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Organic diet intervention significantly reduces urinary pesticide levels in U.S. children and adults

Carly Hyland[a], Asa Bradman[a], Roy Gerona[b], Sharyle Patton[c], Igor Zakharevich[b], Robert B. Gunier[a], Kendra Klein[d,*]

[a] Center for Environmental Research and Children's Health (CERCH), School of Public Health, University of California at Berkeley, Berkeley, CA, United States
[b] Clinical Toxicology and Environmental Biomonitoring Laboratory, University of California at San Francisco, San Francisco, CA, United States
[c] Commonweal Institute, Bolinas, CA, United States
[d] Friends of the Earth U.S., 2150 Allston Way Suite 360, Berkeley, CA 94704, United States

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ABSTRACT

Background: Previous diet intervention studies indicate that an organic diet can reduce urinary pesticide metabolite excretion; however, they have largely focused on organophosphate (OP) pesticides. Knowledge gaps exist regarding the impact of an organic diet on exposure to other pesticides, including pyrethroids and neonicotinoids, which are increasing in use in the United States and globally.

Objective: To investigate the impact of an organic diet intervention on levels of insecticides, herbicides, and fungicides or their metabolites in urine collected from adults and children.

Methods: We collected urine samples from four racially and geographically diverse families in the United States before and after an organic diet intervention (n = 16 participants and a total of 158 urine samples).

Results: We observed significant reductions in urinary levels of thirteen pesticide metabolites and parent compounds representing OP, neonicotinoid, and pyrethroid insecticides and the herbicide 2,4-D following the introduction of an organic diet. The greatest reductions were observed for clothianidin (− 82.7%; 95% confidence interval [95% CI]: − 86.6%, − 77.6%; $p < 0.01$), malathion dicarboxylic acid (MDA), a metabolite of malathion (− 95.0%; 95% CI: − 97.0%, − 91.8%; $p < 0.01$), and 3,5,6-trichlor-2-pyridinol (TCPy), a metabolite of chlorpyrifos (− 60.7%; 95% CI: − 69.6%, − 49.2%; $p < 0.01$). Metabolites or parent compounds of the fungicides boscalid, iprodione, and thiabendazole and the neonicotinoid insecticide imidacloprid were not detected among participants in our study.

Conclusion: An organic diet was associated with significant reductions in urinary excretion of several pesticide metabolites and parent compounds. This study adds to a growing body of literature indicating that an organic diet may reduce exposure to a range of pesticides in children and adults. Additional research is needed to evaluate dietary exposure to neonicotinoids, which are now the most widely used class of insecticides in the world.

1. Introduction

Diet accounts for a significant proportion of total pesticide exposure in the general population (Curl et al., 2015; Riederer et al., 2008). Recent data from the US Food and Drug Administration (FDA) Pesticide Residue Monitoring Program show that approximately 47% of domestic food and 49% of imported foods sampled had detectable pesticide residues in the 2016 examination (United States Food and Drug Administration, 2016). Pesticide residue monitoring data also indicate that organically grown foods have lower pesticide residues compared with conventionally grown foods (Forman and Silverstein, 2012; United States Department of Agriculture, 2016).

Exposure to pesticides has been associated with various adverse health outcomes, including decreased cognitive scores (Bouchard et al., 2011; Engel et al., 2011; Rauh et al., 2011) and increased behavioral and attention problems in children (Quirós-Alcalá et al., 2014), asthma (Hernandez et al., 2011; Raanan et al., 2016), cancer (Bassil et al., 2007), and impacts on the reproductive (Bretveld et al., 2006) and

Abbreviations: ADI, Acceptable Daily Intake; CI, Confidence Interval; RfD, Reference Dose; NHANES, National Health and Nutrition Examination Survey; OP, Organophosphate; U.S. EPA, United States Environmental Protection Agency; U.S. FDA, United States Food and Drug Administration

* Corresponding author.
E-mail address: kklein@foe.org (K. Klein).

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endocrine systems (Mnif et al., 2011). While knowledge gaps exist regarding specific health effects associated with chronic low-level dietary pesticide exposure, a recent longitudinal study of nearly 70,000 adults found that higher frequency of organic food consumption - which is associated with lower pesticide exposure - was protective against several cancers (Baudry et al., 2018).

Pesticide use patterns have shifted in recent decades. While the use of organophosphate (OP) pesticides has declined in the U.S. since the passage of the Food Quality Protection Act in 1996 (United States Environmental Protection Agency, 2017), they are one of the most widely used classes of insecticides in U.S. agriculture (California Department of Pesticide Regulation, 2016; Curl et al., 2015), and diet is the primary source of OP exposure in the general population (Curl et al., 2015). As OP pesticide use has declined, use of pyrethroids and neonicotinoids has increased over the past two decades (Burns and Pastoor, 2018). Neonicotinoids are now the most widely used class of insecticides worldwide (Lu et al., 2018) and residues have been found in food (Chang et al., 2018). Because they are systemic (i.e., readily taken up into the tissue of plants), these residues cannot be washed off food (Abreu-Villaca and Levin, 2017). Biomonitoring data also indicate widespread pyrethroid exposure in the general U.S. population (Saillenfait et al., 2015), with 3-phenoxybenzoic acid (3PBA), a non-specific metabolite of multiple pyrethroids, being detected in over 70% of samples from the 1999–2002 National Health and Nutrition Examination Survey (NHANES) assessment (Barr et al., 2010). Additionally, the latest U.S. Environmental Protection Agency (EPA) report on pesticide use indicates that the herbicide 2,4-D was the fifth most commonly used active pesticide ingredient in the U.S in 2012 (United States Environmental Protection Agency, 2017).

Previous observational and intervention studies have reported decreased urinary pesticide concentrations among people who consume organic food compared with those who follow a conventional diet (Bradman et al., 2015; Curl et al., 2015, 2003; Fenske et al., 2002; Göen et al., 2016; Lu et al., 2008, 2006a; Oates and Cohen, 2011; Oates et al., 2014). However, these studies have primarily focused on exposure to OPs and, to a lesser extent, pyrethroids. In this paper, we address existing data gaps by investigating whether an organic diet intervention reduced urinary levels of OPs, pyrethroids, neonicotinoids, fungicides, and one herbicide among adults and children from four geographically and racially diverse families in the United States.

2. Methods

2.1. Study participants

Four racially diverse families of three to five members were recruited from four locations: Oakland, CA, Minneapolis, MN, Baltimore, MD, and Atlanta, GA. Families in these cities were originally contacted via a brief recruitment email that explained the purpose of the study and study procedures. If interested, families contacted study staff and a phone script was read to screen for eligibility in the study, including: 1) willingness to alter their diet for six days, 2) no more than six family members and with two to three children between the ages of three to eighteen living at home, 3) all children toilet-trained and able to have their breakfast, lunch, and dinner prepared at home during the organic phase of the study, 4) English speaking, 5) no pregnant family members, 6) no family members with severe food allergies, and, 7) the family did not typically consume an organic diet. Families participated in the study between February and May 2017. The Western Institutional Review Board reviewed and approved all study procedures. Written informed consent was obtained from parents before data collection began.

2.2. Data collection

Each family participated in the study over twelve consecutive days. Prior to beginning data collection, participants were contacted via an online video call and provided with instructions on how to collect urine samples and complete food diaries. Food diaries included information about the type of food (produce, grain, dairy, meat, etc.) and portion size. A questionnaire was administered by phone to one adult in each family to collect information about potential pesticide exposure, including pesticide use and storage in and around the home, proximity of the home to locations known to use pesticides, such as golf courses, and potential occupational exposure to pesticides.

2.3. Dietary intervention

During days one through five, study participants were asked to follow their normal conventional diet (conventional phase). During days six through eleven, all family members were provided with certified organic food while at home, work, school, or daycare, including all beverages other than water, all food categories, and oils, condiments, and spices (organic phase). After the participants collected the first morning void urine sample on day twelve, they could choose to eat either organic or conventional food. Organic food was provided to families in two ways: 1) Participants were asked to compile a list of all groceries they would need for six days, and research assistants purchased organic foods from this list and delivered the groceries to the participants' homes for their use, and, 2) dinners were prepared with all organic foods by a licensed chef or caterer and delivered to study participants by the research assistants. All organic foods for the six-day period were provided free of charge to the families.

2.4. Urine collection

Prior to the start of the study, participants were given urine collection instructions and urine collection kits were mailed to their homes. First morning void urine samples were collected into specimen cups and immediately stored in sealed plastic bags in the family's freezer each day during the study period. Research assistants picked up the frozen urine samples for each phase of the study and shipped them overnight on dry ice to the laboratory.

For one family, collection of urine samples from the conventional phase was repeated after the organic phase (with washout time) due to an error in maintaining the samples frozen in the laboratory.

2.5. Laboratory analysis of urine samples

Urine samples were analyzed for eighteen pesticide analytes, including nine specific OP, neonicotinoid, pyrethroid, fungicide, and herbicide analytes, three non-specific pyrethroid analytes, and six non-specific OP dialkylphosphate (DAPS) analytes (Table 1). In choosing pesticides for analysis, we assessed amount of use in U.S. agriculture (United States Environmental Protection Agency, 2017) and frequency of detection as food residues (United States Food and Drug Administration, 2016).

Quantification of the nine pesticide-specific analytes and three non-specific pyrethroid metabolites was performed using liquid-chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent LC 1260-AB Sciex 5500 system. The urine specimens (1 mL) were prepared for LC-MS/MS analysis by solid phase extraction (SPE) using Waters Oasis WAX cartridges (10 mg, 30 μm, 1 cc). All urine samples were deconjugated prior to LC-MS/MS analysis by addition of 450 U H. pomatia glucuronidase (Sigma-Aldrich, St Louis, MO) and incubated at 37 °C for two hours with constant shaking. The compounds were ionized in the negative mode using electrospray ionization (ESI) and monitored by multiple reaction monitoring. Each compound was monitored using two transitions (See Supplementary material S1) along with 2,4-D-d3 and Cloth-d3 as internal standards. Each batch of samples was injected in duplicates and run alongside calibration standards that were run at the beginning, between the duplicate sample injections, and after the

Table 1
Summary of parent compounds and measured metabolites in urine.

Chemical class and precursor compounds	Analyte measured (abbreviation)	LOD (ng/mL)	Overall DF (%)[a]	Conventional DF (%)[b]	Organic DF (%)[c]
Organophosphate Insecticides					
Chlorpyrifos	3,5,6-trichloro-2-pyridinol (TCPy)	0.2	93.05	97.5	82.28
Malathion	Malathion dicarboxylic acid (MDA)	0.02	78.48	87.50	43.04
Azinphos-methyl, chlorpyrifos-methyl, dichlorvos, dicrotophos, dimethoate, fenitrothion, fenthion, isazofos-methyl, malathion, methidathion, methyl parathion, naled, oxydemeton-methyl, phosmet, pirimiphos-methyl, temephos, tetrachlorvinphos, trichlorfon	Total dimethylphosphates (total DMs = DMP + DMTP + DMDTP)	DMP: 0.2 DMTP: 0.4 DMDTP: 0.07	94.34[d]	100.00[d]	88.61[d]
Chlorethoxyphos, chlorpyrifos, coumaphos, diazinon, disulfoton, ethion, parathion, phorate, sulfotepp, terbufos	Total diethylphosphates (total DEs = DEP + DETP + DEDTP)	DEP: 0.25 DETP: 0.09 DEDTP: 0.06	100.00[e]	100.00[e]	100.00[e]
Totals	Total dialkylphosphates (total DMs + total DEs)	–	94.34[f]	100.00[f]	88.61[f]
Pyrethroid Insecticides					
Allethrin, cyhalothrin, cypermethrin deltamethrin, fenpropathrin, permethrin, trialomethrin	3-Phenoxybenzoic acid (3-PBA)	0.02	100.00	100.00	100.00
Cyfluthrin	4-fluoro-3-phenoxybenzoic acid (F-PBA)	0.01	86.39	91.25	77.22
cis-Cypermethrin, *cis*-cyfluthrin, *cis*-permethrin	*cis*-2,2-(Dichloro)-2-dimethylvinylcyclopropane carboxylic acid (*cis*-DCCA; cDCCA)	0.1	95.25	93.75	96.20
trans-Cypermethrin, *trans*-cyfluthrin, *trans*-permethrin	*trans*-2,2-(Dichloro)-2-dimethylvinylcyclopropane carboxylic acid (*trans*-DCCA; tDCCA)	0.05	98.73	96.25	100.00
Neonicotinoid Insecticides					
Imidacloprid	5-Hydroxy-Imidacloprid (5OH-Imd)	0.1	ND	ND	ND
Clothianidin	Clothianidin	0.05	49.37	85.00	13.92
Fungicides					
Thiabendazole	5-Hydroxy-Thiabendazole (5OH-TBZ)	0.2	ND	ND	ND
Boscalid	Boscalid	0.02	ND	ND	ND
Iprodione	Iprodione	0.05	ND	ND	ND
Herbicides					
2,4-Dichlorophenoxyacetic acid	2,4-Dichlorophenoxyacetic acid (2,4-D)	0.01	100.00	100.00	100.00

Abbreviations: DF, detection frequency; LOD, limit of detection; ND, not detected.
[a] Overall analyte DF (%) is the ratio of the total number of urine samples with analyte concentration > LOD during the entire study to the total number of urine samples analyzed in the study (158) multiplied by 100.
[b] Conventional DF (%) is the ratio of the total number urine samples > LOD to the number of samples analyzed during the convectional phase (80).
[c] Organic DF (%) is the ratio of the total number of urine samples > LOD to the number of samples analyzed during the organic phase (78).
[d] Total DM counted as detect if either DMP, DMTP, or DMTP > LOD.
[e] Total DE counted as detect if either DEP, DETP, or DEDTP > LOD.
[f] Total DAPs counted as detect if both total DM and total DE counted as detect.

sample set. Additionally, low and high spiked quality control (QC) samples were run for each analysis batch. A batch run was accepted if both QC samples were within 20% of their target values and had coefficients of variation (CV) ≤ 20%.

The biomarkers for the nine pesticide-specific analytes and three non-specific pyrethroid metabolites were selected based on those that have been previously measured and reported by other studies for the more common analytes (e.g., TCPy, MDA). For novel analytes (e.g., clothianidin, imidacloprid), we conducted a literature search of metabolic studies to identify targets. Because the measurements were conducted in urine, we prioritized metabolites over the parent compound when reference standards of the metabolites were available. The parent compound was used as a biomarker when metabolite reference standards were not available or cost-prohibitive to custom synthesize.

To quantify the six non-specific OP pesticide DAP metabolites (DEP, DETP, DEDTP, DMP, DMTP and DMDTP), 0.1 mL of the urine specimens were enriched with internal standards (DEP-$^{13}C_4$, DETP-$^{13}C_4$, DEDTP-$^{13}C_4$, DMP-d_6, DMTP-d_6, DMDTP-d_6). After adding 1 mL of acetonitrile and 200 mg of potassium carbonate, the samples were derivatized with 15 μL of pentafluorobenzyl bromide (PFBBr) at 70 °C for 2 h. The derivatized products were extracted with 7 mL of a mixture dichloromethane: hexane (8:92), mixed 15 min and centrifuged 5 min at 1500 rpm. The solvent was then evaporated to dryness, taken up in 500 μL of dichloromethane: hexane (20:80) and analyzed for pesticide metabolites on an Agilent 6890 Network gas chromatograph (GC) (Agilent Technologies; Mississauga, Ontario, Canada) coupled to a Waters Quattro Micro GC mass spectrometer in tandem (MS/MS) (Waters; Milford, MA). The GC was fitted with an Agilent 30 m HP-5MS column (0.25 mm i.d., 0.25 μm film thickness) to the MS/MS. The internal reference materials used to control the quality of the analyses were the non-certified reference material ClinChek (Urine Level 1; RECIPE Chemicals; Munich, Germany) and 3 in-house reference materials (Low, Medium, High QC) prepared by the Centre de Toxicologie du Québec (CTQ), Institut national de santé publique du Québec (INSPQ). The overall quality and accuracy of the analytical method was monitored by the participation to the interlaboratory program as the German External Quality Assessment Scheme (G-EQUAS; Erlangen, Germany).

2.6. Data analysis

Urine samples from days one and seven were considered washout days and were excluded from data analysis. We included ten urine samples from each of the participants in the analyses, including five samples each from the conventional diet and organic diet phases. For one participant missing DETP data from the fourth day of the organic

Table 2
Urinary analyte concentrations (ng/mL) and percent change from conventional to organic diet.

Analyte	Conventional			Organic			Percent change (95% CI)	*p*-Value	Median (95% CI) NHANES[a]
	n	Median (IQR)	Max	n	Median (IQR)	Max			
All (n = 16)									
2,4-D	80	0.65 (0.36, 0.95)	2.43	78	0.40 (0.20, 0.78)	1.45	− 36.9 (− 47.0, − 25.0)	0.01	0.28 (0.25, 0.32)
3PBA	80	2.70 (0.93, 4.83)	47.74	78	1.11 (0.67, 1.77)	13.09	− 42.7 (− 57.7, − 22.2)	< 0.01	0.40 (0.35, 0.46)
cDDCA	80	1.98 (0.70, 4.48)	39.64	78	0.68 (0.43, 1.20)	15.58	− 53.2 (− 65.7, − 36.1)	< 0.01	< LOD[d]
Clothianidin	80	0.24 (0.10, 0.52)	6.60	78	< LOD	1.06	− 82.7 (− 86.6, −77.6)	< 0.01	–
FPBA	80	0.04 (0.02, 0.07)	7.11	78	0.02 (< LOD, 0.03)	0.13	− 57.2 (− 66.3, − 45.6)	< 0.01	< LOD[e]
MDA	80	1.03 (0.20, 2.63)	1.04	78	< LOD (< LOD, 0.09)	3.54	− 95.0 (− 97.0, − 91.8)	< 0.01	< LOD[d]
TCPy	80	2.78 (1.91, 4.85)	19.48	78	1.34 (0.64, 2.45)	7.57	− 60.7 (− 69.6, − 49.2)	< 0.01	–
tDCCA	80	2.54 (0.90, 5.58)	47.20	78	1.23 (0.58, 2.31)	20.03	− 46.1 (− 60.9, − 25.6)	< 0.01	< LOD[f]
Adults[b] (n = 7)									
2,4-D	35	0.56 (0.29, 0.86)	2.43	32	0.33 (0.15, 0.81)	1.45	− 41.9 (− 55.4, − 24.4)	< 0.01	0.27 (0.23, 0.31[g]
3PBA	35	3.71 (1.69, 5.57)	47.74	32	0.71 (0.44, 1.47)	3.79	− 71.2 (− 82.0, − 54.0)	< 0.01	0.39 (0.33, 0.47)[g]
cDDCA	35	3.44 (1.36, 8.33)	39.64	32	0.71 (0.41, 1.59)	7.16	− 71.7 (− 83.1, − 52.6)	< 0.01	< LOD[d,g]
Clothianidin	35	0.38 (0.24, 0.73)	5.57	32	< LOD (< LOD, < LOD)	1.06	− 88.0 (− 92.0, − 82.7)	< 0.01	–
FPBA	35	0.03 (0.02, 0.06	0.19	32	< LOD (< LOD, 0.02)	0.06	− 55.3 (− 67.0, − 39.0)	< 0.01	< LOD[e,g]
MDA	35	1.01 (0.14, 2.99)	11.41	32	< LOD (< LOD, 0.10)	3.54	− 94.1 (− 97.3, − 87.1)	< 0.01	< LOD[d,g]
TCPy	35	2.55 (1.79, 3.92)	12.09	32	0.63 (< LOD, 1.25)	7.57	− 77.7 (− 85.8, − 65.1)	< 0.01	–
tDCCA	35	3.35 (1.87, 6.96)	47.20	32	0.97 (0.34, 2.11)	6.14	− 71.2 (− 82.9, − 51.5)	< 0.01	< LOD[f,g]
Children[c] (n = 9)									
2,4-D	45	0.67 (0.38, 0.96)	2.27	46	0.43 (0.25, 0.78)	1.44	− 32.4 (− 46.2, − 15.1)	< 0.01	0.35 (0.29, 0.44)[h]
3PBA	45	1.64 (0.68, 3.89)	19.25	46	1.37 (0.99, 1.91)	13.09	− 4.4 (− 33.9, 38.1)	0.81	0.48 (0.35, 0.70)[h]
cDDCA	45	1.27 (0.35, 3.02)	17.89	46	0.67 (0.44, 1.10)	15.58	− 31.7 (− 52.9, − 1.0)	0.04	< LOD[d,h]
Clothianidin	45	0.17 (0.07, 0.39)	6.60	46	< LOD	0.69	− 77.2 (− 84.0, − 67.3)	< 0.01	–
FPBA	45	0.05 (0.03, 0.08)	7.11	46	0.02 (< LOD, 0.03)	0.13	− 59.0 (− 71.4, − 41.2)	< 0.01	< LOD[e,h]
MDA	45	1.04 (0.37, 2.63)	35.80	46	< LOD (< LOD, 0.08)	0.83	− 95.5 (− 977, − 91.4)	< 0.01	< LOD[d,h]
TCPy	45	2.99 (1.94, 5.32)	19.48	46	1.77 (1.19, 3.03)	7.25	− 41.9 (− 56.2, − 22.9)	< 0.01	–
tDCCA	45	2.35 (0.72, 5.53)	22.41	46	1.45 (0.78, 2.31)	20.03	− 14.1 (− 41.3, 25.6)	0.43	< LOD[f,h]

Abbreviations: LOD, limit of detection; CI, confidence interval; IQR, interquartile range; NHANES, National Health and Nutrition Examination Survey.
[a] Median and 95% confidence interval of non-creatinine adjusted analyte levels in ug/L from 2009 to 2010 NHANES.
[b] Adults in study 36–52 years old.
[c] Children in study 4–15 years old.
[d] LOD = 0.5 μg/L.
[e] LOD = 0.1 μg/L.
[f] LOD = 0.6 μg/L.
[g] Adults from NHANES 20–59 years old.
[h] Children from NHANES 6–11 years old.

diet phase, we imputed the average concentration from their four other organic diet samples. One participant was missing a urine sample from the eighth day of the study (second day of the organic phase) and one participant was missing urine samples from the eighth, tenth, and twelfth days of the study (second, fourth, and sixth days of the organic diet phase). For these two participants, we included the urine sample collected on day seven of the study, which was the first day of the organic phase, and considered a washout day for other participants. These criteria resulted in a total of 158 urine samples included in the analysis.

For the six non-specific OP metabolites, we converted concentrations to their molar equivalents and summed them to produce total DM (DMP + DMTP + DMDTP), DE (DEP + DETP + DEDTP), and DAP (DMs + DEs) concentrations for each sample. All analyte concentrations were log-transformed for statistical analyses, and we computed descriptive statistics for the fourteen analytes detected: specific OPs (MDA, TCPy), six non-specific OPs (summarized as total DMs, total DEs, and total DAPs), pyrethroids (3-PBA, FPBA, cDDCA, tDDCA), neonicotinoid (clothianidin), and herbicide (2,4-D). In order to account for the correlation among repeat urine samples collected from the same individual, we used linear mixed-effects models to estimate the percent change in urinary analyte concentrations from the conventional to organic phase using the formula % Change = [exp(β)-1] * 100, where β is the regression coefficient for organic diet from the mixed effects models.

We also conducted sensitivity analyses to confirm our results: 1) we repeated the statistical analyses with creatinine-adjusted analyte concentrations; and 2) we omitted outliers based on model standardized residuals > 3 or < -3 (Pardoe, 2018).

3. Results

3.1. Demographic characteristics

Seven adults ages 36–52 years and nine children ages 4–15 years participated in this study. The mean (± SD) age for adults and children in our study was 42.3 ± 6.1 years and 8.3 ± 4.1 years, respectively. Nine of the participants were Caucasian, four were Hispanic/Latino, and three were African American. All participants lived above the U.S. federal poverty threshold.

3.2. Household pesticide use during study

None of the families reported using pesticides inside their home within three months of beginning the study or during the study period. One family reported hiring a pest control company to treat termites with imidacloprid (Premise 75, Bayer, Whippany, NJ, USA) on the exterior of their foundation and at entry points one week prior to beginning the study.

3.3. Urinary measurements

Detection frequencies (DFs) of the three non-specific pyrethroid analytes ranged from 91–100% to 77–100% during the conventional and organic diet phases, respectively (Table 1). DFs of the five specific

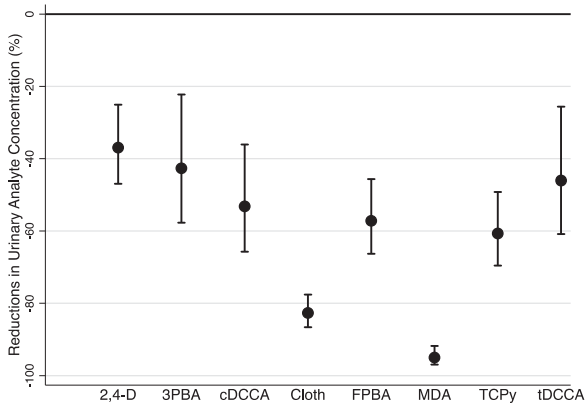

Fig. 1. Percent decrease in urinary metabolite concentration from conventional to organic diet phase from mixed effects models (n = 16 participants; n = 158 urine samples).

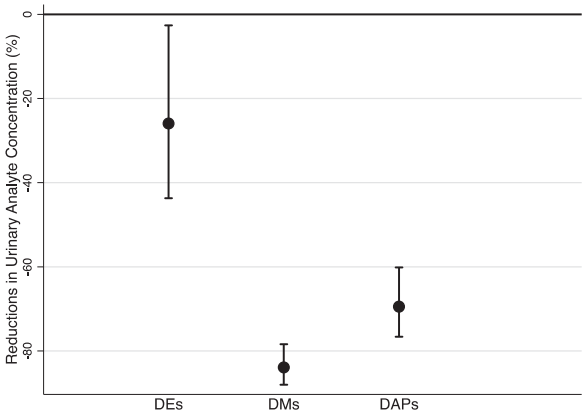

Fig. 2. Percent decrease in urinary DAP metabolite concentrations from conventional to organic diet phase from mixed effects models (n = 16 participants; n = 158 urine samples).

analytes (e.g. MDA, TCPy, clothianidin, FPBA, and 2,4-D) ranged from 85–100% to 14–100% during the conventional and organic diet phases, respectively (Table 1). Parent compounds or metabolites of the insecticide imidacloprid and fungicides thiabendazole, boscalid, and iprodione were not detected in urine samples (Table 1).

3.4. Effect of diet on urinary pesticide levels

Following the organic diet intervention, we observed significant reductions in urinary levels of all analytes detected in the study, except for DEP (percent change from conventional to organic phase: − 16.7% (95% confidence interval [95% CI]: − 37.0%, 10.0%; p = 0.20)). The largest changes were observed for MDA, clothianidin, and TCPy, which decreased by − 95.0% (95% CI: − 97.0%, − 91.8%; p < 0.01), − 82.7% (95% CI: − 86.6%, − 77.6%; p < 0.01), and − 60.7% (95% CI: − 69.6%, − 49.2%; p < 0.01), respectively (Table 2 and Fig. 1). During the organic diet phase, urinary levels of total DE, total DM, and total DAP metabolites decreased by − 26.0% (95% CI: −43.7%, − 2.6%; $p < 0.01$), − 83.9% (95% CI: − 88.0%, − 78.4%; $p < 0.01$), and − 69.5% (95% CI: −76.6%, − 60.2%; $p < 0.01$), respectively (Table 3 and Fig. 2). Fig. 3 presents the mean and 95th CI for urinary levels of clothianidin, MDA, TCPy, total DE, total DM, and total DAPs among all participants, adults, and children.

We also observed significant reductions in levels of 2,4-D, 3PBA, FBPA, cDDCA, and tDCCA among all participants following the organic diet intervention (Table 2). Although statistically significant reductions in 3PBA and tDCCA were observed among all participants and adults, these metabolites did not decrease significantly among children in our study (Table 2).

3.5. Sensitivity analysis

Results from linear mixed-effects models using creatinine-adjusted analytes did not differ appreciably from models not adjusted for creatinine (See Supplementary material Tables S2 and S3). Overall, the results also did not differ appreciably when outliers based on model residuals were excluded from analyses (See Supplementary material Tables S4 and S5). However, among all participants, the percent change in total urinary DE metabolites from the conventional to organic diet phase changed from − 26% to − 23% and the p-value increased from < 0.01 to 0.05 after excluding one outlier with the highest urinary DE concentration. The percent change in total urinary DE metabolites from the conventional to organic diet phase among adults changed from negative to positive after outliers were excluded. While the percent change in total DE exposure was not statistically significant among adults either with or without the outliers, it appears that urinary total DE levels from two samples from one of the participants during the conventional phase may have influenced the overall effect estimate for adults.

4. Discussion

We detected fourteen pesticides and pesticide metabolites in all of

Table 3
Urinary DAP analyte concentrations (nmol/L) during conventional and organic diet.

Metabolite	Conventional			Organic			Percent change (95% CI)	*p*-Value
	n	Median (IQR)	Max	n	Median (IQR)	Max		
All (n = 16)								
DEs	78	31.40 (14.87, 60.21)	819.54	77	24.79 (12.24, 42.08)	223.98	− 26.0 (− 43.7, − 2.6)	< 0.01
DMs	78	101.42 (48.47, 257.98)	1730.72	77	18.34 (9.78, 35.95)	172.50	− 83.9 (− 88.0, − 78.4)	< 0.01
DAPs	78	138.39 (70.93, 315.56)	2039.93	77	45.46 (25.50, 77.43)	310.61	− 69.5 (− 76.6, − 60.2)	< 0.01
Adults (n = 7)								
DEs	34	20.96 (14.11, 36.01)	819.54	32	24.62 (11.71, 34.42)	142.28	− 10.7 (− 42.5, 38.5)	0.61
DMs	34	71.77 (50.45, 135.44)	472.59	32	10.27 (3.88, 19.71)	172.50	− 87.6 (− 91.8, − 81.3)	< 0.01
DAPs	34	93.34 (67.30, 167.10)	927.13	32	39.22 (20.52, 59.07)	230.77	− 68.5 (− 78.7, − 53.4)	< 0.01
Children (n = 9)								
DEs	44	44.01 (22.23, 78.74)	309.21	45	26.00 (12.62, 51.58)	223.98	− 35.2 (− 54.4, − 7.9)	0.02
DMs	44	127.09 (48.07, 315.02)	1730.72	45	28.30 (15.57, 40.49)	86.63	− 80.5 (− 87.0, − 70.6)	< 0.01
DAPs	44	212.98 (85.25, 415.09)	2039.93	45	57.64, 32.03, 110.84)	310.61	− 69.8 (− 79.2, − 56.2)	< 0.01

Abbreviations: LOD, limit of detection; CI, confidence interval; IQR, interquartile range.
DMs = DMP + DMTP + DMDTP; DEs = DEP + DETP + DEDTP; DAPs = DMs + DEs.

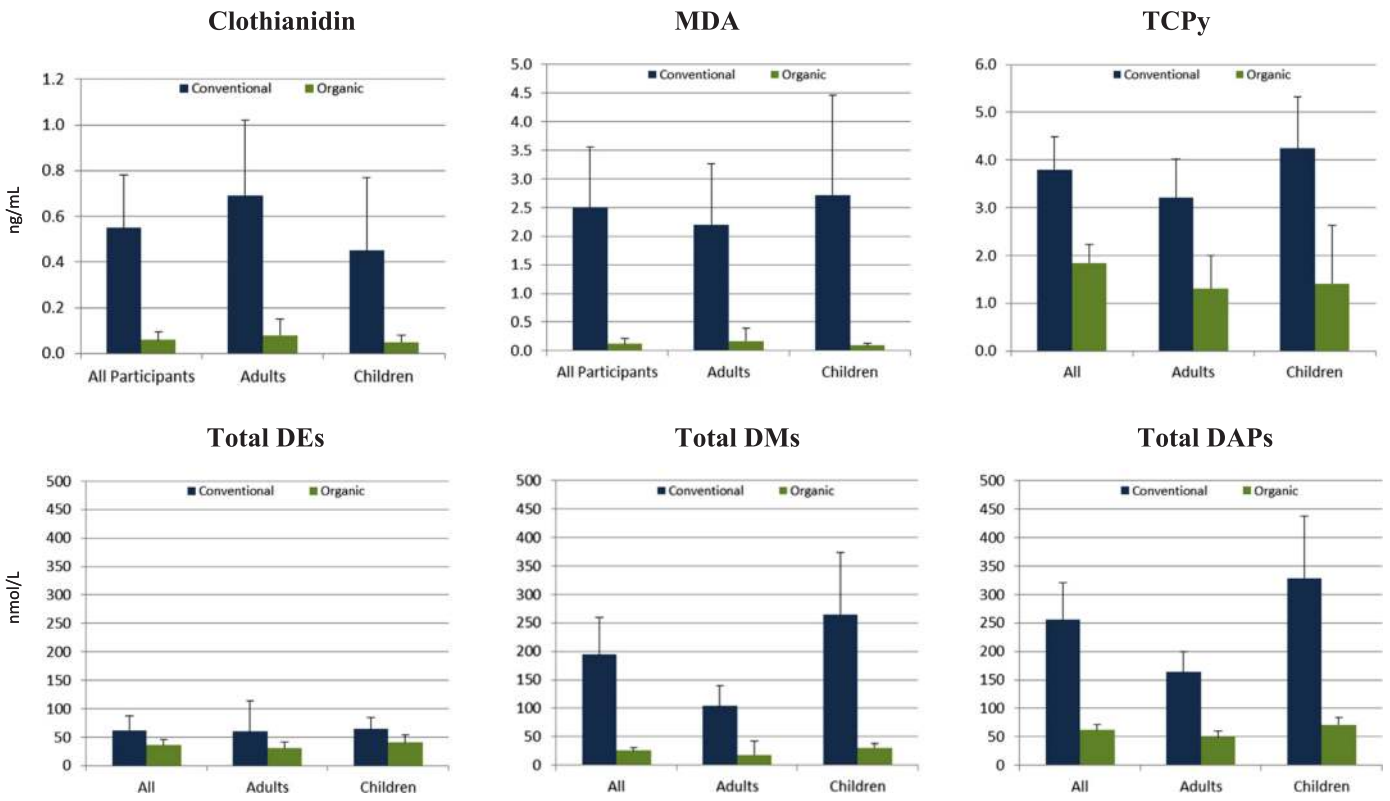

Fig. 3. Estimated mean and 95% CIs for select urinary analytes during conventional and organic diet phase among all participants, adults, and children.

the study participants' urine. These chemicals represent potential exposure to over 40 different pesticides including OP, neonicotinoid, and pyrethroid insecticides as well as the herbicide 2,4-D (Table 1). Following an organic diet intervention, urinary levels of all but one of these exposure biomarkers (DEP) decreased significantly.

4.1. Organophosphates

Previous studies have indicated that dietary ingestion is the primary route of OP pesticide exposure in the general U.S. population (Curl et al., 2015; Lu et al., 2008, 2006a; Smith-Spangler et al., 2012; United States Environmental Protection Agency, 2006), and the results from our study and other investigations have provided consistent evidence that an organic diet may reduce exposure to OP pesticides (Bradman et al., 2015; Göen et al., 2016; Lu et al., 2006a; Oates et al., 2014). For example, in a dietary intervention study assessing OP pesticide exposure among 23 elementary school-aged children, Lu et al. (2006a) observed that median levels of MDA and TCPy immediately fell below the limit of detection following the organic diet intervention and remained nondetectable until a conventional diet was reintroduced. In a crossover study among thirteen adults in Australia, Oates et al. (2014) found that, compared with the conventional diet phase, mean total DAP and DM concentrations were 89% and 96% lower, respectively, during a one-week organic diet phase. Bradman et al. (2015) conducted an organic diet intervention with 40 children living in urban and rural agricultural locations and found that, compared to the two conventional diet phases, an organic diet was associated with a − 39.9% (95% CI: − 58.6, − 12.6%; $p < 0.01$) and − 13.3% (95% CI: − 28.9, 5.8; $p = 0.16$) decrease in mean urinary total DAP and MDA levels, respectively. Most recently, Göen et al. (2016) conducted a pilot organic diet intervention study with two adults in Switzerland and found that levels of DMP, DMTP, DEP, DETP, and TCPy were generally lower during the organic phase.

In our study, levels of all OP metabolites except for DEP decreased significantly following the organic diet intervention. Among the most significant decreases during the organic diet phase were MDA, a metabolite of the insecticide malathion, and TCPy, a metabolite of the insecticide chlorpyrifos. Chlorpyrifos is a neurotoxic pesticide that was effectively banned for residential use in 2001 due to concerns about its health impacts on children (United States Environmental Protection Agency, 2000; Williams et al., 2008). It has been estimated that chlorpyrifos residues on food crops exceed Federal Food, Drug, and Cosmetic Act (FFDCA) safety standards (United States Environmental Protection Agency), and the most recent U.S. EPA data on pesticide sales and usage indicate that chlorpyrifos was the most widely used OP in U.S. agriculture from 2007 to 2012 (United States Environmental Protection Agency, 2017). In 2018 a court ordered the U.S. EPA to ban the insecticide form agricultural use (Dennis, 2018); if implemented, chlorpyrifos food residues would likely decrease.

4.2. Pyrethroids

The role of diet in pyrethroid exposure is not clearly understood because pyrethroids are commonly used in agriculture, as well as for residential and structural pest control purposes (Lu et al., 2006b). Göen et al. (2016) found that levels of cDCCA, tDCCA, and 3-PBA decreased significantly following the introduction of an organic diet, however the study only included two adults, limiting inferences in the broader population. In contrast to these findings, Bradman et al. (2015) found that 3-PBA levels were not associated with an organic diet. We observed significant decreases in 3-PBA and three other pyrethroid metabolites, FPBA, cDDCA, and tDDCA, among all participants following the organic diet intervention. However, levels of 3-PBA and tDCCA did not decrease significantly among children in our study, suggesting potential exposure from other sources.

Notably, we observed significantly higher levels of urinary pyrethroid metabolites than those reported in data from the 2009–2010 NHANES assessment, even during the organic diet phase. Although participants did not report household pyrethroid use during the study, residential use has been identified as the biggest risk factor for

pyrethroid exposure (Lu et al., 2006b) and it is possible that the high analyte concentrations observed in our populations were due to exposure at school or work, or from residential use that was not reported. However, while pyrethroids are commonly used around homes, schools, and other buildings (Williams et al., 2008), the significant decreases in pyrethroid metabolites among participants in our study indicate that exposure may be at least partially attributable to diet. Our findings are consistent with a study that found that dietary intake, seasonal differences, and household pesticide use all contribute to children's pyrethroid exposures (Lu et al., 2009). Additional research is needed to elucidate the relative contributions of the various sources of pyrethroid exposure among both children and adults.

4.3. Neonicotinoids

Neonicotinoids have become one of the most widely used class of insecticides in the world (Lu et al., 2018), and it is estimated that more than four million pounds of neonicotinoids are applied to cropland annually in the United Sates (Cimino et al., 2017). The 2012 FDA Total Diet study indicated that neonicotinoids were among the most commonly reported pesticide residues in infant and toddler foods (Cimino et al., 2017), however little is known about the health effects of chronic low-dose exposure to these insecticides (Lu et al., 2018). We tested two neonicotinoid compounds: the parent compound clothianidin and the imidacloprid metabolite 5-hydroxy-imidacloprid (5OH-Imd). The most recent Pesticide Use Reporting (PUR) data indicate that in 2016, imidacloprid use on crops in California was nearly 26 times higher than clothianidin use (California Environmental Protection Agency Department of Pesticide Regulation, 2018). Yet, clothianidin was detected in all study participants during the conventional diet phase, while 5OH-Imd was below the limit of detection (LOD) for all study participants (0.1 ng/mL). Furthermore, imidacloprid is frequently found as a residue on food (Chang et al., 2018), suggesting that 5OH-Imd may not be the best biomarker to assess dietary imidacloprid exposure. Previous studies have quantified imidacloprid (Ueyama et al., 2014) and 6-chloronicotinic acid (6CN), a non-specific metabolite of imidacloprid and other neonicotinoids (Göen et al., 2016; Nomura et al., 2013), in urine with detection frequencies > 50%. Future studies should consider measuring the parent compound imidacloprid or metabolites such as 6CN, which may be more reliable indicators of dietary imidacloprid exposure.

Despite the widespread use of neonicotinoids and high detection rates of residues on food samples, this is only the second organic diet intervention study to include neonicotinoids, with the first taking place in Switzerland and enrolling only two participants (Göen et al., 2016). To date, the Centers for Disease Control and Prevention (CDC) has not included neonicotinoids in NHANES testing and there are significant data gaps regarding levels of exposure among children and adults in the United States, underscoring the importance of additional research. Future biomonitoring studies should continue to evaluate exposure to imidacloprid and other common neonicotinoids.

4.4. 2,4-D

Similar to Bradman et al. (2015), we found that levels of the herbicide 2,4-D decreased significantly among children, as well as adults, following the introduction of an organic diet. Several studies have detected 2,4-D in food samples and diet has been identified as an important pathway for 2,4-D exposure among children (Wilson et al., 2003), supporting our finding that the lower urinary levels observed during the organic phase in our study were attributable to lower dietary exposure.

4.5. Food choice comparison

Consistent with findings from Bradman et al. (2015) and Oates et al. (2014), fruit and grain consumption increased slightly following the introduction of the organic diet (See Supplementary information Table S6). Given that fruit and grain are potential sources of dietary pesticide exposure, these minor changes in food choices between the diet phases would not have confounded our results.

4.6. Limitations

This study has several limitations. First, the study has a relatively small sample size of sixteen participants, although 158 urine samples were analyzed. Sampling for the families took place at different times of the year between February and May 2017. Differences in seasonal pesticide use could potentially result in different exposures. However, given the global food supply, we do not expect that seasonal or regional differences in agricultural pesticide use had a significant impact on dietary exposures, and our participants lived in urban or suburban areas. Another limitation of our study is that none of the participants were able to identify whether they or their children were exposed to pesticides outside of the home. Additionally, previous studies have noted higher concentrations of OP metabolite among vegetarians (Berman et al., 2016; Van Audenhaege et al., 2009). However, all participants in our study reported consuming meat during both diet phases, and we do not believe that following a vegetarian diet was a predictor of pesticide exposure in this investigation. Notably, urinary concentrations from non-specific pyrethroid and OP insecticides (e.g. 3PBA, cDCCA, tDCCA and DAPs) may reflect exposure not only to a range of parent pesticide compounds, but also to preformed metabolites from diet and the environment (Chen et al., 2012; Lu et al., 2005; Zhang et al., 2008). It is possible that the decreases observed in urinary concentrations of these non-specific pyrethroid and OP insecticide metabolites were at least partially attributable to lower exposure to preformed metabolites on food during the organic diet phase, which may be less toxic than the parent compounds. Finally, our laboratory methods did not allow measurement of spinosad, the single pesticide approved for organic production with a U.S. EPA food tolerance.

5. Conclusion

We observed significant reductions in urinary levels of thirteen pesticide metabolites and parent compounds representing exposure to OP, neonicotinoid, and pyrethroid insecticides and the herbicide 2,4-D following the introduction of an organic diet. The largest changes were observed for clothianidin and metabolites of malathion and chlorpyrifos. Our study builds on prior research by assessing dietary exposure to pesticides that to date have not been examined in diet intervention studies among both children and adults. Additional research is needed to better understand dietary exposure to neonicotinoids, now the most widely used class of insecticides worldwide.

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Author statements

Asa Bradman, Ph.D. is a volunteer member of the Board of Trustees for The Organic Center, a non-profit organization addressing scientific issues about organic food and agriculture and is also a member of the USDA National Organic Standards Board. Bradman also advises organic

and conventional food producers on issues related to pesticides.

Kendra Klein, Ph.D. is Senior Staff Scientist at Friends of the Earth U.S.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2019.01.024.

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