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Assessment of health effects of potato crop phytopharmaceuticals and storage products in a murine model

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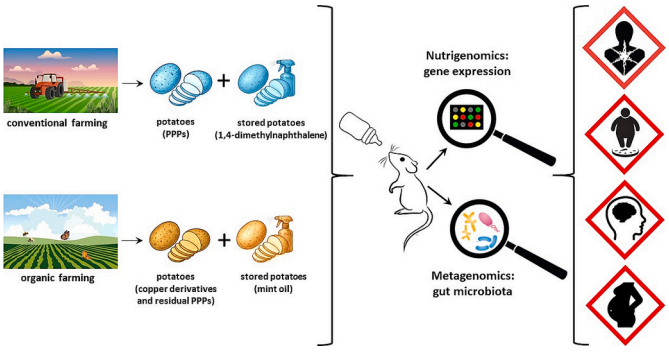
HIGHLIGHTS

- Chronic exposure and PPP mixtures raise serious concerns for long-term health risks.
- Conventional potato and storage treatments alter gene expression and microbiota.
- Risks concern cancer, immunity, metabolism, brain, and reproduction.
- Organic potatoes show minimal effects on transcriptome and gut microbiota.
- Storage and soil residues of PPPs may persist and pose delayed health risks.

GRAPHICAL ABSTRACT



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ABSTRACT

Potatoes are among the most widely consumed staple foods worldwide, but their cultivation and storage frequently involve multiple phytopharmaceutical products (PPPs), raising concerns about the health risks of dietary pesticide residues.

The health effects of multi-residue PPPs used in potato cultivation were assessed in an in vivo murine model, involving 36 mice, and evaluated through omics analyses. Two field cultivation methods (conventional and organic) and two post-harvest storage conditions (using 1,4-dimethylnaphthalene and mint essential oil as sprout inhibitor treatments) were considered. Potato tubers were processed into flour and administered to the animals at a moderate daily dose for 20 consecutive days.

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Nutrigenomic analyses revealed significant gene deregulations, with 70 genes affected in the liver, 56 in the jejunum, and 52 in the brain, suggesting disturbances in cellular proliferation, nervous system functions, lipid and carbohydrate metabolism, reproductive health, and immune responses. Metagenomic analyses indicated microbiota imbalances, including a shift in the Firmicutes/Bacteroidota ratio and changes in 2 bacterial genera with potentially adverse effects.

The main residues suspected of producing these effects include propamocarb, carfentrazone, 1,4-dimethylnaphthalene, copper derivatives, and peppermint essential oil. These findings highlight the importance of large-scale omics approaches in uncovering potential biological disruptions, underscore the health risks associated with chronic dietary exposure to pesticide residues, particularly in mixtures, and emphasize the need to reassess regulatory standards to promote agricultural practices that minimize pesticide residues to better protect the environment and human health.

1. Introduction

The practices and uses of plant protection products (PPPs) implemented by farmers are still sometimes insufficiently understood, despite recent efforts in this direction. Risk assessment related to the use of PPPs for their market authorization is primarily conducted on a substance-by-substance basis but is gradually shifting toward a group-based approach. The fact that PPP formulations applied in the field are mixtures (active substances, co-formulants, adjuvants, impurities, etc.), and that agricultural plots are subjected to multiple treatments throughout a growing season, can lead to low-dose multi-residue contamination of crops, soils, and ecosystems.

The "cocktail effect" of PPPs refers to the combined impact of multiple pesticide residues that may interact synergistically, additively, or antagonistically within plants, the environment, or even in organisms consuming these crops. While individual PPP are assessed for safety, their mixtures can lead to unexpected toxicological outcomes that are not predictable from single-compound studies. These combined effects may alter plant physiology, disrupt microbial communities, or increase risks to non-target organisms and human health. Recent research has emphasized the need to consider mixture toxicity in risk assessments in agricultural contexts (Cedergreen, 2014; Weisner et al., 2021). Several studies have investigated the effects of PPPs on animal models and humans, highlighting potential toxicological impacts beyond their intended agricultural use. In animal studies, chronic exposure to PPPs has been associated with alterations in liver function, hormonal imbalances, neurotoxicity, and disruptions in gut microbiota composition (Mesnage and Antoniou, 2018). In humans, epidemiological data suggest correlations between long-term PPP exposure and increased risks of endocrine disorders, reproductive impairments, neurodevelopmental effects, and certain types of cancer (Mostafalou and Abdollahi, 2017). Exposure to pesticide residues has also been linked to modifications in gut microbial composition, with studies finding alterations in the diversity of gut bacteria among individuals exposed to common agricultural chemicals (Mesnage et al., 2022).

Among cultivated crops that draw significant attention due to their extensive cultivation areas and high levels of human and animal consumption, potato (*Solanum tuberosum* L.) holds a prominent position. Globally, potato ranks as the third most important food crop after rice and wheat, with an estimated 359 million tonnes produced in 2022 over approximately 17.5 million ha (FAO, 2023). In France, potato is a major crop both for fresh consumption and industrial processing, covering around 145,000 ha and with an annual consumption averaging 50 kg per capita (Agreste, 2022). Due to its large-scale production, potato cultivation is among the most pesticide-intensive agricultural systems, involving frequent applications of fungicides, herbicides, and insecticides (Li and Fantke, 2023). Several studies have documented the toxicological effects of pesticide residues from conventionally grown potatoes on animal models. For example, mancozeb, a commonly used fungicide in potato fields, has been shown to induce oxidative stress in rats following chronic exposure, leading to liver damage (Yahia et al., 2014). Similarly, the insecticide chlorpyrifos, used in potato farming, has been associated with significant neurotoxic effects, including deficits in motor functions and cognitive impairments in rats (Wołejko et al., 2022). Moreover, the systemic insecticide imidacloprid has demonstrated neurotoxic effects in rodents, affecting the central nervous system (Hassanen et al., 2023). In humans, epidemiological studies have reported associations between pesticide exposure in potato farming and increased risks of neurodegenerative diseases (e.g. Parkinson's disease), reproductive disorders, certain cancers, and birth defects, especially among agricultural workers and populations living in high-exposure areas (Kamel and Hoppin, 2004; Garry et al., 2002; Devi et al., 2018).

Although these studies have focused on the toxicity of specific PPPs used in potato cultivation, as well as particular molecular or biological markers, no large-scale study as nutrigenomics or metagenomics has yet been conducted to evaluate the full spectrum of responses following the ingestion of potatoes containing PPP residues. Most research has been limited to a narrow focus on a few PPPs, specific biological targets, and localized epidemiological records, without considering the overall or cumulative effects of long-term exposure to combinations of these substances on transcriptomic changes and microbiota composition.

Our study aimed to characterize the effects of PPP mixtures, used in a standardized manner in northern France, on conventionally grown potatoes during the field growing season as well as under storage conditions. Two cultivation methods (conventional – involving PPPs use, and organic – involving the use of specific PPPs such as copper-based products) and two storage treatments (1,4-dimethylnaphthalene and mint essential oil as sprout inhibitor) were considered. The health effects of PPPs used during cultivation and storage were evaluated in vivo using a murine model. A nutrigenomic analysis was conducted to assess the impact of consuming differently treated potatoes on gene expression in various tissues (liver, jejunum, and brain). A metagenomic analysis was also performed to observe changes in the gut microbiota through fecal microbiota metabarcoding (Fig. 1).

2. Materials and methods

2.1. Experimental design

A field experiment was conducted on plots belonging to the Vegetable Production Center of the Northern Region, located in Lorgies, France (50.554995, 2.794980). The experimental site included two plots, each with a surface area of 240 m^2 (40 m × 6 m). The plots were separated by a path bordered with grassy strips (9 m wide). The crop management practices for the organic (PO) and conventional (PC) plots were overseen by the local Agricultural Chamber of Lorgies (Table 1).

2.2. Potato cultivation and sampling

Potato plants (*Solanum tuberosum* var. Allians) were cultivated on the two plots between 25 April 2022 and September 2, 2022. Between 5 and 7 kg of tubers were harvested from each cultivation method for immediate analysis (PC and PO). An additional 5–7 kg of tubers were harvested in the same way to be stored for three months in dedicated

agricultural facilities. For storage purposes, the commercial sprout inhibitor Dormir, containing 1,4-dimethylnaphthalene (DormFresh, Perth, UK), was applied via thermal fogging at the time of entry into the storage facility to conventionally grown potatoes that had been properly cured and dried; these tubers were referred to as the PCS group. In a similar manner, BIOX-M, composed of mint essential oil (Xeda International, Saint-Andiol, France), was applied to organically grown potatoes, which were designated as the POS group. Tubers from both storage groups (PCS and POS) were sampled after two months of storage for subsequent analyses.

The four potato batches (PC, PO, PCS, POS) were washed with water, cut into 1 cm slices, frozen, and then dehydrated by lyophilization (freeze-drying). The potatoes were then ground and reduced to flour.

2.3. PPP residue quantification

PPP residues in the flours obtained from all crop management systems and storage conditions were extracted and quantified. Sample preparation and extraction were carried out according to the QuEChERS method (EN 15662), followed by purification using dispersive solid-phase extraction (d-SPE). Method validation was performed in compliance with the European SANTE guidelines (SANTE/12682/2019), including recovery (70–120 %), repeatability (RSD $<$ 20 %), and confirmation criteria for pesticide residues. The quantification of was performed using GC–MS/MS and LC-MS/MS (Capinov, Landerneau, France), with a high limit of quantification (LOQ $>$ 10 μg/kg). For 50 additional residues, quantification was carried out using GC–MS/MS (Sahyoun et al., 2022), with a low limit of quantification (LOQ $>$ 10 ng/kg).

2.4. Animal experiments

Male BALB/cByJRj 10 weeks old mice were purchased from JanvierLab (Saint Berthevin, France) and conducted on PHExMAR platform of Lille University (France, agreement no. 59-00913). All procedures on mice were performed in accordance with the Directive 2010/63/EEC for the protection of animals used for fine scientists, Law 2012-10 (2012) and 2013-118 (2013), approved by the local ethics committee in charge of animal experiments (no. CEEA75) and certified B5900912. The mice were randomly divided into six groups (n = 12/group) using a computer-generated randomization list, and housed in a controlled environment with a temperature of 22 °C, a 12 h/12 h light/dark cycle (dark cycle 09:00–21:00).

To be administered to mice by oral gavage as a dietary supplement, the potato tuber flour with skin was prepared as an aqueous solution at a concentration of 1 g/mL. The administered dose was calculated to align with human equivalent body weight, following general guidelines derived from traditional practices and pharmacological studies (WHO, 1999; Blumenthal and Busse, 1999). This approach was also consistent with previous nutrigenomic studies that utilized similar dosing strategies (Foure et al., 2018). The volume of each solution was adjusted according to the body weight of the animal (up to a maximum of 10 mL/kg body weight) to ensure a daily gavage corresponding to 30 mg of potato flour per day per mouse. The mice gavage consisted of a daily force-feeding of different treatments (PC, PO, PCS and POS) besides the standard chow (Diet A04C-10, Scientific Animal Food and Engineering, Augy, France) and water provided ad libitum. Controls underwent an equivalent force-feeding with water (Ctr). Finally, another batch of mice was gavaged in parallel with a solution containing a mixture of 10 pesticides used in the technical management of the conventional

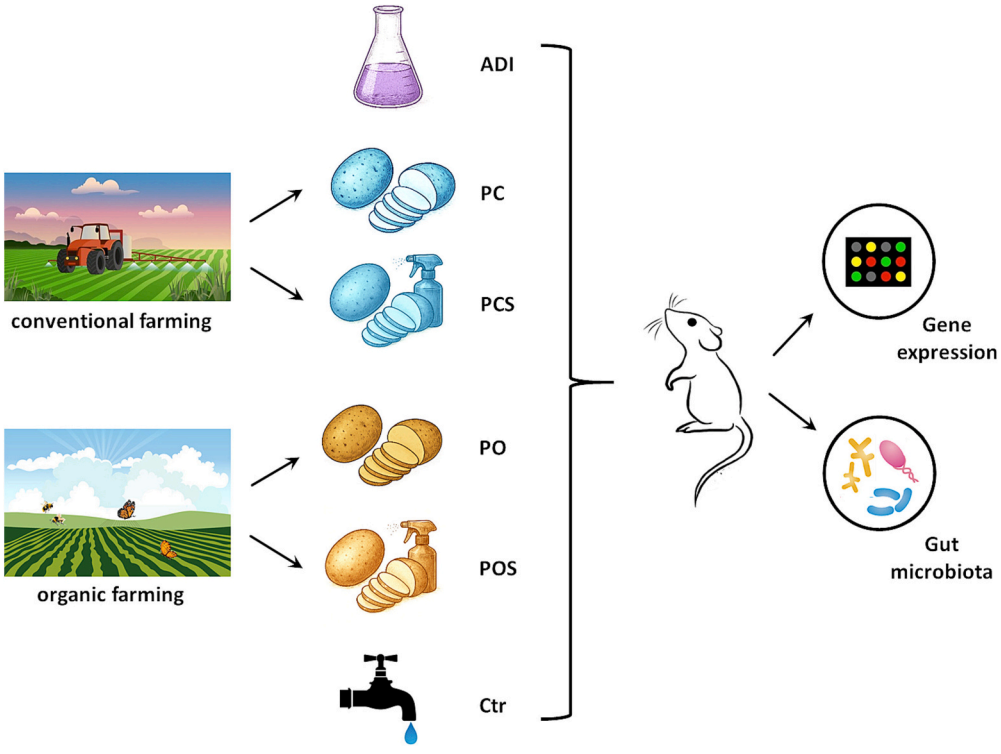

Fig. 1. Schematic representation of the experimental design. Two cultivation methods (conventional and organic) and two storage treatments (1,4-dimethylnaphthalene and essential mint oil as sprout inhibitor treatments) were considered. The potential health effects of plant protection products (PPPs) used during cultivation and storage were evaluated in vivo using a murine model. A nutrigenomic analysis was conducted to assess the impact of consuming differently treated potatoes on gene expression in various tissues. Additionally, a metagenomic analysis of fecal samples was performed to evaluate changes in gut microbiota composition. A control solution containing a mixture of ten pesticides commonly applied in the conventional cultivation system, each at its acceptable daily intake (ADI), was administered. Negative controls (Ctr) underwent equivalent force-feeding with water. Treatment groups included: PC — conventional potato; PO — organic potato; PCS — conventional potato after storage; POS — organic potato after storage.

Table 1
Crop management practices and active substances used in the organic (PO) and conventional (PC) plots.

	Date	Commercial product	Dose	Unit	Active substances
PO	6/7/2022	CHAMP FLO AMPLI	0.85	L/ha	Copper derivative
	6/20/2022	CHAMP FLO AMPLI	0.85		Copper derivative
	6/27/2022	BOUILLIE BORDELAISE NC 20 K	1.5	kg/ha	Copper derivative
	8/19/2022	Crushing of the tops	–	–	–
PC	5/25/2022	RANMAN TOP	0.5	L/ha	Cyazofamid
	6/3/2022	REVUS	0.6		Mandipropamid
	6/7/2022	INFINITO	1.6		Propamocarb and fluopicolide
	6/13/2022	REVUS	0.6		Mandipropamid
	6/20/2022	ZORVEC ENICADE AZULEO	0.15 0.3		Oxathiapiproline Cyazofamid
	6/27/2022	REVUS	0.6		Mandipropamid
	7/8/2022	RANMAN TOP	0.25		Cyazofamid
		PYGMALION	2		Potassium phosphonates
	7/21/2022	OPTIMO TECH	2.5		Pyraclostrobine and dimethomorph
	7/28/2022	GACHINKO	0.5		Amisulbrom
	8/1/2022	ITCAN SL 270	11		Maleic hydrazide
	8/8/2022	RANMAN TOP	0.5		Cyazofamide
	8/16/2022	GACHINKO	0.5		Amisulbrom
		SORCIER	0.8		Pyraflufen-ethyl
	8/16/2022	ACTIROB B	1		Rapeseed oil methyl ester
		SPOTLIGHT PLUS	0.5		Carfentrazone-ethyl
	8/24/2022	RANMAN TOP	0.5		Cyazofamide
	8/24/2022	SPOTLIGHT PLUS	0.7		Carfentrazone-ethyl
	8/24/2022	ACTIROB B	1		Rapeseed oil methyl ester

cultivation system, at their acceptable daily intake (ADI) dose (Table 2).

The six groups of mice (Ctr, ADI, PC, PO, PCS and POS) (Table 3), were nourished for 20 days. At the end of this period (D20), 6 mice from each condition were sacrificed, and the liver, jejunum, and brain were collected. For sampling, upon opening the abdominal cavity of the animals, the central core of the liver left lobe was cut into cubes and immediately frozen in liquid nitrogen. Enterocytes were quickly scraped from the jejunum and immediately immersed in liquid nitrogen. Similarly, the brain was cut into cubes, frozen in liquid nitrogen, and stored

Table 2
Composition of the ADI solution.

Active substance	ADI (mg/kg bw)	Daily dose/mouse (mg)
Carfentrazon-ethyl	0.03	0.0009
Cyazofamid	0.17	0.0051
Pyraflufen-ethyl	0.20	0.0060
Amisulborn	0.10	0.0030
Dimethomorphe	0.05	0.0015
Pyraclostrobin	0.03	0.0009
Fluopicolid	0.08	0.0024
Maleic hydrazide	0.25	0.0075
Mondipropamide	0.15	0.0045
Propamocarb	0.244	0.00732

bw — body weight.

Table 3
Pesticide dosage in potato flours (mg/kg) with a detection threshold of LOQ > 10 μg/kg.

PPP	Organic potato		Conventional potato		MRLs (mg. kg^{-1})
	PO	POS	PC	PCS	
Carfentrazone	ND	ND	D	ND	0.02
Propamocarb	ND	ND	0.012 ± 0.006	0.014 ± 0.007	0.3
Chlorpropham	ND	ND	ND	0.011 ± 0.006	0.04
1,4-dimethylnaphtalene	ND	ND	ND	5.2 ± 2.6	20

ND: not detected; D: detected but not quantified.
MRLs: Maximum Residue Limits established by the European Union in accordance with EU Regulation No 396/2005.

as individual samples at −80 °C for RNA extraction and transcriptomics. Three individual samples from each tissue were randomly chosen for transcriptomic analyses. At D0 and D20, feces were collected from each mouse and frozen at −80 °C for DNA extraction and metagenomics. Five individual samples were randomly selected for metagenomic analyses. The body weight of the mice was regularly recorded during experimental procedure. Investigators performing body weight measurements, tissue collection, and downstream transcriptomic and metagenomic analyses were blinded to the treatment groups to minimize bias.

2.5. RNA extraction and nutrigenomic analysis

Total RNAs were extracted from hepatic tissue, jejunum cells and brain using the NucleoZOL (Macherey-Nagel, Düren, Germany) kit. RNA quality was checked with Nanodrop (Eppendorf, France) and absorbance ratios A260/280 and A260/230 were found between 2.0 and 2.2. RNA quality was also examined by RNA ScreenTape Analysis (Agilent Technologies, France) and a minimal RNA integrity number (RIN) of 0.8 was required for all samples.

For the nutrigenomic analysis, 6 groups of mice were used for each treatment at D20. Three biological replicates per group (n = 3) were used, which is commonly considered sufficient to detect biologically meaningful gene expression changes (Vasiliu et al., 2015; Fernandes et al., 2018). The Agilent Whole Mouse Genome Microarray Sure Print GE 4 × 44 v2, containing 45,220 oligonucleotide probes, was utilized to examine gene expression profiles. Procedures for RNA amplification, staining, hybridization, and washing followed the manufacturer's guidelines. The GenePix 4000B scanner (Molecular Devices Corporation, Sunnyvale, CA, USA) was employed to scan slides at a resolution of 5 μm/pixel. GenePix Pro 6.0 software was used for grid alignment and digitization of expression data. The Quantile algorithm was applied to normalize expression data. To identify genes displaying changes in expression across repetitions, a script utilizing library functions in R was used and genes with a false discovery rate (FDR) of less than 5 % were retained. Fold change (FC) values were determined by comparing individual treated samples to the mean of the controls. Genes were considered differentially expressed if the fold change was >1.5 or <0.5. Functional annotations for differentially expressed genes (DEGs) were sourced from NCBI GenBank, with physiological processes assigned through NCBI, AmiGO 2 Gene Ontology (GO), and UniProt. KEGG pathway analysis was also performed, along with literature data, to identify relevant biological pathways and the role of deregulation in disease for the selected DEGs (Supplementary files 1–3). Microarray data have been submitted to the NCBI GEO: archive for functional genomics data with the accession numbers GSE296688, GSE296690, GSE296772.

2.6. DNA extraction and amplicon sequencing

DNA was extracted from mouse feces, separately collected from 5

individuals per experimental condition, using the NucleoSpin® DNA Stool kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. DNA quantities were measured with a BioSpectrometer (Eppendorf, Hamburg, Germany), and DNA quality was assessed using the 2100 Bioanalyzer (Agilent, Santa Clara, USA). Sequencing was conducted by the FARAH sequencing platform (Liège University, Belgium). For bacterial amplicon sequencing, the V1–V3 region of the 16S rDNA was amplified and libraries were prepared using the following primers: forward (5′-GAGAGTTTGATYMTGGCTCAG-3′) and reverse (5′-GAGAGTTTGGCTCAG-3′). For fungal amplicon sequencing, the Internal Transcribed Spacer (ITS) region 5.8S-ITS2 was amplified and libraries were prepared using universal primers with Illumina overhand adapters targeting the ITS2 region. The forward primer ITS3KYO2 (5′-GATGAAGAACGYAGYRAA-3′) and the reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were chosen for their broad coverage of fungal taxa. Each PCR product was purified with the Agencourt AMPure XP Ball Kit (Beckman Coulter, Pasadena, USA), then subjected to a second PCR round for indexing with Nextera XT index 1 and 2 primers. Following purification, PCR products were quantified using Quant-IT PicoGreen (Thermo-Fisher Scientific, Waltham, USA) and diluted to 10 ng·μL^{-1}. Final quantification of each library sample was performed with the KAPA SYBR FAST qPCR Kit (KapaBiosystems, Wilmington, USA) before standardization, pooling, and sequencing on a MiSeq sequencer with v3 reagents (ILLUMINA, San Diego, USA). Data processing involved the MOTHUR v1.44 package and the VSearch algorithm (Rognes et al., 2016) for alignment, clustering, and chimera detection as described by Gérard et al. (2021). After cleaning, sequences were clustered into operational taxonomic units (OTUs) at 97 % identity. Alignment and taxonomic identification were carried out with MOTHUR using the SILVA v1.32 database of full-length 16S rDNA and 5.8S rDNA gene sequences. A rarefied table of 10,000 reads per sample was used for further analysis. Reads were aggregated into phylotypes at the phylum and genus taxonomic levels. All raw reads from biosamples were deposited at the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA1279032.

2.7. Statistics

OTU counts were first filtered to remove rare taxa (<0.01 % of total reads across all samples) and normalized to relative abundances (total sum scaling). Filtered data were examined for alpha diversity using the Shannon index (SI), which combines richness and evenness, emphasizing the proportional abundance of OTUs (Shannon, 1948; Shannon and Weaver, 1949). Graphical representations of SI and statistical analyses were generated using PRISM 9 (GraphPad Prism 9.0, San Diego, CA, USA). Normality of the data was assessed using the Shapiro–Wilk test, and homogeneity of variances was checked with Bartlett's test. For normally distributed and homoscedastic data, statistical differences were assessed using one-way ANOVA followed by Dunnett's multiple comparisons test. Otherwise, statistical differences were assessed using non-parametric test Kruskal Wallis and Dunn's multiple comparisons test.

Beta diversity was assessed to evaluate dissimilarities in microbial community composition between samples (Gower, 1966). For beta-diversity, counts were Hellinger-transformed, and Bray–Curtis dissimilarities were calculated. Differences between samples were visualized using Principal Coordinates Analysis (PCoA) implemented with the FactoMineR package in R (version 3.5.2).

For differential abundance analysis, relative abundances were subjected to a centered log-ratio (CLR) transformation to account for the compositional nature of microbiota data. Taxon abundance differences between experimental groups and their respective controls were tested using ANOVA (or Kruskal–Wallis when normality assumptions were not met). Normality and homogeneity of variances were assessed as above. Post-hoc comparisons were conducted using two-way ANOVA with Šidák correction ($p < 0.05$), and p-values were further adjusted for multiple testing across all OTUs using the Benjamini–Hochberg procedure (FDR).

3. Results

3.1. Pesticide residues in potato tubers

Potato flours from conventional (PC) and organic (PO) cultivation without storage, as well as flours from stored potatoes (PCS and POS), were subjected to the quantification of 435 pesticide residues using GC–MS/MS and LC-MS/MS with a high detection threshold (LOQ > 10 μg/kg). This analysis showed that PO and POS conditions did not contain detectable pesticides. In the PC samples, carfentrazone was detected but not quantified (LD > 0.01 mg/kg), while propamocarb was quantified in PC and PCS samples, and chlorpropham in PCS samples. The storage compound 1,4-dimethylnaphthalene was detected and quantified only in PCS samples. (Table 3).

The analysis of 50 other residues was conducted at a lower detection threshold (LOQ > 10 ng/kg) using the QuEChERS extraction method. The presence of pesticide residues in all conditions and for all 3 repetitions was detected (Table 4). For instance, dimethomorph and fluopicolide, two pesticides used in the technical management of conventional potato cultivation (PC), were found in both PC, PCS, and PO, POS conditions, though concentrations were higher in potatoes from conventional cultivation. For example, dimethomorph was detected at 0.27 and 0.24 μg/kg in PC and PCS, respectively, compared to 0.19 and 0.17 μg/kg in PO and POS, respectively; fluopicolide was detected at 0.30 and 0.45 μg/kg in PC and PCS, compared to 0.02 and 0.03 μg/kg in PO and POS, respectively. Surprisingly, other pesticides were found in the potato flours, even though they were not applied in the technical crop management practices of any condition (e.g., lenacil, mesosulfuron, chloridazone, silthiofam, etc.). Given the location of the site, it seems difficult to accept that there was drift of pesticides during spraying, as the application was very localized to the potato rows in the PC condition. However, residue persistence in the soil is plausible because these substances are not easily leachable, and the field had a 10-year history of conventional cultivation prior to this study.

3.2. Nutrigenomic analyses in food safety assessment

The liver, jejunum, and brain were selected for analysis due to their critical functions in metabolic processing, nutrient handling, and neurological regulation, providing insight into systemic impacts of dietary PPP exposure, though effects on additional organs cannot be excluded. Comparative whole transcriptomics of the liver, jejunum and brain tissues from mice force-fed with potato flour issue from different conditions (PC, PO, PCS and POS) as well as with ADI, allowed us to identify differently expressed genes (DEGs) that showed differences in transcript accumulation (Fig. 2 and Supplementary files 1–3).

After 20 days of gavage, 20 genes were up-regulated in liver tissue compared to controls following a diet supplemented with PC potato flour, compared to only 8 genes for PO flour and 4 genes for the ADI solution. The PCS and POS conditions showed a similar response, with 50 up-regulated genes (Fig. 2A).

In jejunum cells, 4 genes were up-regulated and 23 down-regulated following a diet supplemented with PC potato flour, compared to only 2 up-regulated genes for PO flour and 9 up-regulated and 13 down-regulated genes for the ADI solution. The PCS and POS conditions showed a similar response, with 22 up-regulated genes (Fig. 2A).

In brain tissue, one single gene was found up-regulated following a diet supplemented with PC flour and any other deregulation was observed for PO and ADI treatments. The PCS and POS conditions showed a similar response, with 51 up-regulated genes (Fig. 2A).

To estimate the effects of the different gene deregulations observed under the various experimental conditions, a heatmap was generated for each tissue (Fig. 2B and Supplementary files 1–3), illustrating the up-

Table 4
Pesticide dosage in potato flours (µg/kg) with a detection threshold of LOQ > 10 ng/kg.

PPP	Organic potato		Conventional potato		MRLs ($mg \cdot kg^{-1}$)
	PO	POS	PC	PCS	
Amisulbrom	ND	5.42	ND	ND	0.01
Dimethomorph	0.19	0.17	0.26	0.24	0.05
Fluopicolide	0.02	0.03	0.30	0.45	0.03
Pyraclostrobin	2.78	5.59	9.03	5.07	0.02
Aclonifen	0.02	0.06	0.01	0.03	0.02
Azoxystrobin	0.38	0.38	0.38	0.37	7.0
Benoxacor	<0.01	<0.01	<0.01	<0.01	0.1
Benzovindiflupyr	0.32	0.31	0.31	0.38	0.02
Bixafen	0.26	0.25	0.25	0.25	0.06
Boscalid	<0.01	0.01	0.04	0.03	2.0
Bromuconazole	0.04	0.15	0.04	0.02	0.01
Chloridazone	5.32	7.10	6.61	7.71	0.03
Chlortoluron	1.90	2.44	1.91	0.88	0.01
Cypermethrin	ND	ND	0.03	ND	0.05
Cyproconazole	0.05	0.01	<0.01	0.01	0.05
Cyprodinil	0.17	0.18	0.17	0.17	0.02
Deltamethrin	0.49	0.49	0.49	0.48	0.01
Difenoconazole	0.08	0.04	0.00	0.07	0.1
Diflufenicanil	0.06	0.14	0.09	0.08	0.01
Epoxiconazole 1 (enantiomer A)	0.01	0.01	0.06	0.04	0.01
Epoxiconazole 2 (enantiomer B)	0.18	0.19	0.17	0.17	0.01
Ethofumesate	0.14	0.15	0.14	0.14	0.03
Fenpropidine	0.51	0.51	0.51	0.52	0.01
Fluazinam	0.01	0.05	0.02	0.09	0.02
Fludioxonil	0.39	0.48	0.50	0.47	5.0
Flufenacet	0.14	0.15	0.03	0.53	0.15
Fluopyram	0.02	0.01	0.07	0.05	0.08
Fluoxastrobin	0.96	1.35	0.75	0.66	0.1
Fluxapyroxad	0.12	0.15	0.12	0.13	0.3
Imazalil	0.06	0.03	0.15	0.05	0.01
Lambda-cyhalothrin	0.20	0.20	0.19	0.19	0.01
Lenacil	0.51	0.27	0.19	0.28	0.1
Mesosulfuron methyl	1.12	0.47	0.67	1.07	0.01
Metconazole	1.30	1.31	1.30	1.30	0.04
Metobromuron	0.76	0.76	0.76	0.76	0.1
Napropamide	0.37	0.58	0.43	0.56	0.01
Pencycuron	0.10	0.09	0.09	0.11	0.02
Pendimethalin	0.02	0.07	0.07	0.07	0.05
Prochloraz	0.10	0.09	0.07	0.09	0.03
Propyzamide	0.37	0.26	0.30	0.27	0.01
Pyrimethanil	0.25	0.25	0.25	0.25	0.05
S-metolachlor	0.25	0.24	0.24	0.25	0.05
Sedaxane	0.05	0.04	0.12	0.04	0.15
Silthiofam	0.50	0.50	0.51	0.50	0.01
Spiroxamine	0.19	0.17	0.18	0.27	0.01
Tebuconazole	ND	ND	ND	ND	0.02
Tefluthrin	0.18	0.20	0.20	0.19	0.01
Terbuthylazine	0.31	0.31	0.31	0.31	0.01
Tetraconazole	0.13	0.11	0.14	0.13	0.01
Tri-allate	0.06	0.06	0.06	0.06	0.1

ND: not detected.
MRLs: Maximum Residue Limits established by the European Union in accordance with EU Regulation No 396/2005.

and down-regulated genes compared to the control. The biological functions affected by these deregulations, as well as their putative effects, were documented through database consultation (NCBI, GO, UniProt, KEGG) and literature review.

To assess the potential effect of potato as a dietary matrix, we examined the genes commonly deregulated under the conditions PC vs Ctr, PO vs Ctr, PCS vs Ctr, and POS vs Ctr. Only five DEGs were identified in the liver (Parva, Cdh1, Ercc8, Ighv2-3, and Ighv5-9-1), which may reflect the effect of potato consumption itself. Although these deregulations could also result from residual PPPs persisting in the soil from prior agricultural treatments and subsequently present in both plant types (PC and PO, and by extension PCS and POS), they were excluded from the final interpretation concerning the effects of PPPs.

The ADI condition reflects the combined (cocktail) effect of several PPPs used in mixture. This treatment triggered the deregulation of 26 genes (4 DEGs in the liver and 22 in the intestine).

Potato flour from conventional farming (PC) exhibited a stronger nutrigenomic effect than ADI, with 20 DEGs in the liver and 27 in the intestine, along with a single DEG in brain tissue. This is likely due to residues of PPPs used during conventional crop management practices, which remained in the tuber tissues.

Potato flour from organic farming had a much weaker impact on gene expression, than ADI, with only 8 DEGs in the liver and 2 in the jejunum. This limited effect is likely attributable either to residual PPPs persisting in the soil from previous treatments and subsequently absorbed by the plants, or to copper-based treatments applied as part of the specific agricultural practices used for this type of cultivation.

Storage conditions appear to be highly disruptive, as they significantly altered the expression of 50 genes in the liver, 22 in the intestine, and 51 in the brain. Similar patterns were observed for both PC and POS treatments, suggesting that the anti-sprouting agents used during storage were retained in the tubers regardless of the cultivation method.

The analysis of all found DEGs suggests potential disturbances in several key biological processes, including: cell proliferation (activation of cell division, cell migration, tumor transformation), the nervous system (synaptic and neurohormonal alterations associated with psychological disorders, various forms of autism, schizophrenia, and depression), metabolism (disruptions in lipid and glucose metabolism potentially leading to obesity and diabetes), immunity (alterations in immune regulation mechanisms and stimulation of inflammatory responses), and the reproductive system (fertility and reproductive tissue development).

3.3. Gut microbiota modifications in food safety assessment

Metagenomic analysis was performed on fecal samples from each individual (n = 5) for each dietary group, both before the start of the treatment (D0) and at the end of the treatment period (D20). Alpha and beta diversity were calculated, and the composition of bacterial phyla, genera, and species was recorded for each condition (Fig. 3).

A total of 1861 OTUs were identified. The Shannon index showed stable diversity levels ranging from approximately 4.3 to 4.8 across all groups at D0, with no significant changes observed following the potato-supplemented diets (D20). Notably, the diet derived from organically grown potatoes (PO) exhibited the highest alpha diversity value at D20 (Fig. 3A).

Beta diversity was assessed by principal coordinates analysis (Fig. 3B), which did not reveal clear separation among the conditions, indicating that any differences are likely to be more subtle and condition-specific.

The identified phyla (Fig. 3C) included Actinobacteriota, Bacteroidota, Cyanobacteria, Desulfobacterota, Firmicutes, Patescibacteria, Proteobacteria, and Verrucomicrobiota, with Firmicutes and Bacteroidota being the most abundant. After 20 days of dietary intervention, a significant reduction in Bacteroidota abundance was observed in the POS and PCS groups and also an increase of Verrucomicrobiota was observed in PC, PCS and POS samples.

At the bacterial genus level, considerable variability was observed among the mouse groups at D0. *Lachnospiraceae* UCG-001, *Lactobacillus*, *Limnosilactobacillus*, and *Ruminococcaceae* UBA 1819 showed significantly different abundances across the groups. This variability evolved toward a more uniform distribution after 20 days of diet (Fig. 3D). Few differences were observed between groups at D20. Thus, *Akkermansia* abundance significantly increased in the PC, PCS, and POS groups, which may be due to the initial microbiota composition, as these differences were already observed at D0. In contrast, *Faecalibaculum*, which was not detected at D0, developed by D20 and showed an increased abundance in the PC, PCS, and POS groups, with a significant increase

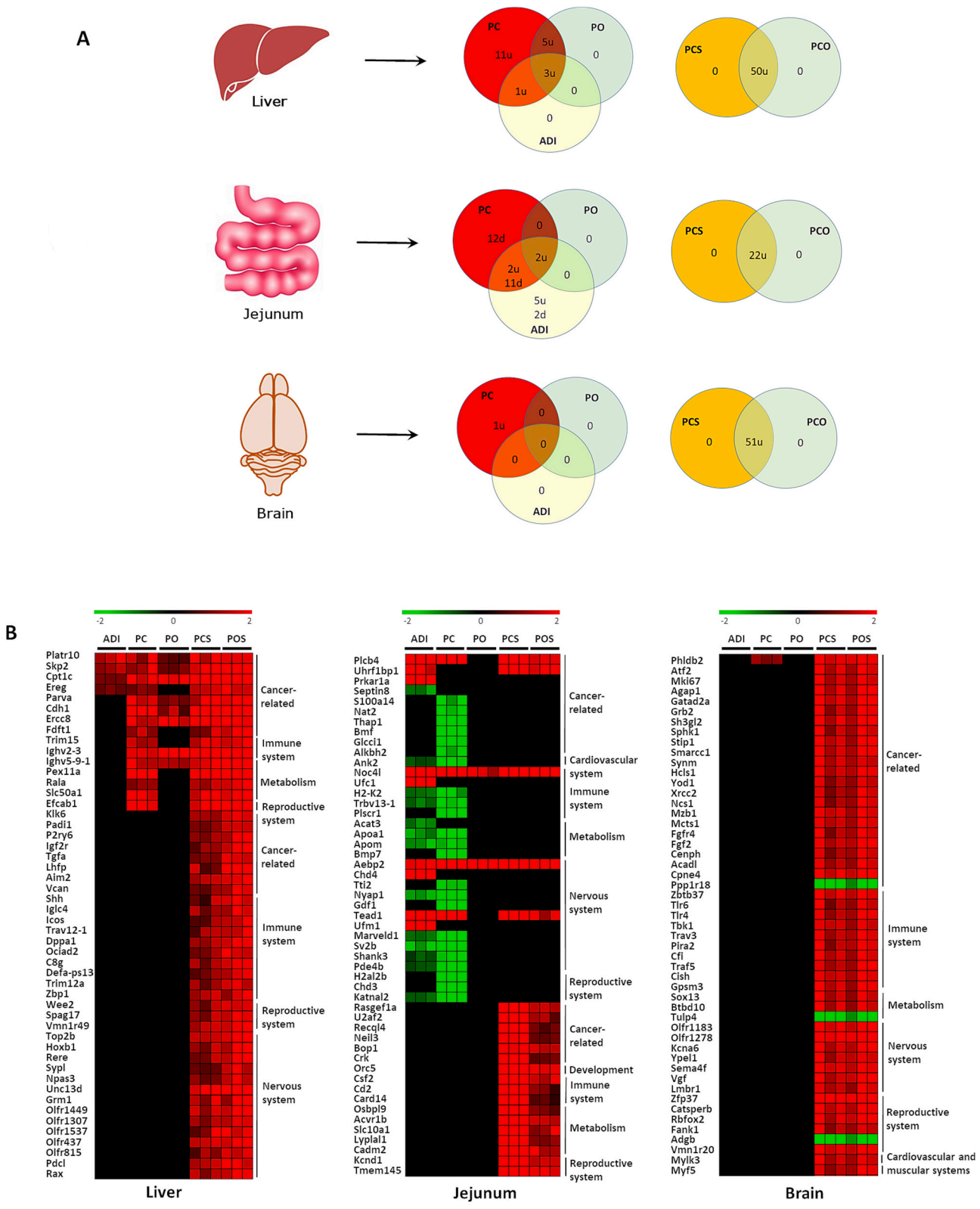

Fig. 2. Nutrigenomic analyses.

A. Venn diagrams showing the number of differentially expressed genes (DEGs) in liver, jejunum, and brain tissues for the PC, PO, and ADI treatments, as well as for the PCS and POS treatments. Overlapping areas indicate DEGs shared between treatments.

B. Heatmaps of gene expression profiles in mouse liver, jejunum, and brain tissues following 20 days of daily ingestion of diets supplemented with ADI, PC, PO, PCS, or POS. Only genes with fold change >2 or <0.5 (log_2FC relative to the control condition, n = 3 mice per group) are shown. Functional gene groupings and potential relevance to disease are shown on the right side of each heatmap.

ADI — acceptable daily intake; PC — conventional potato; PO — organic potato; PCS — conventional potato after storage; POS — organic potato after storage; u — upregulated genes compared to control; d — downregulated genes compared to control.

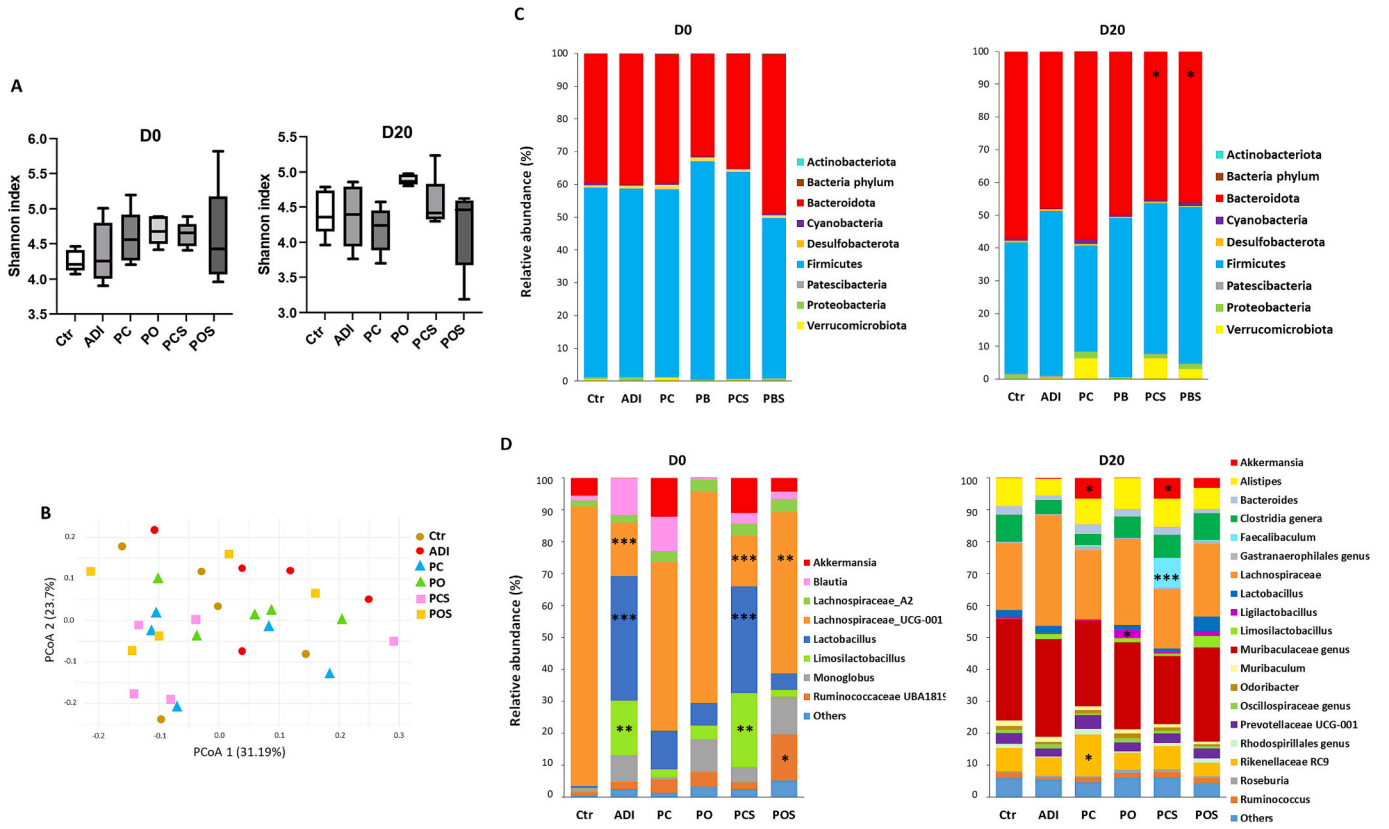

Fig. 3. Changes in gut microbiota composition.
A. Alpha diversity of the fecal microbiota in mice subjected to different dietary regimens. Shannon Index remained stable across all groups. Comparisons to the control group (Ctr) were performed using one-way ANOVA or non-parametric Kruskal Wallis test with Dunnett's multiple comparisons or Dunn's multiple comparison and no significant differences were observed.
B. Principal Coordinates Analysis (PCoA) illustrating the overall differences in microbial composition among treatment groups.
C. Relative abundance of bacterial phyla before (D0) and after 20 days of dietary intervention (D20). Values represent group means (n = 5). Statistical comparisons to the control group (Ctr) were performed using two-way ANOVA with Šidák post hoc correction (*$p < 0.05$).
D. Relative abundance of bacterial genera before (D0) and after 20 days of dietary intervention (D20). Values represent group means (n = 5). Statistical comparisons to the control group (Ctr) were performed using two-way ANOVA with Šidák's multiple comparisons (*$p < 0.05$, **$p < 0.01$, **$p < 0.001$).
Ctr — control group; ADI — acceptable daily intake; PC — conventional potato; PO — organic potato; PCS — conventional potato after storage; POS — organic potato after storage.

observed in PCS. Two other genera identified at D0 with very low abundance (below 1 %) also expanded by D20: *Ligilactobacillus*, which was found to be significantly increased in PO samples, and *Rikenellaceae* RC9, which significantly increased in PC samples. Overall, although alterations were observed in the gut microbiota of mice fed different diets, there was no significant effect on mouse weight (Supplementary file 4).

4. Discussion

4.1. Impact of ADI-level pesticide mixtures on gene expression

The ADI condition demonstrates that, even at acceptable daily intake levels, a mixture of plant protection products (carfentrazone-ethyl, cyazofamid, pyraflufen-ethyl, amisulbrom, dimethomorph, pyraclostrobin, fluopicolide, maleic hydrazide, mandipropamid, and propamocarb), commonly used in various crop treatments, can exert a « cocktail effect », significantly impacting gene expression in both hepatic and intestinal tissues. Specifically, 4 genes were deregulated in the liver and 22 in the intestine (Fig. 2A). Although different sets of genes were affected in each organ and are involved in distinct biological functions, all have been previously associated with carcinogenic processes (Fig. 2B and Supplementary files 1 and 2).

In the liver, Platr10, which encodes a long non-coding RNA crucial for somatic cell reprogramming (Du et al., 2021), has been linked to tumor development when dysregulated (Jiang et al., 2019). Skp2 encodes a component of the cyclin A-CDK2 complex, which is involved in S-phase progression and is a recognized proto-oncogene (Chen et al., 2011). Cpt1c, a gene involved in β-oxidation, has been identified as a prognostic marker in carcinomas (Zhao et al., 2024), while Ereg, associated with inflammatory and proliferative processes, is known to promote tumor progression in various human tissues (Shigeishi et al., 2008).

Similarly, in the intestinal tissue, genes such as Plcb4, Uhrf1bp1, Prkar1a, and Septin8 were modulated in response to the ADI treatment and have also been linked to carcinogenesis (Fig. 2B and Supplementary file 2). Plcb4, encoding a phospholipase C, has been found overexpressed in tumors (Cai et al., 2017). Uhrf1bp1, involved in cell cycle regulation, has been found upregulated in cancers, while Prkar1a, a regulatory subunit of protein kinase A, has been found involved in apoptosis inhibition and tumor growth (Djari et al., 2021). Septin8, which encodes a protein involved in the regulation of cytokinesis and cytoskeletal dynamics, has been identified as a cancer-associated gene when deregulated (Russell and Hall, 2005). Although our dataset does not allow us to establish direct causal links between these deregulations and tumor development, the observed associations point to biologically relevant pathways that could guide future mechanistic studies.

Additional gene deregulations induced by the ADI treatment in

intestinal cells suggest potential perturbations in multiple physiological systems, including the cardiovascular and immune systems, metabolic regulation, particularly obesity-related pathways, as well as neural development, synaptic transmission, and reproductive function. Cardiovascular effects were suggested by the deregulation of Ank2, a gene implicated in the regulation of cardiac contraction (Sucharski et al., 2020). Immune system alterations were indicated by the deregulation of several key genes: Noc4l, involved in regulatory T cell (Treg) activation and immune tolerance (Zhu et al., 2019); Ufc1, which regulates type II interferon production (Balce et al., 2021); H2-K2, responsible for presenting foreign antigens to the immune system (Kvist et al., 1983); and Trbv13-1, which contributes to antigen recognition (Koop et al., 1994). Metabolic disturbances were suggested by the altered expression of Acat3 (acetyl-coenzyme A acetyltransferase 3), involved in fatty acid β-oxidation (Lee et al., 2013); Apoa1 (apolipoprotein A-I), which plays a key role in cholesterol and lipid transport (Gorshkova and Atkinson, 2011); and Apom (apolipoprotein M), also essential for cholesterol efflux (Elsøe et al., 2013). These genes are crucial for metabolic homeostasis, and their dysregulation is known to impact obesity and related metabolic disorders (Huang et al., 2023). Regarding the nervous system, nutrigenomic alterations were suggested by the deregulation of Nyap1 and Aebp2, involved in neural developmental processes (Wang et al., 2020; Kim et al., 2015); Tead1 and Ufm1 contributing to myelin formation (Grove et al., 2024; Muona et al., 2016), or Sv2b, which is involved in synaptic transmission (Paulussen et al., 2024). Additionally, Shank3 and Pde4b, two genes known to be associated with neurodevelopmental and psychiatric disorders, were also affected. Shank3 has been linked to autism spectrum disorders (Tao et al., 2022), while Pde4b has been implicated in schizophrenia and bipolar disorder (Numata et al., 2008). The downregulation of the Katnal2 gene, which is involved in germ cell development, suggests a potential infertility-related perturbation (Dunleavy et al., 2017). All these results should serve as hypotheses, guiding future research toward the mechanistic basis of the observed deregulations.

4.2. Impact of potato flour derived from organically grown tubers on gene expression

The PO condition also induced changes in gene expression, with eight DEGs identified in hepatic tissue and two DEGs in intestinal cells (Fig. 2). Notably, several of these genes displayed similar deregulation patterns as those observed in the ADI and/or PC treatments (Fig. 2A and Supplementary files 1 and 2), suggesting that the effects may be attributed to residual pesticides in the soil from previous conventional crop cycles, which are common to both PC and PO conditions. These are probably among the 47 residues found at a low detection threshold (Table 4). Additionally, the potential contribution of copper-based compounds, applied as part of the organic farming practices (Table 2), cannot be excluded as a possible explanation for these effects. In hepatic tissue, the deregulation of Platr10, Skp2, and Cpt1c, already affected in the ADI condition, have shown a potential association with cancer development (Fig. 2B and Supplementary file 1). In intestinal cells, Noc4l, which plays a role in immune regulation, and Aebp2, involved in neuronal developmental processes, were deregulated in a similar manner as in the ADI condition (Fig. 2B and Supplementary file 2).

4.3. Impact of potato flour derived from conventionally grown tubers on gene expression

The PC condition revealed a more pronounced effect than both the ADI and PO treatments, with 20 differentially expressed genes (DEGs) detected in the liver, 27 in intestinal cells, and 1 DEG in brain tissue (Fig. 2A). While some of these DEGs showed similar deregulation patterns to those observed in the ADI or PO conditions, a substantial number of genes were exclusively deregulated under the PC treatment (11 in the liver, 12 in the jejunum, and 1 in the brain). This suggests that pesticide residues present in flour derived from conventionally grown potatoes have a greater impact on gene expression. Carfentrazone and propamocarb residues were detected in PC flour at a high detection limit (Table 3) and also a number of 48 residues at a low detection limit (Table 4).

In terms of biological relevance, the deregulated genes in the PC condition also affected the same functional groups previously identified in the ADI and PO conditions, including those putatively related to carcinogenesis, metabolism, immune response, neurological development, and reproductive health (Fig. 2B and Supplementary Files 1–3). Among the genes specifically deregulated by the PC treatment in the liver, Fdft1 has been shown to play a critical role in cancer development, particularly in metabolic reprogramming, cell proliferation, and invasion (Ha and Lee, 2020). Trim15, another PC-specific DEG, has been described to promote tumor invasion and metastasis (Sun et al., 2021). Additionally, Pex11a, Rala, and Slc50a1, which are involved in lipid metabolism and glucose homeostasis, have been associated with obesity and overweight when dysregulated (Opaliński et al., 2011; Skorobogatko et al., 2018; Fiorentino et al., 2023). Efcab1, also specifically deregulated in the PC condition, has been described to be implicated in infertility (Hjeij et al., 2023).

In the intestinal tissue, several PC-specific DEGs have been described as being related to carcinogenic processes. For example, S100a14, found to be downregulated, has been characterized as a tumor suppressor, and its reduced expression has been described to be associated with cancer progression and metastasis (Pandey et al., 2020). Plscr1, involved in interferon-induced antiviral responses, was also downregulated, suggesting potential immune system disturbances (Sadanari et al., 2022). Bmp7, a gene essential for brown adipose tissue development, also showed decreased expression (Tseng et al., 2008), while Tti2, which plays a role in intellectual development, was similarly downregulated (Langouët et al., 2013). Furthermore, deregulation of H2al2b, involved in spermiogenesis (Rathke et al., 2014), and Chd3, essential for normal gonadal development (de Castro et al., 2022), suggests possible adverse effects on reproductive system function. In brain tissues, the single gene found upregulated in PC treatment was Phldb2 which has been characterized as a proto-oncoprotein (Han et al., 2024).

4.4. Nutrigenomic responses to potato flour treated with anti-sprouting agents

The treatments with flour derived from potatoes stored and treated with the anti-sprouting agents (PCS and POS) induced effects 2 to 3 times stronger compared to the other conditions, with 50 DEGs detected in the liver, 22 DEGs in the jejunum, and 51 DEGs in the brain. These effects were consistently observed in both PCS and POS treatment groups, and some of these gene expression changes were also identified in the ADI, PO, and/or PC treatments. Interestingly, even though the anti-sprouting treatments differ for PCS and POS, the same genes were deregulated in both conditions with similar expression patterns. Although the deregulated genes varied across tissues, their functions converged, and their up-regulation pointed to the same putative health effects as those observed previously with PC, PO, or ADI treatments: carcinogenesis, immune system deregulation, metabolism dysregulation, neurodevelopmental disturbances, and reproductive function deregulation. It would be useful to verify whether tubers stored without any anti-sprouting treatment show nutrigenomic deregulations, in which case aging or the degradation of certain molecules could be implicated.

Collectively, these findings support the diverse effects observed following the administration of various PPPs in different animal models (rodents, swine, and rams), as well as in humans, through targeted studies focusing on specific physiological impacts. These include tumorigenic effects (Cavalier et al., 2023), neurological disorders (Villaorduña et al., 2024), immune responses (Afolabi et al., 2019), metabolic alterations (Noppakun and Juntarawijit, 2021), and

reproductive system dysfunctions (Falero et al., 2025). The differences observed among the three analyzed tissues may be explained by variations in the availability of compounds that are progressively released from the food matrix or digested at different stages along the digestive tract. Since the effects of PC and PO flours, as well as ADIs, appear to be minimal in the brain but more pronounced in the liver and intestine, differences in the absorption and/or metabolism of various PPPs can be suspected. The pathways and mechanisms of action of these substances remain to be elucidated.

4.5. Effects of PPPs on the gut microbiota

The comparative analysis of gut microbiota composition under different dietary regimens revealed that, after 20 days of ingestion, the diet derived from organically grown potatoes (PO) exhibited the highest alpha diversity (Fig. 3A), the greatest OTU dispersion (Fig. 3B), and no disruption in microbiota phyla abundance (Fig. 3C). Moreover, it did show a significant increase in the relative abundance of *Ligilactobacillus* (Fig. 3D), a genus known for its probiotic and functional properties, as well as its various health-promoting roles (Yang et al., 2024).

The administration of PPPs as a cocktail (ADI) produced results similar to the control group in terms of alpha diversity and relative abundance of bacterial taxa (Fig. 3). While the ADI group showed a distinct metagenomic profile at D0, microbial taxa stabilized to levels comparable to the control group after 20 days of exposure.

The most disruptive dietary regimens were found to be PC, PCS, and POS. The phylum Bacteroidota was significantly reduced under PCS and POS conditions after 20 days of daily ingestion (Fig. 3C). Animal model and human studies have shown that the overall Firmicutes/Bacteroidota ratio (F/B) is higher in obese individuals compared to lean individuals (Koliada et al., 2017; Barlow et al., 2015). Diet-induced obesity has also been shown to alter this ratio due to a marked increase in the abundance of Firmicutes in the distal gut microbiota (Turnbaugh et al., 2008). Furthermore, this ratio has been reported to be significantly increased in patients developing type 1 diabetes (Wen et al., 2008). We also found that the abundance of *Faecalibaculum* significantly increased in the PCS group (Fig. 3D). The genus *Faecalibaculum*, particularly *Faecalibaculum rodentium*, contributes to gut homeostasis through its influence on epithelial cell renewal and bile acid metabolism, although its direct role in food digestion remains unclear (Cao et al., 2022). Additionally, it has been implicated in modulating gut–brain communication and depressive-like behavior in mice via vagus nerve signaling (Wang et al., 2021). *Rikenellaceae* RC9 gut group was found to be significantly increased in the PC mice group after 20 days of the diet (Fig. 3D). This taxon is an important component of the gut microbiota, playing roles in metabolism and having potential associations with health and disease states (Sun et al., 2019).

Overall, our gut microbiota analysis revealed that the most detrimental dietary conditions were those involving conventionally grown potatoes (PC) and potatoes subjected to storage with anti-sprouting treatments (PCS and POS). These results are consistent with previous studies conducted on the gut microbiota using various PPPs administered separately in solution (Sharma et al., 2023), and demonstrate the disruptive effects on intestinal microbial balance following the ingestion of mixed PPPs contained in food, even at moderate doses.

4.6. What treatment for potatoes?

Very few nutrigenomic effects have been attributed to potato consumption, with only five potentially responsive DEGs identified in liver tissue. However, an extent of gene expression alterations and changes in the gut microbiota observed in mice were related to the presence of PPP residues detected in both conventionally and organically grown potatoes (PC and PO).

Potatoes cultivated using conventional agricultural practices induce significant nutrigenomic effects, with 15 DEGs in the liver, 27 in the jejunum, and 1 in the brain (Fig. 2), and also affect the gut microbiota (Fig. 3). Carfentrazone and promocarb, which are used in conventional cultivation practices (Table 1) and were detected in PC samples (Table 3), appear to be the most impactful compounds. Their repeated ingestion over time could potentially contribute to long-term metabolic disturbances (Table 5).

Carfentrazone-ethyl is a contact herbicide belonging to the aryl triazolinone chemical class. It is primarily used for controlling broadleaf weeds in various crops by inhibiting the enzyme protoporphyrinogen oxidase, leading to membrane disruption and cell death in the target plants (NCBI, National Center for Biotechnology Information, 2025). Although it is considered to have a relatively low application rate and minimal residual activity in the soil, its toxicological profile has been thoroughly studied. Carfentrazone-ethyl shows low acute toxicity to mammals, birds, and bees but exhibits moderate toxicity to aquatic organisms, particularly fish and invertebrates (EFSA, European Food Safety Authority, 2016). Due to its rapid environmental degradation and low potential for bioaccumulation, it is generally regarded as posing a low long-term environmental risk when used according to recommended guidelines.

Promocarb is a systemic fungicide whose active ingredient, metalaxyl-M (also known as mefenoxam), is commonly used to control soil- and seed-borne diseases caused by oomycetes. Metalaxyl-M disrupts ribosomal RNA synthesis in target fungi, impairing protein production and ultimately leading to pathogen death (Davidse et al., 1981). Toxicological assessments indicate that metalaxyl-M has relatively low acute toxicity to mammals, birds, and bees, but is moderately toxic to aquatic organisms, especially under conditions of repeated exposure or runoff (EFSA, European Food Safety Authority, 2023).

Diets based on organically grown potatoes (PO) appeared to have the least nutrigenomic impact (Fig. 2) and did not induce unfavorable changes in the gut microbiota. On the contrary, they seemed to support a favorable shift in microbial diversity (Fig. 3). However, risks remain, as indicated by the dysregulation of 3 genes in the liver tissue and 2 genes in intestinal cells (Fig. 2). These risks, although much lower compared to conventionally grown potatoes, could be attributed to treatments with copper-based derivatives (Table 5), which were not quantified and were used during organic cultivation (Table 1). There is also the possibility of residual contamination from previous crops or neighboring crops, which was detected in PO samples at a low detection threshold (Table 4).

Copper-based compounds, such as copper sulfate, copper oxychloride, and copper hydroxide, are commonly used in organic farming as fungicides and bactericides to control plant pathogens, especially in crops like grapes, potatoes, and tomatoes. These compounds work by disrupting enzymatic systems in fungi and bacteria, making them effective against a broad range of diseases (Lamichhane et al., 2016). Despite being approved for organic farming, concerns have been raised about the environmental and toxicological impacts of copper accumulation in soils. High concentrations of copper can pose risks to human health and aquatic ecosystems, leading regulatory agencies to set limits on its use and promote alternative disease management strategies (EFSA, European Food Safety Authority, 2018).

For stored conditions (PCS and POS), the disruptive effects were remarkable, being 2 to 3 times more significant in terms of the number of deregulated genes (50 DEGs in liver, 22 in jejunum and 1 in brain) and 3 affected microbial genera (Figs. 2 and 3). The anti-sprouting agent 1,4-dimethylnaphthalene used for PCS and detected in PCS flour samples (Table 3), as well as the mint essential oil (not measured) used for POS, seem to induce a very disruptive yet similar effect (Table 5).

1,4-Dimethylnaphthalene is an aromatic hydrocarbon commonly used as a sprout suppressant, particularly in the post-harvest storage of potatoes. It effectively inhibits sprouting by modulating hormonal pathways in tubers, helping to maintain dormancy and reduce spoilage during storage (Campbell et al., 2010). This compound has garnered interest due to its natural occurrence in potato volatiles and its effectiveness at low concentrations. Regarding its toxicity, 1,4-

Table 5
Effects related to PPPs used in potato cultivation and storage.

Culture type	PPP	Action	Nutrigenomic changes	Gut microbiota changes	Putative health effects
PC	Carfentrazone Promocarb	Herbicide Fungicide	20 DEGs in the liver 27 DEGs in the jejunum 1 DEG in the brain	1 affected genus	• Carcinogenesis • Immune system deregulation • Metabolism deregulations • Reproductive function deregulation
PO	Copper derivatives	Fungicides and bactericides	8 DEGs in the liver 2 DEGs in the jejunum	1 affected genus	• Carcinogenesis • Immune system deregulation • Neurodevelopment deregulation
PCS	1,4-Dimethyl-naphthalene	Sprout inhibitor	50 DEGs in the liver 22 DEGs in the jejunum 51 DEGs in the brain	F/B change 1 affected genus	• Carcinogenesis • Immune system deregulation • Metabolism deregulations • Neurodevelopment deregulations • Reproductive function deregulation
POS	Mint essential oil	Sprout inhibitor	50 DEGs in the liver 22 DEGs in the jejunum 51 DEGs in the brain	F/B change	• Carcinogenesis • Immune system deregulation • Metabolism deregulations • Neurodevelopment deregulation • Reproductive function deregulation

PC: conventional potato; PO: organic potato; PCS: conventional potato after storage; POS: organic potato after storage; NI: not identified; DEG: deregulated gene.

dimethylnaphthalene has been evaluated in several studies for potential environmental and health risks. Toxicological assessments have examined its effects on aquatic organisms, mammals, and soil microbiota, with most studies indicating a relatively low acute toxicity profile when used at recommended levels (EPA, U.S. Environmental Protection Agency, 2003; EFSA, European Food Safety Authority, 2012). Our study adds another layer of concern regarding the use of this product.

Mint essential oil has been explored as an alternative to synthetic sprout inhibitors like chlorpropham for use in post-harvest potato storage. This oil, primarily derived from species such as *Mentha piperita*, contains active compounds like menthol and menthone, which have been shown to inhibit sprouting in stored tubers (Gómez-Castillo et al., 2013). These compounds act by disrupting cellular respiration and enzymatic activity in sprout tissues, effectively reducing sprouting rates. While mint essential oil offers a more natural option, its toxicity profile is not well understood, and concerns have been raised regarding its potential effects on human health and the environment. Studies suggest that high doses could be hepatotoxic and nephrotoxic (Nath et al., 2012), but a comprehensive evaluation of its toxicity is still necessary (Vigan, 2010). For this product, our study also raises concerns, but additional detection and quantification studies are needed to validate our interpretation.

Intriguingly, we detected chlorpropham in the PCS flours (Table 3), and amisulbrom in the POS flours (Table 4), even though these products were not used in the storage treatments. Chlorpropham is a synthetic sprout inhibitor commonly used in post-harvest potato storage to suppress sprouting and maintain tuber quality during extended storage periods. It works by disrupting cell division in meristematic tissues, thereby preventing sprout development. Despite its effectiveness, chlorpropham has raised concerns due to its toxicological profile and the persistence of its residues in potatoes and storage environments. Repeated exposure to chlorpropham may pose risks to human health, including potential effects on the liver, thyroid, and blood parameters, as well as possible carcinogenicity (EFSA, European Food Safety Authority et al., 2017). In 2020, the European Commission decided not to renew the approval of chlorpropham, citing insufficient data to confirm consumer safety and environmental protection (Official Journal of the European Union, 2019).

Amisulbrom is a systemic fungicide used primarily in agriculture to control a broad spectrum of fungal diseases (FAO/WHO, 2009). It belongs to the chemical class of benzoylureas and works by inhibiting the biosynthesis of chitin, a critical component of fungal cell walls (Merzendorfer, 2013). The compound has moderate toxicity to humans, but long-term exposure can lead to adverse health effects such as skin irritation, eye damage, or potential reproductive toxicity, although these risks are generally considered low when used according to recommended guidelines (EPA, U.S. Environmental Protection Agency, 2011).

Since these two products were not applied to the PCS and POS tubers respectively, we can explain their presence by the fact that the containers used for storage, being part of an agricultural structure that had been dedicated to storage for several years, were likely contaminated with chlorpropham and amisulbrom from prior treatments. This observation highlights the critical need for thorough and systematic decontamination of spaces and tools associated with potato storage.

A time-course study was not conducted in this work, which limits our ability to assess the temporal dynamics of PPP-induced effects; however, the present findings provide a comprehensive snapshot of the biological impact after chronic exposure. Our findings also support the need to move beyond single-compound assessments and to integrate cumulative and mixture-based toxicity frameworks into PPP risk assessments, particularly for compounds that commonly co-occur in crop production and storage (EFSA Scientific Committee et al., 2019; Boobis et al., 2008; Hernández and Tsatsakis, 2017). Such approaches are essential to better capture potential synergistic or additive effects of multiple pesticide residues on transcriptomic and microbiota alterations.

Although our study used ADI-based doses, it reflects aspects of real-world dietary exposure to multiple pesticide residues. EFSA monitoring data indicate that humans are frequently exposed to mixtures of residues, often at levels below the ADI but chronically and simultaneously across compounds (EFSA, 2020). In addition, commercial pesticide formulations contain co-formulants (e.g., surfactants, solvents, adjuvants) that can exert independent biological effects or enhance the toxicity of active substances (Mesnage et al., 2019). Therefore, even low-level exposures may produce additive or synergistic 'cocktail effects,' underscoring the importance of considering mixture toxicity in risk assessment.

5. Conclusion

This study provides compelling evidence that chronic dietary exposure to PPP residues, even at levels considered acceptable for daily intake (ADI), can exert significant biological effects. Diets based on conventionally grown potatoes and those stored with anti-sprouting agents revealed that PPPs induce distinct yet functionally convergent transcriptomic alterations, particularly affecting genes associated with carcinogenesis, immune modulation, metabolic regulation, neurodevelopment, and reproductive function. Complementary gut microbiota analyses further supported these conclusions, indicating altered microbial diversity and composition, which have been linked to metabolic disorders, inflammatory diseases, and neuropsychiatric conditions, suggesting a broader spectrum of potential systemic effects. Taken

together, the parallel transcriptomic alterations observed in the liver, jejunum, and brain, combined with the microbial shifts, point toward possible disruptions of the gut–liver–brain axis, an integrative pathway mediating metabolic regulation, immune responses, and neurophysiological functions. Our results also raise critical questions about the adequacy of current safety thresholds for PPPs and highlight the importance of considering the cumulative or synergistic "cocktail effect" of multiple pesticide residues and long-term dietary exposure in risk assessments.

In contrast, diets derived from organically grown potatoes (PO) preserved microbial diversity and showed minimal transcriptomic impact, emphasizing the protective role of pesticide-free food sources. However, the fact that some organic crops do not necessarily guarantee health benefits underscores the importance of studying more closely the impact of copper derivatives used in the agricultural process, as well as investigating the cleanliness of the soil. These areas should be the focus of dedicated studies and appropriate measures. It is also essential to avoid storage methods involving plant protection products that may pose health risks. Both 1,4-dimethylnaphthalene and mint essential oil should be avoided or better dosed, and further studies are required in this regard. Additionally, particular attention should be paid to residual contamination in storage environments.

Overall, these results highlight, on one hand, the importance of large-scale omics analyses in providing a comprehensive overview of potential biological disruptions, and on the other hand, emphasize the need to revisit regulatory standards and promote agricultural practices that minimize pesticide residues to protect public health.

Although murine models are highly informative for studying the effects of dietary PPP exposure, caution is needed when extrapolating to humans. Species-specific differences in physiology, metabolism, and gut microbiota may affect pesticide biotransformation and host responses. Therefore, complementary human-relevant studies are required to confirm and extend these findings.

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CRediT authorship contribution statement

Sandy Theysgeur: Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation. **Camille Dugardin:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation. **Brice Louvel:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Sébastien Lemière:** Writing – review & editing, Project administration, Funding acquisition. **Jérôme Muchembled:** Writing – review & editing, Resources, Methodology. **Bernard Taminiau:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Georges Daube:** Writing – review & editing, Supervision, Resources. **Ali Siah:** Writing – review & editing, Validation, Supervision. **Rozenn Ravallec:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Jean-Louis Hilbert:** Writing – review & editing, Supervision, Resources. **Anca Lucau-Danila:** Writing – review & editing, Writing – original draft, Validation, Project administration, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Data availability

Data will be made available on request.

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