic pattern analysis, adduct and elemental composition annotation, finalized by a mining of the elemental compositions for possible annotations of pesticide metabolites. The obtained tentative annotations (n = 498) were used for acquiring representative data-dependent tandem mass spectra (MS^2) and verified by spectral comparison to reference spectra generated from commercially available reference standards or produced through human liver S9 *in vitro* incubation experiments. 14 parent pesticides and 71 metabolites

Abbreviations: CEC, Chemicals of emerging concern; dd-MS^2, data-dependent tandem mass spectrometry; ESI+, positive mode electrospray ionization; ESI, negative mode electrospray ionization; HCD, higher-energy collision-induced dissociation; HBM, human biomonitoring; HBM4EU, Human biomonitoring for Europe; SPECIMEn, Survey on PestiCIde Mixtures in Europe; LC-HRMS, Liquid chromatography coupled to high resolution mass spectrometry; RT, retention time; TIC, total ion current; MS^2, tandem mass spectrometry; MRM, multiple reaction monitoring; NCE, normalized collision energy.

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(including 16 glucuronide and 11 sulfate conjugates) were detected. Collectively these related to 46 unique pesticides.

For the remaining tentative annotations either (i) no data-dependent MS^2 spectra could be acquired, (ii) the spectral purity was too low for sufficient matching, or (iii) RTs indicated a wrong annotation, leaving potential for more pesticides and/or their metabolites being confirmed in further studies. Thus, the reported results are reflecting only a part of the possible pesticide exposure.

1. Introduction

Human biomonitoring (HBM) allows the assessment of population exposure (either general or occupational) to a wide range of chemicals including compounds which are known or suspected to cause adverse health effects (Kolossa-Gehring et al., 2017). Among the chemicals of emerging concern (CEC) are currently used pesticides, many of which are extensively metabolized in plants, animals and humans (Meijer et al., 2021; Aizawa, 2001).

Food consumption is a major route of exposure of the general population to pesticides (Hamilton et al., 2004). In addition, there is evidence that residents living close to agricultural fields are additionally exposed through inhalation of outdoor air or contaminated house dust (Deziel et al., 2015; Dereumeaux et al., 2020; Figueiredo et al., 2021). Multiple adverse health effects connected with an exposure to pesticides have been reported, including the increased risk of cancer, (Gilden et al., 2010) neurological disorders, (Loser et al., 2021) reproductive disorders (Bretveld et al., 2006) and respiratory diseases (Mamane et al., 2015).

Currently, >400 active substances are approved for use as pesticides in the European Union. (European Commission, 2016) While information on possible toxicological effects and current use generally is available, information on human exposure patterns to individual pesticides or to pesticide mixtures is limited (Giddings et al., 2016; Abu-Qare and Abou-Donia, 2001; Abdel-Rahman et al., 2001). Therefore, strategies are required to analyze the concurrent presence of different pesticides and their metabolites in human matrices, such as urine (Louro et al., 2019). Conventional pesticide analysis mainly focuses on parent pesticides, for which analytical reference standards are readily available. However, since most pesticides are extensively metabolized, HBM of pesticides in urine samples is more challenging and analytical approaches are required to measure the formed metabolites. The number of possible pesticide metabolites is high and analytical reference standards are rarely available. This calls for the application of suspect screening strategies based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to obtain a list of tentative annotations of pesticides and pesticide metabolites present in a sample set.

Suspect screening approaches have previously been applied in a few HBM studies (Pellizzari et al., 2019; Wang et al., 2018; Plassmann et al., 2015; Gerona et al., 2018; Pourchet et al., 2020) to gain insight into the exposure of humans to chemicals not covered in targeted monitoring programs.

With regard to pesticides, to our knowledge Bonvallot et al., 2021 performed the largest targeted study (338 urine samples of pregnant women in France; 27 detected compounds related to 17 pesticides). This targeted study was extended with the application of suspect screening. The latter together with subsequent confirmation efforts, resulted in tentative detection of metabolites from seven additional pesticides. Most other screening studies performed the final confirmation only for a limited number of compounds for which reference standards were available (Sierra-Diaz et al., 2019; Norén et al., 2020; Hill et al., 1995; Llop et al., 2017).

To enhance the scope of detection, there is a need to establish reliable high throughput methodologies to detect marker signals for pesticide metabolites for which standards are unavailable. Preferably, this needs to be applicable for large-scale cohort studies. Furthermore, generating metabolites *in vitro* together with adequate confirmation strategies for annotation with high-level confidence should be in place.

This study presents a suspect screening workflow applied in the Survey on PestiCIde Mixtures in Europe (SPECIMEn) that conducted harmonized LC-HRMS analysis of 2,088 urine samples across five laboratories to study human population exposure to pesticides (Vlaanderen et al., 2019). The applied data analysis workflow described here results in a list of verified occurrences of pesticides and pesticide metabolites. It comprises the following steps: (i) full scan LC-HRMS data analysis, (ii) prioritization of putative metabolites, (iii) generation of a list of representative samples for tandem mass spectrometry acquisition and (iv) final confirmation of putative metabolites by spectral comparison with the reference standard either purchased/synthesized or generated *in vitro* by human liver S9 incubations.

2. Material and methods

2.1. Fullscan MS^1 suspect screening

2.1.1. Study population and sample collection

First morning urine samples were collected from adult/child pairs as part of the SPECIMEn study that was conducted within the HBM4EU project (https://www.hbm4eu.eu/). The sample collection was performed in five countries (Czech Republic, Hungary, Latvia, Spain, and the Netherlands) and samples were shipped on dry ice to the corresponding laboratories. From each country, about 100 adult/child pairs (200 individuals) participated, who provided a urine sample in winter 2019/2020 and summer 2020. The total number of collected urine samples was 2,088. The samples of each country were analyzed by one of five participating laboratories, each analyzing approx. 400 samples (Czech Republic, Germany, France, Spain, and the Netherlands). Further information on the study design and sample handling is provided in the project deliverable (Vlaanderen et al., 2019).

2.1.2. Harmonized sample preparation and instrumental analysis

All five laboratories were equipped with LC-Orbitrap-MS instrumentation and used the same harmonized sample preparation and measurement methodology. Quality control urine samples, spiked with standards at two concentrations (2 and 20 ng/mL), quality control external standard mixes and internal standard mixes (isotopically labelled compounds) were distributed among all five laboratories. Sequence orders for sample injections were in a predefined format allowing for quality assessment prior to data analysis. Sample preparation consisted of buffering the urine samples to pH 6.8, followed by 96-well plate solid phase extraction resulting in a fivefold preconcentration. All samples were stored at −20 °C or below prior and between the instrumental analysis and at −80 °C for longer storage periods. Internal standard mixtures containing 21 isotope-labeled compounds (including seven pesticides and metabolites) were added to the samples before the extraction and during reconstitution. HRMS full-scan analysis was performed in positive and negative electrospray ionization (ESI+/ESI-) using optimized chromatographic conditions for each mode. In both cases a C18 column (2.1 mm × 100 mm, 1.7 μm particle size, Waters BEH C18) was used at 50 °C and a flow rate of 0.3 mL/min. For ESI+, we applied a water/methanol (containing 0.1 % formic acid and 2 mM ammonium formate) gradient elution 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. For negative mode, a gradient elution of water and water/

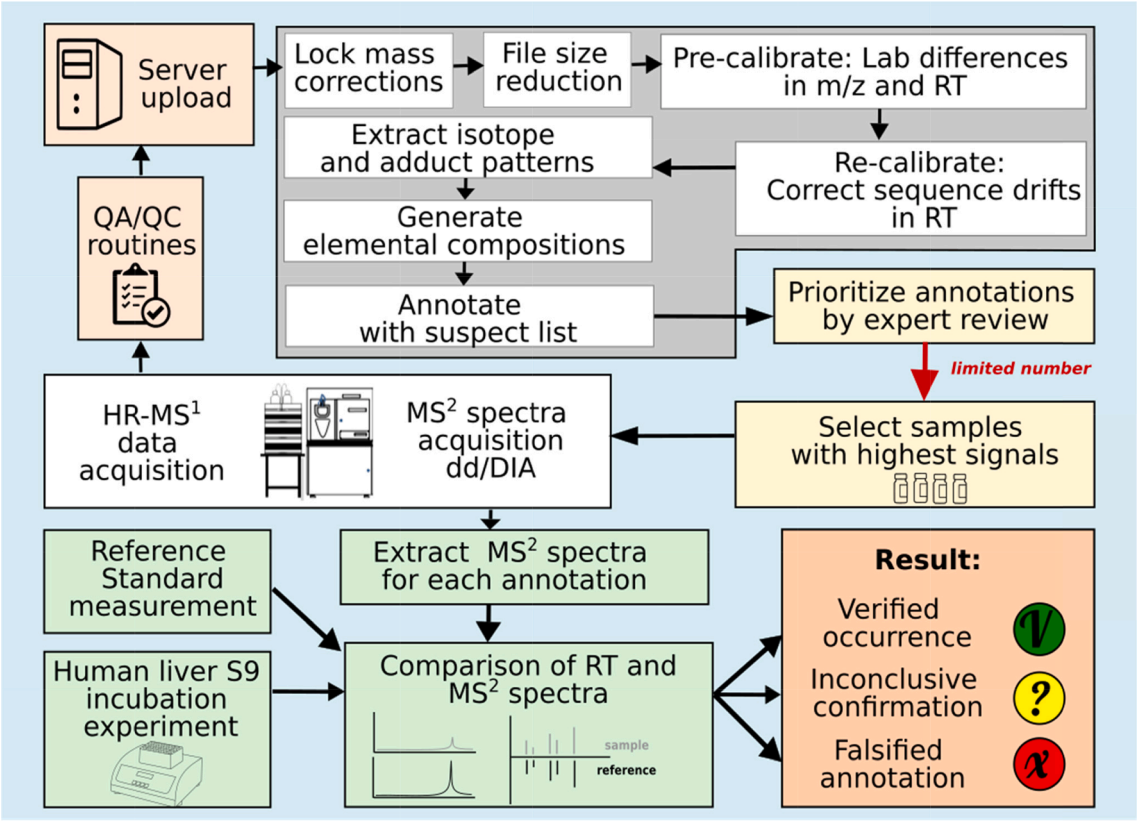

Fig. 1. Schematic strategy of the annotation and confirmation workflow applied in this study. The grey area describes the MS^1 data analysis performed within MetAlign. The resulting tentative annotations were prioritized by manual review and the sample with highest signal intensity was chosen for MS^2 spectra acquisition for the confirmation workflow.

methanol (90:10 v/v), both containing 10 mM ammonium bicarbonate, was used with the same program as described above. Full scan HRMS analysis was performed at a nominal resolving power of R ≥ 100,000 at *m/z* 200 in five laboratories. In total, twelve sequences from each laboratory resulted in >5,200 full scan data files including QC samples. In total, two injections of a quality control standard and ten of a spiked pooled sample matrix with two concentration levels (2 and 20 ng/mL) were included in each sample batch. Further details of the methods can be found in the Supplementary Material, Tables S1-2 and in a previously published manuscript describing the QA/QC methodology and assessment applied for this study (Vitale et al., 2022).

2.1.3. Suspect database generation

Previously curated suspect lists of pesticides and their metabolites were aggregated among the five laboratories. Phase I metabolites were mainly retrieved from the registration dossiers of the respective compounds provided by the European Food Safety Authority (https://www.efsa.europa.eu/en/calls/consultations), which are typically derived from exposure studies using animals as a proxy for humans. The final aggregated list contained >4,600 possible pesticide metabolites. Additionally, for all generated suspect list entries, the sulfate conjugate [$+SO_3$] for negative mode (if O was present in the molecular formula) and glucuronide [$+C_6H_8O_6$] conjugates for both ionization modes (if N or O was present in the molecular formula) were added. The aggregated suspect list is available for download from zenodo (https://doi.org/10.5281/zenodo.6530623).

2.1.4. Fullscan MS^1 data analysis workflow

The MetAlign software suite (Lommen and Kools, 2012; Lommen et al., 2011; Lommen et al., 2019; Lommen, 2014; Lommen, 2009) was used for processing and comparison of single-stage full-scan accurate mass data on a HP Z820 workstation with two Intel® Xeon® E5-2690 CPU 2.90 GHz processors (2x8 cores, 2x16 virtual) and 64 GB RAM with 64-bit Windows 10 operating system. The data pre-processing workflow (software and workflow description on zenodo, https://doi.org/10.5281/zenodo.6530623), for retention time (RT) alignment and mass calibration corrections is summarized in Fig. 1 and contained the following steps:

1 A lock mass correction during format conversion using multiple lock masses corresponding to persistent mobile phase ions (see Table S3 for *m/z* values) (Lommen et al., 2011).
2 Data reduction (ca. 100–500 fold) using MetAlign (Lommen and Kools, 2012; Lommen, 2009).
3 Interlaboratory alignment of the RT using quality control external standard mixes and transferring all RTs to that of the reference laboratory (see Figures S1-2).
4 Corrections for mass calibration errors in the reduced-size data files per sequence using multiple *m/z* from quality control samples, quality control external standard mixes, internal standard mixes and some known, ubiquitous system contaminants (see Figures S3-4) (Lommen et al., 2011).
5 Sequence retention drift corrections using *m/z* values of internal standards and some ubiquitous known abundant signals.

Further details on the processing steps are given in the Supplementary Materials, Table S4. After this processing stage, all datasets from different laboratories are assumed to be comparable on the RT scale and to possess a mass precision ≤ 1.5 ppm. This corrected data was subsequently used for automated isotope pattern recognition, adduct and elemental composition analysis followed by annotation using the suspect database (Lommen, 2014). No normalization was performed on the

signal intensity, as all different types of normalization have some drawbacks and can significantly influence the results (Cook et al., 2020).

Elemental composition analysis based on the elements C, H, N, O, P, S, Cl, F, Br was performed on the isotope patterns in the dataset between RT of 2.0 and 18.5 min, a *m/z* range of 100–800 m.u., using a mass error $\leq$ 1.5 ppm and a threshold of signal intensity (centroid mode) in a range between 10^3-10^4 a.u. For elemental compositions containing Cl and Br, the detection of the characteristic ^{37}Cl and ^{81}Br isotope pattern was mandatory.

The calculation of elemental compositions results typically in about 800,000 elemental compositions per LC-HRMS data file. Because this is an unmanageable number of tentative detects, a further data reduction step was required focusing on subsets based on the presence of F, Cl, Br and PO_3. However, a few metabolite annotations (i.e., thiabendazole, pyrimethanil, cyprodinil, propamocarb and ametoctradin) without these elemental composition traits were kept, as their RT information for the LC system used was available from other unpublished studies (relating confirmed high pesticide intake from food vs potential metabolites in human urine). This resulted directly in an increased confidence for these annotations. For Cl, Br and F containing elemental compositions, a higher likelihood of xenobiotic origin is assumed. However, there are a few exceptions, such as compounds originating from marine environments (e.g., bromotryptophan metabolism (Bittner et al., 2007) and certain vegetables (e.g., chlorotryptophan metabolism (Anderson and Chapman, 2006). The presence of PO_3 was included as a typical sub-feature for organophosphorus pesticides since phosphates in natural compounds are often cleaved prior to excretion in urine.

2.1.5. Prioritization of tentative annotations for confirmation through manual review by an expert

For practical reasons (i.e., time and budget), the resulting output from Cl, Br, F and PO_3 sub-feature selection required further reduction before confirmation/identification procedures could commence. This was performed by manual review procedures based on expert judgement and knowledge:

a) Since the consumption of pharmaceuticals was not excluded in the SPECIMEn study, halogenated pharmaceuticals and their metabolites may be present in urine. Pesticide metabolites are expected to occur at much lower signal intensities than those of pharmaceuticals due to orders of magnitude lower intake rates. Yet, in some cases minor pharmaceutical metabolites can possess the same elemental composition as a pesticide metabolite. Examples of probable ambiguous chemical formulas found are for instance that of dihydroxy-diclofenac identical to the herbicide chlorazifop and that of 3,5-dibromo anthranilic acid (metabolite of bromhexine/ambroxol) identical to 3,5-dibromo-4-hydroxybenzamide (metabolite of the herbicide bromoxynil). In practice, pesticide metabolites can be distinguished from minor pharmaceutical metabolites based upon the simultaneous presence of high intensity signals of the parent pharmaceutical and/or its main metabolites. A pre-screening using a list of elemental compositions of commonly used halogen-containing pharmaceuticals (subset of a list of 200 most commonly used medicines in 2019 (see dataset on https://doi.org/10.5281/zenodo.6530623) was performed as a first step in the analysis. If these particular pharmaceuticals were found at high signal intensities (cut-off > 5x10^6) the corresponding samples were flagged. Flagged samples were typically not used as a primary source for discovery if elemental compositions of pesticide metabolites had some similarity with the pharmaceutical present.

b) Small molecules such as halogenated phenols, cresols, benzoates, anilines (most of which are conjugated) were put in a separate list and not used as potential pesticide metabolites for human biomonitoring because they were regarded as not specific enough. Metabolites related to bromotryptophan, chlorotryptophan and sucralose were listed separately and omitted from confirmation. These annotated but excluded compound categories are made available (see folder Work_folder_search_templates_HBM4EU/Miscellaneous_small_chlorinated_or_brominated_compounds, https://doi.org/10.5281/zenodo.6530623).

c) The remaining tentative pesticide metabolite annotations were sorted by parent name per sequence. The isotope patterns and the RT consistency were then manually checked and anomalies filtered out. The resulting RT and isotope pattern combinations were searched over all processed sequences. If blank samples contained the RT-isotope pattern combination, the occurrence was noted as a background contamination.

d) Parent pesticides with more than one marker (parent and/or multiple metabolites) detected in the overall dataset and showing (partial) co-occurrence with each other were prioritized (examples are given in Figure S5-S10). If multiple metabolites occurred for the same pesticide, their relative RTs were checked with regard to each other. In general, metabolites that are more polar than the parent compound are expected to elute earlier than the parent. Therefore, conjugated metabolites should elute earlier than the corresponding unconjugated metabolite; hydroxylated or demethylated earlier than the parent. If information on primary metabolites in literature was available, their presence was checked against the other metabolites.

A final check in the expert manual review was based on the plausibility of the observed ionization mode vs the proposed structure of the metabolite. The resulting annotations are given per pesticide (see Work_folder_search_templates_HBM4EU, https://doi.org/10.5281/zenodo.6530623).

2.2. Confirmation procedures

2.2.1. General considerations

Confirmation procedures were set up to compare RT and MS^2 spectra of tentative annotations found in the original urine samples (and in selected cases also deconjugated samples) with RT and MS^2 information acquired from commercially available reference standards or produced through human liver S9 incubation experiments. Due to predominantly low signal intensities for pesticide metabolites, confirmatory experiments were performed on samples in which the highest signals were observed.

2.2.2. Deconjugation of urine samples

To confirm the identification of glucuronide and sulfate conjugates, enzymatic deconjugation was performed for a selection of urine samples. The urine samples were 1:1 diluted with a 50 mM phosphate buffer pH 6.8 containing the enzymatic mixture. A combination of two enzymes was used, β-glucuronidase/arylsulfatase (Merck) from *Helix pomatia*, corresponding to 0.01 U/mL of urine β-glucuronidase and 0.03 U/mL of urine arylsulfatase, and β-glucuronidase from *Escherichia coli* (Sigma-Aldrich), corresponding to 1570 U/mL of urine. Samples were incubated overnight at 37 °C. After incubation, internal standards were added and the regular suspect screening sample preparation procedure was performed.

2.2.3. Reference standards

All commercially available reference standards of pesticides and pesticide metabolites considered in this study are listed in Table S5. The measurements of all standards were performed at a level of 100 ng/mL using the same instrumental method as described above for the urine samples.

2.2.4. MS^2 acquisition

All urine samples selected for MS^2 measurement (based on representative high signal intensity for a prioritized annotation) were remeasured using the same LC conditions as described above. The 60 samples in ESI- and 64 in ESI+ originated from two different laboratories which participated in the confirmatory work and contained samples from Hungary and the Netherlands, respectively. The acquisition method consisted of a combination of a full scan MS^1 at R = 70,000 and four data-dependent dd-MS^2 scans at R = 35,000 in two parallel

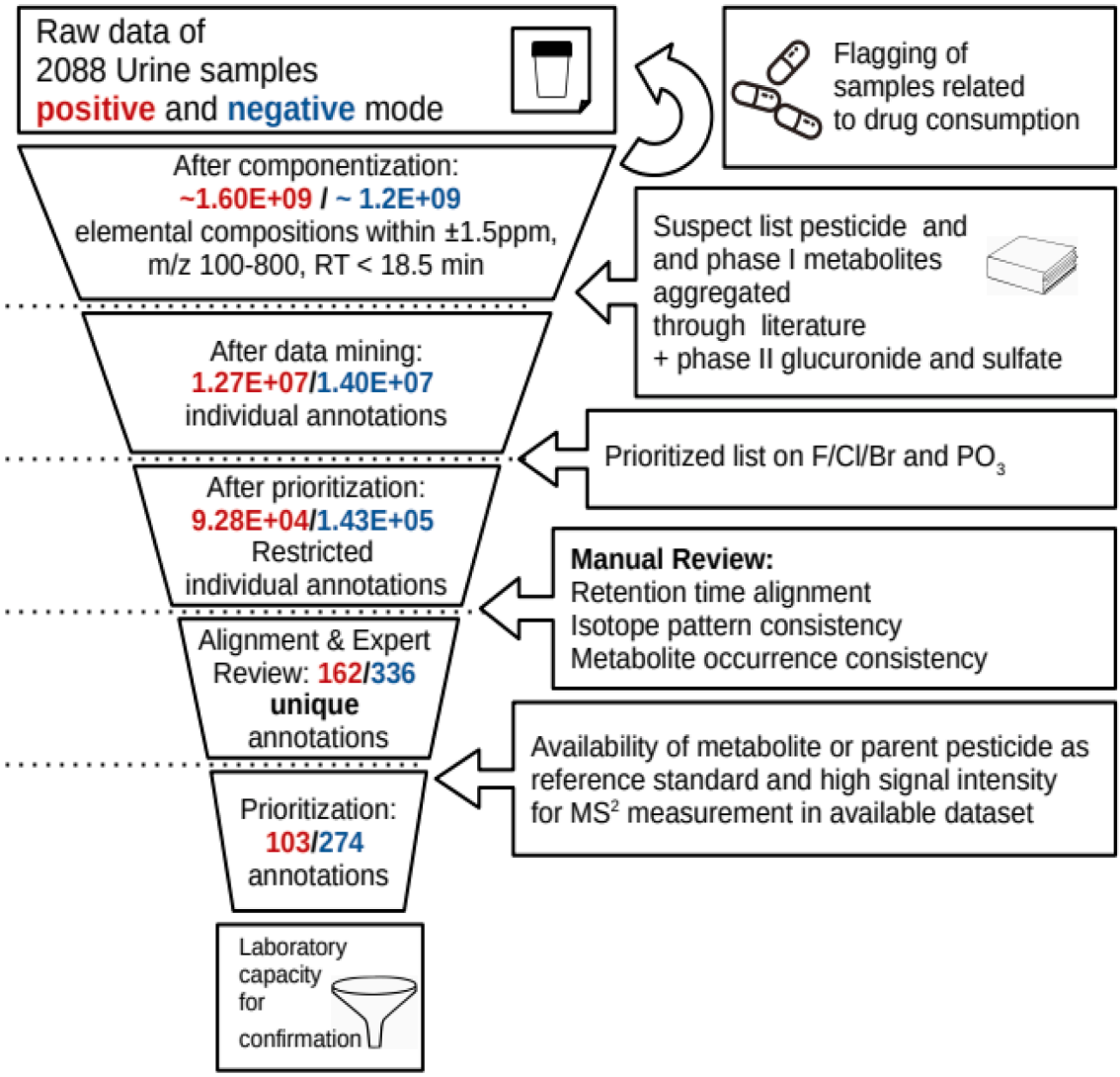

Fig. 2. Data processing workflow applied to prioritize and annotate pesticide exposure markers detected by the applied suspect screening approach based on the first-stage full-scan MS^1 dataset.

experiments (higher-energy collision-induced dissociation (HCD) at normalized collision energies (NCE) of 35 and 50 %) to generate diagnostic fragmentation patterns. An inclusion list was applied triggering the corresponding *m/z* at RT (±1 min) and a precursor window of 1 mass unit of the tentative annotations. If a dd-MS^2 could not be triggered due to low signal intensity, the sample was re-injected using the multiple-reaction monitoring (MRM) MS^2 acquisition mode with the same inclusion list.

2.2.5. Human liver S9 incubation

Human liver S9 incubation experiments were performed for 69 pesticides. Pesticides were selected based on what was found in the suspect screening and what was commercially available (See Table S6). The human liver incubation procedure was performed as previously described (Huber et al., 2021). The chromatography as well as the instrumental settings for the LC-Orbitrap-MS measurements are described in Section 2.2.4. The inclusion list was based on the precursor ion masses of the metabolites originating from the suspect screening. The reference information extraction from the acquired raw files (RT, MS^2 spectra at two different collision energies) was performed with the same workflow as described for the spectral database generation (Huber et al., 2021).

2.2.6. MS^2 data processing

All sample files were converted into .mgf and .mzML files using the ProteoWizard version v3.0.18265 function msconvert (Kessner et al., 2008). The data analysis workflow was performed in R version 4.0.3. Spectra extraction was performed using the mzR package (Chambers et al., 2012) and the msPurity package (Lawson et al., 2017) was used for evaluating the purity of all spectra related to the tentative annotations within a RT window of ±30 s. The Spectra package (Rainer et al., 2022) was used to generate head-to-tail plots and to calculate forward and reverse dot product scores between the spectra measured from the urine samples and spectra acquired from reference standards or human liver S9 incubations of parent pesticides. A mass tolerance of 10 ppm was used for matching the fragment ions. Spectra were cleaned using the command line tool Genform (Meringer et al., 2011), applying the molecular formula of the suspect and a threshold of 10 ppm. A confirmation was automatically set if at least three fragment ions were matched and a reverse dot product score >0.4 for dd-MS^2 observed. Furthermore, all spectral comparisons were manually reviewed to check if isobaric contamination was causing low dot product scores. Sulfate and glucuronide conjugate metabolites were confirmed based on an in-silico deconjugation procedure (Huber et al., 2022). To this end, the spectra of the conjugate metabolite were compared with the reference spectra of either the parent pesticide or the phase I metabolite, also stemming from

different collision energies, however without applying a strict dot-product score threshold. For further verification of the method, we evaluated a selection of deconjugated samples.

In cases when experimental MS^2 spectra could be acquired for a tentative annotation but without a corresponding reference metabolite generated by the human liver S9 experiment, a MS^2 data modeling approach was used to identify the corresponding marker based on the fragmentation tree computation, chemical fingerprint prediction and compound class prediction of the java-based software framework SIRIUS (Dührkop et al., 2019) version 4.9.9, further details see Table S7.

2.2.7. Quantitative analysis on selected metabolites and samples

Within the context of the SPECIMEn study, a limited quantitative analysis was performed in parallel to the suspect screening approach and the obtained data was available for comparison. The subset contained 107 enzymatic deconjugated samples. The targeted method included the measurement of pyrethroid metabolites *cis*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCCA), 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) and *cis*-3-(2-chloro-3,3,3trifluoroprop-1-enyl)-2,2dimethylcyclopropanecarboxylic acid (ClF3CA), as well as the metabolite 3,5,6-trichloro-2-pyridinol (TCPy) of chlorpyriphos(-methyl). The glucuronides of these four compounds are known for exhibiting low fragmentation (except for the loss of glucuronide) on a LC-HRMS-Orbitrap system. Therefore, the analysis was carried out by LC-MS/MS using a triple quadrupole instrument in the multiple reaction monitoring acquisition mode. Further details of the analytical method can be found in Tables S8-S10. The concentrations of these analyzed compounds were compared with the signal abundances of their glucuronides annotated from the suspect screening approach, both for confirmatory purpose and for QA/QC consolidation of the suspect screening workflow.

3. Results and discussion

3.1. Full scan MS^1 suspect screening and prioritization

The workflow steps described in Section 2.1.4 and Section 2.1.5 were performed by one expert to exclude any difference in data handling between the batches. Section 2.1.5 was done as an iterative approach, in which the annotation list increased while analyzing the sample batches. Section 2.1.4 together with Section 2.1.5 required 8 months work on a full time basis.

The result of the elemental composition analysis based on the annotated isotope patterns extracted from 2,088 urine samples is presented in Fig. 2. A total of $1.6x10^9$ (ESI+) and $1.2x10^9$ (ESI-) possible elemental compositions were found. During a prescreening of the data, 207 of the 2,088 urine samples contained very high signals of halogenated compounds that could be linked to commonly used pharmaceuticals. In the present study, halogenated pharmaceuticals - for instance aceclofenac/diclofenac and bromhexine/ambroxol - were found to have signals around saturation levels (signal intensity $> 10^9$ in centroid mode). Therefore, signals of minor metabolites of these pharmaceuticals may still be observable even if they are at a 1.000–100.000 fold lower intensity (i.e. ca. 10^4-10^6). This is within the expected intensity range for pesticide metabolites, which is much lower than for pharmaceuticals due to orders of magnitude lower intake rates. Since these samples could contain elemental compositions from pharmaceutical metabolites that also match pesticide metabolites, these samples were flagged to avoid false-positive annotations (see Section 2.1.5). The selection of elemental compositions based on the suspect list left $1.27x10^7$ (ESI+) and $1.4x10^7$ (ESI-) possible annotations for 2,088 samples. The majority of these are assumed to be false-positive annotations not related to the molecular structures of the suspect list entries. After a selection of Cl, Br, F and PO_3-containing features, the remaining dataset consisted of $9.28x10^4$ (ESI+) and $1.43x10^5$ (ESI-) tentative annotations in 2,088 samples. Many of these annotations originate from the same compounds in different urine samples.

These remaining annotations were used in the next prioritization step, referred to as manual review by an expert. Figures S5-S10 (Spearman rank correlation) are given to illustrate the co-occurrence of different known metabolites of the same pesticide in the same sample. A direct correlation between metabolites of the same pesticide is often - but not always - present. One example is desnitro-imidacloprid, which is known to mostly originate from plants and environmental processes (Loser et al., 2021). As expected, the annotation of desnitro-imidacloprid does not correlate well with the other human metabolites of imidacloprid (see Figure S6). A second example is desmethyl-chlorpyrifos-methyl (and possibly other organophosphates), which may be a result of degradation during long-term crop storage or food processing (Brancato et al., 2017). The desmethyl-chlorpyrifos-methyl annotation does not correlate well with TCPy-glucuronide annotation (see Figure S5). Therefore, only relying on Spearman rank correlations without expert curation based on published knowledge is not advisable.

The manual expert review resulted in 162 and 336 individual tentative annotations (most occurring in many samples), in ESI+ and ESI- respectively, covering about 80 pesticides. Among the 498 candidates, there were 198 glucuronide and 105 sulfate conjugates annotated. A final pragmatic selection was based on the commercial availability of reference standards and the maximum signal intensity (related to likelihood of being able to perform MS^2 experiments), which led to 103 and 274 annotations prioritized for confirmatory analysis in ESI+ and ESI- modes, respectively.

3.2. Confirmed annotations of pesticide metabolites by tandem mass spectrometry

Based on the observed signal intensities for pesticide metabolites spiked in the QC samples at a concentration of 2 ng/mL and the comparison with results obtained from targeted analysis (Figures S11-14), most detected pesticide metabolites in urine samples are roughly estimated to be in a range of 0.05–10 ng/mL. Consequently, the signal intensities measured were often low ($<10^5$), leading to challenges in MS^2 acquisition and the extraction of high-quality MS^2 spectra.

Only samples with the highest signal intensity available (samples from two of five laboratories) for each tentative annotation were used for confirmation analysis. In total, 60 samples were re-analyzed in ESI+ and 64 samples in ESI- to generate MS^2 information for the prioritized annotations. In practice, MS^2 information could only be acquired for about one third of selected tentative annotations ($N = 377$; see Fig. 2) in urine samples. For annotations with low signal intensities there were clear difficulties in MS^2 data-dependent acquisition even when applying an inclusion list to force triggering of the precursor ions. In some cases, data was acquired by targeted MRM acquisition. However, these spectra contained impurities, which decreased the ability to draw conclusions from the spectral matching to the reference spectra. Therefore, lower scores for spectral comparisons were accepted in three cases acquired by MRM following manual review.

The commonly accepted Schymanski confidence scale (Schymanski et al., 2014) was used to attribute a level of confidence to each annotated exposure marker. All tentative annotations start out as level 4/5 (unique elemental composition or more than one elemental composition possible, respectively). To further categorize or upgrade levels, all tentative annotations that underwent the confirmation workflow are further divided into three categories:

Confirmed: Information is present to increase the identification level to 1, 2b or 3. For level 1, there is a RT and MS^2 match with a reference standard. For level 2b there is a RT and MS^2 match with data obtained from the S9 incubation experiment. For level 3, there is no reference standard or S9 incubation MS^2 available but there is a conclusive explanation of the MS^2 spectra obtained by the fragmentation tree computation software SIRIUS.

Inconclusive: There is a RT match with a reference standard or with a

Table 1
Overview of the annotated pesticides and pesticide metabolites in the overall dataset. The numbers of associated features are given for each ionization mode (ESI+/ESI-). Detection criteria are marked (+) if they hold true for the metabolite and if the comparison was performed with the human liver S9 incubation experiment or a reference standard.if the comparisson.

	Pesticide	Suspect Features ESI+	Suspect Features ESI -	Detected metabolites	Precursor ion	Exact *m/z*	RT urine [min]	*m/z* match (< ±5 ppm)	Cl/Br pattern match	RT match (<±0.1 min)	MS^2 match (DP > 0.4)	In-silico verif. of spectra	Aglycon after decon.	ID level [a]
Fungicides	**Iprodione**	0	13	-C3H6 (RP32490)	[M−H]-	285.979	12.9	+	+	S9				4
	Boscalid	9	5	+O +SO3	[M−H]-	436.977	10.3	+	+		S9			2b
				+O +SO3	[M + H]+	438.992	10.5	+	+		S9			2b
				+O (M510F01)	[M−H]-	357.020	11.9	+	+	Std				4
				+O (M510F01)	[M + H]+	359.035	11.7	+	+	Std				4
	Fenhexamid	3	9	+O +C6H8O6	[M + NH3]+	511.124	9.3	+	+			+		3
	Propiconazole	6	9	-C5H10O + H2 +C6H8O6	[M−H]-	432.037	9.0	+	+			+		3
				-C5H10O (CGA91304)	[M−H]-	253.989	12.3	+	+	S9				4
	Tebuconazole	6	11	–2H + 2O	[M−H]-	336.112	12.2	+	+	S9	S9			2b
				+O + C6H8O6	[M + H]+	500.179	12.7	+	+			+		3
	Tolclofos-methyl	0	6	–CH2	[M−H]-	284.931	10.3	+	+	S9				4
	Pyrimethanil	3	5	+O	[M + H]+	216.113	11.7	+	n.a	S9	S9			2b
				+O +SO3	[M−H]-	294.056	9.2	+	n.a		S9			2b
	Cyprodinil	6	5	+O +SO3	[M−H]-	320.071	11.9	+	n.a		S9			2b
				+2O +SO3	[M−H]-	336.066	9.2	+	n.a			+		3
	Fludioxonil	1	5	+O +C6H8O6	[M−H]-	439.061	11.8	+	n.a.		S9			2b
	Imazalil	9	9	+C6H8O6	[M + H]+	473.087	11.5	+	+	S9				2b
				+H2O2 +C6H8O6	[M + H]+	507.095	9.15	+	+			+		3
	Propamocarb	2	0	(parent compound)	[M + H]+	189.160	6.0	+	n.a	Std	Std			1
				+O	[M + H]+	205.155	6.5	+	n.a	S9	S9			2b
	Thiabendazole	5	2	+O (5-hydroxy)	[M + H]+	218.038	6.8	+	n.a	Std				5
				+O +C6H8O6	[M−H]-	392.055	6.0	+	n.a		S9			2b
	Fluopyram	11	17	–2H (in source fragment)	[M + H]+	395.039	13.1	+	+	S9	S9			2b
				+O +SO3	[M−H]-	490.991	12.7	+	+		S9			2b
				+O +C6H8O6	[M + H]+	589.081	13.1	+	+	S9	S9			2b
	Myclobutanil	4	9	–H2 +2O	[M−H]-	317.081	9.0	+	+			+		3
	Flutolanil	10	13	-C3H6 +O +SO3	[M−H]-	376.011	8.2	+	n.a.			+		3
	Penconazole	5	7	–2H +2O	[M + H]+	314.046	11.9	+	+			+		3
				+O +C6H8O6	[M + H]+	476.098	11.5	+	+					2b
	Trifloxystrobin	4	5	–CH2 –CH2	[M−H]-	379.091	13.1	+	n.a.	S9	S9			2b
				–CH2	[M + H]+	395.121	14.9	+	n.a.	Std				5
	Ametoctradin	1	1	-C2H6 +2O	[M−H]-	276.147	8.17	+	n.a	Std				5
				-C2H6 +2O	[M + H]+	278.161	9.47	+	n.a	Std	Std			1
Insecticides	**Deltamethrin**	1	2	DBCA +C6H8O6	[M−H]-	470.930	11.4	+	+				+	4
	Acetamiprid	2	1	–CH2	[M−H]-	207.044	8.7	+	+	Std	Std			1
				–CH2	[M + H]+	209.059	8.6	+	+	Std				4
				(parent compound)	[M + H]+	223.075	8.7	+	+	Std				4
	Chlorpyrifos (/methyl)	4	8	TCPy	[M−H]-	195.913	10.1	+	+	Std				4
				–CH2	[M−H]-	305.872	10.7	+	+	Std	Std			1

(*continued on next page*)

Table 1 (*continued*)

	Pesticide	Suspect Features ESI+	Suspect Features ESI -	Detected metabolites	Precursor ion	Exact *m/z*	RT urine [min]	*m/z* match (< ±5 ppm)	Cl/Br pattern match	RT match (<±0.1 min)	MS^2 match (DP > 0.4)	In-silico verif. of spectra	Aglycon after decon.	ID level [a]
				TCPy +C6H8O6	[M−H]-	371.945	8.4	+	+				+	4
	Imidacloprid	5	3	–NO2 +H	[M + H]+	211.074	6.0	+	+	Std	Std			1
				–2H	[M + H]+	254.044	7.3	+	+	Std				4
				(parent compound)	[M + H]+	256.060	8.0	+	+	Std				4
				+O	[M + H]+	272.054	7.5	+	+	Std				4
	Cypermethrin, Cyfluthrin, Permethrin, Transfluthrin	6	11	DCCA	[M−H]-	206.999	10.7	+	+	Std				4
				DCCA +C6H8O6	[M−H]-	383.031	11.0	+	+				+	4
	Clothianidin (or thiamethoxam)	2	2	–NO2 +2H	[M + H]+	205.031	5.8	+	+	Std	Std			1
				–CH2	[M−H]-	233.986	7.5	+	+	S9	S9			2b
				(parent compound)	[M−H]-	248.002	8.1	+	+	Std	Std			1
				(parent compound)	[M + H]+	250.016	8.1	+	+	Std				4
	Thiamethoxam	2	0	–NO2 +H	[M + H]+	247.041	6.2	+	+	S9				1
				(parent compound)	[M + H]+	292.026	7.1	+	+	Std	Std			1
	Thiacloprid	0	4	+O	[M−H]-	267.011	9.2	+	+	S9	S9			2b
				+H2 +O	[M−H]-	269.027	7.1	+	+	S9				4
	Flupyradifurone	5	4	-C2H2F2	[M + H]+	225.043	7.5	+	+	S9				4
				(parent compound)	[M + H]+	289.056	8.8	+	+	Std	Std			1
	Flonicamid	3	4	–C2HN	[M + H]+	191.043	6.10	+	n.a.	S9	S9			2b
				(parent compound)	[M−H]-	228.040	6.9	+	n.a.	Std	Std			1
				(parent compound)	[M + H]+	230.054	6.8	+	n.a.	Std				4
	Chlor-antraniliprole	0	1	+O	[M−H]-	497.956	12.7	+	+	S9	S9			2b
Insecticides/ Acaricides	**Pirimiphos-methyl**	4	3	–CH2 -C2H4	[M−H]-	262.042	7.5	+	n.a	S9				5
				–CH2 -C2H4	[M + H]+	264.056	6.2	+	n.a	S9				5
				–CH2	[M−H]-	290.073	10.8	+	n.a	Std	Std			1
	Fipronil	1	5	(parent compound)	[M−H]-	434.931	15.0	+	+	Std				4
				+O	[M−H]-	450.926	15.4	+	+	Std				4
	Bifenthrin/ Cyhalothrin	1	3	F3CCA +C6H8O6	[M−H]-	417.057	12.0	+	+				+	4
	Fluvalinate	1	5	-C14H9NO	[M−H]-	294.051	13.9	+	+	S9	S9			2b
				-C14H9NO	[M + H]+	296.066	14.4	+	+	S9				4
				-C14H9NO +O	[M−H]-	310.046	12.8	+	+			+		3
	Chloropropylate	0	2	-C3H6 –CO2	[M−H]-	251.004	12.9	+	+	S9				4
				-C3H6	[M−H]-	294.993	12.9	+	+	S9				4
Herbicides	**2,4-D**	0	2	(parent compound)	[M−H]-	218.962	9.93	+	+	Std	Std			1
	Propyzamide	0	2	+H2O3	[M−H]-	304.014	11.4	+	+	S9	S9			2b
	MCPA	0	4	(parent compound)	[M−H]-	199.017	10.0	+	+	Std				4
				+O	[M−H]-	215.012	7.6	+	+			+		3
	Haloxyfop	1	1	–CH2	[M−H]-	360.026	13.4	+	+	Std				4
	Fluazifop	1	1		[M−H]-	326.065	11.7	+	n.a.	Std	Std			1

(*continued on next page*)

Table 1 (*continued*)

	Pesticide	Suspect Features ESI+	Suspect Features ESI -	Detected metabolites	Precursor ion	Exact *m/z*	RT urine [min]	*m/z* match (< ±5 ppm)	Cl/Br pattern match	RT match (<±0.1 min)	MS^2 match (DP > 0.4)	In-silico verif. of spectra	Aglycon after decon.	ID level [a]
				(parent compound)										
				(parent compound)	[M + H]+	328.079	13.6	+	n.a.	Std	Std			1
	Clopyralid	1	1	(parent compound)	[M−H]-	189.947	3.5	+	+	Std	Std			1
	Fluroxypyr	1	0	(parent compound)	[M + H]+	254.973	10.5	+	+	Std				4
	Quinmerac	1	1	(parent compound)	[M−H]-	220.017	8.5	+	+	Std				4
	Diuron	3	7	–CH2 –CH2	[M−H]-	202.979	12.0	+	+	S9				4
				–CH2	[M−H]-	216.994	12.5	+	+	S9				4
				–CH2	[M + H]+	219.008	12.1	+	+	S9				4
	Chlorpropham	11	16	-C4H6O +SO3	[M−H]-	221.963	6.2	+	+			+		3
				+O	[M−H]-	228.043	11.0	+	+	Std				4
				+O +SO3 (4-HSA)	[M−H]-	308.000	9.5	+	+	Std	Std			1
				+2O +SO3	[M−H]-	323.995	7.5	+	+			+		3
				+O +C6H8O6	[M−H]-	404.076	8.6	+	+					4
Others	**Triclosan**	2	7	+SO3	[M−H]-	366.901	13.9	+	+	Std				4
				+C6H8O6	[M−H]-	462.976	13.2	+	+	Std	Std			1
				+O +C6H8O6	[M−H]-	478.971	9.4	+	+			+		3
				+C6H8O6	[M + NH3]+	482.017	14.0	+	+	Std				4
	Pentachloro-phenol	0	2	in source fragment of +SO3	[M−H]-	264.837	13.2	+	+	S9				4

[a] Confidence levels: 1- confirmed (MS^2 and RT match with reference standard); 2b - circumstantial evidence (MS^2 and RT match with incubation experiment); 3 – tentative candidate (in-silico predicted); 4 -unequivocal molecular formula (adduct match).

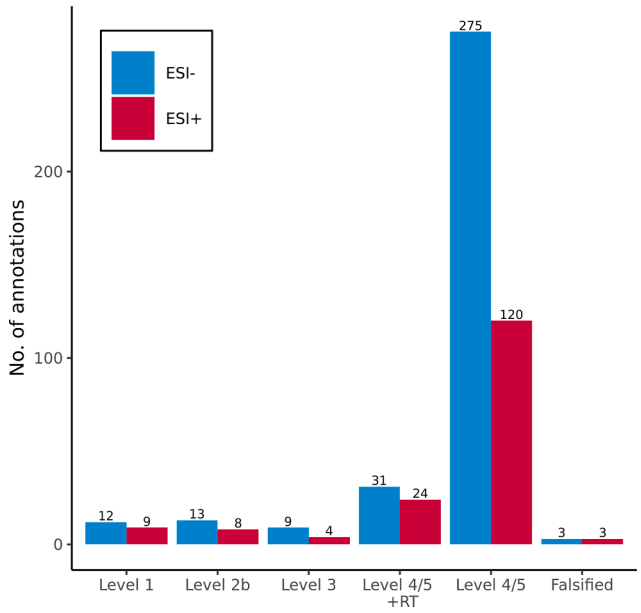

Fig. 3. Level of identification confidence according to Schymanski et al. (Schymanski et al., 2014) and falsified annotations (see text for explanation) achieved for all tentative annotations from the generated suspect screening data (N = 498). The numbers may include duplicate confirmations of the same compounds in ESI+ and ESI-. Confidence levels: 1- confirmed (MS^2 and RT match with reference standard); 2b - circumstantial evidence (MS^2 and RT match with incubation experiment); 3 – tentative candidate (in silico predicted); 4 -unequivocal molecular formula (adduct match); 5- exact mass only.

metabolite in a S9 incubation, but the spectral similarity is deemed to be too unclear to draw further conclusions (i.e., noise and/or isobaric interferences vs wrong identity). In this case level 4/5 is kept and the RT match is indicated in Table 1. It should be noted, that some parent pesticide compounds for which reference standards were available had too low signal intensities to obtain good quality MS^2 spectra and therefore also remained inconclusive (level 4/5) even if confirmed metabolites were also present in the same samples.

Falsified: There is no RT match with a reference standard or a corresponding metabolite in the human S9 incubation. It is therefore presumed that the identities do not match.

Fig. 3 summarizes the numbers of annotations for each confirmation level with the applied confirmation efforts. It should be noted that the Schymanski scale (Schymanski et al., 2014) does not consider co-occurrences of known metabolites/annotations of the same pesticide as additional confirmatory evidence, nor does it consider compounds with rarer elemental compositions (e.g., assigned elements like Cl and Br) to have an additional confirmation value. To be in line with the commonly accepted Schymanski confidence scale and for clarity of presentation, all tentative annotations were treated as individual entities, in contrast to the selection mechanism used in the manual review by an expert.

Table 1 summarizes the results for pesticides and pesticide metabolites, the fulfilled criteria and the assigned confirmation level. Included are all level 1, 2b, 3 assignments as well as level 4/5 with correct RTs (but missing MS^2 information in either S9 incubation or urine sample).

Level 1/2b: The corresponding head-to-tail plots can be found in the SI, Figures S15-S61. Average dot-product score (forward matches) achieved for confirmation were 0.56 in ESI- and 0.54 for ESI-.

Level 3: For 54 annotations, MS^2 spectra information could be acquired from the urine samples but the corresponding reference information was missing (i.e., the same metabolite was not formed in the human liver S9 experiment or only in low concentrations, so that no MS^2 spectra was extracted). Presence of a consistent neutral loss for phase II glucuronides and sulfates (if applicable) was used as first indication of their identities. Further evaluation was performed by applying the chemical fingerprint prediction tool included in SIRIUS (Dührkop et al., 2019). If SIRIUS achieved a successful molecular formula assignment and if at least 10 significant chemical fingerprints matched with the molecular structure of the suspect, the annotation was assigned to level 3. This resulted in a total of 13 level 3 annotations.

Level 4 or 5 with correct RT: For all pesticides and pesticide metabolites without any acquired MS^2 information for the urine samples, the RT was compared to the available reference standard or the corresponding assigned signal in the human liver S9 experiment. The RT correlation for 43 tentative annotations in urine vs the human liver S9 incubation can be found in Figures S62-S63. If a deviation of more than ±0.8 min (from the fitted regression lines) was observed, the annotation was falsified. In total, five annotations were falsified based on this criterion, and 55 results with confirmed RT (either S9 or reference standard) were added to the reporting.

3.3. Comparison with targeted analysis

For DCCA-glucuronide (common metabolite for cis and trans cyfluthrin, cypermethrin, permethrin or transfluthrin), DBCA-glucuronide (common metabolite for deltamethrin), F3CCA-glucuronide (common metabolite for bifenthrin, cyhalothrin), TCPy-glucuronide, (common metabolite for chlorpyrifos-methyl) only the loss of the glucuronide was observed in MS^2 spectra acquired on the Orbitrap system. In the confirmation efforts, this was considered an insufficient fragmentation pattern for a successful spectral match and would therefore only lead to an assignment level of 4/5 according to the workflow and identification level criteria. This shows one limitation of generic screening approaches in the confirmatory procedure for compounds with either weak fragmentation or low ionization efficiency.

Thanks to the availability of targeted quantitative analyses of a subset of 107 samples performed in-parallel by LC-MS/MS on a triple quadrupole instrument employing multiple reaction monitoring (see section 2.2.7), a comparison was possible with the annotated feature signal intensities. Figures S11-S14 show the correlation of the quantitative results for DCCA, DBCA, F3CCA and TCPy in those samples vs their glucuronide intensities in the LC Orbitrap data. Although variations in ion suppression may be expected, a clear correlation is observed suggesting that the annotations are correct.

3.4. Detection frequencies

For all annotations with at least a retention time confirmation (see Table 1), the preprocessed (cleaned), RT and mass corrected LC-HRMS data were searched (exact mass from Table 1; RT threshold ±0.2 min; mass error <1.5 ppm). The search results were used to calculate the number of detections in the 2,088 samples. For each parent, the annotation with the highest observed detection rate is displayed in Fig. 4.

The number of detections is a summary of how often certain pesticide metabolites were found and thus reflects an estimated minimum number of human exposures at two time points. High detection frequencies were – among other metabolites - observed for desmethyl-acetamiprid, hydroxy-chlorpropham sulfate and the glucuronides of resp. DCCA, DBCA, F3CCA, TCPy; these are in good agreement with high detection frequencies reported in previous studies (Jamin et al., 2014; Taira et al., 2021; Marfo et al., 2015; Glorennec et al., 2017; Buckley et al., 2022; Norén et al., 2020). The exposure to pyrimethanil, thiamethoxam, clothianidin, 2,4-D, imidacloprid, acetamiprid, desmethylacetamiprid and pentachlorophenol have also been reported for pregnant women in the USA (Wang et al., 2018).

The number of detections is influenced by MS sensitivity, proficiency in protocols, ionization efficiency (sensitivity) of metabolites, ion suppression, software capabilities, isobaric interferences and sometimes even cross-contamination of standards used in quality control samples.

The five laboratories involved used harmonized methods in which

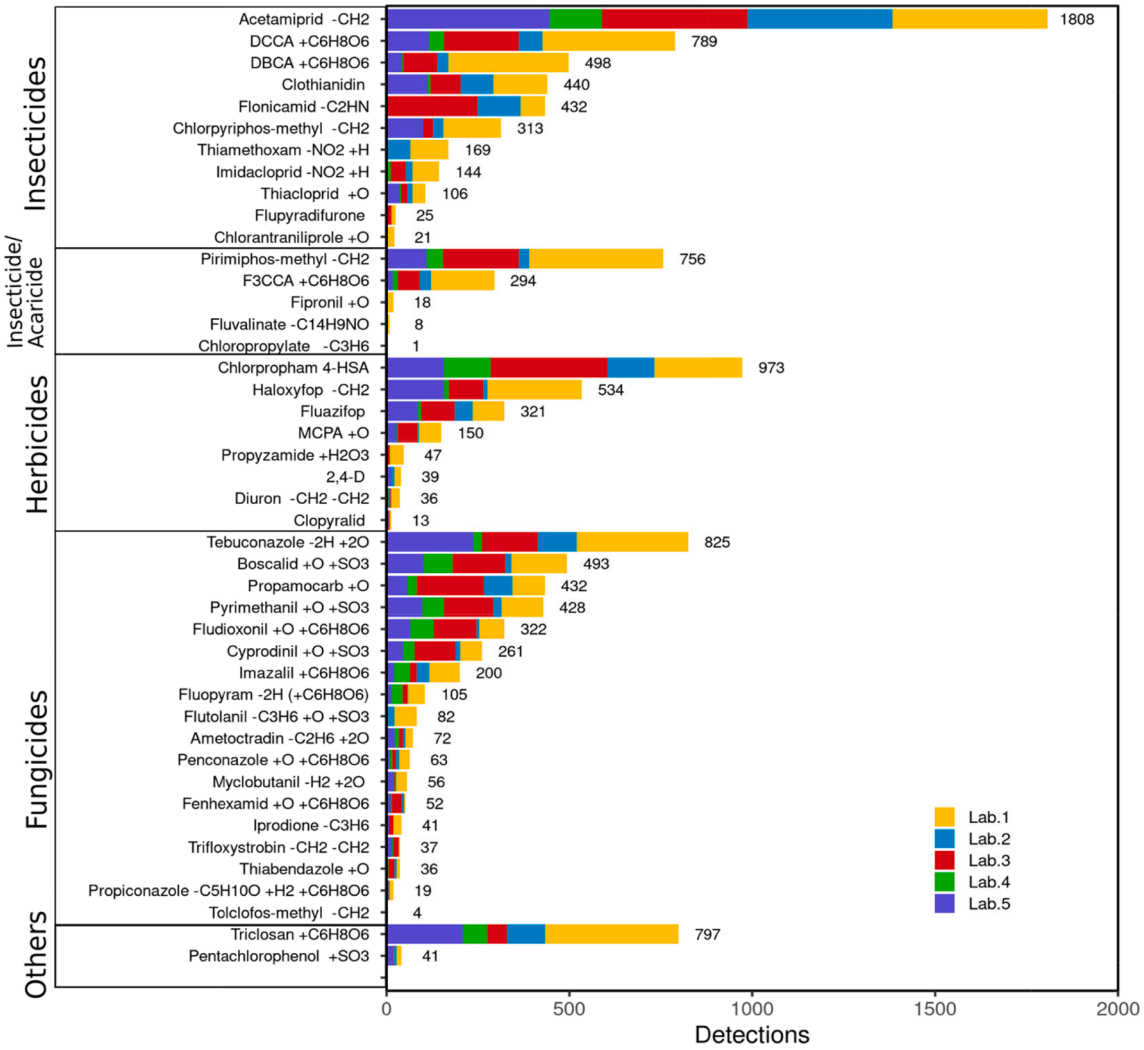

Fig. 4. Accumulated number of detections among the aligned datasets of the different laboratories (total N = 2,088) for one marker for each pesticide (chosen by highest detection frequency).

the hardware was relatively comparable, but differences in sensitivity did occur. Further details of the (inter-)laboratory comparability specific to this study and on the harmonized Quality Assurance/Quality Control provisions are available in Vitale et al., 2022. Ionization efficiency of metabolites depends strongly on the chemical structure. Ion suppression depends on co-elution of compounds competing for the charge and therefore also on ionization efficiency.

With regards to the software capabilities and peak detection (one software suite for all data), the following observations were made:

a) Large mass defects (as is the case for many of our tentative annotations with Cl and Br) give relatively clean mass traces and easily detected peaks.
b) For the automated peak detection, state-of-the-art noise estimations based on dynamic range and TIC values and well-estimated low thresholds were used, which provide high confidence in peak detection and supports accurate estimates of detection frequencies.
c) In rare cases, such as that observed for the insecticide flupyradifurone, some of the smaller signal intensities were in fact due to isobaric contamination (see comparison of MS^2 spectra in Figure S55 vs S64). To avoid overestimation of the fluradipyrone detection rate, this was resolved by co-searching for the ^{37}Cl isotope. It cannot be excluded that this may also occur to some extent with other compounds.
d) Fipronil and its metabolite fipronil sulfone were successfully confirmed by our approach. A problem with these two analytes is that they were present as reference standards in the quality control samples of this study and are extremely sensitive in ESI-. In a few sequences fipronil and fipronil-sulfone were recognized as cross-contamination signals at very low, constant levels. These cross-contamination signals were removed from detection. This shows that it is necessary to be careful and alert when calculating detection frequencies if compounds are also used as reference standards in the sequence.

4. Conclusions

This study illustrates the application of a multicentric suspect screening approach in the context of a large-scale human biomonitoring study. The application of harmonized and quality-controlled sample preparation and LC-HRMS analysis methods, together with harmonized data processing has enabled the reporting of comparable and transferable confirmation results for datasets analyzed in five different laboratories. Overall, joint efforts allowed us to confirm 46 pesticides and their metabolites from a prioritized sub-set of 498 tentative assignments (representing about 80 pesticides) despite the analytes having low signal intensities against high matrix background. We were unable to further substantiate many of the tentative annotations because the low-level

signals meant no data-dependent MS^2 spectra could be acquired, or these were severely affected by impurities from coeluting isobaric compounds. Additionally, since the identification efforts were prioritized for halogenated and PO_3-containing compounds (together with a few other tentative annotations from prior studies), a wide range of further suspect pesticide metabolites remain to be evaluated. Thus, the reported results are reflecting only a part of the pesticide exposure of humans.

However, to our knowledge our study represents the first suspect screening approach for pesticides handling a high number of tentative annotations from thousands of datasets and leading to a high number of confirmations. Further automation of data handling would facilitate dealing with these large numbers of datasets in a routine fashion. The number of confirmed annotations may be enhanced in the future with better availability of reference standards and additional *in vitro* incubation experiments to generate reference data for metabolites. Besides generating human metabolites through *in vitro* incubation, it might be advantageous to also investigate possibilities to generate plant, environment, storage and food processing metabolites.

Our study reports data that is qualitative in nature due to the highly variable matrix effects encountered in urine samples. Nevertheless, we consider the suspect screening approach as promising for chemical risk assessment because it provides a first glimpse of the potential overall exposure and can guide the prioritization of pesticide (metabolites) to be further elucidated for their suitability as biomarkers of exposure using targeted and quantitative methods. The metabolites found at high frequencies should also be considered as candidates for synthesis efforts.

Further investigation within the HBM4EU project is underway to investigate possible pesticide mixture exposure in the population sampled in this study. In combination with the metadata received from questionnaires, this will give us a better understanding of the exposure pathways as well an preliminary evaluation of the total burden to human health.

CRediT authorship contribution statement

Carolin Huber: Writing – original draft, Methodology, Visualization, Formal analysis, Validation, Investigation. **Rosalie Nijssen:** Investigation, Methodology, Writing – review & editing. **Hans Mol:** Conceptualization, Methodology, Validation, Writing – review & editing. **Jean Philippe Antignac:** Project administration, Conceptualization, Writing – review & editing. **Martin Krauss:** Writing – review & editing, Conceptualization, Methodology, Supervision. **Werner Brack:** Supervision, Writing – review & editing. **Kevin Wagner:** Investigation, Writing – review & editing. **Laurent Debrauwer:** Investigation, Writing – review & editing. **Chiara Maria Vitale:** Investigation, Writing – review & editing. **Elliott James Price:** Investigation, Writing – review & editing. **Jana Klanova:** Supervision, Writing – review & editing. **Borja Garlito Molina:** Investigation, Writing – review & editing. **Nuria Leon:** Investigation, Writing – review & editing. **Olga Pardo:** Resources, Investigation, Writing – review & editing. **Sandra F. Fernández:** Resources, Writing – review & editing. **Tamás Szigeti:** Resources, Writing – review & editing. **Szilvia Középesy:** Resources, Writing – review & editing. **Libor Šulc:** Resources, Writing – review & editing. **Pavel Čupr:** Resources, Writing – review & editing. **Inese Mārtiņsone:** Resources, Writing – review & editing. **Läsma Akülova:** Resources, Writing – review & editing. **Ilse Ottenbros:** Conceptualization, Writing – review & editing. **Roel Vermeulen:** Conceptualization, Writing – review & editing. **Jelle Vlaanderen:** Conceptualization, Writing – review & editing. **Mirjam Luijten:** Conceptualization, Methodology, Writing – review & editing. **Arjen Lommen:** Writing – original draft, Methodology, Software, Formal analysis.

Declaration of Competing Interest

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

Data availability

Data and software was made available at DOI:10.5281/zenodo.6530623

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107452.

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