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Effects of an organic diet intervention on the levels of organophosphorus metabolites in an adult cohort

Neus González, Carla Pàmies, Paula Martinez, Laura Martí, José L. Domingo, Martí Nadal, Montse Marquès *

Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain

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ABSTRACT

Pesticides are a group of organic compounds used to control weeds or insect infestations in agriculture. Diet is the major route of human exposure to these compounds, which can cause serious health problems, even when the intake occurs at low concentrations. Hence, the consumption of organic food is an appropriate strategy to minimize the exposure to pesticides. A prospective, randomized study was conducted to assess the impact of an organic dietary intervention on the levels of urinary dialkyl phosphates (DAP). A screening of 204 pesticides was also carried out in order to confirm the absence of these compounds in organic food. The analytical results showed that only 20 of the 204 pesticides (9.8 %) had concentrations above the limit of quantification in one or more samples of the organic food consumed by the participants. It is substantially lower than the levels of pesticides found in other studies analysing conventional food, confirming the diet as suitable for the organic dietary intervention. A general reduction of most DAP metabolites in urine was found, being significant ($p < 0.05$) the decrease of dimethyl phosphate (DMP) (0.49 μg/g creatinine in Day 1 vs. 0.062 μg/g creatinine in Day 6), dimethyl thiophosphate (DMTP) (0.49 μg/g creatinine in Day 1 vs. 0.093 μg/g creatinine in Day 6) and diethyl phosphate (DEP) (0.28 μg/g creatinine in Day 1 vs. 0.12 μg/g creatinine in Day 6). In addition, the molar score for the total dimethyl DAP (ΣMP) and total dialkyl phosphate (ΣDAP) also showed significant differences after changing a conventional diet by an organic diet, being reduced from 0.008 μmol/g to 0.002 μmol/g for ΣMP and from 0.012 μmol/g to 0.003 μmol/g for ΣDAP. To the best of our knowledge, this is the first study that evaluates both the impact of an organic diet in the exposure to DAP and the levels of 204 pesticides in the organic food provided to the participants. In summary, the consumption of organic products decreases the dietary intake of pesticides, thus reducing also the potential adverse effects on human health.

1. Introduction

Pesticides are a group of organic compounds, which are used to control weeds or insect infestations in agricultural systems, as well as in residential areas (Wei et al., 2023; Kim et al., 2017). Pesticides are beneficial to increase crop yields and improving the efficiency of food production systems (Straw et al., 2023; Suratman et al., 2015; Wang et al., 2019). However, most of these chemicals ultimately affect non-target plants and species, being also spread into the environment (Tudi et al., 2021). Moreover, some pesticides can be bioaccumulated and biomagnified along the food chain, thus reaching high concentrations in tissues of animals located at the highest trophic levels (Mrema et al., 2013; Sharma et al., 2020).

In humans, exposure to pesticides can occur through the diet, inhalation, and dermal absorption (Wei et al., 2023). Exposure at even low levels may lead to serious adverse effects, such as cancer, asthma, allergies, hormone disruption, or cognitive effects (Shiny Raj & Anoop Krishnan, 2023; Amoatey et al., 2020; Kim et al., 2017; Mrema et al., 2013). In this sense, farmworkers and pesticide handlers are highly exposed to these compounds, being inhalation and dermal absorption the main routes for occupational exposure (Amoatey et al., 2020). Activities like mixing, loading, cleaning equipment or spraying are potential sources of contamination (Monger et al., 2023; Silva Pinto et al., 2020). The correct use of personal protection equipment and appropriate handling of pesticide products is crucial to reduce the risk of occupational exposure (Silva Pinto et al., 2020). In turn, due to the harmful effects for the environment, plants, and animal and human (EPAH) health, the European Commission (EC) under Regulation (EC)

* Corresponding author.
E-mail address: montserrat.marques@urv.cat (M. Marquès).

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No 396/2005, has controlled the use of these compounds in the conventional agriculture, also setting maximum residue levels (MRL) for each compound in specific crops. MRL is the highest level of a pesticide residue that is legally tolerated when pesticides are correctly applied (European Commission, 2005).

Pesticides entail various groups of compounds, namely organochlorines (OC), carbamates, pyrethroids, and organophosphates (OPs), among others (Narenderan et al., 2020). OPs are among the most commonly applied pesticides, accounting for the 33 % of total insecticide use in the United States and the 34 % in China (Shiny Raj & Anoop Krishnan, 2023; Atwood & Paisley-Jones, 2017; Tudi et al., 2021). In Europe, OPs represent more than 40 % of the total sales of herbicides, mainly due to glyphosate, which is a widely used pesticide in the EU (EUROSTAT, 2022). The biomonitoring of OPs is usually done by analysing OPs metabolites in urine. Dialkyl phosphates (DAP) are non-specific OPs metabolites widely used to evaluate exposure to these pesticides (Aguilar-Garduño et al., 2017; Barr et al., 2004; Figueroa et al., 2015; Ye et al., 2016; Yucra et al., 2006). The six DAP include: dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP).

Since the production of organic food is free of pesticides, a good strategy to reduce exposure to pesticides is the alternative consumption of organic food products. According to the Regulation (UE) No 2018/848 (European Commission, 2018), organic foods do not contain traces of synthetic fertilizers and genetically modified organisms (GMO). Recently, there has been growing interest on organic products in the general population, due to a higher-quality and safety perception (De Canio & Martinelli, 2021; Schouteten et al., 2019; Sesini et al., 2023). In the scientific literature, a number of studies have reported a decrease of pesticide exposure after consumption of an organic diet (Berman et al., 2016; Curl et al., 2019; Göen et al., 2017; Hyland et al., 2019; Lu et al., 2006; Oates et al., 2014).

The present study was aimed at assessing the effects of a dietary intervention with organic food products on the exposure to OPs pesticides, while a screening of a large number of pesticides was also performed. More specifically, the levels of the six DAP metabolites were determined in urine samples collected before and after the organic dietary intervention. In order to certify that the diet was organic, the content of 204 pesticide compounds – including OPs pesticides, but also OCs, carbamates and pyrethroids – was also determined in food composites from the duplicate diet.

2. Materials and methods

2.1. Study design

A prospective, randomized study was conducted to analyse the urinary levels of DAP metabolites in an adult cohort after a 5-day organic diet intervention. The study was conducted in Tarragona County (Catalonia, Spain) between the 1st and the 5th of March of 2021, being approved by the Ethics Committee concerning Research into People, Society and the Environment of the Universitat Rovira i Virgili (CEIPSA-URV; Ref: CEIPSA-2020-PR-0003).

2.2. Study population

The study population comprised 32 subjects who met the eligibility criteria: a) older than 18 years; b) native speaker of Catalan and/or Spanish; and c) without food allergies. In order to complete the information about the cohort, the participants filled in a questionnaire with their lifestyles and dietary habits (Table 1).

2.3. Dietary intervention

Before the dietary intervention, participants were asked for following a conventional diet containing -as much as possible- food products from the conventional agriculture/farming. After a week of conventional diet, the 5-day dietary intervention with organic food was conducted. The varied and nutritional organic diet was elaborated by nutritionists, being all the required food products delivered to the participants one day before starting the intervention. The 5-days organic diet is summarized as Supplementary Information (Table S1). A duplicate food basket was processed in the laboratory in order to analyse the concentrations of the 204 pesticides.

Table 1
Cohort characteristics (n = 32).

Characteristics	%	Characteristics	%
Sex		*Sport*	
Men	38	Yes	59
Women	62	No	41
Age (years)		*BMI (kg/m^2)*	
18–25	22	Underweight (<19)	0
26–40	31	Normal (19–25)	66
41–50	25	Overweight (>25–30)	22
>50	22	Obesity (>30)	12
Smoker		*Usual consumer of organic food products*	
Yes	19	Yes	12
No	81	No	88

2.4. Chemicals

Dimethyl phosphate-d_6, Diethyl phosphate-d_{10} and O,O-Dimethyl dithiophosphate-d_6 were purchased from TRC Canada (Toronto, Canada), while O,O-dimethyl thiophosphate-d_6 and O,O-Diethyl dithiophosphate-$^{13}C_4$ were obtained from LGC Standards (Teddington, UK). In turn, dimethyl phosphate was purchased from Fisher Scientific (Hampton, NH, USA), and O,O-dimethyl thiophosphate, diethyl phosphate and O,O-dimethyl dithiophosphate were obtained from TRC Canada (Toronto, Canada). O,O-diethyl thiophosphate and diethyl dithiophosphate were purchased from Sigma Aldrich (West Chester, PA, USA). Dried acetonitrile, diethyl ether, 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr), n-hexane, toluene and acetonitrile were purchased from Sigma Aldrich (West Chester, PA, USA).

Potassium carbonate was obtained from Panreac Quimica (Barcelona, Catalonia, Spain). Formic acid Optima™ LC/MS Grade and Ammonium Formate 10 M HPLC-MS were acquired from Fisher Scientific (Hampton, NH, USA). Three types of QuEChERS containing: a) 4 mg of $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate and 0.5 g of disodium citrate; b) 150 mg $MgSO_4$ and 25 mg of C18, and c) 50 mg of primary-secondary amine (PSA), 50 mg of C18 and 150 mg of $MgSO_4$ were purchased from Agilent (Little Falls, DE, USA). Oxyfluorfen-(ethoxy-d_5), pyraclostrobin-(N-methoxy-d_3), metribuzin-(S-methyl-d_3), linuron-(methyl-d_3, methoxy-d_3), fipronil (pyrazole-$^{13}C_3$, cyano-^{13}C) and MCPA-(methyl-d_3) were purchased from Merck (Darmstadt, Germany). Hexachlorobenzene-$^{13}C_6$, glyphosate PESTANAL® and amino phosphonic acid (AMPA) were obtained from Sigma Aldrich (West Chester, PA, USA). Lindane-d_6, fenvalerate-d_5, isoproturon-d_3 and fenbuconazole-d_5 were acquired from TRC Canada (Toronto, Canada). Propyzamide-d_3, 2,4′-DDT-d_8, metalaxyl-M-d_3, methiocarb-d_3, and acetamiprid-d_3 (N-methyl-d_3) were purchased from Cymit Química (Barcelona, Catalonia, Spain). Finally, dimethoate-d_6, chlorpyrifos-d_{10}, AMPA-^{13}C-^{15}N and glyphosate 1,2-^{13}C-^{15}N were obtained from LGC Standards (Teddington, UK).

Standards for the 204 analysed pesticides were obtained from Wageningen University (Wageningen, Netherlands) in six different mixtures: 1) GC compounds, 2) P LC POS-1 compounds, 3) P LC-POS-2 compounds, 4) special compounds, 5) P/M LC-NEG compounds, and 6) M LC-POS compounds. All the mixtures were prepared at a concentration of 1 μg/mL in acetonitrile (0.1 % of formic acid).

2.5. Urine sampling and DAP analysis

On the first day, before starting the dietary intervention, the first void urine was collected in a coded container. Then, the day after the last day of the intervention, the first void urine was also collected in a different coded container. Hence, two urine samples were provided by each participant. Samples were stored at −20 °C until the analysis of DAP metabolites.

The method to analyse DAP metabolites (DMP, DMTP, DMDTP, DEP, DETP and DEDTP) in urine was adapted from Bravo et al. (2004). Briefly, urine was lyophilised overnight to eliminate the water present in the sample. Then 50 μL of the spiking solution of the internal standard (IS), 2 mL of dried acetonitrile and 2 mL of diethyl ether were added to the residue of the lyophilised urine sample. Each screw-capped glass jar was tightly sealed with the corresponding screw cap, being the sample vigorously mixed on the laboratory shaker (10 min, 200 rpm). Samples were allowed to stand for 5 min and the supernatant was carefully removed with a glass Pasteur pipette to a new 25 mL screw-capped glass jar. Subsequently, 1 mL of dried acetonitrile was added to the residue, the screw-capped glass jar was tightly sealed, and it was mixed by the laboratory shaker (10 min, 200 rpm). After 5 min, the supernatant was carefully withdrawn and added to the first extract. The combined extracts were evaporated to approx. 1.5 mL under a stream of nitrogen. About 25 mg of potassium carbonate and 100 μL of the PFBBr solution were added.

The screw-capped glass jar was tightly sealed and shaken for a few seconds on a vortex shaker. Samples were subsequently derivatised for 15 h at 40 °C in a drying cupboard. After the sample cooled down to room temperature, 5 mL of water (LC-MS grade) and 5 mL of hexane were added, being the sample tightly sealed with the corresponding screw cap and extracted on the laboratory shaker (10 min, 200 rpm). After centrifugation (5 min, 300 rpm) the upper phase was transferred to a new 25 mL screw-capped jar. The sample was extracted a second time with 5 mL of n-hexane on the laboratory shaker (10 min, 200 rpm). The hexane phase was withdrawn and added to the first hexane extract. The combined extracts were evaporated to approximately 1 mL under a gentle stream of nitrogen and transferred to a threaded 1.5 mL vial.

Finally, 200 μL of toluene were added to rinse the 25 mL screw-capped glass jar, being the glass walls rinsed several times. A pipette was used to transfer the toluene to the evaporated hexane extract. The solution was evaporated in a gentle stream of nitrogen and reconstituted with 100 μL of n-hexane. The solution was mixed for 10 min in the vortex and placed in a 1.5 mL chromatographic vial with a micro-insert, which was then tightly sealed. Samples were stored at −20 °C until analyses, which were conducted by means of gas chromatography coupled to triple quadrupole (GC-QqQ).

2.6. Determination of pesticides in food

Food composites were done by equally weighting the individual food products that were consumed according to the diet (Table S1), until a final weight of 50 g per composite. A total of 5 composites were analysed for 204 pesticides (Table S2).

2.6.1. Multi-residue extraction

Extraction of pesticides for the multi-residue analysis in food samples was done according to the QuEChERS methodology (Anastassiades et al., 2003). Briefly, 10 g of sample were weighted and transferred to a 50-mL centrifuge tube. Twenty μL of IS solution were then added to the samples. Ten mL of acetonitrile were added, being the samples vortexed for 30 s. Subsequently, 4 mg of $MgSO_4$, 1 g of NaCl, 1g of sodium citrate and 0.5 g of disodium citrate, were added to the samples, which were vigorously vortexed for 1 min. Subsequently, samples were centrifuged 5 min at 4700 rpm. One mL of the supernatant was transferred to a tube containing 150 mg $MgSO_4$ and 25 mg of C18 while another mL was transferred to a tube containing 50 mg of PSA, 50 mg of C18 and 150 mg of $MgSO_4$. Samples were vortexed for 30 s and rapidly centrifuged for 5 min at 13500 rpm. Supernatant was carefully transferred to a clean 2-mL vial and injected to analysis.

2.6.2. Glyphosate and AMPA analyses

For glyphosate and AMPA analyses, 2 g of sample were weighted and 10 mL of solvent extraction (MeOH:H_2O 1:1 0.1 % Formic acid) were added and shaken in an automatic shaker for 15 min, being then centrifuged at 4600 rpm and 4 °C for 1.5 min. After this, 300 μL of the supernatant were passed through an Oasis HLB cartridge (30 mg), previously conditioned with 200 μL methanol and 2 mL of the extracting solvent, being 200 μL of the extract collected into an Eppendorf. Finally, 100 μL of the collected sample were passed into an autosampler vial and 100 μL of water containing the IS at a concentration of 200 ng/mL were added. A 2-μL volume of sample were injected into the LC-MS/MS system.

2.7. Equipment

2.7.1. Instrumentation for urine analysis of DAP metabolites

A gas chromatograph 7890A with a detector QqQ 7000 Series (Agilent, Little Falls, DE, USA) equipped with split/splitless injector, autosampler and data processing system for evaluation was used. GC separation was performed on a DB-35MS column (60 m × 0.250 mm × 0.25 μm; Agilent, Little Falls, DE, USA). Helium was the carrier gas with a constant flow of 1.5 mL/min. The injection was made in splitless mode at 250 °C. The MS transfer line was held at 280 °C.

2.7.2. Instrumentation for food analysis

2.7.2.1. LC-MS/MS positive and negative modes. An UHPLC 1290 Infinity II Series coupled to a triple quadrupole QQQ/MS 6490 Series were used, both from Agilent Technologies (Santa Clara, CA, USA). The chromatographic column used was a Zorbax Eclipse Plus C18 1.8 μm (150 × 2.1 mm) (Agilent Technologies). Chromatographic separation was performed using water 5 mM ammonium formate (mobile phase A) and methanol 5 mM ammonium formate (mobile phase B), both complemented with 0.1 % formic acid. Temperature of the column was set at 40 °C and the injection volume was 2 μL.

2.7.2.2. GC–MS/MS methods. Pesticides were separated on an HP 5MSUI chromatographic column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed as follows: (i) initial temperature 60 °C, (ii) linearly raised at 40 °C/min to 170 °C (0 min), and (iii) then linearly raised at 10 °C/min to 310 °C (3 min). The column flow was set at 1 mL/min using He as carrier gas. The injector was set at 280 °C and the extracts were injected in solvent mode. The ionisation was carried out by electronic impart (70 eV) and mass analyser operated on Multi Reaction Monitoring (MRM).

2.7.2.3. Glyphosate and AMPA method. An HPLC 1260 Infinity Series coupled to a triple quadrupole QQQ/MS 6490 A Series was used, both from Agilent Technologies (Santa Clara, CA, USA). Chromatographic column used was a InfinityLab Poroshell 120 HILIC-Z(100x2.1 mm), PEEK Lined (Agilent Technologies). Chromatographic separation was performed using water (mobile phase A) and acetonitrile (mobile phase B), both complemented with 0.04 % formic acid and 5 μM medronic acid. Temperature of the column was set at 35 °C and the injection volume was 2 μL.

2.8. Determination of creatinine

Creatinine was analysed in all urine samples. A Cobas Mira automatic analyser (Roche Pharmaceuticals, Basel, Switzerland) was used according to the instructions provided by the manufacturer.

2.9. Quality control/quality assessment

The method was validated on a reference homogenized sample as stated in the EU SANTE document (SANTE/2020/12830). Spiked samples of reference homogenized sample were used to validate the selective multiresidue method. The method validation performance criteria were evaluated by assessing the linearity of the calibration curve, trueness (as % recovery), precision (as repeatability % RSD) and limit of quantification (LOQ), following the EU SANTE Guideline on analytical quality control and validation procedures. The trueness and intraday precision in matrix were determined, via injecting spiked reference homogenized sample at three concentration levels: either 0.2 μg/kg, 1 μg/kg and 10 μg/kg, and 1 μg/kg, 10 μg/kg and 50 μg/kg, based on their respective MRLs and the method LOQ (LOQm) obtained for each pesticide. The trueness (as % recovery, n = 5) and inter-day precision (as % RSD, n = 5) of the recovery experiments were determined. For validation purposes, recoveries were calculated using the isotope-labelled internal standards for quantification. Reproducibility (inter-day precision) data was obtained via the on-going validation of the method by collection of recovery and RSD data from the QC-samples in routine analysis series, as stated in the SANTE document (SANTE/2020/12830). The LOQm has been defined as the lowest spike level of the recovery study that can be quantified with acceptable trueness (recovery, 70–120 %) and precision (RSD < 20 %), and limit of detection (LOD) estimated as LOQ/3.33.

2.10. Data treatment

Urinary concentrations of DAP were expressed as creatinine-adjusted concentrations (μg/g creatinine), which were calculated as follows:

$$\textit{Adjusted DAP concentration}\ (\mu g/\text{g creatinine}) = \frac{\text{Urinary DAP concentration}\ (\mu g/L)}{\text{Creatinine concentration}\ (g/L)}$$

In line with previous studies (Hyland et al., 2019; Oates et al., 2014), the molar sum of the dimethyl-containing and diethyl-containing metabolites was calculated in order to give each participant a score for the combined DAPs (ΣDAP), total dimethyl DAPs (ΣMP), and total diethyl DAPs (ΣEP). Total molar metabolite quantities (μmol/g) were obtained by dividing each individual result (μg/g) by its molecular weight (g/mol), as follows:

$$\Sigma MP = [\text{DMP}]/125 + [\text{DMTP}]/141 + [\text{DMDTP}]/157$$

$$\Sigma EP = [\text{DEP}]/153 + [\text{DETP}]/169 + [\text{DEDTP}]/186$$

$$\Sigma DAP = \Sigma\text{MP} + \Sigma\text{EP}$$

For calculations, values below the LOD were assumed to be one-half of that limit (<LOD = ½ LOD).

Data treatment was performed by means of the statistical package SPSS 27.0. The Shapiro-Wilk test was used to compare the homogeneity of the variances. Subsequently, significant differences of the data were computed by an ANOVA or the Kruskal-Wallis test. Significance was set at $p < 0.05$.

3. Results and discussion

3.1. Characteristics of the cohort

Cohort characteristics are summarized in Table 1. Participants had a mean age of 39 ± 19 years old. Most participants were female (62 %), non-smokers (81 %), with normal body mass index (BMI) (66 %) and non-consumers of organic produce (88 %).

3.2. Analysis of pesticide residues in the organic diet

A screening of 204 pesticide residues in food composites was conducted in order to corroborate the absence of pesticides in the foods available in the organic basket and, therefore, to confirm whether these food items could be used for the dietary intervention (Table S2). Table 2 summarizes the results corresponding to OPs. Only one sample showed a concentration of pirimiphos-methyl (0.09 μg/kg) above the LOQ, while the rest of compounds were below their respective LOQ and LOD in all the samples. Because of the low number of compounds detected, it was confirmed that the planned diet was suitable as basis to conduct a dietary intervention aimed at assessing the potential decrease exposure to OPs in individuals following an organic diet. In previous studies where the levels of OPs were assessed in conventional food, up to 45 % of the analysed samples showed detectable levels of at least one OP (Caldas et al., 2011; Tsuchiyama et al., 2022).

Table 3 shows the individual concentrations of the compounds with one or more food samples with levels above their respective LOQ. Only 20 of the 204 pesticides (9.8 %) had concentrations above the LOQ in one or more samples. Five compounds were detected in all samples (metalaxyl (M), metamitron, pirimicarb-desmethyl, tebuconazole and trifloxystrobin). In turn, metamitron and 2,4-dichlorophenoxyacetic acid (2,4-D) showed the highest mean concentrations (3.93 ± 2.49 and 1.54 ± 0.16 μg/kg, respectively). Unexpectedly, two of the 20 compounds detected (carbendazim and thiacloprid) were not approved by the EC. On one hand, carbenzadim is highly persistent in soil and water systems, meaning that it is resistant to degradation and stable in the environment. On the other hand, although thiacloprid is a non-persistent compound, its approval expired in 2020. Therefore, it could be still being used before harvesting the food from the field. Anyway, although the number of detected compounds in the current survey is substantially lower than those found in other total diet studies screening a high number of pesticides in food (Ingenbleek et al., 2019; Nougadère et al., 2020), it is important to highlight that a number of pesticide residues were found in organic food products. In accordance to our results, previous studies assessing the presence of pesticide residues in organic products also found pesticide contamination in a low percentage of samples (Cressey et al., 2009; Kazimierczak et al., 2022; Schusterova et al., 2021).

3.3. DAP metabolites in urine

Because of their wide use, DAP metabolites were selected as the best representative compounds to assess exposure to OPs of the general population. In addition, DAP metabolites provide information about the cumulative exposure to OPs, because most OPs are metabolized into one or more of the six DAP metabolites (Berman et al., 2013).

The frequency of detection and median values of the six DAP metabolites in urine are summarized in Table 4. All metabolites were detected in -at least- one urine sample. The highest detection rates were found in day 1 for DMTP (88 %), DMP (81 %) and DEP (81 %), with

Table 2
Concentrations (μg/kg) of selected OPs in food composites from the organic diet.

Compound	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*
Chlorpyrifos	<1	<0.30	<0.30	<0.30	<0.30
Chlorpyrifos-methyl	<0.06	<0.06	<0.06	<0.06	<0.06
Chlorpyrifos-methyl-desmethyl	<3	<3	<3	<3	<3
Dimethoate	<0.03	<0.1	<0.03	<0.03	<0.03
Phosmet	<0.1	<0.1	<0.1	<0.1	<0.1
Phosmet oxon	<0.03	<0.03	<0.03	<0.03	<0.03
Pirimiphos-methyl	0.09	<0.003	<0.003	<0.003	<0.003
Pirimiphos-methyl metabolite DEAMPY	<3	<3	<3	<3	<3
Pirimiphos-methyl-N-desethyl	<0.03	<0.03	<0.03	<0.03	<0.03

Table 3
Individual concentrations (μg/kg), percentage of detection and mean concentrations of pesticide residues in an organic diet.

Compound	Day 1	Day 2	Day 3	Day 4	Day 5	% detection (n)	Mean ± SD (μg/kg)	Approved by the EC
2,4-D (free)	1.36	<0.30	1.63	1.62	<0.30	60 (3)	1.54 ± 0.16	Yes
Acetamiprid	<0.03	<0.03	<0.10	<0.10	0.15	20 (1)	0.15*	Yes
Carbendazim	<0.05	0.05	<0.02	<0.02	<0.02	20 (1)	0.05*	No
Cyproconazole	<0.06	2.08	0.52	0.75	0.23	80 (4)	0.90 ± 0.82	Yes
Diflufenican	<0.10	0.21	0.20	0.14	0.11	80 (4)	0.17 ± 0.05	Yes
Diflufenican AE-B107137	<0.15	0.72	<0.15	<0.50	<0.15	20 (1)	0.72*	–
MCPA	<0.20	<0.06	<0.20	<0.20	0.35	20 (1)	0.35*	Yes
Metalaxyl (M)	0.17	0.12	0.16	0.12	0.14	100 (5)	0.14 ± 0.02	Yes
Metamitron	4.35	7.97	3.48	1.78	2.07	100 (5)	3.93 ± 2.49	Yes
Pendimethalin	<0.20	0.43	<0.20	0.21	0.24	60 (3)	0.29 ± 0.12	Yes
Piperonyl butoxide	0.14	<0.10	0.11	<0.10	0.20	60 (3)	0.15 ± 0.04	Not assessed
Pirimicarb desmethyl-	0.02	0.02	0.02	0.02	0.02	100 (5)	0.02 ± 0.00	–
Pirimiphos-methyl	0.09	<0.003	<0.003	<0.003	<0.003	20 (1)	0.09*	Yes
Propamocarb (hydrochloride)	<0.02	<0.02	<0.02	0.07	<0.02	20 (1)	0.07*	Yes
Pyrethrin I	<0.06	<0.06	<0.06	<0.06	0.24	20 (1)	0.24*	Yes
Pyrimethanil	0.28	0.24	<0.03	<0.03	<0.10	40 (2)	0.26 ± 0.02	Yes
Pyroxsulam	<0.03	0.43	0.26	0.28	0.13	80 (4)	0.28 ± 0.12	Yes
Tebuconazole	0.41	0.61	0.43	0.40	0.23	100 (5)	0.42 ± 0.14	Yes
Thiacloprid	<0.02	<0.02	0.12	0.08	0.08	60 (3)	0.09 ± 0.03	No
Trifloxystrobin	0.02	0.02	0.02	0.03	0.02	100 (5)	0.02 ± 0.00	Yes

* Calculation of standard deviation was not possible because only one sample was positive.

Table 4
Frequency of detection and median adjusted values (μg/g creatinine) for 6 DAP urinary metabolites before (Day 1) and after (Day 6) dietary intervention.

Metabolite	Frequency of detection (n, %)		Median (P25-P75)*		*p* value
	Day 1	Day 6	Day 1	Day 6	
DMP	26 (81)	12 (38)	0.49 (0.20–0.95)	0.062 (0.038–0.25)	**<0.001**
DMTP	28 (88)	13 (41)	0.49 (0.17–0.64)	0.093 (0.042–0.30)	**0.001**
DMDTP	7 (22)	11 (34)	0.054 (0.037–0.081)	0.050 (0.036–0.14)	0.814
DEP	26 (81)	20 (63)	0.28 (0.099–0.58)	0.12 (0.049–0.26)	**0.035**
DETP	11 (34)	6 (19)	0.051 (0.037–0.093)	0.050 (0.033–0.10)	0.893
DEDTP	1 (3)	6 (19)	0.004 (0.003–0.007)	0.005 (0.003–0.012)	0.298

* In the median values, levels < LOD were assumed to be ½ LOD.

values of 0.49, 0.49 and 0.28 μg/g creatinine, respectively. On the other hand, DMDTP, DETP and DEDTP showed lower detection rates (22, 34 and 3 %, respectively) and lower concentrations (0.054, 0.051 and 0.004 μg/g creatinine for DMDTP, DETP and DEDTP, respectively).

On the other hand, the levels of DAP metabolites in urine were compared according to the cohort characteristics shown in Table 1. Only DEP concentrations before starting the dietary intervention (day 1) were significantly higher in females when compared to males ($p < 0.002$). This significant difference was not found after the dietary intervention (day 6). For other variables (age, BMI, smoke, sport and consumption of organic food), no significant differences were found in DAP levels in urine. This absence of significant differences between cohort characteristics may be caused by the low number of samples collected during the dietary intervention.

The median concentrations before the dietary intervention (baseline concentrations) are substantially lower than levels reported elsewhere, which could be related to the low number of participants in the current survey compared to other biomonitoring studies. In Israel, Berman et al. (2013) recruited 247 adults and analysed the levels of six DAP metabolites in their urine. Creatinine-adjusted median values for DEP, DETP, DEDTP, DMP, DMTP and DMDTP were 1.5, 0.5, 0.02, 10.0, 5.2 and 0.2 μg/g creatinine, respectively. In turn, the concentrations reported in studies performed in Norway, the Netherlands and the USA are also higher than those here observed. The mean concentrations of DMP, DMTP, DMDTP, DEP, DETP and DEDTP in urine of Norwegian people were 12.9, 13.2, 0.98, 2.54, 1.51 and 0.02 μg/g creatinine, respectively. In the Netherlands, the urinary values of the same compounds were 19.8, 19.4, 0.85, 5.02, 2.98 and 0.12 μg/g creatinine, respectively. Finally, Ye et al. (2009) reported concentrations of 2.33 μg/g creatinine for DMP, 6.47 μg/g creatinine for DMDTP, 1.22 μg/g creatinine for DMDTP, 2.36 μg/g creatinine for DEP, 1.22 μg/g creatinine for DETP and 0.18 μg/g creatinine for DEDTP in the USA.

3.4. Impact of the dietary intervention in the urinary levels of DAP metabolites

In general, the detection rates and median concentrations of DAP metabolites in urine were higher before the organic dietary intervention (day 1) than at the end of the study (day 6). Specifically, median levels of DMP ($p < 0.001$), DMTP ($p = 0.001$) and DEP ($p = 0.035$) were significantly higher before than after the dietary intervention (Table 4). Median levels of DMP decreased from 0.49 (0.20–0.95) μg/g creatinine (Day 1) to 0.062 (0.038–0.25) μg/g creatinine (Day 6), while DMTP decreased from 0.49 (0.17–0.64) μg/g creatinine (Day 1) to 0.093 (0.042–0.30) μg/g creatinine (Day 6). DEP median levels lowered from 0.28 (0.099–0.58) μg/g creatinine (Day 1) to 0.12 (0.049–0.26) μg/g creatinine (Day 6). In turn, two DAP (DMDTP and DETP) were also lower after the dietary intervention, decreasing from 0.054 (0.037–0.081) to 0.050 (0.036–0.14) μg/g creatinine and from 0.051 (0.037–0.093) to 0.050 (0.033–0.10) μg/g creatinine, respectively. Only DEDTP was slightly higher after the dietary intervention, with an increase from 0.004 (0.003–0.007) to 0.005 (0.003–0.012) μg/g creatinine. However, this could be because only 3 % of the urine samples had detectable levels of this compound.

These findings are in agreement with those reported in various studies where an organic dietary intervention has been carried out. Some of these studies evaluated not only the levels of DAP metabolites, but also the content of other OP metabolites and pesticides. In the USA, three studies have been conducted. In two of them, 23 children were recruited. In both cases, median urinary concentrations of malathion dicarboxylic acid (MDA) and 3,5,6-trichlor-2-pyridinol (TCPy) were significantly reduced to non-detected levels after introduction of organic diets (Lu et al., 2006, 2008). Other metabolites (2-isopropyl-6-methyl-pyrimidin-4-ol (IMPY), 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY) and 3-chloro-7-hydroxy-4-methyl-2H-chromen-2-one (CMHC)) were also lower, but the differences were not statistically

significant (Lu et al., 2006, 2008). On the other hand, in a recent study conducted in the USA, 16 adults and children were recruited. In that case, significant reductions were found for 13 metabolites and parent compounds belonging to OPs, neonicotinoids and pyrethroids, with the highest reductions corresponding to clothidianin, MDA and TCPy (Hyland et al., 2019). In Australia, the mean total DAP levels in urine from 13 adults were 89 % lower in the organic phase when compared to the conventional phase. A reduction of 96 % was found for dimethyl metabolites (DMP, DMTP and DMDTP), while diethyl metabolites (DEP, DETP and DEDTP) decreased to 50 % (Oates et al., 2014). In Europe, two studies have been recently performed. Göen et al. (2017) reported higher levels of 15 compounds (DMP, DMTP, DEP, DETP, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxyl acid (*cis*-Cl_2CA), *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxyl acid (*trans*-Cl_2CA), 3-phenoxybenzoic acid (PBA), TCPy, CHMC, 6-chloronicotinic acid, 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), dichlorprop, trichlopyr and glyphosate) in urines collected during the conventional diet. In turn, a study conducted in the United Kingdom reported a reduction of 91 % in the total urinary pesticide excretion during the organic intervention (Rempelos et al., 2022). Specifically, a decrease in chlormequat (95 %), herbicide (26 %), insecticide (66 %), OPs (72 %) and pyrethroid (53 %) residues was found in the organic phase. In that survey, DAP metabolites were not evaluated.

Differences in the molar sum of ΣMP, ΣEP and ΣDAP are summarized in Table 5. Median ΣMP results were lower after the organic dietary intervention (0.008 μmol/g (0.005–0.012) vs. 0.002 μmol/g (9.29E−04–0.006)). Similar results were obtained for ΣDAP, with a reduction from 0.012 μmol/g (0.007–0.016) to 0.003 μmol/g (0.002–0.011). Both of them were significantly reduced after the dietary intervention ($p = 0.002$ and 0.003, respectively). In turn, ΣEP was also lower after the organic diet consumption (0.002 μmol/g (0.001–0.004) vs. 0.001 μmol/g (6.68E−04–0.004)), but the difference was not statistically significant ($p = 0.068$).

Oates et al. (2014) also found significant differences in ΣMP and ΣDAP when comparing conventional and organic phase, while the differences in ΣEP did not reach the statistical significance. Similarly, Hyland et al. (2019) also found significant differences in ΣMP and ΣDAP, but not in ΣEP, after an organic diet intervention.

It must be remarked that food was here considered as the only exposure pathway. However, drinking water is also another important source of exposure to pesticides, with more than 100 pesticide residues found in samples from 31 countries (El-Nahhal & El-Nahhal, 2021). These same authors (El-Nahhal & El-Nahhal, 2021) estimated the hazard index associated to exposure to those residues, finding values above one in many cases, thus meaning the exposure could mean a health risk for adults, children and infants. In turn, other potential routes of exposure such as inhalation and dermal absorption were not considered. However, these routes could play an important role in non-occupationally exposed populations when applying domestic pesticides. Although the dietary exposure – mainly through food consumption – is the major route of exposure to pesticide residues, the fact of not considering other potential sources and routes of exposure could explain why traces of all DAP metabolites were detected even after the organic dietary intervention. Another major limitation of the study is the low number of samples collected during the organic intervention. Only two samples (before and after the dietary intervention) were obtained, thus precluding the capacity to perform stratified analyses and unavoidably lowering the precision of the effect estimates.

4. Conclusions

The present study evaluated the effects of consumption of an organic diet for 5 days by assessing the levels of urinary DAP metabolites. A screening of 204 pesticides in the diet consumed by the participants in the study was also carried out. Although the current results showed a substantial lower number of pesticide residues in organic foods than in conventional foodstuffs, traces of a number of compounds were detected, even when the food was organically produced.

Table 5
Median molar score (μmol/g) for the combined DAP, total dimethyl phosphates and total diethyl phosphates before (Day 1) and after (Day 6) organic dietary intervention.

Molar score	Median (P25-P75)		*p* value
	Day 1	Day 6	
∑MP	0.008 (0.005–0.012)	0.002 (9.29E−04–0.006)	**0.002**
∑EP	0.002 (0.001–0.004)	0.001 (6.68E−04–0.004)	0.068
∑DAP	0.012 (0.007–0.016)	0.003 (0.002–0.011)	**0.003**

The concentrations of most DAP metabolites in urine were lower after an organic dietary intervention. In fact, the levels of DMP, DMTP and DEP were significantly lower only six days after the change of diet. Moreover, the score for the molar sum of ΣMP and ΣDAP also showed significant differences after consuming an organic diet. These results suggest that consumption of organic products decreases exposure to pesticides, reducing also the potential adverse effects on human health. However, the role of other potential sources of exposure, such as drinking water, and domestic use of pesticides, should be extensively evaluated for an accurate health risk assessment.

CRediT authorship contribution statement

Neus González: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Carla Pàmies:** Investigation. **Paula Martinez:** Investigation. **Laura Martí:** Investigation. **José L. Domingo:** Writing - review & editing. **Martí Nadal:** Funding acquisition, Project administration, Writing - review & editing. **Montse Marquès:** Funding acquisition, Project administration, Supervision, Conceptualization, Writing - review & editing.

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

The data that has been used is confidential.

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Appendix A. Supplementary material

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References

Aguilar-Garduño, C., Blanco-Muñoz, J., Roxana Antonio, K., Escamilla-Nuñez, C., Juárez-Pérez, C. A., Schilmann, A., … Lacasaña, M. (2017). Occupational predictors of urinary dialkyl phosphate concentrations in Mexican flower growers. *International Journal of Occupational and Environmental Health, 23*(2), 151–159. https://doi.org/10.1080/10773525.2018.1441676

Amoatey, P., Al-Mayahi, A., Omidvarborna, H., Baawain, M. S., & Sulaiman, H. (2020). Occupational exposure to pesticides and associated health effects among greenhouse

farm workers. *Environmental Science and Pollution Research, 27*(18), 22251–22270. https://doi.org/10.1007/s11356-020-08754-9

Atwood, D., & Paisley-Jones, C. (2017). Pesticides industry sales and usage. 2008–2012, market estimates. US EPA.

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*, 412–431.

Barr, D. B., Bravo, R., Weerasekera, G., Caltabiano, L. M., Whitehead, R. D., Olsson, A., … Needham, L. L. (2004). Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population. *Environmental Health Perspectives, 112*(2), 186–200. https://doi.org/10.1289/ehp.6503

Berman, T., Göen, T., Novack, L., Beacher, L., Grinshpan, L., Segev, D., & Tordjman, K. (2016). Urinary concentrations of organophosphate and carbamate pesticides in residents of a vegetarian community. *Environment International, 96*, 34–40. https://doi.org/10.1016/j.envint.2016.08.027

Berman, T., Goldsmith, R., Göen, T., Spungen, J., Novack, L., Levine, H., … Grotto, I. (2013). Urinary concentrations of organophosphate pesticide metabolites in adults in Israel: Demographic and dietary predictors. *Environment International, 60*, 183–189. https://doi.org/10.1016/j.envint.2013.08.008

Bravo, R., Caltabiano, L. M., Weerasekera, G., Whitehead, R. D., Fernandez, C., Needham, L. L., … Barr, D. B. (2004). Measurement of dialkyl phosphate metabolites of organophosphorus pesticides in human urine using lyophilization with gas chromatography-tandem mass spectrometry and isotope dilution quantification. *Journal of Exposure Analysis and Environmental Epidemiology, 14*(3), 249–259. https://doi.org/10.1038/sj.jea.7500322

Caldas, E. D., de Souza, M. V., & Jardim, A. N. O. (2011). Dietary risk assessment of organophosphorus and dithiocarbamate pesticides in a total diet study at a Brazilian university restaurant. *Food Additives and Contaminants - Part A, 28*(1), 71–79. https://doi.org/10.1080/19440049.2010.538935

Cressey, P., Vannoort, R., & Malcolmb, C. (2009). Pesticide residues in conventionally grown and organic New Zealand produce. *Food Additives and Contaminants: Part B Surveillance, 2*(1), 21–26. https://doi.org/10.1080/02652030802684096

Curl, C. L., Porter, J., Penwell, I., Phinney, R., Ospina, M., & Calafat, A. M. (2019). Effect of a 24-week randomized trial of an organic produce intervention on pyrethroid and organophosphate pesticide exposure among pregnant women. *Environment International, 132*(July), Article 104957. https://doi.org/10.1016/j.envint.2019.104957

De Canio, F., & Martinelli, E. (2021). EU quality label vs organic food products: A multigroup structural equation modeling to assess consumers' intention to buy in light of sustainable motives. *Food Research International, 139*(February 2020), 109846. doi: 10.1016/j.foodres.2020.109846.

El-Nahhal, I., & El-Nahhal, Y. (2021). Pesticide residues in drinking water, their potential risk to human health and removal options. *Journal of Environmental Management, 299*(August), Article 113611. https://doi.org/10.1016/j.jenvman.2021.113611

European Commission. (2005). Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Official Journal of the European Union 2005, L 70/1.

European Commission. (2018). Regulation (EU) 2018/848 on organic production and labelling of organic product. *Official Journal of the European Union, 2018*(1151), 1–92. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0848&from=EN.

EUROSTAT. (2022). Agri-environmental indicator - consumption of pesticides. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agri-environmental_indicator_-_consumption_of_pesticides. Accessed on: 27/04/2023.

Figueroa, Z., Young, H. A., Meeker, J. D., Martenies, S. E., Barr, D. B., Gray, G., & Perry, M. J. (2015). Dialkyl phosphate urinary metabolites and chromosomal abnormalities in human sperm. *Environmental Research, 143*, 256–265. https://doi.org/10.1016/j.envres.2015.10.021.Dialkyl

Göen, T., Schmidt, L., Lichtensteiger, W., & Schlumpf, M. (2017). Efficiency control of dietary pesticide intake reduction by human biomonitoring. *International Journal of Hygiene and Environmental Health, 220*(2), 254–260. https://doi.org/10.1016/j.ijheh.2016.11.008

Hyland, C., Bradman, A., Gerona, R., Patton, S., Zakharevich, I., Gunier, R. B., & Klein, K. (2019). Organic diet intervention significantly reduces urinary pesticide levels in U. S. children and adults. *Environmental Research, 171*(January), 568–575. https://doi.org/10.1016/j.envres.2019.01.024

Ingenbleek, L., Hu, R., Pereira, L. L., Paineau, A., Colet, I., Koné, A. Z., … Le Bizec, B. (2019). Sub-Saharan Africa total diet study in Benin, Cameroon, Mali and Nigeria: Pesticides occurrence in foods. *Food Chemistry: X, 2*(December 2018), 100034. doi: 10.1016/j.fochx.2019.100034.

Kazimierczak, R., Średnicka-Tober, D., Golba, J., Nowacka, A., Hołodyńska-Kulas, A., Kopczyńska, K., … Gnusowski, B. (2022). Evaluation of Pesticide Residues Occurrence in Random Samples of Organic Fruits and Vegetables Marketed in Poland. *Foods, 11*(13), 1–18. https://doi.org/10.3390/foods11131963

Kim, K. H., Kabir, E., & Jahan, S. A. (2017). Exposure to pesticides and the associated human health effects. *Science of the Total Environment, 575*, 525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009

Lu, C., Barr, D. B., Pearson, M. A., & Waller, L. A. (2008). Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. *Environmental Health Perspectives, 116*, 537–542. https://doi.org/10.1289/ehp.10912

Lu, C., Toepel, K., Irish, R., Fenske, R. A., Barr, D. B., & Bravo, R. (2006). Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. *Environmental Health Perspectives, 114*(2), 260–263. https://doi.org/10.1289/ehp.8418

Monger, A., Mahat, K., Dorjee, O. M., Mongar, P., Dorji, T., Jamtsho, A., Wangdi, K., Wangdi, C., Jamtsho, T., & Chettri, V. (2023). Assessment of exposure to pesticides and the knowledge, attitude and practice among farmers of western Bhutan. *PLoS ONE, 18*(5), Article e0286348. https://doi.org/10.1371/journal.pone.0286348

Mrema, E. J., Rubino, F. M., Brambilla, G., Moretto, A., Tsatsakis, A. M., & Colosio, C. (2013). Persistent organochlorinated pesticides and mechanisms of their toxicity. *Toxicology, 307*, 74–88. https://doi.org/10.1016/j.tox.2012.11.015

Narenderan, S. T., Meyyanathan, S. N., & Babu, B. (2020). Review of pesticide residue analysis in fruits and vegetables. Pre-treatment, extraction and detection techniques. *Food Research International, 133*(February), Article 109141. https://doi.org/10.1016/j.foodres.2020.109141

Nougadère, A., Sirot, V., Cravedi, J. P., Vasseur, P., Feidt, C., Fussell, R. J., … Hulin, M. (2020). Dietary exposure to pesticide residues and associated health risks in infants and young children – Results of the French infant total diet study. *Environment International, 137*(December 2019), 105529. doi: 10.1016/j.envint.2020.105529.

Oates, L., Cohen, M., Braun, L., Schembri, A., & Taskova, R. (2014). Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. *Environmental Research, 132*, 105–111. https://doi.org/10.1016/j.envres.2014.03.021

Rempelos, L., Wang, J., Baranski, M., Watson, A., Volakakis, N., Hoppe, H-W., … Leifert, C. (2022). Diet and food type affect urinary pesticide residue excretion profiles in healthy individuals: results of a randomized controlled dietary intervention trial. *American Journal of Clinical Nutrition, 115*, 364–377. https://doi.org/10.1093/ajcn/nqab308

Schouteten, J. J., Gellynck, X., & Slabbinck, H. (2019). Influence of organic labels on consumer's flavor perception and emotional profiling: Comparison between a central location test and home-use-test. *Food Research International, 116*(September 2018), 1000–1009. doi: 10.1016/j.foodres.2018.09.038.

Schusterova, D., Hajslova, J., Kocourek, V., & Pulkrabova, J. (2021). Pesticide residues and their metabolites in grapes and wines from conventional and organic farming system. *Foods, 10*(2). https://doi.org/10.3390/foods10020307

Sesini, G., Castellini, G., Iannello, P., Lombi, L., Lozza, E., Lucini, L., & Graffigna, G. (2023). Determinants of the willingness to buy products certified by omics technology: Differences between regular and occasional consumers of organic food. *Food Research International, 164*(September 2022), 112324. doi: 10.1016/j.foodres.2022.112324.

Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., … Singla, N. (2020). Global trends in pesticides: A looming threat and viable alternatives. *Ecotoxicology and Environmental Safety, 201*(May), Article 110812. https://doi.org/10.1016/j.ecoenv.2020.110812

Shiny Raj, R., & Anoop Krishnan, K. (2023). A comprehensive review on the impact of emerging organophosphorous pesticides and their remedial measures: Special focus on acephate. *Environmental Nanotechnology, Monitoring & Management, 20*, Article 100813. https://doi.org/10.1016/j.enmm.2023.100813

Silva Pinto, B. G., Marques Soares, T. K., Azevedo Linhares, M., & Castilhos Ghisi, N. (2020). Occupational exposure to pesticides: Genetic danger to farmworkers and manufacturing workers – A meta-analytical review. *Science of the Total Environment, 748*, Article 141382. https://doi.org/10.1016/j.scitotenv.2020.141382

Straw, E. A., Kelly, E., & Stanley, D. A. (2023). Self-reported assessment of compliance with pesticide rules. *Ecotoxicology and Environmental Safety, 254*, Article 114692. https://doi.org/10.1016/j.ecoenv.2023.114692

Suratman, S., Edwards, J. W., & Babina, K. (2015). Organophosphate pesticides exposure among farmworkers: Pathways and risk of adverse health effects. *Reviews on Environmental Health, 30*(1), 65–79. https://doi.org/10.1515/reveh-2014-0072

Tsuchiyama, T., Ito, Y., Oya, N., Nomasa, K., Sato, H., Minato, K., … Kamijima, M. (2022). Quantitative analysis of organophosphate pesticides and dialkylphosphates in duplicate diet samples to identify potential sources of measured urinary dialkylphosphates in Japanese women. *Environmental Pollution, 298*(October 2021), 118799. doi: 10.1016/j.envpol.2022.118799.

Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., … Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health, 18*(3), 1–24. https://doi.org/10.3390/ijerph18031112

Wang, X., Zhou, L., Zhang, X., Luo, F., & Chen, Z. (2019). Transfer of pesticide residue during tea brewing: Understanding the effects of pesticide's physico-chemical parameters on its transfer behavior. *Food Research International, 121*(September 2018), 776–784. doi: 10.1016/j.foodres.2018.12.060.

Wei, Y., Wang, L., & Liu, J. (2023). The diabetogenic effects of pesticides: Evidence based on epidemiological and toxicological studies. *Environmental Pollution, 331*, Article 121927. https://doi.org/10.1016/j.envpol.2023.121927

Ye, M., Beach, J., Martin, J. W., & Senthilselvan, A. (2016). Urinary dialkyl phosphate concentrations and lung function parameters in adolescents and adults: Results from the Canadian health measures survey. *Environmental Health Perspectives, 124*(4), 491–497. https://doi.org/10.1289/ehp.1509745

Ye, X., Pierik, F. H., Angerer, J., Meltzer, H. M., Jaddoe, V. W. V., Tiemeier, H., … Longnecker, M. P. (2009). Levels of metabolites of organophosphate pesticides, phthalates, and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). *International Journal of Hygiene and Environmental Health, 212*, 481–491. https://doi.org/10.1016/j.ijheh.2009.03.004

Yucra, S., Steenland, K., Chung, A., Choque, F., & Gonzales, G. F. (2006). Dialkyl phosphate metabolites of organophosphorus in applicators of agricultural pesticides in Majes - Arequipa (Peru). *Journal of Occupational Medicine and Toxicology, 1*(1), 1–8. https://doi.org/10.1186/1745-6673-1-27