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Simultaneous detection of mycotoxins and pesticides in human urine samples: A 24-h diet intervention study comparing conventional and organic diets in Spain

Jose A. Gallardo-Ramos^{a,1}, Jesús Marín-Sáez^{b,c,1}, Vicente Sanchis^a, Laura Gámiz-Gracia^b, Ana M. García-Campaña^b, Maykel Hernández-Mesa^{b,2}, German Cano-Sancho^{d,2,*}

^a Department of Food Technology, Engineering and Science. Applied Mycology Group, AGROTECNIO-CERCA Center, University of Lleida, 25198, Lleida, Spain

^b Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Campus Fuentenueva S/n, E-18071, Granada, Spain

^c Department of Chemistry and Physics, Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria,

Agrifood Campus of International Excellence, CeiA3, E-04120, Almeria, Spain

^d Oniris, INRAE, LABERCA, 44300, Nantes, France

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ABSTRACT

Pesticides and mycotoxins, prominent chemical hazards in the food chain, are commonly found in plant-based foods, contributing to their pervasive presence in the human body, as evidenced by biomonitoring programs. Despite this, there is limited knowledge about their co-occurrence patterns. While intervention studies have demonstrated that organic diets can significantly reduce pesticide levels, their impact on mycotoxin exposure has been overlooked.

To address this gap, this study pursued two objectives: first, to characterize the simultaneous presence of mycotoxins and pesticides in human urine samples by means of the control of the biomarkers of exposure, and second, to investigate the influence of consuming organic foods on these co-exposure patterns. A pilot study involving 20 healthy volunteers was conducted, with participants consuming either exclusively organic or conventional foods during a 24-h diet intervention in autumn 2021 and spring 2022 to account for seasonal variability. Participants provided detailed 24-h dietary records, and their first-morning urine samples were collected, minimally treated and analysed using LC-Q-ToF-MS by means of a multitargeted method in order to detect the presence of these residues.

Results indicated that among the 52 screened compounds, four mycotoxins and seven pesticides were detected in over 25% of the samples. Deoxynivalenol (DON) and the non-specific pesticide metabolite diethylphosphate (DEP) exhibited the highest frequency rates (100%) and concentration levels. Correlations were observed between urine levels of mycotoxins (DON, ochratoxin alpha [OT α], and enniatin B [ENNB]) and organophosphate pesticide metabolites DEP and 2-diethylamino-6-methyl-4-pyrimidinol (DEAMPY). The pilot intervention study suggested a reduction in ENNB and OT α levels and an increase in β -zearalenol levels in urine after a short-term replacement with organic food. However, caution is advised due to the study's small sample size and short duration, emphasizing the need for further research to enhance understanding of the human chemical exposome and refine chemical risk assessment.

1. Introduction

The widespread presence of pesticides and mycotoxins in the food chain remains among the top chemical concerns in the European Union (EU) as highlighted by the Rapid Alert System for Food and Feed (RASFF, 2023). Mycotoxins are chemical substances produced by filamentous fungi like *Aspergillus, Penicillium,* or *Fusarium* that can produce a variety of harmful acute and chronic effects on the organism (Kihal

* Corresponding author.

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E-mail address: German.CanoSancho@inrae.fr (G. Cano-Sancho).

¹ Both authors have participated equally as first authors.

 $^{^{2}\,}$ Both authors have equally contributed as last authors

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et al., 2023; Tjallinks et al., 2023; P. J. Wang et al., 2023). Mycotoxins are mainly produced in a variety of plant-based raw feed and foods, and they are most widely detected in nuts, cereals, and cereal-based products. The main mycotoxins are among other aflatoxins, zearalenone, patulin, fumonisins, ochratoxin A and nivalenol/deoxynivalenol, strictly regulated by European legislation. The presence of mycotoxins in food and feed could produce adverse health effects in animals and are mainly related to gastrointestinal and kidney disorders, immune deficiency or cancer (European Commission, 2023). Also, emerging mycotoxins are lesser-known newer forms of mycotoxins that are neither routinely determined, nor legislatively regulated in foods (Mihalache et al., 2023). Emerging mycotoxins include enniatin B (ENNB), which lacks regulated limits in food despite the growing toxicological evidence associated (De Felice et al., 2023; Prosperini et al., 2017).

To avoid fungal growth and improve crop production, the agrifood industry has developed an array of phytochemical strategies including fungicides and pesticides strongly regulated in the EU by Regulation (EC) No 540/2011, with maximum residue levels established by Regulation (EC) No 396/2005, based on their toxicological effects (European Commission, 2005). According to EuroStat, the sales of pesticides in the EU during 2021 were led by Spain (76174 annual tonnes) followed by France, Turkey and Italy (Eurostat, 2023). Modern non-persistent pesticides can be classified depending on their mechanism of action or chemical composition, including organophosphates (OPs, e.g. chlorpyrifos, dimethoate, or pirimiphos-methyl), carbamates (e.g. carbendazim), neonicotinoids (e.g. acetamiprid, clothianidin) or pyrethroids (e.g. cypermethrin), among others. These pesticides can access the organism in different ways, being the diet the main way (Scheepers et al., 2023; Xiao et al., 2023). However, dermal absorption or inhalation can also be an access to pesticides (Kluxen et al., 2023; Kuster et al., 2022), and can exert a variety of toxic effects like cancer, cognitive effects, hormone disruption, or asthma (Bai et al., 2023; Shiny Raj and Anoop Krishnan, 2023). These compounds are quickly metabolized and excreted in the urine in a few hours up to a few days, either as specific or non-specific metabolites, such as diethyl phosphate (DEP) (Bradman and Whyatt, 2005).

The simultaneous exposure to mycotoxins and pesticides could be suspected by the likely co-occurrence in similar raw food (da Luz et al., 2017), but also the concurrent consumption of different plant food in plant-based dietary patterns (Traoré et al., 2016). The co-exposure of multiple chemical hazards can result in toxic effects at lower concentrations due to synergistic effects (Martin, 2023; Y. P. Wang et al., 2023), hence challenging conventional risk assessment. Biomonitoring studies have shown the widespread presence of pesticides and mycotoxins in urine among the general EU population. In these studies, the aim is to monitor the biomarkers of exposure, which are the actual chemicals, or their metabolites, that can be measured in the body or after excretion from the body to determine different characteristics of an organism's exposure. In the case of mycotoxins, the most widely present mycotoxin DON has been found in 96.5% of adults from the HBM4EU European-aligned studies (Govarts et al., 2023). Likewise, the same multi-country study showed the widespread presence of pesticides in urine. Non-specific dialkyl phosphate pesticide metabolites (DAPs), such as DEP or dimethylthiophosphate (DMTP) have been widely detected in urine from France (82.5-100% detection frequency) and Spain (65-100%) (Tagne-Fotso et al., 2023; Yusà et al., 2022).

Despite, the growing evidence on the likely co-occurrence of pesticides with mycotoxins in human matrices, few studies have assessed their levels simultaneously. In turn, an increased consumption of organic food during the last decades has been observed in European countries likely motivated by health consciousness and government policies (J. Y. Wang et al., 2023). For instance, the consumption of organic food among the Spanish population increased by 7% in 2021 (MAPA, 2021). Dietary interventions support that organic food replacement may decrease pesticide exposure and urinary levels (Göen et al., 2017; Hyland et al., 2019; Oates et al., 2014); however, there is no evidence concerning the impacts of such replacements on mycotoxin exposure. For this reason, the present study aims to characterize the simultaneous presence of mycotoxins and pesticides in human urine as biomarkers of exposure and to evaluate the impact of organic food replacement on these co-exposure profiles. Hence, we have conceived a pilot interventional study with healthy adults following a full 24-h organic or conventional diet, repeated in autumn and spring to account for seasonal variability. Then a comprehensive analysis of urine was conducted to simultaneously characterize the biomarkers of exposure to pesticides and mycotoxins.

2. Material and method

2.1. Reagents and chemicals

Pesticide standards of dimethyl dithiophosphate (DMDTP) and DMTP were obtained from Cambridge Isotope Laboratories (Andover, USA). Acephate, acetamiprid-desmethyl, azoxystrobin, azoxystrobin acid, clothianidin, clothianidin-desmethyl, cypermethrin, permethrinic acid (DCCA), 2-diethylamino-6-methyl-4-pyrimidinol (DEAMPY), diethyl dithiophosphate (DEDTP), DEP, desnitro-imidacloprid, dimethoate, dimethyl phosphate (DMP), 5-hydroxycarbendazim, hydroxyimidacloprid, imidacloprid-olefin, 3-phenoxybenzoic acid (PBA), pirimiphos-methyl, 3,5,6-trichloro-2-pyridinol (TCPy), tebuconazole, and tebuconazole-buthylhydroxy were purchased from LGC (Augsburg, Germany). Acetamiprid, carbendazim, chlorpyrifos, chlorpyrifosmethyl, and imidacloprid were obtained from Merck (Darmstadt, Germany).

Mycotoxin standards of 15-acetyl-deoxynivalenol (15-AcDON), 3acetyl-deoxynivalenol (3-AcDON), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), α -zearalenol (α -ZOL), β -zearalenol (β -ZOL), deepoxydeoxynivalenol (DOM-1), DON, enniatin A (ENNA), enniatin A1 (ENNA1), ENNB, enniatin B1 (ENNB1), HT-2 toxin, T-2 toxin, ochratoxin α (OT α), ochratoxin A (OTA), ochratoxin B (OTB), zearalenone (ZEN), and zearalanone (ZAN) were purchased from Techno Spec (Barcelona, Spain).

The individual standard solution of each compound was prepared at 1000 mg/L by dissolving 1 mg of the solid standard with 1 mL of methanol. These standard solutions were kept at -20 °C and were used to prepare the working standard solutions at concentrations of 10 and 100 mg/L. The β -glucuronidase enzyme (from *Helix pomatia* >100,000 units/mL) was obtained from Merck (Darmstadt, Germany).

2.2. Study participants, design, and sampling

Twenty healthy volunteers (sex ratio of 1:1) were enrolled at the University of Lleida (Spain) in 2021, with eligibility criteria specifying an age between 20 and 65 years, the absence of renal dysfunctions and/ or under medication. The protocol for volunteer recruitment and the procedure for gathering their personal information was approved by the Arnau de Vilanova University Hospital (Lleida) Ethical Committee on September 23, 2021, with the code CEIC-2521. Prior to interventions and sample collections, volunteers were informed about the protocol and provided written informed consent.

The study design comprised 24-h dietary interventions, during which all the participants were instructed to substitute all foods from their regular diets with certified organic food or conventional food, henceforth referred to as "organic" or "conventional" diets, respectively. Each volunteer conducted an organic and conventional diet intervention spaced one week apart, during autumn (November 2021) and the same volunteers repeated the process in spring (April 2022). Volunteers should maintain their usual food intake choices throughout the study, especially between organic food-replacements, matching the food types and quantities with those consumed without replacement.

First-morning urine samples were collected the morning following

each intervention. A total of 80 samples (4 samples from each of the 20 volunteers) were aliquoted and stored at -20 °C until the analysis. Participants completed a basic questionnaire providing personal information at the beginning of the study and reported the weight of the portion intakes during the 24-h dietary record for each intervention day.

2.3. Procedure for the determination of biomarkers of exposure to pesticides and mycotoxins in urine samples

A novel multitarget method specifically developed and validated for mycotoxins and pesticides in urine (Marín-Sáez et al., 2024) has been applied in the present study. The method showed good analytical performance in urine according to SANTE guidelines (Pihlström et al., 2024).

2.3.1. Sample preparation

Urine samples were initially thawed and centrifuged to eliminate solid particles. Subsequently, 1 mL of the urine samples was pre-treated with 60 μ L β -glucuronidase resulting in a final 6000 units/mL concentration, for 12 h. After the 12-h pretreatment, a salting-out assisted liquid-liquid extraction (SALLE) procedure was performed. First, 1 mL of acetonitrile was added and then vortexed for 10 min in a multi-tube vortexer model BV1010 (Benchmark Scientific, Savreville, USA). Then 0.8 g of ammonium sulphate was added and vortexed again for 5 min. After vortex agitation, the mixture was centrifuged at 9000 rpm (7690 g) for 10 min at 4 °C in a Universal 320R centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was transferred to a glass vial and evaporated under a gentle stream of N₂ in a nitrogen dryer EVA-EC System (VLM GmbH, Bielefeld, Germany). The evaporated extract was reconstituted with 600 µL of methanol:H2O (50:50 v/v with 0.1% of formic acid). A volume of 100 μL of the solution was transferred to a 500 µL Amicon centrifugal filter and centrifuged at 12000 rpm (10250 g) for 2 h at 4 $^{\circ}$ C.

2.3.2. Biomarker determination by LC-Q-ToF

The filtered samples were injected into an Agilent 1290 Infinity II Ultra-High Pressure Liquid Chromatography (UHPLC) system (Santa Clara, CA, USA) coupled to an Agilent 6550 iFunnel Q-ToF LC/MS model (Santa Clara, CA, USA), equipped with a JetStream electrospray ion source (ESI). The system was operated in full scan mode with a fragmentation energy of 0V and in all ion fragmentation (AIF) mode, selecting a fragmentation energy of 40 V. Chromatographic separation was performed with a Hypersil Gold aQ column ($100 \times 2.1 \text{ mm}, 1.9 \text{ µm}$ particle size) provided by Thermo Fisher Scientific (Les Ulis, France).

The mobile phase consisted of water MilliQ with 0.2% (ν/ν) of formic acid and ammonium formate 4 mM (solvent A), and MeOH with 0.2% (ν/ν) of formic acid and ammonium formate 4 mM (solvent B). The column temperature was set to 40 °C and the mobile phase flow rate during the separation was constant at 0.3 mL/min. Firstly, 10 µL of the sample were injected selecting 10% of solvent B as the initial conditions of the separation. They were held for 1 min before changing gradually until reaching 50% of phase B at minute 5. After being held for 1 min, the mobile phase B was increased gradually until it reached 90% at minute 11 and was held for 1 min. At minute 13 the gradient returned to the initial conditions, which were kept until finish at minute 16.

2.3.3. Creatinine determination

The analytical results were normalised considering the creatinine concentration in the urine samples. Creatinine was determined by LC-MS as previously described (Fraselle et al., 2015) with some modifications. Firstly, 1 mL of thawed and centrifuged urine samples were diluted at a factor of 1:10000 using water with 0.1% NH₄OH (ν/ν). The final dilution also contained 100 µL of creatinine-D3 at 1 mg/L. The external standard calibration curve ranged from 5 to 250 µg/L and it was carried out using the same chromatographic column as cited above. The mobile phase consisted of MilliQ water with 0.1% (ν/ν) of a solution of

ammonium hydroxide (25%, ν/ν) (solvent A), and acetonitrile (solvent B). The elution gradient followed the details described by Fraselle et al. (2015), which were 100% of mobile phase A at 0.2 mL/min flow rate from minute 0 to minute 2.6, then 100% of mobile phase B at 0.3 mL/min flow rate from minute 2.7 to minute 3.6, and finally return to initial conditions of 100% mobile phase A 0.2 mL/min from minute 3.7 to minute 6.

2.4. Data analysis

The 24-h dietary records were abstracted and tabulated in Microsoft Excel sheets (v2019). Individual food items were categorized into 24 main food groups based on their composition for simplicity, like milk, yoghurt or butter included in the group of "dairy", or oranges and orange juice in the group of "citrics", as detailed in Table S1.

Exposure biomarker data were described in terms of detection and quantification frequencies, median, and interquartile ranges (IQR). Biomarkers were excluded from the subsequent statistical analysis when the frequency of quantification was below 25% and/or the frequency of detection was below 75%. For values between the LOD and LOQ, we used the chromatographic values provided by the instrument, and the values below the LOD were substituted by $\text{LOD}/\sqrt{2}$. Bivariate correlations were analysed using Spearman correlations, a comparison of biomarker levels between groups by Mann-Whitney Wilcox test. The associations between biomarker levels and the probability of being in a diet group adjusting for confounding variables were conducted using multivariate logistic regression. All statistical analyses were conducted using R software version 4.2.1, and a p-value <0.05 was considered statistically significant.

3. Results

3.1. Population characteristics

The median age and body mass index (BMI) and their IQR were 39 (31–44) years and 23.5 (21.9–26.0) kg/m², with no statistically significant differences between sexes. All participants were Caucasian living in the same city (Lleida, Spain) during the interventions. Creatinine concentration in urine was slightly higher among males (median [IQR] 174 [149–193] mg/dL) than females (median [IQR] 123 [77–160] mg/dL), p = 0.03.

3.2. Occurrence and co-occurrence of biomarkers in urine

Eleven compounds (out of 52 chemicals screened) were quantified above 25% of all urine samples, which are presented in Table 1 with their creatinine-adjusted and non-adjusted contamination levels. Their histograms of the frequency distribution are presented in Fig. S1. Details of compounds quantified <25% of samples can be found in Table S2. The pesticide carbendazim, the non-specific metabolite DEP, and the mycotoxin DON were detected in all samples. The metabolite hydroxycarbendazim was the unique congener with some values between the LOD and LOQ (15% of values). The highest median concentration level was observed for DON, followed by DEP and ENNB, with 67.9 $\mu g/g$ creatinine, 18.7 μ g/g creatinine, and 2.4 μ g/g creatinine, respectively. The Spearman correlation analysis showed a cluster of 6 positively associated compounds, including dimethoate, DON, DEAMPY, OTa, DEP, and ENNB (Fig. 1). Among them, OTα, DEP and ENNB present the strongest correlation ($\rho = 0.7$ –0.8). It was remarkable the presence of some negative correlations, for instance between $OT\alpha$ and $\beta\mbox{-zearalenol}$ or ENNB with hydroxycarbendazim ($\rho = -0.2$).

3.3. Influence of organic diet on biomarker levels

We first explored sources of variability of biomarkers including sex, season, age, and BMI (Tables S3–S6). For instance, DEP, $OT\alpha$, and ENNB

Table 1

Levels of pesticides and mycotoxins as exposure biomarkers in first-morning urine samples.

Contaminant category	Parent compound	Metabolite	Creatinine-adjusted median (IQR) (µg/g creatinine)	Non-creatinine adjusted median (IQR) (ng/mL)	FQ (%)	LOD (ng/ mL)	LOQ (ng/ mL)
Pesticides							
Pyrethroid insecticides		Permethrinic acid	0.21 (0.21-3.16)	0.21 (0.21-6.53)	28.75	0.3	0.9
Non-specific organophosphate		Diethyl phosphate	18.7 (13.1–25.3)	23.0 (18.9–30.6)	100	0.2	0.7
Organophosphate		DEAMPY	0.46 (0.01–1.00)	1.3 (0.01–1.36)	51.25	0.015	0.05
	Dimethoate		0.13 (0.05-0.17)	0.18 (0.13-0.19)	75	0.005	0.015
Neonicotinoid	Imidacloprid		0.01 (0.01–1.50)	0.01 (0.01-2.03)	42.5	0.015	0.05
Fungicides	Carbendazim		0.08 (0.05–0.17)	0.09 (0.06–0.18)	100	0.007	0.025
		Hydroxycarbendazim*	0.002 (0.001-0.004)	0.003 (0.001–0.005)	65	0.015	0.005
Mycotoxins							
Trichothecenes	Deoxynivalenol		67.9 (33.8–102.5)	75.6 (53.0–118.0)	100	0.75	2.5
Zearalenones		β-zearalenol	1.10 (0.04–2.15)	2.45 (0.04-2.61)	55	0.05	0.16
Ochratoxins		Ochratoxin a	0.18 (0.18–1.43)	0.18 (0.18-2.75)	30	0.25	0.8
Enniatins	Enniatin B		2.42 (0.007–3.97)	4.36 (0.07–4.45)	68.75	0.01	0.03

Abbreviations: DEAMPY: 2-diethylamino-6-methyl-4-pyrimidinol. FQ, frequency of quantification. IQR: interquartile range. LOD: limit of detection. LOQ: limit of quantification.

*, 15% of the values were between the LOD and the LOQ.



Fig. 1. Heatmap depicting the Spearman correlation coefficients between chemical biomarkers. *Abbreviations: DCCA, permethrinic acid. DEAMPY, 2-diethylamino-6*methyl-4-pyrimidinol. DEP, diethyl phosphate. DON, deoxynivalenol. ENNB, enniatin B. OTα, ochratoxin alpha.

were found significantly higher among women than men (Table S3). In turn, ENNB was found at the highest concentrations among the older group \geq 40 years (Table S5). Regarding season (Table S4), only imidacloprid showed significant differences between autumn and spring (p-value 0.03), and no differences in any compound were found concerning BMI (Table S6). The median levels of biomarkers were mostly similar according to the organic diet replacement except for β -zearalenol, which increased among the organic group (Table S7). These trends were robust to the multivariate regression adjusting for potential confounders such as sex or season, as seen in Fig. 2, which shows the differences between



Fig. 2. Change in urine biomarker levels after an organic diet replacement compared to the conventional diet expressed as a coefficient regression and 95% confidence intervals from a multivariate linear regression adjusted for season and age using creatinine-adjusted biomarkers.

the chemical compounds regarding their probability to be associated with consumption of an organic diet, being ENNB and Ot α the most significantly probable to consume, while β -zearalenol is significantly the least. ENNB and OT α concentrations were also found slightly higher after consumption of conventional diets than organic, but the p-value did not reach significance (p-value 0.06 and 0.09 respectively).

3.4. Food consumption and urine biomarkers

The summary of consumption of 24 major food groups can be found in Table S8 and the bivariate correlation between foods in Fig. S2. The consumption was similar among diet interventions based on food production (organic or conventional, Table S9). Slight differences were found between intervention seasons, for instance, dairy products, eggs, and stocks were more highly consumed in autumn, while pulses were more consumed in spring (Table S10).

The Spearman correlation analysis revealed strong positive associations between the intake of whole bread during dinner and the levels of dimethoate ($\rho = 0.42$), OT α ($\rho = 0.39$), DEAMPY ($\rho = 0.33$), DEP ($\rho =$ 0.32), DON ($\rho = 0.30$), ENNB ($\rho = 0.29$), and carbendazim ($\rho = 0.24$), but the highest correlation was found between the fish food group and DEAMPY, and the vegetable fruit group and carbendazim (both correlations with $\rho = 0.43$). The fruit group also presented a positive correlation with β -zearalenol ($\rho = 0.31$) (Fig. 3). The same trends but attenuated were found when the aggregated consumption during the entire intervention day was considered, except for the fish group, which showed an enhanced correlation with carbendazim ($\rho = 0.48$) (Fig. S3).

4. Discussion

To the best of our knowledge, this is one of the first studies to simultaneously assess human co-exposure to mycotoxins and pesticides with urine biomarkers. Using an LC-ESI-Q-ToF-MS method, 4 main mycotoxins and 7 pesticide biomarkers in above 25% of urine samples were determined among 52 compounds screened. We found strong correlations between the mycotoxins OT α and ENNB and the non-

specific metabolite DEP. The pilot 24-h intervention study showed that organic diets may decrease exposure to the mycotoxin $OT\alpha$, mostly related to the consumption of whole bread.

4.1. Pesticides

The most widely detected pesticide biomarkers were carbendazim and the non-specific metabolite DEP of organophosphate pesticides such as chlorpyrifos (Table S2), followed by dimethoate. The non-specific metabolic DEP has been widely detected in the present study (100% detection) and showed the highest median concentration levels among detected pesticide biomarkers (18.7 µg/g creatinine creatinine-adjusted or 23 µg/mL non-adjusted creatinine). Those results are in line with previous studies conducted in Spain, where DEP was among the most detected pesticide metabolites, with detection frequencies ranging from 65 to 92% (Yusa et al., 2022). The median concentrations of DEP in the present study are in general slightly higher than those found in most of the previous studies, where the studies with creatinine-adjusted values show a median between 0.28 μ g/g creatinine and 27 μ g/g creatinine (González et al., 2023; Llop et al., 2017), while the studies with non-adjusted creatinine values show a median between 1.9 ng/mL and 61.6 ng/mL (López et al., 2016), all of them compiled in Table S12.

Despite the interdiction in 2016 for plant protection, cypermethrin is still in use as a biocidal product, which can be found on some foods mostly below established maximum residue levels such as fruits and vegetables (Quijano et al., 2016) or cereals (Rodríguez-Ramos et al., 2023). Although in our study cypermethrin was detected in a low frequency (2.5% detection), the detection frequency of its metabolite DCCA was 29%. In previous studies, DCCA has rarely been detected, with a detection frequency between 4% and 26%, at slightly lower detection rates than in our study (Yusà et al., 2022).

Carbendazim is the main metabolite of thiophanate-methyl, a fungicide widely used in wine grapes, beans with pods, wheat, or vegetables such as tomatoes (Arena et al., 2018). In our study, carbendazim was found in 100% of the samples at median levels of 0.09 ng/mL, yet this fungicide and its metabolites have been poorly studied in



Fig. 3. Heatmap depicting the Spearman correlation coefficients between food consumption at dinner before sampling and the contaminant concentration found in the urine samples the subsequent morning.

biomonitoring studies in Spain (Yusà et al., 2022). Nevertheless, it was widely found (100% detection) in hair from infants from Luxembourg at concentrations of 0.867 pg/mg (Iglesias-González et al., 2022) or urine from rural citizens from the Netherlands (Oerlemans et al., 2021).

The pirimiphos-methyl metabolite, DEAMPY, was detected in 51% of samples at median levels of 1.3 ng/mL (non-adjusted creatinine), in the line of detection frequencies of previous studies (range 40–80%) or mean levels around 0.8–1.8 ng/mL (Yusà et al., 2022).

4.2. Mycotoxins

DON was the most broadly detected mycotoxin, showing the highest concentration levels (median of 68 μ g/g creatinine), followed by two other Fusarium mycotoxins such as the emerging mycotoxin ENNB and the ZEN metabolite, β -zearalenol. The widespread presence of Fusarium mycotoxins among the analysed compounds enhanced the importance of monitoring this fungal genre, not only because it can produce several mycotoxins, but also because these mycotoxins are found in different types of commonly consumed cereal-based food, including wheat and maize, pulses like soybeans, and even oils (Li et al., 2024;

Terada-Nascimento et al., 2023; Zhou et al., 2023).

The median concentration of total DON found in the present study (75.6 ng/mL) is in the line of our previous findings in the same region using a direct method (sum of mean DON + DON-3-glucuronide = 58.1 ng/mL) (Vidal et al., 2016). Nevertheless, these values were higher than those found in other studies conducted in Spain (mean = 9.07 ng/mL) (Carballo et al., 2021) or the EU aligned biomonitoring studies (geometric mean = 5.09 μ g/g creatinine) (Govarts et al., 2023). Our findings confirmed the widespread presence of ENNB with median values of 2.42 μ g/g creatinine, consistent with median concentrations reported in Spain in the range 3.5–4.8 μ g/g creatinine (Dasí-Navarro et al., 2022, 2023). Noteworthy, we found strong correlations between DON and ENNB in urine, supporting our previous findings where co-occurrence and co-exposure were also found in a diet-based approach (Gallardo et al., 2023).

The levels of quantification of OTA (15%) are in the same range of those previously reported in same region of Catalonia (12.5%), nevertheless we have found 30% lower frequencies of quantification of OT α despite the concentrations were comparable (Coronel et al., 2011). Our present method covered other major mycotoxins and metabolites (e.g. aflatoxins B1, B2, 15-AcDON, DOM1) and emerging mycotoxins (e.g. beauvericin); however, they were scarcely detected in our samples at frequencies below 25%, in line with other previous studies (Carballo et al., 2021; Dasí-Navarro et al., 2022, 2023; Vidal et al., 2016).

4.3. Impact of dietary intervention

Excretion of non-persistent pesticides occurs between 24 and 48 h after intake depending on the compound (Huen et al., 2012). Hence the present short-time intervention did not reveal differences among pesticide levels, but more than 24 h would imply losses in polar compounds like DON, which is excreted at 49-86% in 24-h (Sun et al., 2022). A robust body of evidence supports the efficiency of organic diet interventions on the exposure levels of pesticides, mostly conducted over 5–7 days with reductions up to 95% (Fagan et al., 2020; Lu et al., 2008; Oates et al., 2014). Conversely, following a 24-h organic food replacement, we observed a statistically significant reduction of ENNB, a mycotoxin with a low elimination half-live, estimated at 1.57 h in pigs (Devreese et al., 2014). Although storage may play an important role in the mycotoxin content (Wang et al., 2024), in general, there is no difference regarding mycotoxin content between conventional and organic production systems (Pleadin et al., 2017). However, the mycotoxins DON and the precursor of β -zearalenol ZEN are more likely to be found in conventional production (Remža et al., 2016), which is in contrast to the results found in this study, where DON and β -zearalenol are more likely to be consumed in an organic diet (Fig. 2). The presence of ENNB in food depending on organic practices is divergent. A higher concentration of ENNB has been found in organic pasta from a Spanish market (Serrano et al., 2013), but another recent study showed that there was more ENNB in conventional flours than in organic ones (Giannioti et al., 2023), which is related to the results shown in Fig. 2.

This study has some methodological limitations that should be considered with caution. First, this is a pilot study with a small sample size, hence the results may not be generalizable to large populations. Second, we have considered a short 24-h intervention and first-morning urine to focus on those chemicals with short half-lives such as DON. Hence, longer diet interventions and pooling urine samples (e.g. 24h or multiple spot) or blood matrices, will be more adequate to assess the impact of intervention on chemicals with a longer half-life such as OTA, aflatoxins or certain pesticides. Finally, the analytical method used in the present study has its own limitations. As all multi-target methods, it may be challenging finding a good performance balance between coverage, selectivity and sensitivity for all chemicals. Hence, more targeted methods ensuring higher sensitivity may be more convenient in future studies interested on specific biomarkers.

5. Conclusions

The present study confirmed the co-occurrence of patterns of mycotoxins and pesticides in urine samples. The mycotoxin DON remains the most detected mycotoxin, strongly correlated with the emergent mycotoxin ENNB and the pesticide metabolite DEP and dimethoate supporting the need for mixture toxicological analysis and refined risk assessment. The pilot intervention study revealed a reduction of ENNB and OT α and an increase of β -zearalenol levels in urine after a short-term organic food replacement. Taken together with the established body of evidence on organic diet reduction of pesticides, these results suggest that an organic food replacement could simultaneously reduce mycotoxin and pesticide risks. Nevertheless, intervention studies with a larger sample size should confirm these findings with a longer timeframe and multiple sampling windows to capture the diverse toxicokinetic properties of mycotoxins and pesticides.

CRediT authorship contribution statement

Jose A. Gallardo-Ramos: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis. Jesús Marín-Sáez: Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft. Vicente Sanchis: Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Writing – review & editing. Laura Gámiz-Gracia: Data curation, Investigation, Methodology, Validation, Writing – review & editing. Ana M. García-Campaña: Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing. Maykel Hernández-Mesa: Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. German Cano-Sancho: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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