

## Article

# A Snapshot of Pesticide Residues in Human, Animal, and Environmental Samples Across 10 European Countries

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## Abstract

Intensive pesticide use raises concerns for environmental, human, and animal health, yet Europe-wide data on exposure remain fragmented. In this study, 224 pesticide residues were analysed in environmental and biological samples collected during the 2021 growing season across 10 European sites, covering conventional and organic farms. Samples

included human (farmers, neighbours, consumers) and animal (cattle, goats, pigs, sheep, chickens, cats) matrices—urine, plasma, faeces, and silicone wristbands—as well as farm feed and environmental matrices including soil, earthworms, crops, water, sediment, outdoor air/dust, and indoor dust. Across all matrices, the top ten rankings based on detection frequency identified 63 priority compounds; 62 were detected in at least two matrices. Notably, non-approved pesticides accounted for 10–70% of the residues in the top ten across human, animal, and environmental samples. Detection frequencies and concentrations were generally higher in conventional than in organic systems, although differences were smaller than expected. The most frequently detected residues were glyphosate (in 9 of 16 matrices) and the banned persistent DDT metabolite p,p' DDE (in 11 of 16 matrices). Overall, pesticide residues were widespread, mostly at low levels, across humans, animals, and environments in Europe. A One-Health-based harmonised monitoring and risk assessment approach is needed, including both approved and non-approved compounds.

**Keywords:** environmental pollution; plant protection products; biological monitoring; contaminants mixture; crops; farm

## 1. Introduction

Pesticides are substances designed to kill, repel, or control certain plants, animals, or fungi that are considered pests or diseases. They are widely used, especially in conventional farming, to secure crop and livestock yields. Due to their intensive and ubiquitous application, coupled with the high persistence and mobility of many compounds, evidence on off-site contamination by pesticide residues has substantially grown over the years. Pesticides and their transformation products (hereafter referred to as metabolites) are increasingly detected, partly due to advances in sensitive detection methods. Pesticides are also detected in the environment, animals and humans in farming systems with more regulated pesticide use, such as integrated pest management and organic farming, although generally at lower levels compared to conventional farming [1,2]. Among the 425 compounds approved in the EU for plant protection, almost half (207) were synthetic pesticides [3]. According to the Food and Agriculture Organization (FAO), between 2000 and 2020, overall pesticide use in Europe increased by 12% with a substantial increase (89%) in insecticide use [4]. National trends vary considerably: Spain reported a 112% overall increase in pesticide use during this period, most pronounced for fungicides (+260%), whereas France reported a substantial decrease (−34%), mainly due to a 51% decrease in [4] fungicide and bactericide use. Globally, pesticide use increased by 62% between 2000 and 2020, with herbicides showing the largest increase (+114%) [4].

Many approved active substances meet one or more of the hazard criteria related to mobility, bioaccumulation, persistence, or toxicity to non-target species, including farm animals and humans, as reflected in the hazard classes introduced by the EC Delegated Regulation amending the CLP Regulation [5]. Acute and chronic neurologic diseases and neuropsychological disorders are well established for some groups of pesticides, particularly organophosphates and carbamate insecticides [6–9], and are also documented or suspected for other groups [9–14]. Emerging evidence also links pesticide exposure to cancer [9,15], respiratory diseases [9,16–20], endocrine disorders including diabetes [21–23], reproductive adverse health effects [24–26] and kidney disease [27].

Overall, pesticide monitoring in Europe remains fragmented across sectors, species and countries, which hampers a comprehensive understanding of pesticide occurrence, exposure pathways, and associated risks across humans, animals, and the environment.

Recognising these gaps, the European Food Safety Authority, EFSA [28], and four other European agencies have published a joint framework to support the implementation of the One Health agenda in the EU [29]. While a general framework for a more sustainable use of pesticides exists [30], there is an urgent need for harmonised, large-scale monitoring of pesticide levels across humans, animals and the environment. The European Sustainable Plant Protection Transition (SPRINT) project was designed to address this need. Its overarching aim was to develop a Global Health Risk Assessment Toolbox to assess the potential impacts of plant protection products on environmental, human and animal health [31], including determining the presence of pesticide residues and levels across humans, animals and environmental matrices.

To our knowledge, SPRINT represents the first harmonised European assessment of pesticide occurrence across humans, livestock and environmental matrices using a common analytical framework. In this overview paper, the objective was to describe the assessment of more than 200 synthetic pesticides and metabolites collected in SPRINT to be used for future risk assessment modelling. We measured across 16 matrices: humans (urine, plasma, faeces, wristbands), farm animals (urine, plasma, faeces, wristbands, feed) and the environment (soil, water, sediment, outdoor air and dust, indoor dust, earthworms, crops). Samples were collected during a field campaign in 10 SPRINT case study sites (CSSs) covering conventional farming practices, involving the use of pesticides and synthetic fertilisers and organic farming practices in Europe (for more details please see [32]).

All relevant ethical approvals and written informed consent were obtained from all study participants across the 10 case study sites, in accordance with the requirements of the relevant national or regional scientific committees. Details including the ethical approval number, approval dates, and contact information are provided in Supplementary Section S1, Tables S1 and S2. There was no public involvement at any stage of the study.

## 2. Materials and Methods

### 2.1. SPRINT Field Campaign

The sampling campaign took place during the growing season, corresponding to the period when most pesticide applications had already been carried out. Ten European CSSs were included: Spain (ES-1), Portugal (PT-2), France (FR-3), Switzerland (CH-4), Italy (IT-5), Croatia (HR-6), Slovenia (SL-7), Czech Republic (CZ-8), the Netherlands (NL-9), and Denmark (DK-10) [32]. Collectively, these covered 20 different crops (cereals, corn, rapeseed, sunflowers, poppies, mustard, grapes, olives, potatoes, apples, pears, cherries, plums, strawberries, red cabbages, green peppers, red peppers, lettuce, cabbages, and broccoli), and six farm animal species (cattle, goats, pigs, sheep, chickens and cats). For most crops and all animals, both conventional and organic production systems were covered.

The timing of the growing season varied across the CSSs depending on the crop and climate zone. For CSSs 1, 3, 4, and 6–10, mid-season corresponded to March–June 2021, whereas for CSS2 and CSS5, which had a later growing season, sampling took place from September–October 2021.

Altogether, 669 participants were included across the 10 CSSs: 116 from organic farms (53 females and 63 males), 109 from conventional farms (48 females and 61 males), 208 neighbours living next to or close to the farm or farm field (107 females and 101 males) and 236 non-farming consumers (129 females and 107 males) living further from fields and not exposed professionally to farming, as seen in Table 1.

**Table 1.** The number of human and animal samples per sample matrix across all CSSs by conventional and organic farming systems. The numbers of human samples varied slightly among matrices and do not always meet the total number of participants.

| Sample Type | Total Human | Number of Samples, Human Types |     |     |     | Total Animal | Cattle |    | Goat |    | Sheep |    | Pig |   | Chicken |    | Cat |    |
|-------------|-------------|--------------------------------|-----|-----|-----|--------------|--------|----|------|----|-------|----|-----|---|---------|----|-----|----|
|             |             | OF                             | CF  | N   | CO  |              | O      | C  | O    | C  | O     | C  | O   | C | O       | C  | O   | C  |
| Plasma      | 658         | 113                            | 108 | 204 | 233 | 198          | 49     | 40 | 3    | 18 | 18    | 24 | 0   | 4 | 8       | 15 | 10  | 9  |
| Urine       | 664         | 116                            | 108 | 204 | 236 | 95           | 25     | 54 | 11   | 1  | 3     | 0  | 1   | 0 | -       | -  | -   | -  |
| Faeces      | 649         | 114                            | 102 | 200 | 233 | 167          | 30     | 61 | 1    | 18 | 8     | 11 | 0   | 4 | 9       | 15 | 3   | 7  |
| Feed        | -           | -                              | -   | -   | -   | 66           | 20     | 15 | 1    | 6  | 7     | 6  | 0   | 1 | 3       | 6  | 0   | 1  |
| Wristband   | 639         | 115                            | 105 | 201 | 218 | 169          | 40     | 31 | 0    | 18 | 17    | 23 | 0   | 4 | 8       | 12 | 5   | 11 |

–: Not relevant. OF = Organic farmer; CF = Conventional farmer; N = Neighbour; CO = Consumer; O = Organic; C = Conventional.

The following matrices were collected for pesticide determination:

*Human:* blood (plasma), urine, faeces, and silicone wristbands (all CSSs).

*Animal:* cattle: blood (plasma), urine, faeces, feed, and wristbands (CH, CZ, DK, NL, and SL); goat: blood (plasma), urine, faeces, feed, and wristband (CH and ES); sheep: blood (plasma), urine, faeces, feed, and wristbands (CZ, HR, and NL); pig: blood (plasma), urine, faeces, feed, and wristbands (PT); chicken: blood (plasma), faeces, feed, and wristbands (CZ and PT); cat: blood (plasma), faeces, and wristband (PT, CZ and DK).

*Environment:* Soil, sediment, water, outdoor dust/air, home indoor dust, crops, and earthworms (all CSSs).

An overview of the number of samples collected per matrix across all CSSs according to the conventional or organic farming system is given in Table 1 for human and animal samples and in Table 2 for the environmental samples.

**Table 2.** Total number of environmental samples per matrix across all CSSs and by conventional/organic farming system.

| Matrix           | Total | Farm Type |              |
|------------------|-------|-----------|--------------|
|                  |       | Organic   | Conventional |
| Soil             | 201   | 101       | 100          |
| Water            | 58    | -         | -            |
| Sediment         | 38    | -         | -            |
| Outdoor air/dust | 20    | 10        | 10           |
| Indoor dust      | 115   | 57        | 58           |
| Earthworm        | 152   | 78        | 74           |
| Crop             | 193   | 95        | 98           |

–: Not classified as either conventional or organic.

## 2.2. Collection and Analysis of Samples

A target list of 208 compounds consisting of 164 parent compounds, 43 metabolites and one co-formulant and synergist (piperonyl butoxide) was established during the initial phase of the SPRINT project [33]. The selection was based on pesticides potentially used for plant protection in each CSS, pesticides known to occur in the environment and food, and results from a pesticide screening of soil, sediment, and dust samples collected from the CSS regions prior to the sampling campaign. In January 2021, 113 of the 164 active substances were approved for plant protection purposes in Europe. This list was targeted in all matrices but urine, and it was possible to analyse close to 200 substances in each matrix. For urine samples, the focus was on the corresponding urinary pesticide biomarkers of exposure which are often metabolites. Here the scope was restricted to 44 compounds

(28 from the original target list and 16 metabolites measured only in urine) due to limited availability of analytical reference standards.

For a detailed description of sampling and analysis, please see Section S2. Most of the analytical methods used have been published [34–40]. The limit of detection (LOD) was used as the analytical limit for the descriptive analysis of concentration levels and varied across the different matrices and pesticides.

#### 2.2.1. Human and Animal Samples

Morning spot *urine* samples were analysed using a multi-residue method based on liquid chromatography–tandem mass spectrometry (LC-MS/MS). Glyphosate and AMPA were analysed using a dedicated method based on solid-phase extraction and derivatisation followed by LC-MS/MS [39]. Isotopically labelled internal standards were added to the urine sample prior to extraction and derivatisation.

In *blood plasma*, following extraction based on an adapted quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, all pesticides were analysed using LC-MS/MS.

One scoop of *faeces* from each participant or animal was divided between tubes. Residues were analysed using LC-MS/MS and/or gas chromatography–tandem mass spectrometry (GC-MS/MS) analysis. Glyphosate and AMPA were analysed using LC-MS/MS [39].

One *feed sample* (100–500 g) was collected from each farm. Following extraction, LC-MS/MS and/or GC-MS/MS analysis was applied. For glyphosate and AMPA assessment, isotopically labelled internal standards were added before extraction, and LC-MS/MS analysis was applied.

Participants and animals continuously wore a *silicone wristband* for 7 consecutive days. These wristbands passively absorb pesticides from the surrounding environment [41] and 169 pesticide residues were analysed by LC-MS/MS while the remaining 25 were analysed by GC-MS [40].

#### 2.2.2. Environmental Samples

One composite *soil sample* (~2 kg) comprising 5 subsamples was randomly collected from each sampling field. Samples were dried, sieved and homogenised before being analysed for multi-residue pesticides, glyphosate and AMPA by LC-MS/MS and GC-MS/MS [33].

*Water samples* were collected from surface water bodies like small rivers, small lakes, or ponds connected to or located close to the sampled fields. Between 3 and 8 water bodies were sampled per CSS (around 2 L). The samples were analysed using three different methods [35]. Solid-phase extraction cartridges followed by either LC-MS/MS or GC-MS/MS were applied for multi-residue analyses of pesticides. Glyphosate and AMPA were analysed by LC-MS/MS. Finally, organochlorine pesticides were analysed by gas chromatography–high-resolution mass spectrometry (GC-HRMS).

*Sediment samples* were collected at the water sampling locations across the CSSs, with one composite sediment sample per water body. Sediment samples were analysed using methods like those applied to soil samples with minor adjustments [42–44].

Sampling of *outdoor air/dust* was carried out over a period of approximately 2 months during the growing season using a TIEM passive air sampling device installed 1.5 m above the soil surface using polyurethane foam (PUF) disks to absorb (semi)volatile pesticides and polyester filters (PEFs) to collect particulate matter. After extraction of the TIEM-PUF disks, the extracts were analysed by LC-MS/MS and GC-MS/MS. Glyphosate and AMPA were extracted from the PEF materials and analysed by LC-MS/MS.

For *indoor dust*, farmers were asked to replace their vacuum cleaner bag one month before the field campaign and to continue with their usual household cleaning practices. The vacuum cleaner bag was collected after one month and the dust was sieved and subsequently the fine fraction was analysed using three complementary methods [35]. Multi-residues were analysed via the QuEChERS approach for extraction and analysed using GC and LC-MS/MS. Glyphosate and AMPA were after extraction analysed by LC-MS/MS. The organochlorine pesticides were extracted by sonication and centrifugation and analysed by GC-HRMS.

For *earthworms*, a 25 cm × 25 cm × 25 cm monolith was dug from a representative plot of the sampling field, and a mustard solution was added to facilitate the emergence of earthworms which were then collected. For the analysis of glyphosate and AMPA, earthworm subsamples were spiked with isotopically labelled standards, extracted and analysed by LC-MS/MS [45]. For the analysis of pesticide residues, the QuEChERS approach was used for extraction [44,46,47]. One fraction was analysed using LC-MS/MS with electrospray ionisation and another fraction was analysed using GC-MS/MS.

One *crop* class was selected per CSS. At harvest five plants were collected and combined into a composite sample for analysis. Glyphosate and AMPA were determined by a method involving isotopically labelled standards and LC-MS/MS analysis [43]. Multi-residues were extracted using an adaptation of the QuEChERS approach followed by GC analyses.

### 2.3. Data Analysis

Pesticide residues in all matrices were ranked according to their detection frequency ( $\geq$ LOD) among the 208 targeted residues. In addition, 16 urine metabolites were included in the analysis of the urine matrix. The ten most frequently detected residues were then identified for each matrix. If more residues had the same detection frequency around the cut point of ten residues, the one first mentioned on the list was included. The top ten residues from each matrix were compiled into a single list for further analysis. The approval status of these compounds was retrieved from the EU pesticide database, based on their status prior to the initiation of the sampling campaign (1 January 2021).

For each matrix, median pesticide residue levels among samples with concentrations  $\geq$  LOD were classified into 10 log<sub>10</sub> levels (−5, −4, −3, −2, −1, 0, 1, 2, 3 and 4) corresponding to concentration ranges from 0.00001 to 10,000 ng/mL or µg/kg, depending on the matrices. Each level was represented by a different colour on the heatmaps, ranging from blue to red. The four lowest levels (−5 to −2) were referred to as low, the three intermediate levels (−1 to 1) as medium, and the three highest levels (2 to 4) as high. Results are displayed as heatmaps by matrix, sex, and participant type for humans (conventional farmers, organic farmers, neighbours, and consumers). For animals, data are presented by matrix, animal species and farm type (organic or conventional). For environmental samples, data is presented by matrix and farm type (organic, conventional). All data processing and visualisation were conducted in R version 4.3.3.

## 3. Results

### 3.1. Top Frequently Detected Compounds Across Matrices

The 10 most frequently detected compounds in each matrix are listed in Tables 3–5. Overall, this resulted in a compiled list of 63 compounds (43 parent compounds, 19 metabolites and one co-formulant). Of these, three pyrethroid metabolites were only measured in urine (DCCA-cis, DCCA-trans, and 3-PBA).

**Table 3.** Top ten most frequently detected pesticide compounds (parent compounds or their metabolites) in human matrices (urine, plasma, faeces, and wristband). Orange colour: non-approved compounds. Green colour: approved compounds. White colour: approval status not applicable. The blue colour scale illustrates the detection frequency scales, with the darker the blue, the higher the detection frequency. I = insecticide; M = metabolite; F = fungicide; H = herbicide; C = adjuvant component of pesticide formulations for synergy.

| Urine (N = 664)                                       |     | Plasma (N = 658)               |     |
|-------------------------------------------------------|-----|--------------------------------|-----|
| TCPy <sup>a</sup> (I, M)                              | 99% | p,p' DDE (I, M)                | 51% |
| 3-PBA (M) <sup>b</sup>                                | 92% | Fluopyram benzamide (F, M)     | 19% |
| Tebuconazole-OH (M)<br>[tebuconazole, F] <sup>c</sup> | 86% | Bromoxynil (H)                 | 17% |
| DCCA-trans (M) <sup>d</sup>                           | 81% | Fipronil sulfone (I, M)        | 12% |
| DEAMPY (M)<br>[pirimiphos-methyl, I] <sup>e</sup>     | 56% | Fluazifop (H)                  | 11% |
| DCCA-cis (M) <sup>f</sup>                             | 48% | Hexachlorobenzene (F)          | 10% |
| 2,4-D (H)                                             | 39% | Piperonyl butoxide (C)         | 9%  |
| DBCA (M) [Deltamethrin, I] <sup>g</sup>               | 37% | S-Metolachlor (H)              | 7%  |
| Glyphosate (H)                                        | 35% | 2,4-D (H)                      | 7%  |
| Boscalid-OH (M) [boscalid, F] <sup>h</sup>            | 20% | Acetamiprid-N-desmethyl (I, M) | 7%  |
| Faeces (N = 649)                                      |     | Wristband (N = 639)            |     |
| Glyphosate (H)                                        | 71% | Permethrin (I)                 | 97% |
| p,p' DDE (I, M)                                       | 54% | Chlorpyrifos (I)               | 91% |
| Fipronil sulfone (I, M)                               | 24% | p,p' DDE (I, M)                | 88% |
| Cypermethrin (I)                                      | 23% | Piperonyl butoxide (C)         | 87% |
| Hexachlorobenzene (F)                                 | 23% | Azoxystrobin (F)               | 74% |
| Deltamethrin (I)                                      | 17% | Fipronil (I)                   | 73% |
| Lambda-Cyhalothrin (I)                                | 16% | Boscalid (F)                   | 72% |
| Spirotetramat enol (I, M)                             | 10% | p,p' DDT (I)                   | 71% |
| Piperonyl butoxide (C)                                | 9%  | Fludioxonil (F)                | 63% |
| Cyprodinil CGA304075 (F, M)                           | 5%  | Cypermethrin (I)               | 62% |

<sup>a</sup>: 3,5,6 trichloro-2-pyridinol, a metabolite of chlorpyrifos and chlorpyrifos-methyl. <sup>b</sup>: Metabolites from multiple pyrethroids including permethrin, cypermethrin, cyfluthrin, cyhalothrin, fenvalerate and deltamethrin. <sup>c</sup>: Tebuconazole-OH was measured reflecting exposure to tebuconazole. <sup>d,f</sup>: Metabolites from multiple pyrethroids including permethrin, cypermethrin, cyfluthrin. <sup>e</sup>: DEAMPY was measured, reflecting exposure to pirimiphos-methyl. <sup>g</sup>: DBCA was measured reflecting exposure to deltamethrin. <sup>h</sup>: Boscalid-OH was measured reflecting exposure to boscalid.

**Table 4.** Top ten most frequently detected pesticide compounds in animal matrices (urine, plasma, faeces, wristband, and animal feed). Orange colour: non-approved compounds. Green colour: approved compounds. White colour: approval status not applicable. The blue colour scale illustrates the detection frequency scales, with the darker the blue, the higher the detection frequency. I = insecticide; M = metabolite; F = fungicide; H = herbicide; C = adjuvant component of pesticide formulations for synergy.

| Urine (N = 95)                                                  |     | Plasma (N = 198)                        |     |
|-----------------------------------------------------------------|-----|-----------------------------------------|-----|
| DEAMPY (M) [pirimiphos-methyl, I] <sup>a</sup>                  | 88% | p,p' DDE (I, M)                         | 40% |
| Glyphosate (H)                                                  | 77% | Bromoxynil (H)                          | 26% |
| Tebuconazole-OH (M) [tebuconazole, F] <sup>b</sup>              | 65% | p,p' DDT (I)                            | 9%  |
| 2,4-D (H)                                                       | 39% | MCPA (H)                                | 9%  |
| MCPA (H)                                                        | 38% | Pyrethrin II (I)                        | 8%  |
| AMPA (H, M)                                                     | 29% | Metolachlor ethane sulfonic acid (H, M) | 7%  |
| TCPy <sup>c</sup> (I, M)                                        | 20% | Triallate (H)                           | 5%  |
| Fluazifop (H)                                                   | 18% | Fluopyram benzamide (F, M)              | 5%  |
| DCCA-trans (M)                                                  | 16% | o,p' DDT (I)                            | 5%  |
| Trifloxystrobin CGA321113 (M) [trifloxystrobin, F] <sup>d</sup> | 4%  | Metconazole (F)                         | 4%  |

Table 4. Cont.

| Faeces (N = 167)       |     | Wristband (N = 169)    |     | Feed (N = 66)           |     |
|------------------------|-----|------------------------|-----|-------------------------|-----|
| Glyphosate (H)         | 44% | Pendimethalin (H)      | 70% | Glyphosate (H)          | 92% |
| AMPA (H, M)            | 34% | Permethrin (I)         | 61% | Cypermethrin (I)        | 39% |
| p,p' DDE (I, M)        | 25% | Piperonyl butoxide (C) | 60% | Bifenthrin (I)          | 35% |
| Cypermethrin (I)       | 19% | Chlorpyrifos (I)       | 56% | Piperonyl butoxide (C)  | 35% |
| p,p' DDD (I, M)        | 11% | p,p' DDE (I, M)        | 54% | Chlorpyrifos (I)        | 32% |
| Deltamethrin (I)       | 11% | Cypermethrin (I)       | 41% | p,p' DDE (I, M)         | 32% |
| Pyroxsulam (H)         | 9%  | Terbutylazine (H)      | 39% | Deltamethrin (I)        | 32% |
| Permethrin (I)         | 8%  | Boscalid (F)           | 38% | Pirimiphos methyl (I)   | 21% |
| Lambda-Cyhalothrin (I) | 6%  | Fluopyram (F)          | 35% | Chlorpyrifos methyl (I) | 18% |
| Chlorpyrifos (I)       | 5%  | Difenoconazole (F)     | 27% | p,p' DDD (I, M)         | 17% |

<sup>a</sup>: DEAMPY was measured, reflecting exposure to pirimiphos-methyl. <sup>b</sup>: Tebuconazole-OH was measured reflecting exposure to tebuconazole. <sup>c</sup>: 3,5,6 trichloro-2-pyridinol, metabolite of chlorpyrifos and chlorpyrifos-methyl. <sup>d</sup>: Trifloxystrobin CGA321113 was measured reflecting exposure to trifloxystrobin.

**Table 5.** Top ten most frequently detected pesticide compounds in environmental matrices (Soil, water, sediment, outdoor air/dust, indoor dust, earthworm, and crop). Orange colour: non-approved compounds. Green colour: approved compounds. White colour: approval status not applicable. The blue colour scale illustrates the detection frequency scales, with the darker the blue, the higher the detection frequency. I = insecticide; M = metabolite; F = fungicide; H = herbicide; C = adjuvant component of pesticide formulations for synergy.

| Soil (N = 201)                     |      | Water (N = 58)                   |      | Sediment (N = 38) |     |
|------------------------------------|------|----------------------------------|------|-------------------|-----|
| p,p' DDE (I, M)                    | 90%  | Glyphosate (H)                   | 100% | AMPA (H,M)        | 76% |
| Hexachlorobenzene (F)              | 40%  | Hexachlorobenzene (F)            | 100% | Glyphosate (H)    | 61% |
| AMPA (H, M)                        | 38%  | Lindane (I)                      | 100% | p,p' DDE (I, M)   | 50% |
| Chlorpyrifos (I)                   | 34%  | Dieldrin (I)                     | 98%  | p,p' DDD (I, M)   | 37% |
| p,p' DDT (I)                       | 25%  | p,p' DDE (I, M)                  | 95%  | Fludioxonil (F)   | 34% |
| Boscalid (F)                       | 24%  | AMPA (H, M)                      | 81%  | Azoxystrobin (F)  | 32% |
| Glyphosate (H)                     | 22%  | p,p' DDD (I, M)                  | 76%  | Fluopicolide (F)  | 32% |
| o,p' DDT (I)                       | 18%  | p,p' DDT (I)                     | 76%  | Boscalid (F)      | 29% |
| p,p' DDD (I, M)                    | 16%  | Terbutylazine (H)                | 72%  | Prosulfocarb (H)  | 26% |
| Azoxystrobin (F)                   | 16%  | o,p' DDD (I, M)                  | 71%  | o,p' DDD (I, M)   | 24% |
| Outdoor air/dust (N = 20)          |      | Indoor dust (N = 115)            |      |                   |     |
| Glyphosate (H)                     | 100% | Fludioxonil (F)                  | 100% |                   |     |
| AMPA (H, M)                        | 75%  | Hexachlorobenzene (F)            | 100% |                   |     |
| Pendimethalin (H)                  | 70%  | Imidacloprid (I)                 | 100% |                   |     |
| S-Metolachlor (H)                  | 50%  | Piperonyl butoxide (C)           | 100% |                   |     |
| Terbutylazine (H)                  | 50%  | Azoxystrobin (F)                 | 99%  |                   |     |
| Terbutylazine_desethyl (H, M)      | 50%  | Carbendazim (F)                  | 99%  |                   |     |
| Folpet (F)                         | 45%  | Fipronil (I)                     | 99%  |                   |     |
| Prosulfocarb (H)                   | 45%  | Fipronil sulfone (I, M)          | 99%  |                   |     |
| Prothioconazole-desthio (F, M)     | 45%  | Lindane (I)                      | 99%  |                   |     |
| Chlorpyrifos (I)                   | 35%  | Tebuconazole (F)                 | 99%  |                   |     |
| Earthworm (N = 152)                |      | Crop (N = 193)                   |      |                   |     |
| p,p' DDE (I, M)                    | 47%  | Dieldrin (I)                     | 15%  |                   |     |
| Imidacloprid (I)                   | 36%  | Metalaxyl-M (F)                  | 10%  |                   |     |
| Acetamiprid (I)                    | 19%  | Metolachlor oxanilic acid (H, M) | 10%  |                   |     |
| Thiacloprid (I)                    | 17%  | Dimethomorph (F)                 | 9%   |                   |     |
| Azoxystrobin (F)                   | 15%  | Boscalid (F)                     | 9%   |                   |     |
| p,p' DDD (I, M)                    | 14%  | Fluopyram (F)                    | 8%   |                   |     |
| Clothianidin (I, (M)) <sup>a</sup> | 13%  | Tebuconazole (F)                 | 8%   |                   |     |

Table 5. Cont.

| Earthworm (N = 152)                    |     | Crop (N = 193)   |    |
|----------------------------------------|-----|------------------|----|
| Metolachlor ethane sulfonic acid (H,M) | 13% | Acetamiprid (I)  | 8% |
| Difenoconazole (F)                     | 12% | Azoxystrobin (F) | 8% |
| Azoxystrobin-O-demethyl (F,M)          | 10% | Deltamethrin (I) | 8% |

<sup>a</sup>: Clothianidin is a parent compound and a metabolite of thiamethoxam.

### 3.2. PPP Frequency and Levels in Human, Animal and Environmental Matrices

#### 3.2.1. Humans

In *urine*, among the top ten detected compounds, six were insecticides (one non-approved (NA) metabolite TCPy, three pyrethroids, DEAMPY and deltamethrin), as seen in Table 3. The remaining four compounds included two fungicides (boscalid, tebuconazole) and two herbicides (glyphosate and 2,4-D). TCPy was the most frequently detected (99%), followed by 3-PBA (92%) and 5-hydroxy-tebuconazole (86%), with similar detection frequencies across all groups (males/females; farmers/neighbours/consumers; organic/conventional), see Figure 1. Detection frequencies for the other seven compounds ranged from 20% to 81%. Glyphosate was most frequently detected among male conventional farmers, as seen in Figure 1. The pirimiphos-methyl metabolite DEAMPY had the highest median levels (around 1 ng/mL) relatively consistently across all groups, see Figure 2, and was found in at least 50% of the samples in all groups, see Figure 1. MCPA concentrations were lower among female farmers than in the other groups. Otherwise, the levels were similar across groups, see Figure 2.

In *plasma samples*, four top ten compounds were insecticides (p,p' DDE (NA), fipronil sulfone (NA), acetamiprid-N-desmethyl, and piperonyl butoxide, a co-formulant used in pyrethroid-based products). Four were herbicides (bromoxynil (NA), fluazifop-P, S-metolachlor, and 2,4-D). The remaining two compounds were fungicides, hexachlorobenzene (HCB) (NA) and fluopyram benzamide, see Table 3. Detection frequencies and levels for all 10 compounds were rather similar across groups (Table 3, Figures 1 and 2), with detection frequencies below 25% (apart from p,p' DDE found in 51%), and plasma levels below 1 ng/mL.

In *faeces samples*, the majority (seven out of ten) were insecticides (p,p' DDE (NA), fipronil sulfone (NA), cypermethrin, deltamethrin, lambda-cyhalothrin, spirotetramat, and the co-formulant piperonyl butoxide), Table 3. Two were fungicides (cyprodinil metabolites CGA304075 and hexachlorobenzene (NA)). Only one herbicide, glyphosate, was among the top ten compounds, but with the highest detection frequency, 71%. p,p' DDE had a 54% detection frequency. Glyphosate and p,p' DDE were more frequently detected in conventional compared to organic farmers, with the highest glyphosate frequency observed among male conventional farmers and male neighbours. The remaining top ten compounds had detection frequencies below 25%, with generally no major differences between groups. Exceptions included permethrin and p,p' DDD which were detected only in males whereas chlorpyrifos was detected only in female participants, see Figure 1. Median levels were also highest for glyphosate, reaching approximately 100 µg/kg, with no substantial differences between groups, see Figure 2. For the other pesticides, the levels were substantially lower.

In *wristband samples* six of the top ten identified compounds were insecticides (permethrin, chlorpyrifos (NA), p,p' DDE (NA), fipronil (NA), p,p' DDT (NA), and cypermethrin), three were fungicides (azoxystrobin, boscalid and fludioxonil), and one was the insecticide co-formulant piperonyl butoxide, see Table 3. All the top ten compounds were detected in at least 88% of the samples, with permethrin detected in as many as 97%. For several compounds, detection frequencies were highest for male conventional farmers, including some of the top compounds (azoxystrobin, fludioxonil). For permethrin, levels were

high across all groups, up to around 100 µg/kg, see Figure 2. Concentrations of several other residues were also relatively high, including chlorpyrifos, lindane, cypermethrin and tebuconazole. Boscalid, cypermethrin, chlorpyrifos, and fludioxonil levels were highest among conventional male farmers. No apparent sex differences were observed for the top ten compounds.



**Figure 1.** Frequency of >LOD values in human urine, plasma, faeces, and wristbands, stratified by different participant types and sex. F\_CF: Female conventional farmers, F\_OF: Female organic farmers, F\_Neighbour: Female neighbours, F\_Consumer: Female consumers, M\_CF: Male conventional farmers, M\_OF: Male organic farmers, M\_Neighbour: Male neighbours, M\_Consumer: Male consumers. A-I: approved insecticides, A-H: approved herbicides, A-F: approved fungicides, A-MT: metabolites of approved compounds, NA-I: non-approved insecticides, NH: non-approved herbicides, NA-F: non-approved fungicides, NA-MT: metabolites of non-approved compounds, C: co-formulant (piperonyl butoxide), UM: Metabolites only detected in urine matrix. Coloured fields in the heatmap are included for compounds which were detected for at least five individuals per group, otherwise the field is white. Grey colour: not measured.



**Figure 2.** Median levels of frequently detected compounds across all matrices in human samples (plasma and urine: ng/mL, faeces and wristband: µg/kg) stratified by participant types and sex. F\_CF: Female conventional farmers, F\_OF: Female organic farmers, F\_Neighbour: Female neighbours, F\_Consumer: Female consumers, M\_CF: Male conventional farmers, M\_OF: Male organic farmers, M\_Neighbour: Male neighbours, M\_Consumer: Male consumers. A-I: approved insecticides, A-H: approved herbicides, A-F: approved fungicides, A-MT: metabolites of approved compounds, NA-I: non-approved insecticides, NH: non-approved herbicides, NA-F: non-approved fungicides, NA-MT: metabolites of non-approved compounds, C: co-formulant (piperonyl butoxide), UM: Metabolites only detected in urine matrix. Coloured fields in the heatmap are included for compounds which were detected for at least five individuals per group, otherwise the field is white. For urine, tebuconazole-OH was measured reflecting exposure to tebuconazole; DEAMPY was measured reflecting exposure to pirimiphos-methyl; DBCA was measured reflecting exposure to deltamethrin; boscalid-OH was measured reflecting exposure to boscalid; and trifloxystrobin CGA321113 was measured reflecting exposure to trifloxystrobin.

In summary, multiple pesticide residues were widely detected across all human matrices. Insecticides were more prominently represented among the ten most frequently detected compounds, whereas herbicides were less frequently represented. No consis-

tent patterns emerged across participant groups. Nevertheless, certain residues—such as glyphosate, p,p' DDE, and chlorpyrifos—were more frequently detected and/or present at higher concentrations among conventional farmers than among other groups in one or more matrices. Some compounds, including glyphosate and p,p' DDD, also exhibited sex-specific differences in distribution. While most detected residues are currently approved as pesticides, notable exceptions were identified in plasma where pesticides banned by the end of the growing season in 2021 or earlier were present (p,p' DDE, bromoxynil, HCB), wristbands (chlorpyrifos, cypermethrin, p,p' DDE, p,p' DDT), faeces (p,p' DDE, HCB), and urine (TCPy).

### 3.2.2. Animals

For *urine samples*, three top ten compounds were insecticides (pirimiphos-methyl, TCPy (NA), DCCA-trans), five were herbicides (glyphosate, 2,4-D, MCPA, AMPA, fluazifop-P) and two were fungicides (tebuconazole, trifloxystrobin), see Table 4. The top three were pirimiphos-methyl (88%), glyphosate (77%) and tebuconazole (65%). The remaining compounds were detected in 39% of samples or fewer. Pirimiphos-methyl and glyphosate were more frequently detected in conventional than in organic cattle, whereas glyphosate and tebuconazole were more frequently detected in organic than in conventional goats, see Figure 3. Furthermore, fluazifop-P, DCCA-trans and trifloxystrobin were only detected in livestock from conventional farms. In general, median levels of pesticide residues were in the range of 0.1–1 ng/mL, see Figure 4. Pirimiphos-methyl levels were higher, with the highest median concentrations observed in cattle from conventional farms (approximately 10 ng/mL) compared with other livestock. Moreover, median levels were higher in samples from conventionally farmed livestock for glyphosate, 2,4-D, MCPA, TCPy, and AMPA.

In *plasma samples* from livestock and cats, four top ten residues were insecticides (p,p' DDE (NA), o,p' DDT (NA), p,p' DDT (NA), pyrethrin II), three were herbicides (metolachlor ethane, triallate, bromoxynil (NA)), and two were fungicides (metconazole, fluopyram benzamide), see Table 4. The two most frequently detected compounds were p,p' DDE (40%) and bromoxynil (26%). The remaining compounds were detected in 9% of samples or fewer. Detection frequencies were generally similar across groups, except for MCPA and fluopyram benzamide, which were more frequently detected from conventional farms, see Figure 3. Across animal types, eight compounds were only found in samples from organic farms whereas eight were only found in samples from conventional farms. Generally, median levels were in the low to medium range (0.01–1 ng/mL). However, high levels of MCPA (above 100 ng/mL) were found in conventionally farmed livestock (cattle and sheep), see Figure 4. The highest p,p' DDE levels were found in pigs and chickens from both farming systems, whereas the lowest concentrations were observed in cattle, goats, and sheep. p,p' DDE was not detected in plasma from organically farmed goats. Bromoxynil levels were higher in organic cats and cattle than in conventionally farmed animals, whereas bromoxynil concentrations were higher in conventionally than organically farmed sheep. Bromoxynil was not detected in chickens, organically farmed goats or conventionally farmed pigs. Fluopyram benzamide was only detected in plasma samples from conventionally farmed animals.

In *faeces samples*, seven top ten compounds were insecticides (cypermethrin, deltamethrin, lambda-cyhalothrin and four NA (p,p' DDE, p,p' DDD, permethrin, chlorpyrifos)). The remaining three were herbicides (glyphosate, AMPA, pyroxsulam). Glyphosate was detected most frequently (44%) followed by AMPA (34%). The remaining compounds were detected in 25% or below, see Table 4. Detection frequencies were generally higher in animals from conventional than from organic farms, see Figure 3. Median levels detected were in the

medium to high range (0.1–1000 µg/kg), see Figure 4. No clear differences in levels were seen between organic and conventional farms.



**Figure 3.** Frequency of detection of commonly detected compounds across all matrices in animal samples, stratified by farm and animal types (OF: organic farm, CF: conventional farm). A-I: approved insecticides, A-H: approved herbicides, A-F: approved fungicides, A-MT: metabolites of approved compounds, NA-I: non-approved insecticides, NH: non-approved herbicides, NA-F: non-approved fungicides, NA-MT: metabolites of non-approved compounds, C: co-formulant (piperonyl butoxide), UM: metabolites only detected in urine matrix. Coloured fields in the heatmap are included for compounds which were detected for at least five individuals per group, otherwise the field is white. For urine, grey colour: not measured. For urine, tebuconazole-OH was measured reflecting exposure to tebuconazole; boscalid-OH was measured reflecting exposure to boscalid; and trifloxystrobin CGA321113 was measured reflecting exposure to trifloxystrobin.

For *wristbands* worn by farm animals, four top ten compounds were insecticides (permethrin (NA), chlorpyrifos (NA), p,p' DDE (NA), cypermethrin), three were fungicides (boscalid, fluopyram, difenoconazole), one was the co-formulant and synergist piperonyl butoxide, and the remaining two were herbicides (pendimethalin, terbutylazine), see Table 4. Detection frequencies were high: pendimethalin was detected in 70% of samples, permethrin in 61%, piperonyl butoxide in 60% and the remaining compounds of the top

ten compounds in at least 27% of samples. Detection frequencies were highest among animals from conventional farms, see Figure 3. Most levels detected in animal wristbands were in the medium range (0.1–10 µg/kg), with some exceptions, such as permethrin and cypermethrin with some high range levels (10–100 µg/kg). Overall, similar levels were seen in animals from conventional and organic farms (Figure 4).

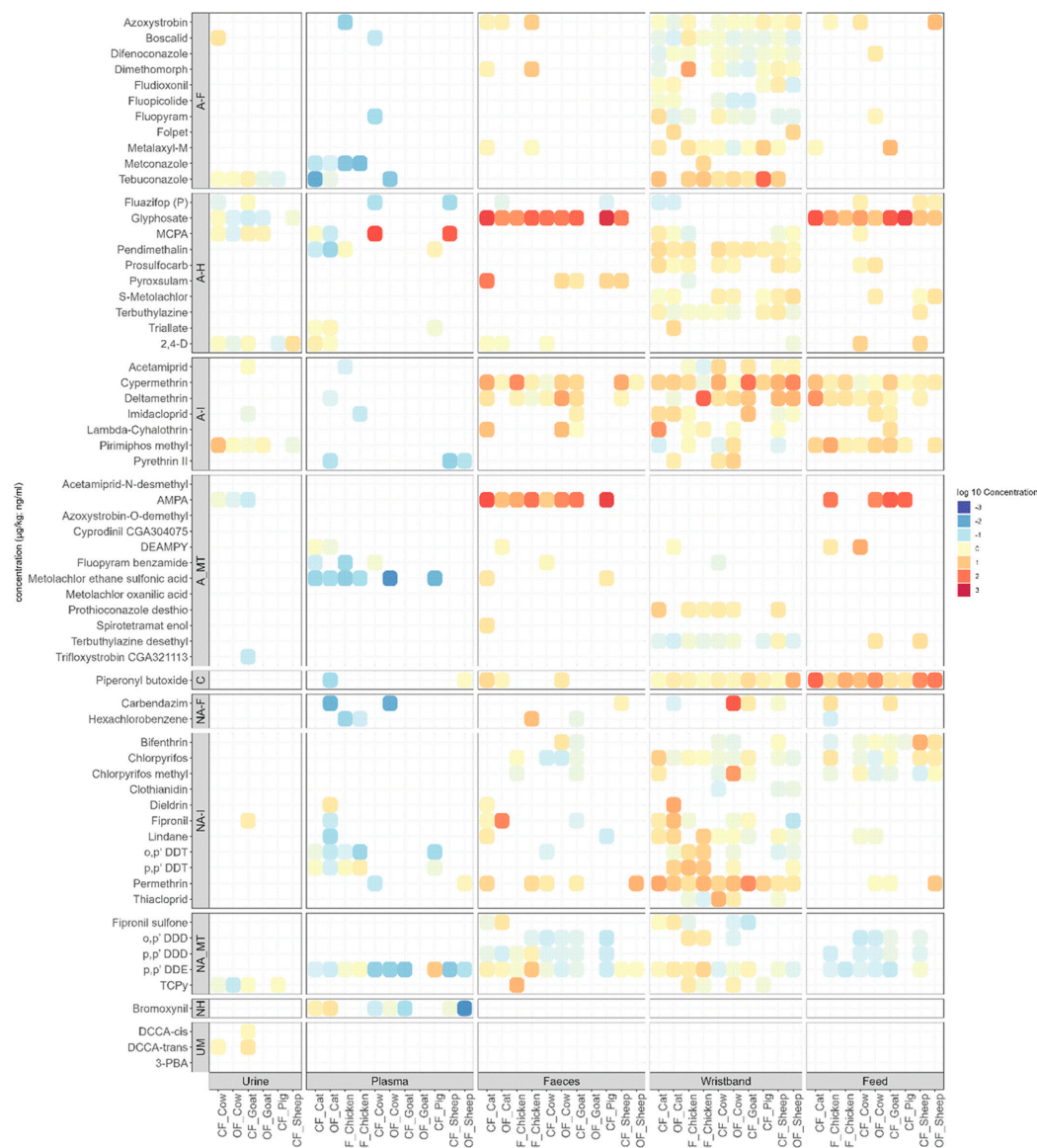


**Figure 4.** Median levels of frequently detected compounds across all matrices in animal samples (plasma and urine: ng/mL, faeces and wristband: µg/kg), stratified by farm and animal types (OF: organic farm, CF: conventional farm). A-I: approved insecticides, A-H: approved herbicides, A-F: approved fungicides, A-MT: metabolites of approved compounds, NA-I: non-approved insecticides, NH: non-approved herbicides, NA-F: non-approved fungicides, NA-MT: metabolites of non-approved compounds, C: co-formulant (piperonyl butoxide), UM: metabolites only detected in urine matrix. Coloured fields in the heatmap are included for compounds which were detected for at least five individuals per group, otherwise the field is white. For urine, tebuconazole-OH was measured reflecting exposure to tebuconazole; boscalid-OH was measured reflecting exposure to boscalid; and trifloxystrobin CGA321113 was measured reflecting exposure to trifloxystrobin.

In livestock *feed samples*, nine out of the top ten were insecticide residues (cypermethrin, deltamethrin, pirimiphos methyl, bifenthrin (NA), chlorpyrifos (NA), p,p' DDE (NA), p,p' DDD (NA), chlorpyrifos-methyl (NA), and piperonyl butoxide). One of the top ten was the

herbicide glyphosate. The top three detected were glyphosate (92%), cypermethrin (39%) and bifenthrin (35%) (Table 4). Detection frequencies were comparable or slightly lower in organic than in conventional feed samples, see Figure 3. Median levels in feed samples were generally in the medium range (0.1–10 µg/kg) although higher levels for glyphosate and AMPA were identified (10–1000 µg/kg), see Figure 3. Slightly higher levels were detected in the feed samples from conventional than from organic farms. Glyphosate levels were particularly lower in feed samples from organically farmed chickens and cattle feed than in corresponding samples from conventionally farmed animals. The highest levels of cypermethrin were found in feed from organically farmed chickens and conventionally farmed goats. For bifenthrin, the highest levels were found in sheep feed, particularly from conventional sheep farms.

In summary, insecticides predominated across animal matrices, whereas fungicides were less represented. A substantial number of the identified compounds—primarily insecticides—were not approved and detected in multiple matrices. Overall, more compounds were detected in samples from conventional farms, often with higher detection frequencies than in organic systems. Median concentrations in plasma, faeces, and wristbands were generally comparable between conventional and organic farms and fell within similar ranges, albeit with some exceptions. In contrast, feed and urine samples showed a tendency toward higher median concentrations in conventional farming systems.

### 3.2.3. Environment

For *soil samples*, five top ten compounds were NA insecticides (p,p' DDE, p,p' DDT, o,p' DDT, p,p' DDD, chlorpyrifos), three were fungicides (azoxystrobin, boscalid, HCB (NA)), and the remaining two were herbicide residues (glyphosate and AMPA), see Table 5. p,p' DDE was detected in 90%, HCB in 40%, and AMPA in 35% of the samples. Detection frequencies were generally similar between conventional and organic farms except for AMPA detected more frequently in conventional fields, see Figure 5. Of note, outside the top ten compounds more pesticides had higher detection frequencies in conventional compared to organic farms. Median levels were in the medium to high range (10–1000 µg/kg). Generally, similar or higher levels were observed for conventional than in organic soil samples. Similar levels were found for the most frequently detected compounds, p,p' DDE and HCB, across conventional and organic fields (Figure 6) whereas median levels of AMPA were slightly higher in conventional fields.

In *surface water samples*, six top ten compounds were NA insecticides (lindane, dieldrin, p,p' DDE, p,p' DDD, p,p' DDT, o,p' DDD), one was an NA fungicide (HCB) and three were herbicides (glyphosate, AMPA, terbuthylazine). Detection frequencies were high: the top three compounds glyphosate, HCB, and lindane were detected in 100%, while the remaining top ten compounds were detected in at least 71% of samples, see Table 5 and Figure 5. However, levels were at the low end (0.000001–0.1 ng/mL), see Figure 6. Of note, organochlorine pesticides were quantified at lower LODs (1–20 pg/L) than the other pesticides (0.08–14.6 ng/L).

For *sediment samples*, three top ten compounds were NA insecticides (p,p' DDE, p,p' DDD, o,p' DDD), four were fungicides (fludioxonil, azoxystrobin, fluopicolide, boscalid) and three were herbicides (glyphosate, AMPA, prosulfocarb). AMPA, glyphosate, and p,p' DDE were detected in 76%, 61%, and 50% of the samples, respectively, and the other compounds in the top ten were detected in 24–37% of the samples (Table 5, Figure 5). Pesticide levels were in the medium range (1–10 µg/kg), see Figure 6.



**Figure 5.** Frequency of detection of frequently detected compounds across all matrices in environmental sample, stratified by farm types (OF: organic farm, CF: conventional farm, CSS: case study sites without distinction of organic or conventional fields). A-I: approved insecticides, A-H: approved herbicides, A-F: approved fungicides, A-MT: metabolites of approved compounds, NA-I: non-approved insecticides, NH: non-approved herbicides, NA-F: non-approved fungicides, NA-MT: metabolites of non-approved compounds, C: co-formulant (piperonyl butoxide), UM: metabolites only detected in urine matrix. Coloured fields in the heatmap are included for compounds which were detected for at least five individuals per group, otherwise the field is white. Grey colour: not measured.

For *outdoor air/dust samples*, three of the top ten compounds were fungicides (folpet, phthalimide, prothioconazole-desthio), the remaining seven were herbicides (glyphosate, AMPA, pendimethalin, S-metolachlor, terbuthylazine, terbuthylazine desethyl, prosulfocarb). The top three detected compounds were herbicides: glyphosate (100%), AMPA

(75%), and pendimethalin (70%). The remaining compounds in the top ten were detected in 35–50% of samples (Table 5). Detection frequencies were similar across farming systems and only three additional compounds were detected in outdoor air sampled from conventional compared with organic fields (Figure 5). Similar levels (0.0001–10 µg/kg) were seen in conventional and organic outdoor air/dust samples, see Figure 6.

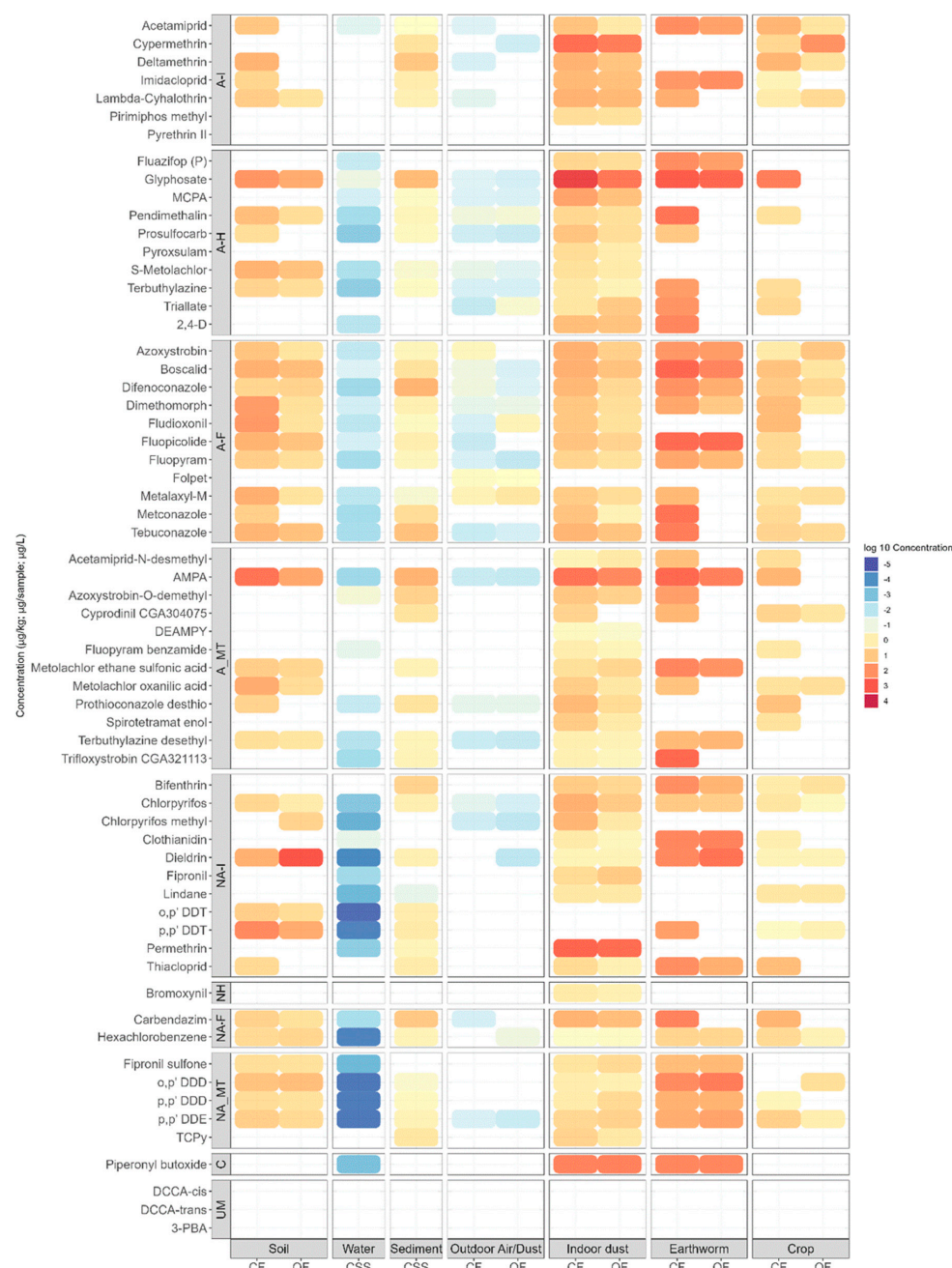


**Figure 6.** Median levels of frequently detected compounds across all matrices in environmental samples (soil, water, sediment, outdoor air/dust, indoor dust, earthworm, and crop), stratified by farm types (OF: organic farm, CF: conventional farm, CSS: case study sites without distinction of organic or conventional fields). A-I: approved insecticides, A-H: approved herbicides, A-F: approved fungicides, A-MT: metabolites of approved compounds, NA-I: non-approved insecticides, NH: non-approved herbicides, NA-F: non-approved fungicides, NA-MT: metabolites of non-approved compounds, C: co-formulant (piperonyl butoxide), UM: metabolites only detected in urine matrix. Coloured fields in the heatmap are included for compounds which were detected for at least five individuals per group, otherwise the field is white.

Among *indoor dust samples* in farmhouses, five top ten compounds were insecticides or insecticide-related compounds (fipronil (NA), fipronil sulfone (NA), lindane (NA), imidacloprid, and the co-formulant and synergist piperonyl butoxide). The remaining five were fungicides (fludioxonil, azoxystrobin, tebuconazole, HCB (NA), carbendazim (NA)). Among the 63 compounds included in the compiled list, 60 were detected in indoor dust, with no major differences in detection frequencies between conventional and organic farming systems, see Figure 5. Median levels were generally in the medium range (10–100 µg/kg) and similar between conventional and organic farming systems, see Figure 6. A few compounds detected in 25–100% of samples had higher levels, in the range of 100–1000 µg/kg (cypermethrin, glyphosate, AMPA, permethrin).

For *earthworm samples*, six top ten compounds were insecticides (acetamiprid, imidacloprid, p,p' DDE (NA), p,p' DDD (NA), thiachloprid (NA), clothianidin (NA)). Three were fungicides or fungicide metabolites: azoxystrobin, difenoconazole, azoxystrobin-O-demethyl, and the last one was the herbicide metabolite S-metolachlor ethane sulfonic acid. p,p' DDE was detected in 47%, imidacloprid in 36%, and acetamiprid in 19% of the samples, the rest was detected in below 17%, see Table 5. Levels for conventional and organic earthworm samples were similar and in the medium to high range (1–1000 µg/kg). However more compounds were detected in the earthworm samples from conventional fields (Figures 5 and 6).

In *crop samples*, three top ten compounds were insecticides (acetamiprid, deltamethrin, dieldrin (NA)). Six were fungicides (metalaxyl-M, tebuconazole, dimethomorph, azoxystrobin, fluopyram, boscalid), and one was the herbicide metabolite S-metolachlor-oxanilic acid. Detection frequencies were rather low: the top three detected compounds were dieldrin (15%), metalaxyl-M (10%) and S-metolachlor-oxanilic acid (10%), see Table 5. Dieldrin was more frequently detected in crop samples from organic farming. Conversely, metalaxyl-M and S-metolachlor-oxanilic acid were more frequently detected in crop samples from conventional farming, see Figure 5. For those three compounds, levels were in the median range (1–10 µg/kg) and were similar between crops from conventional and organic farming, see Figure 6. For other compounds detected in crops, levels were also largely comparable between conventional and organic crops.

In summary, among environmental samples, indoor dust contained by far the highest number of detected compounds. Across most matrices, insecticides and fungicides were the most frequently identified residues. The greatest numbers of non-approved compounds were observed in surface water and soil. In matrices for which comparisons between farming systems were possible (soil, crops, earthworms, outdoor air/dust, indoor dust), detection frequencies and/or concentrations were generally similar or higher in conventional than in organic farms.

### 3.3. Compounds Across Humans, Animals, and Environment

We compared the compounds commonly detected across human, animal and environmental matrices as seen in Table 6. The percentage of co-occurring compounds was highest between human and animal wristbands (78%), followed by indoor dust (79% with human wristband and 71% with animal wristband). Compounds detected in urine showed, as expected, limited overlap with all other matrices, ranging from 11% to 22% due to the different and narrower scope in urine. Environmental matrices showed substantial overlap with an average 51% similarity across crop, earthworm, indoor dust, outdoor air/dust, sediment, soil, and water matrices.

**Table 6.** Co-occurrence matrix showing the percentage of common compounds detected across every pair of matrices, from the complete list of the 63 compounds. A-: Animal compartment, H-: Human compartment. The darker the blue, the smaller number of common compounds between two matrices. The darker the red, the greater number of common compounds between two matrices.

|                  | A-Faeces | A-Feed | A-Plasma | A-Urine | A-Wristband | Crop | Earthworm | Indoor Dust | Outdoor Air/Dust | Sediment | Soil | Water | H-Faeces | H-Plasma | H-Urine |
|------------------|----------|--------|----------|---------|-------------|------|-----------|-------------|------------------|----------|------|-------|----------|----------|---------|
| A-Feed           | 37%      |        |          |         |             |      |           |             |                  |          |      |       |          |          |         |
| A-Plasma         | 29%      | 24%    |          |         |             |      |           |             |                  |          |      |       |          |          |         |
| A-Urine          | 13%      | 13%    | 14%      |         |             |      |           |             |                  |          |      |       |          |          |         |
| A-Wristband      | 43%      | 43%    | 41%      | 17%     |             |      |           |             |                  |          |      |       |          |          |         |
| Crop             | 33%      | 32%    | 29%      | 11%     | 49%         |      |           |             |                  |          |      |       |          |          |         |
| Earthworm        | 33%      | 35%    | 30%      | 16%     | 49%         | 51%  |           |             |                  |          |      |       |          |          |         |
| Indoor dust      | 51%      | 49%    | 41%      | 22%     | 71%         | 60%  | 63%       |             |                  |          |      |       |          |          |         |
| Outdoor Air/Dust | 24%      | 32%    | 22%      | 11%     | 44%         | 40%  | 37%       | 48%         |                  |          |      |       |          |          |         |
| Sediment         | 37%      | 40%    | 32%      | 16%     | 56%         | 52%  | 52%       | 65%         | 44%              |          |      |       |          |          |         |
| Soil             | 32%      | 33%    | 27%      | 11%     | 49%         | 48%  | 49%       | 56%         | 43%              | 54%      |      |       |          |          |         |
| Water            | 37%      | 38%    | 35%      | 17%     | 57%         | 44%  | 51%       | 63%         | 41%              | 54%      | 49%  |       |          |          |         |
| H-Faeces         | 41%      | 38%    | 33%      | 13%     | 49%         | 44%  | 44%       | 62%         | 35%              | 49%      | 40%  | 46%   |          |          |         |
| H-Plasma         | 37%      | 32%    | 35%      | 17%     | 52%         | 40%  | 46%       | 60%         | 35%              | 49%      | 43%  | 48%   | 41%      |          |         |
| H-Urine          | 17%      | 16%    | 17%      | 22%     | 22%         | 21%  | 21%       | 30%         | 14%              | 21%      | 14%  | 21%   | 21%      | 22%      |         |
| H-Wristband      | 44%      | 43%    | 43%      | 19%     | 78%         | 52%  | 54%       | 79%         | 44%              | 59%      | 49%  | 60%   | 56%      | 60%      | 25%     |

## 4. Discussion

This paper provides an overview of pesticide residue occurrence and levels across 16 matrices: four human matrices (plasma, faeces, urine, and silicone wristbands); five matrices for six animal species (cattle, goat, pig, sheep, chicken, and cat) including plasma, faeces, urine, silicone wristbands, and feed; and 7 environmental matrices (soil, earthworms, crops, water, sediment, outdoor air/dust, and indoor dust from farmhouses) in 10 European countries, covering both conventional and organic production and residential settings. The frequency and levels of pesticide residues varied substantially according to the compartment (human, animal, or environmental), matrix, and farming system, whereas they appeared to be less strongly influenced by sex. Most levels identified across matrices were in the parts per billion to parts per trillion range.

### 4.1. Humans

Our findings confirm widespread, albeit generally low-level, human exposure to multiple pesticide residues. Insecticides dominated the top ten detected compounds, while herbicides were less represented. Detection frequencies were highest in urine and wristbands, where the top three compounds were present in at least 86% of samples, and lowest in plasma (17–51%), consistent with the role of urine as a major route of pesticide excretion.

Detection frequencies for key urinary metabolites were comparable to those reported in the European Human Biomonitoring Initiative (HBM4EU), including TCPy (99% vs. 100%), 3-PBA (92% vs. 91%), and DCCA isomers (48–81% vs. 45–75%) [46]. Glyphosate detection was somewhat higher in SPRINT (35%) than in HBM4EU cohorts (17–30%) [48] despite relatively stable use of glyphosate over the last decade, although substantial regional differences have been reported [48].

Notably, several pesticides banned in the EU for decades (e.g., *p,p'* DDE, HCB) were still detected across multiple matrices. Their continued detection may reflect their environmental persistence and, in some cases, bioaccumulative properties. *p,p'* DDE was found in 51% of plasma samples, in line with earlier European studies ([49,50]), although generally at lower levels, suggesting a gradual decline in exposure over time. However, differences between studies may also reflect temporal trends and methodological variations, including differences in analytical methods and limits of detection. Median plasma levels were also somewhat lower than those previously reported [51].

HCB was detected in 10% of the plasma samples, within the wide range reported in previous studies [49,50]. Although previous research observed sex-related differences in HCB and *p,p'* DDE levels [49,50], no clear sex differences emerged in the present study.

TCPy was detected in 99% of the urine samples, consistent with the high prevalence reported in other European cohorts [51]. Bromoxynil was present in 17% of the plasma samples, likely reflecting overlap between its regulatory phase-out around 2021 and the sampling period, as well as possible temporary exemptions.

Fipronil and its metabolite were also among the most frequently detected compounds in several matrices. Their presence may reflect both environmental persistence following past agricultural use and ongoing non-agricultural applications, including veterinary treatments and household pest control. A likely source is the use of fipronil as a biocide in veterinary medicine, particularly in flea and tick treatments for cats and dogs, as well as in biocidal products used to control ants, termites, cockroaches and other pests. Such uses are not restricted to agricultural settings. Although some compounds were more prevalent among conventional farmers, overall differences between occupational groups were modest. Similarly, no consistent sex differences were observed, apart from a higher glyphosate detection frequency in male urine samples. Occasional sex-specific detections

of low-frequency compounds may reflect chance findings [52]. However, this might also reflect genuine differences related to specific work tasks or work practices between males and females, as well as sex-specific biological factors or gender-specific dietary habits [53].

#### 4.2. Animals

Insecticides were more widely represented among the animal matrices, whereas fungicides were less frequently represented. Overall, more compounds were detected in animals from conventional than from organic farms. Median levels were generally comparable between farming systems, but with some exceptions. Taken together, these findings indicate the pesticide exposure was higher among animals from conventional farming systems but by no means restricted to them.

In feed, glyphosate, cypermethrin and bifenthrin were the most frequently detected pesticides across all types of animals. Glyphosate residues were also among the top three for animal faeces and urine, highlighting the widespread presence of glyphosate-related residues across animal matrices [54,55]. Cypermethrin has previously been reported to be abundantly present in cow and goat feed and water, especially shortly after application on crops [56] at levels comparable to those observed in the present study. In the same study cypermethrin was present in cattle and goat milk more than 35 days after application of cypermethrin.

A considerable number of the top ten compounds detected in animals were not approved for use in the EU and many of these compounds were also detected in humans (including p,p' DDE, p,p' DDT, chlorpyrifos, and bromoxynil). These findings highlight the importance of continuously monitoring both approved and non-approved pesticide residues.

It is important to acknowledge that some substances detected in animal matrices may originate from sources other than pesticides for plant protection. For example, permethrin is also used as a biocide for pest control in livestock settings [57]. Consequently, the detection frequencies and concentrations observed for compounds such as permethrin, including its detection in 61% of animal wristbands, may partly reflect such non-agricultural or dual use applications rather than exposure exclusively related to plant protection.

#### 4.3. Environment

Glyphosate and AMPA were in the top three for several environmental matrices, consistent with previous reports in the US, Argentina, and Europe, although concentrations were lower in the present study [58–60]. Conventional fields generally showed higher median levels and frequencies than organic fields, particularly in soil and crops.

The studies in the US [58], Argentina [59] and the EU [60] have found glyphosate and AMPA in agricultural soils and water. Reported concentrations ranged from 35 to 1502 µg/kg in cultivated soils in Argentina and reached up to 2000 µg/kg in EU agricultural topsoil, compared to our finding of 10–100 µg/kg. Additionally, glyphosate and AMPA have been detected in wind-eroded sediment, with higher levels in the finest particle fractions [61]. In surface water, glyphosate and AMPA were detected in 12–15% of samples from Argentina [59] and in 45% of the topsoil collected in the EU [60]. These frequencies are lower than those observed in the present study (76% AMPA and 61% glyphosate in sediment samples). Furthermore, glyphosate and AMPA were also detected in sediment, with levels ranging from 85 µg/kg to 1000 µg/kg in Sri Lanka [62] and reached up to 7219 µg/kg in Argentina [63], substantially higher than the concentration observed in the present study (0.001–0.1 µg/kg). While direct comparisons across locations and systems remain challenging, the overall body of evidence clearly indicates that glyphosate and its metabolite AMPA are pervasive in the environment, both across Europe and globally.

A considerable number of the detected compounds are not approved in EU. In line with previous findings [1], six out of ten compounds frequently detected in soil were not approved. *p,p'* DDE was represented within the top three pesticides in soil, earthworms, and water. This is corroborated by earlier findings [64,65].

In outdoor air/dust samples, detection frequencies and levels were highest for herbicides, with glyphosate detected in 100% of samples. No clear difference was observed between organic and conventional farms. These results are consistent with previous findings from European studies measuring pesticides in outdoor air/dust [66,67].

Indoor dust was the matrix with the highest detection frequency of pesticides, i.e., the top ten pesticides were found in 99–100% of all samples. Similar detection frequencies were found in conventional and organic farmhouses, suggesting that sources other than agricultural activities may contribute substantially to pesticide residues detected indoors, for example, diet and application of household pest products. More details on the findings in indoor dust can be found in [35].

Direct comparisons between pesticide detection frequencies and levels in crops across studies are limited by differences in the crops investigated, different sampling periods, and analytical methods. Furthermore, the crop results in the present study are presented on a dry-weight basis and therefore cannot be directly compared with EU maximum residue levels (MRLs), which are based on fresh weight [68]. Despite these limitations, our results point towards crop pesticide pollution, including in organic systems.

Most water samples contain a mixture of pesticide residues. The water samples were collected from surface water bodies, most of which were expected to be used in the irrigation of agricultural fields, although some may also be used recreationally. An EU directive from 2026 [69] sets for groundwater a limit of 0.1 µg/L for individual pesticides/metabolites, and this limit was exceeded in fewer than 1% of the samples.

#### 4.4. Pesticides Across Human, Animal, and Environmental Compartments

For the 63 pesticide residues included in the compiled list and across all 16 investigated matrices, glyphosate and AMPA were detected in nine matrices and in all three investigated compartments (human, animal, and environment). The highest detection frequencies of glyphosate were found in water and outdoor air/dust (100%), feed (92%), animal urine (77%), and human faeces (71%). The highest detection frequencies of AMPA were found in sediment (76%) and outdoor air/dust (75%). The frequent detection of glyphosate in water and outdoor air/dust support its potential for long-distance transport beyond the original site of application as suggested by previous studies [70]. *p,p'* DDT and its metabolites were detected in 11 different matrices across the three compartments. Their presence in a large proportion of the investigated SPRINT matrices demonstrates that, even though DDT has not been approved for use in European agriculture since the 1980s, residues and metabolites remain widely present in the environment.

Of all 43 parental compounds included in the compiled list, nine are used both as plant protection products and as biocides (acetamiprid, cypermethrin, clothianidin, deltamethrin, fipronil, lambda-cyhalothrin, pyrethrin II, tebuconazole, thiacloprid). Consequently, pesticide residues detected in the different compartments may also originate from biocidal use which is important to consider when interpreting the results.

Compounds detected in human and animal wristbands had a substantial co-occurrence (Table 6), as did compounds in indoor dust and human and animal wristbands. This may indicate common sources or mechanisms of cross-contamination that are not well understood and require further investigation. However, co-occurrence may also partly be explained by characteristics of the matrices or by the physicochemical properties of the analytes of interest. This may be particularly relevant for passive sampling devices such as

silicone wristbands known to accumulate certain lipophilic chemicals such as fipronil but they may be less suitable for sampling highly water-soluble and zwitterionic compounds such as glyphosate [71]. Compounds detected in urine showed limited overlap with those detected in other matrices. This may reflect the fact that the analytical target list for urine differed from those for the other matrices, as many pesticides are metabolised before excretion, and analytical standards are only available for a limited number of these urinary metabolites. In contrast, the environmental matrices showed a substantial overlap in the compounds detected across crops, earthworms, indoor dust, outdoor air/dust, sediment, soil, and water. This may indicate common routes of transport and deposition across environmental matrices.

## 5. Strengths and Limitations

The SPRINT study is by far the most comprehensive study to date in terms of the number of pesticides analysed and the number of matrices investigated across conventional and organic farming systems and across different human participant groups. The comprehensive data collection in SPRINT across humans, animals and the environment support the One Health concept where each compartment is not separately interpreted but integrated, as a connected exposure system in which environmental contamination, livestock exposure, and human biomonitoring reflect different indicators of the same underlying pesticide mixture.

An important limitation of the study is the cross-sectional design and the collection of pesticide measurements during only the 2021 growing season. The results should therefore be regarded as a snapshot of pesticide residues in human, animal, and environmental samples across the 10 European study sites. Furthermore, the increasing analytical sensitivity for identifying pesticides to some extent hampers comparisons of detection frequencies with earlier studies, and the current results serve as only one part of the input data for subsequent risk assessment modelling. We did not identify any major 2021 climatic event that could have accounted for our finding on detection frequencies and levels. Pesticide use and environmental contamination may vary considerably over time depending on crop type, weather conditions, pest pressure, agricultural practices and the timing and intensity of pesticide applications. Similarly, human and animal exposure may fluctuate according to work activities, dietary habits, contact with treated environments, and the timing of sampling relative to pesticide application. The timing of sampling may also have influenced the results differently across matrices. Whereas some pesticides or metabolites may be rapidly eliminated from the human or animal body, others may persist and accumulate in environmental matrices, particularly in soil, sediment, indoor dust, or biological tissues. Consequently, the levels and detection frequencies observed during the sampling period may not necessarily reflect average or long-term exposure levels. In addition, a single sampling campaign cannot capture inter-annual variability or longer-term changes resulting from changes in pesticide use, regulatory restrictions, agricultural practices, or environmental degradation processes. Longitudinal studies involving repeated sampling across several growing seasons would be needed to assess temporal variability, identify persistent exposure patterns, and better distinguish short-term exposure events from more sustained contamination.

Previous studies focused mainly on wristbands and urine and have generally covered fewer compounds in the analysis. The present study thereby provides crucial insights into the levels and occurrence of a broad range of pesticide compounds, extending beyond the limited set of substances commonly investigated such as persistent organochlorine compounds (e.g., HCB and p,p' DDT and its degradation products) and widely used herbicides (e.g., glyphosate).

This overview focuses on the top ten compounds identified in each of the 16 investigated matrices as it was not feasible to meaningfully present all 224 compounds included in the prioritised list. A potential limitation of this approach is that some of the compounds may occur at relatively high levels in some of the matrices but with low detection frequencies. Such compounds would not be included in the current overview. Therefore, comparisons between categories of samples (e.g., conventional versus organic farming systems) should be interpreted as comparisons based on the compounds included in the respective top ten lists and should not be considered representative across the entire prioritised pesticide list.

Some selection may also have occurred in the passive sampling of outdoor air/dust and wristbands due to the physicochemical properties of pesticides and their metabolites. For example, glyphosate was not detected in human silicone wristbands, consistent with its high polarity and limited affinity for the silicone matrix. More comprehensive descriptions of the analytical methods, results and associated limitations are provided in the corresponding matrix-specific publications.

## 6. Conclusions

Our findings demonstrate that insecticides, fungicides, and herbicides are widely distributed across the 10 SPRINT European case study sites. Both pesticides approved and not approved in the EU are frequently detected in human, animal, and environmental matrices. These results highlight the need to systematically include non-approved compounds in monitoring programmes and in the assessment of risks, although risk management and mitigation will mostly be possible for approved substances. Beyond providing a comprehensive snapshot of exposure prevalence and concentration levels, the data presented here offer valuable input for exposure and risk assessment modelling. The findings also illustrate the interconnectedness of human, animal, and environmental exposure and highlight the importance of considering these compartments together when investigating pesticide contamination using a One Health approach. Ultimately, the SPRINT data contributes to advancing our understanding of the potential impacts of pesticides and their metabolites—individually and as mixtures—on environmental, animal, and human health.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/environments13100545/s1>. Section S1 (Ethical applications in SPRINT) including Table S1 and Table S2; Section S2 (A description of the sampling and analysis of pesticides for humans, animals and for environmental samples across various matrices).

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