

Supplementary material

Section S1. Ethical applications in SPRINT

All 10 centres who have contributed to the manuscript have approved ethical applications with informed consent from all participants. Table 1 shows approval number, contact person and contact e-mail for each centre. Table 2 display date for letter of approval and data of recruitment of first participant.

Table S1. Approval number, contact person and contact e-mail for each of the 10 study centres

	Spain	Portugal	France	Switzerland	Italy	Croatia	Slovenia	Czech Republic	The Netherlands	Denmark
Ethical permission, id number	CEI 21_002	03-CED/2021	2021-A00205-36	2021-00774	473-2021	KLASA 003-05/21-1/35 UR BROJ 2170-29-02/1-21-2	0120-146/2021/3	(C)ELSPAC/EK/1/2021	NL-OMON50994 (see also https://onderzoekmetmensen.nl/en/trial/50994)	1-10-72-47-21
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Table S2. Date for letter of approval and data of recruitment of first participant

Case study in	Date of letter of approval	Date of recruitment of first participant
Croatia	17-03 -2021	14-04-2021
Czech Republic	23-03-2021	19-04-2021
Denmark	03-05-2021	07-05-2021
France	11-03-2021	04-05-2021
Italy	20-05-2021	13-10-2021
Portugal	12-04-2021	11-05-2021
Spain	09-04-2021	20-10-2021
Slovenia	12-05-2021	24-05-2021
Switzerland	15-06-2021	22-06-2021
The Netherlands	01-03-2021	18-03-2021

Section S2. A description of the sampling and analysis of pesticides for humans, animals and for environmental samples across various matrices

Human samples

Urine sampling

Each participant collected a urine sample of approximately 60-125 mL and placed it in a cooling box (2-10°C) until pickup by a member of the research team. Within 24 hours of the sampling, the urine was aliquoted into different 12 ml Greiner tubes. After aliquoting, tubes were stored in the freezer (-18°C or lower) until transport to Wageningen Food Safety Research (WFSR) in the Netherlands, whereafter it was stored at -80°C until analysis.

Blood sampling

A maximum of 30 mL blood was drawn by venipuncture from each participant by trained healthcare personnel. After fractionating and aliquoting, plasma was stored at -80°C until transportation to Radboud University Medical Centre in the Netherlands where it was stored at -80°C until further analysis.

Faeces sampling

Pre-labelled faeces sample collection tubes (5 in total) together with the provided collection materials were handed out to the participants before the pick-up visit by the SPRINT data collector. Participants were asked to collect a faeces sample of the first bowel movement of the day (1 scoop of faeces sample per tube collection). Faeces samples were kept at 2-10°C in a pre-delivered cooling bag until pick-up by the SPRINT data collector and were stored at -18 °C until transportation to WFSR in the Netherlands, whereafter it was stored at -80°C until analysis.

Wristband sampling

Participants were asked to wear a silicon wristband that absorbs pesticides from the surroundings (continuously for 7 consecutive days) to analyse which pesticides, the person had been in contact with. After that the wristbands were placed inside a zip-lock bag and placed in the freezer. After collection of wristbands from the participants, the wristbands were stored at -20°C and sent to the analysis laboratory as one batch.

Animal samples

Urine sampling

Depending on the type of animal (dairy, sheep, goat, or pig), 100 mL urine was collected and aliquoted the same day into 12 ml Greiner tubes. After aliquoting, all tubes were stored in the freezer (-18°C or lower) until transport to WFSR.

Blood sampling

Depending on the size of the animal, blood was drawn from by venipuncture by a trained veterinarian. After fractioning, whole blood, serum, and plasma were stored at -80°C until transportation to Radboud University Medical Centre, Nijmegen, the Netherlands for further analysis.

Faeces sampling

Depending on the type of animal, faeces samples were taken directly from the animal (dairy, sheep, goat, chicken, pig) or identified in the close vicinity (cats) and one scoop of faeces was divided into 5 prelabelled collection tubes. Faeces samples were thereafter kept at -18 °C until transportation to WFSR for analysis.

Wristband sampling

On the same animals considered for urine and blood collection, a silicon wristband was attached to the animal collar or leg the day of urine/blood sampling and removed again after one week. The wristbands were kept separately in zip-lock bags at -20 °C until transportation to Radboud Umc, Nijmegen, the Netherlands.

Feed sampling

One feed sample (100-500g) was collected from each farm. Dry sample material was stored at 2-10°C or at ambient temperature (in the dark). Perishable/wet sample material was stored in the freezer (-18°C) and kept frozen until pesticide analysis at WFSR.

Environmental samples

Soil sampling

One composite soil sample (~2 kg) comprising 5 sub-samples was randomly collected across each sampling field. The sampling depth was between 0 - 5 cm in permanent crops and 0 - 20 cm in arable land with tillage. The soil samples were stored at -20°C until shipment to RECETOX, Brno, Czech Republic, for pesticide analysis.

Water sampling

Water samples were collected from CSS with surface water bodies like small rivers, stream channels, small lakes, or ponds connected to or close to the sampled fields.

Between 3 and 8 water bodies were sampled per CSS. One composite water sample of ~2L was collected per water body. Water samples were collected sub-superficially (directly collected with a polypropylene bottle) at three points of the water body at least 10 meters from each other. After sampling, the water samples were stored at -20°C and sent to CIEMAT in Madrid, Spain for pesticide analysis.

Sediment sampling

Sediments were collected among CSSs with water sampling locations. One composite sediment sample was collected per water body consisting of 3 sub-samples collected at sites distancing at least 10 m from each other. Samples were collected at a depth of 0-10 cm using a core sampler, stainless-steel scoop, or an auger, at the sediment surface. After thorough mixing, 1 kg of composite sediment sample was placed in a polypropylene container and stored at -20°C until pesticide analysis at WFSR-WUR.

Outdoor air sampling

Sampling of outdoor air and dust was carried out during a period of approximately 2 months during the growing season using a TIEM passive air sampling device (71). Two devices were installed per CSS and were installed one month before the mid growing season/sampling campaign ensuring overlap with

sampling of the other environmental samples. The TIEM head collector was installed 1.5 meters from the soil surface. The device uses a polyurethane foam (PUF) disk to absorb (semi) volatile pesticides from the gas phase by diffusion, and polyester foam filters (PEF), to collect particulate matter by diffusion to analyse glyphosate and AMPA. After 8 weeks, the filters were removed from each device and sent to the KWALIS, Fulda, Germany for analysis.

Indoor dust sampling

Farmers were requested to replace their vacuum cleaner bag in the farmhouse with a new one at the time of one month prior to the start of the field campaign and proceed with their normal house cleaning. The vacuum cleaner bag was collected after one month and stored in a zip lock bag, in the dark at -20°C until pesticide analysis at CIEMAT, Madrid, Spain.

Earthworm sampling

A 25x25x25 cm monolith was dug in a representative plot of the sampling field. All the vegetation was cut, and the leaf litter was removed from the sample area, and a mustard solution was added to facilitate that the earthworms come to the surface, where they were collected. Collected earthworms were then deep frozen and sent to RECETOX laboratory, Czech Republic, for pesticide analysis.

Crop sampling

One crop class was sampled per CSS, and one plant species per field at the time of harvest. CSS 1, 2, 3, 6, 7, and 9 covered a single plant species whereas CSS 4, 5, 8, and 10 crop samples covered multiple plant species. A composite sample contained crop-specific harvesting product from a combination of 5 plants/trees. The crop-specific harvesting product was collected in areas of the field representative of soil and crop, and at least 5 m away from the edge of the field. Crop samples stored at -20°C until shipment to RECETOX, Czech Republic, for pesticide analysis.

Target pesticides

A target list of 208 analytes consisting of 164 active substances, 43 metabolites and one co-formulant (piperonyl butoxide) was established during the initiation phase of the SPRINT project (33). The selection was based on information on possible used pesticides for plant protection in each CSS, pesticides known to occur in 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. This list was targeted in all matrices but urine. In urine samples we measured known urinary pesticide biomarkers, for which analytical reference standards were available. A total of 44 pesticides and metabolites were measured in urine (28 from the original target list and 16 metabolites that were only measured in urine (Ametoctradin-M650F06, 4-F-3-PBA, Chlothianidin desmethyl, DBCA, Difenoconazole CGA205375, 4-HAS, Imidacloprid olefin, Myclobutanil-OH, DCCA-cis, DCCA-trans, Propamocarb, 3-PBA, CIF3CA, Quinmerac, Thiametoxam-desnitro).

The analytes were measured by quantitative multi-methods based on LC-MS/MS and GC-MS/MS. Separate dedicated methods were used for the analysis of glyphosate and AMPA. All methods were validated before sample analysis. The limit of detection (LOD) was used as the analytical limit.

Assessment of pesticide levels

Urine (human and animal)

Multi residue assessment involved overnight enzymatic deconjugation using β -glucuronidase/aryl sulfatase. After that, the QuEChERS method was applied to 3 mL sample by adding acetonitrile containing acetic acid and a mixture of magnesium sulphate and sodium acetate. After shaking and centrifugation, the acetonitrile phase was evaporated and redissolved in a mixture of methanol/water prior to LC-MS/MS analysis. The LOQ range was from 0.1 to 2.5 ng/mL. For determination of glyphosate in urine, we applied a method with solid phase extraction (SPE) and derivatization using FMOC-Cl (9-Fluorenylmethoxycarbonyl chloride). After passing 1 mL urine through Strata SAX cartridges previously conditioned, the samples were eluted with a sodium chloride solution prepared in hydrochloric acid. The samples were, then, neutralized with sodium hydroxide and derivatized with FMOC-Cl in the presence of borate solution at 5%. After 30 min, formic acid was added, and the samples injected into the LC-MS/MS system. For AMPA, the same method but without the SPE cleanup was used. Isotopically labelled internal standards were added to urine before extraction/derivatization. The LOQ for glyphosate was 0.1 ng/mL and for AMPA 0.2 ng/mL.

Plasma (human and animal)

The sample preparation method for plasma pesticide analysis is based on an adapted QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method. In short, 1 mL of plasma was added to an extraction tube together with 3 mL of 0.1% formic acid in acetonitrile. After vortexing, 1.5 g of QuEChERS salts were added (1.2 g magnesium sulphate (MgSO_4), 0.3 g sodium acetate (CH_3COONa) and vortexed. Samples were then centrifuged for 5 minutes at 3200 RCF. Supernatant was collected and divided in separate tubes (1 mL for LC analysis and 1 mL for GC analysis). Ten μL of dimethyl sulfoxide (DMSO) was added to the 1 mL LC-MS/MS fraction prior to evaporation under a stream of nitrogen at 40 °C. Samples were evaporated till dryness and subsequently reconstituted into 5 mM ammonium formate (NH_4HCO_2) 50:50 (v/v) Ultrapure water: methanol (CH_3OH) solution. An aliquot of 1 mL GC-MS fraction was inserted into a DisQue QuEChERS tube (150 mg MgSO_4 : 25 mg primary secondary amine (PSA): 25 mg C_{18}) vortexed and centrifuged for 5 minutes at 21000 RCF. From this an aliquot of 500 μL was taken and placed in an Eppendorf tube for further GC-MS pretreatment and analysis at Limoges CHU.

Faeces (human and animal)

Before handling, the samples for multi residue assessment were allowed to reach room temperature. Thereafter, 2 g of sample were weighed in an extraction tube followed by addition of water. Next, acetonitrile containing 1% of acetic acid was added. The tubes were shaken followed by addition of 1.6 g of magnesium sulphate and 0.4 g of sodium acetate. The tubes were once again shaken for 10 minutes followed by centrifugation. For LC-MS/MS analysis, the extracts were diluted in a vial with methanol containing 0.1% acetic acid. For GC-MS/MS analysis, 1 mL of extract was cleaned with a mixture of C_{18} , PSA and magnesium sulphate and, after centrifugation, 200 μL was placed in an autosampler vial for analysis. The LOQ range was 1 $\mu\text{g}/\text{kg}$ - 10 $\mu\text{g}/\text{kg}$ faeces.

For Glyphosate and AMPA assessment, 2 g sample were weighed followed by the addition of isotopically labelled internal standards, ethylenediaminetetraacetic acid (EDTA), water and methanol containing formic acid (39). The mixture was shaken and transferred to the freezer at -80 °C for 20 minutes. After centrifugation the extracts were diluted in vial with a mixture of methanol and water before LC-MS/MS measurement. The LOQ for glyphosate was 8 $\mu\text{g}/\text{kg}$ and for AMPA 25 $\mu\text{g}/\text{kg}$ faeces.

Wristbands (human and animal)

Before starting with the extraction, the wristband was cut into four pieces. Those four pieces were replaced into a 50 mL Greiner tube and followed by adding 20 mL of ethyl acetate and an internal standard (diazinon-D10 and bentazone-D6). The tubes were shaking for 1 hour using a head-over-head shaker. The aliquot was transferred into a 12 mL glass tube containing 100 µL of DMSO as keeper and evaporated under nitrogen at 40 °C using Turbovap. The residue was redissolved in 2 mL acetonitrile containing 1% of formic acid. For LC-MS/MS analysis, 250 µL of the extract was diluted with 250 µL of water containing 0.1% formic acid and injection internal standards (2,4-D-D3 and diuron-D6). 169 compounds were then analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and the other 25 compounds were analysed by gas chromatography–mass spectrometry (GC-MS). For more details we refer to (40)

Feed

The same methods for multi-residues and glyphosate/AMPA assessments were used for feed as for the faeces.

Soil

Soil samples were thawed and homogenized. Thereafter 2 grams were used for the determination of glyphosate and AMPA, and 5 grams was used for analyses of multi-residue (LC-MS/MS & GC-MS/MS). The first aliquot was analysed using the procedure described by Bento et al (62). and Yang et al. (44) with 0.6 M KOH as extraction solvent, isotopically labelled standards, and borate buffer and FMOC-Cl as derivatization agent. The second aliquot was analysed using the modified QuEChERS approach, with acetonitrile as extraction solvent, and a PSA-based clean-up step, added to part of the extraction supernatant used for the GC analyses (34).

Water

Water samples were thawed, filtered and spiked with labelled standards and analysed by three different methods: One for the determination of multi-residue analysis of pesticides, one for glyphosate and AMPA determination, and one for organochlorinated pesticides. Multi-residue analysis was based on Solid-Phase Extraction (SPE) cartridges, conditioned with methanol and ultrapure water and eluted with methanol. The extract was evaporated under a stream of nitrogen and divided into two aliquots, one for HPLC-MS/MS and one for GC-MS/MS. To analyse glyphosate and AMPA, water samples were treated with KH_2PO_4 and $\text{Na}_2\text{B}_4\text{O}_7$ (0.1M, pH=9) and derivatized with FMOC-Cl (1mg/mL). The extract was then passed through Oasis HLB cartridge, eluted with methanol, and finally analysed by HPLC-MS/MS. Organochlorine pesticides were extracted by liquid-liquid extraction with dichloromethane and analysed by GC-HRMS. The details of the three methods can be found in Navarro et al. (36)

Sediment

Sediment samples were analysed like the soil samples, with two minor exceptions. Sample preparation involved a centrifugation step at 3500 rpm for 5 min, the solid portion was decanted, and water was discarded. The aliquot for determination of glyphosate and AMPA and the aliquot for multi-residues was both 2 grams. The methods used were as described by Bento et al. (62,43), Yang et al. (44), and Silva et al. (45).

Outdoor air

Extraction of the TIEM-PUF disks was carried out with dichloromethane in a Soxhlet extractor. Samples were extracted for 24 hours using at least 16 extraction cycles. After concentrating the extract to 1 mL under a stream of nitrogen, the extract was analysed by LC-MS/MS and GC-MS/MS. Glyphosate and AMPA were extracted from the PEF materials with 0.125 N hydrochloric acid and derivatized with fluorenylmethoxycarbonyl. The analysis was conducted by LC-MS/MS.

Indoor dust

Each vacuum clean bag material was analysed by three methods: One for the determination of multi-residue analysis of pesticides, one for glyphosate and AMPA determination, and one for organochlorinated pesticides. Multi-residues were analysed via the QuEChERS approach, involving surrogate labelled standards, acetonitrile containing 1% of acetic acid as the extraction solvent, and a salt-induced phase separation. Glyphosate and AMPA were analysed using labelled standards, but $\text{KH}_2\text{PO}_4/\text{Na}_2\text{B}_4\text{O}_7$ as extraction solvent, and a FMOC-based derivatization overnight. The extraction was done by sonication and centrifugation with a ^{13}C labelled surrogate standards with a 3:1 hexane:acetone solution and purified by a 1 g florisil column eluted with 16 mL of hexane (35).

Earthworm

Frozen earthworm samples were lyophilized, homogenized and split into two parts: one part for glyphosate and AMPA determination and one for the multiresidue screening. Glyphosate and AMPA was determined using the method described by Oulkar et al.(46). Earthworm subsamples were spiked with the labelled standards and extracted with water containing 0.1% formic acid. Derivatization was carried out following Yang et al. (44). The remaining pesticide residues were extracted using the QuEChERS approach, as described by Silva et al. (45), Svobodová et al. (47) and Lehotay et al. (48). Earthworm subsamples were spiked with D6-tebuconazole and mixed with Millipore water and acetonitrile. After agitation, a mixture of MgSO_4 , NaCl, NaCitate, disodium citrate was added. The tube was then vortexed and centrifuged, and the supernatant collected, and one part analysed using LC-MS/MS with electrospray ionization (ESI) and one part analysed using GC-MS/MS.

Crop

Crop samples were thawed, homogenized, and divided into two aliquots: 5g for glyphosate and AMPA determination and 10g for multi-residue. Glyphosate and AMPA were determined using the modified method described by Yang et al. (44) involving isotopically labelled standards, 0.1% formic acid and dichloromethane-based extraction, and a FMOC-Cl based derivatization. Multi-residues were extracted using an adaptation of the QuEChERS approach, as described by Silva et al. (45) using acetonitrile as extraction solvent, and dispersive sorbents for the extra clean-up step done before GC analyses.