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

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A R T I C L E I N F O

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A B S T R A C T

Background: A growing set of studies show that an organic diet is associated with reduced levels of urinary pesticide analytes. However, with the exception of one pilot study of two individuals, diet intervention studies to date have not analyzed glyphosate, the most commonly used herbicide in the United States and globally.
Objective: To investigate the impact of an organic diet intervention on levels of glyphosate and its main metabolite, AMPA (aminomethyl phosphonic acid), in urine collected from adults and children.
Methods: We analyzed urine samples from four racially and geographically diverse families in the United States for five days on a completely non-organic diet and for five days on a completely organic diet ($n = 16$ participants and a total of 158 urine samples).
Results: Mean urinary glyphosate levels for all subjects decreased 70.93% (95% CI -77.96, −61.65, $p<0.010$) while mean AMPA levels decreased by 76.71% (95% CI -81.54, −70.62, $p < 0.010$) within six days on an organic diet. Similar decreases in urinary levels of glyphosate and AMPA were observed when data for adults were examined alone, 71.59% (95% CI -82.87, −52.86, $p < 0.01$) and 83.53% (95% CI -88.42, −76.56, $p < 0.01$) and when data for children were examined alone, 70.85% (95% CI -78.52, −60.42, $p < 0.01$) and 69.85% (95% CI -77.56, −59.48, $p < 0.01$).
Conclusion: An organic diet was associated with significantly reduced urinary levels of glyphosate and AMPA. The reduction in glyphosate and AMPA levels was rapid, dropping to baseline within three days. This study demonstrates that diet is a primary source of glyphosate exposure and that shifting to an organic diet is an effective way to reduce body burden of glyphosate and its main metabolite, AMPA. This research adds to a growing body of literature indicating that an organic diet may reduce exposure to a range of pesticides in children and adults.

1. Introduction

Glyphosate is a systemic herbicide first introduced in 1974 (Benbrook, 2016). The uses of this herbicide have expanded rapidly over the years, resulting in a rapid increase in volume sold. Today, it is used as a pre-emergent herbicide with conventional crops and as an over-the-top herbicide with genetically modified glyphosate-resistant crops (Duke and Powles, 2009). It is also used widely as a desiccant, primarily for grains and legumes (Baig et al., 2003; Cessna et al., 2002) and to control weeds both in perennial crops and in urban areas (Richmond, 2018). Today, it is the most widely used pesticide worldwide, including both developed and developing countries (Benbrook, 2016).

Recent research indicates that the increase in use of glyphosate has been paralleled by an increase in exposure of the human population, at least in the US. It was reported that urine glyphosate levels increased more than five-fold from the mid-1970s to 2014, and that the percent of the population with detectable urine glyphosate levels increased nearly 600%, representing more than 70% of the populations (Mills et al., 2017).

Evidence of glyphosate's toxicity has emerged in recent years. The International Agency for Research on Cancer (IARC), an intergovernmental agency which is part of the World Health Organization, classified

Abbreviations: AMPA, Aminomethyl phosphonic acid; IARC, International Agency for Research on Cancer; UN FAO, United Nations Food and Agriculture Organization; LOD, Limit of detection; LOQ, Limit of quantification.

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glyphosate as a probable human carcinogen in 2015 (IARC, 2015). In addition to carcinogenicity, glyphosate has been implicated as an important contributor, among other pesticides, to kidney toxicity, which has led to fatalities among sugarcane workers in Sri Lanka (Jayasumana et al., 2014) as well as Latin America and China (Scammell et al., 2019). Recent animal studies have implicated Roundup®, the herbicide formulation in which glyphosate is the active ingredient, in fatty liver disease (steatosis); endocrine disruption mechanisms may be involved since early signs of steatosis were observed in rats at even ultra-low doses of Roundup® (Mesnage et al., 2017). Additional connections to lipid dysregulation have been highlighted in recently published chemoproteomic and metabolomic studies that were carried out in an in vivo murine model, although tests at lower glyphosate concentrations are required to assess impacts at levels consistent with environmental exposures (Ford et al., 2017). Studies in animal developmental models have implicated the retinoic acid signaling pathway as a route by which glyphosate may act teratogenically (Paganelli et al., 2010). Endocrine disruptive effects have also been observed in male rats, where glyphosate-based herbicides were found to stimulate mammary gland development (Altamirano et al., 2018; Gomez et al., 2019).

Consistent with glyphosate's known antimicrobial effects and with earlier reports of effects on the gut microbiota of livestock eating feed produced from Roundup-treated crops (Krüger et al., 2013; Shehata et al., 2013), recent research has shown that exposure to glyphosate and Roundup significantly alters the gut microbiome of rat pups relative to controls (Mao et al., 2018). Significantly more work will be required in order to interpret these differences, but evidence demonstrates that both glyphosate and Roundup have substantial effects on the developing microbiome that could lead to significant impacts on health.

Several researchers have reported evidence linking glyphosate with oxidative stress. It has been reported that in rats, glyphosate activates the antioxidant defense system (Astiz et al., 2009) and causes lipoperoxidation (Beuret et al., 2005). Similarly, it has been shown that exposure to Roundup also triggers oxidative stress (El-Shenawy, 2009). The mechanism of these effects is suggested by papers demonstrating that glyphosate uncouples mitochondrial energy transduction (Olorunsogo, 1990; Olorunsogo et al., 1979), although later work comparing glyphosate and Roundup observed uncoupling effects only with Roundup (Peixoto, 2005). Similarly, oxidative damage was found to be much greater with Roundup than with glyphosate alone (Gehin et al., 2005). These and a number of other toxic effects of glyphosate and AMPA, including neurotoxicity and reproductive toxicity, have been reviewed (Mesnage et al., 2015).

Although AMPA is much less studied than glyphosate, several researchers have found that AMPA is potentially toxic. AMPA was shown to be genotoxic to human cells in culture (Mañas et al., 2009) and in the fish Anguilla Anguilla L. causing both DNA and chromosomal damage (Guilherme et al., 2014). AMPA was also shown to alter cellular and biochemical parameters of the mussel *Mytilus galloprovincialis* (Matozzo et al., 2018) and to cause damage to the gills as well as steatosis and chromosomal damage to the liver of the fish *Poecilia reticulata* (Antunes et al., 2017). In earthworms, AMPA reduced growth at environmentally relevant concentrations (Domínguez et al., 2016).

In recent years, dietary interventions examining urinary levels of a number of pesticides have been carried out in which the subjects' standard diet was partially or entirely replaced with organic food (Bradman et al., 2015; Curl et al., 2019; Fenske et al., 2002; Göen et al., 2017; Hyland et al., 2019; Lu et al., 2008, 2006; Oates et al., 2014). Other studies have compared urinary pesticide levels in participants that self-report eating either a primarily conventional or primarily organic diet (Curl et al., 2015, 2003). This approach has been taken because a growing body of evidence indicates that organically grown foods have lower levels of pesticide residues compared to conventionally produced foods (Baker et al., 2002; United States Department of Agriculture, 2016). Organic foods are produced in accordance to strict regulations governed by the U.S. Department of Agriculture which prohibit use of approximately 900 pesticide active ingredients allowed in conventional production (USDA National Organic Program, 2020).

Although much more needs to be learned regarding the health impacts associated with chronic dietary exposure to specific pesticides, longitudinal diet intervention studies have identified statistically significant improvements in health outcomes linked to diet modification. For instance, an investigation of nearly 70,000 adults reported that increased frequency of organic food consumption was correlated with reduced incidence of various types of cancer (Baudry et al., 2018). Other studies have reported decreased risk of diabetes (Sun et al., 2018) and improved fertility treatment outcomes (Chiu et al., 2018) associated with higher frequency of organic food consumption.

Diet interventions have provided consistent evidence that an organic diet reduces exposure to pesticides. The present study extends this research model to glyphosate and its main metabolite, AMPA (aminomethyl phosphonic acid).

2. Methods

This is the second part of a two-part study in which the same urine samples were analyzed. In the first part, a set of fourteen pesticide analytes were measured, representing organophosphates, pyrethroids, neonicotinoids and 2,4-D (Hyland et al., 2019). The same research procedures were used in both studies for selection of study participants, data collection, dietary intervention and urine collection, and are described in Methods Sections 2.1 through 2.4.

2.1. Study participants

We recruited four racially diverse families in four locations: Oakland, CA, Minneapolis, MN, Baltimore, MD, and Atlanta, GA. Each family had between three to five members. Families were originally contacted via a recruitment email that explained the study purpose and procedures. Families contacted study staff if they were interested in participating, and a phone script was read to screen for eligibility, including: 1) willingness to alter the family's diet for six days, 2) two to three children between the ages of three to eighteen living at home, 3) all children toilet-trained, 4) ability to prepare all family members' breakfast, lunch, and dinner at home during the organic diet phase, 5) English speaking, 6) no pregnant family members, 7) no severe food allergies, and 8) not typically organic food consumers. Families participated in the study between February and May 2017. The study was approved by the Western Institutional Review Board. Written informed consent was obtained from parents before data collection began. Families were provided with a gift card to a grocery store of their choice after participating.

2.2. Data collection

Families participated in the study over the course of twelve consecutive days (see Fig. 1). Before beginning, researchers conducted online video calls with each family and provided them with instructions on urine collection and food diaries. Food diaries included information about food type and portion size. One adult in each family also completed a questionnaire by phone to collect information about pesticide use and storage in and around the home, proximity of the home to golf courses and other locations known to use pesticides, and possible occupational exposure to pesticides.

2.3. Dietary intervention

During days one through five, study participants followed their typical conventional diet (conventional phase). During days six through eleven, participants were provided with certified organic food while at home, work, school, or daycare (organic phase). Diet substitution included all beverages other than water, all food categories, and oils,

Day of Study	1	2	3	4	5	6	7	8	9	10	11	12
Participant Diet*	C	C	C	C	C	O	O	O	O	O	O	**
Samples Analyzed		X	X	X	X	X		X	X	X	X	X

Fig. 1. Schedule of organic diet intervention. *C = conventional diet phase, O = organic diet phase. ** Participants were free to choose their diet on day 12 since urine samples were collected first thing in the morning.

condiments, and spices. The final urine samples were collected on the morning of day twelve, after which participants could choose to eat either organic or conventional food. Organic food was provided to families in two ways: 1) research assistants delivered organic groceries to participants' homes based on shopping lists compiled by each family for one week's worth of groceries, and 2) a licensed chef or caterer prepared dinners during the organic phase, and these were delivered to participants' homes by research assistants. All food during the organic phase was provided free of charge to the families.

2.4. Urine collection

Participants were given urine collection instructions prior to the start of the study, and 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 home freezer. Research assistants picked up the frozen urine samples and shipped them overnight on dry ice to the laboratory for each phase of the study.

One family repeated the conventional phase of the study after the organic phase (with washout time) because the first batch of samples thawed as a result of an error in the chain of custody.

2.5. Laboratory analysis of urine samples

Analysis of glyphosate and AMPA in urine specimens was performed at Health Research Institute, Fairfield, IA using an isotope dilution methodology accredited according to ISO/IEE 17025 and to the US EPA CLIA program. Separations were carried out using a Shimadzu Nexera X2 ultra high-performance liquid chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) linked to a Bio-Rad (Hercules, CA, USA) Cation-H guard column (30 mm by 4.6 mm). Mass spectrometry was carried out using a QTRAP 5500 triple quadrupole instrument from AB Sciex (Framingham, MA, USA).

Quantification was carried out using a previously described method (Jensen et al., 2016) modified to achieve greater sensitivity. The modifications lowered the limit of quantification for glyphosate and AMPA from 0.100 to 0.050 ng/ml and the limit of detection from 0.023 to 0.020 ng/ml for glyphosate and from 0.033 to 0.013 ng/ml for AMPA. In brief, isotopically labeled internal standards of glyphosate (^{13}C,^{15}N) (Cambridge Isotope Laboratories, Andover, MA, USA) and AMPA (^{13}C, ^{15}N, D_2) (Sigma-Aldrich, St Louis, USA) were added to urine samples, which were also adjusted to 0.05% formic acid. Samples were centrifuged at 14,800 rpm for 10 min to sediment particulates and were transferred to polypropylene vials for LC-MS/MS analysis. Samples were corrected for dilution using specific gravity using a BlueTooth enabled refractometer (ATAGO, Tokyo, Japan).

Quality control (QC) procedures included the following: (1) analysis of certified reference material (LGC, Lancashire, UK)) diluted into control urine at moderate concentrations (~0.4 ng glyphosate/ml and ~0.80 ng AMPA/ml) at the beginning and end of the run, and at low concentrations (~0.04 ng glyphosate/ml and ~0.08 ng/ml AMPA) at the beginning and end of each run and following every 10 samples during the run, with ±20% agreement between measured and declared values; (2) matrix-matched calibration curve for glyphosate and AMPA (certified reference materials from Sigma-Aldrich, St. Louis, USA) from 0.025 ng/ml to 50.0 ng/ml placed at the beginning of each run, with a repeat of the 2.5 and 0.25 ng/ml points at the end of the run, with ±20% agreement between measurements; (3) duplicate analysis of 10% of samples and repeat of analyses in cases where duplicates diverged by more than 30%; (4) verification that the raw counts of the highest and lowest points in the calibration curve met criteria for between-run consistency for both glyphosate and AMPA.

For samples with glyphosate and AMPA concentrations below the LOD, concentrations were set at the LOD divided by the square root of 2 (Hornung and Reed, 1990). For samples with glyphosate and AMPA concentrations below the LOQ but above the LOD, concentrations were set at 50% of the LOQ.

This method has been accredited to ISO 17025 by a third-party accreditation body and is also included under Health Research Institute's high-complexity CLIA certification, managed through the Centers for Disease Control and Prevention.

2.6. Data analysis

Days one and seven were considered washout days, therefore, urine samples from these days were excluded from the analysis. We analyzed ten urine samples from each of the participants — five from the conventional phase and five from the organic phase. One participant was missing a sample from the eighth day (organic phase) and one was missing samples from the eighth, tenth, and twelfth days of the study (organic phase). For these two participants, we included the urine sample from day seven, the first day of the organic phase. This resulted in a total of 158 urine samples in the analysis.

Log-transformed analyte concentrations were used for statistical analyses. We used linear mixed effects models to calculate the percent change in analyte concentrations from the conventional to organic diet phase, which accounts for correlation among repeated urine samples from the same individual. Percent change was calculated using the formula % Change = [exp(β)-1]*100; β is the regression coefficient for organic diet from the mixed effects models.

2.7. Sensitivity analysis

We conducted sensitivity analyses in which we 1) used specific gravity-adjusted analyte concentrations, and 2) omitted outliers based on model standardized residuals >3 or < -3, and 3) excluded residuals. Results from these sensitivity analyses did not differ appreciably from results in our main analyses (See Supplementary Information Tables S1, S2, and S3).

3. Results and discussion

3.1. Demographic characteristics

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

3.2. Household pesticide use during study

Within three months of beginning the study or during the study period, none of the families reported using pesticides inside their home. One week prior to beginning the study, one family reported hiring a pest control company to treat termites on the exterior of their foundation and at entry points. The company sprayed Premise 75, which has imidacloprid as the active ingredient.

3.3. Urinary measurements

Glyphosate was detected in 93.7% of urine samples tested, and AMPA was detected in 96.9% of samples. Glyphosate levels ranged from non-detected to 6.22 ng/ml and AMPA levels ranged from non-detected to 1.96 ng/ml.

3.4. Effect of diet on urinary pesticide levels

Following the organic diet intervention, we observed significant decreases in urinary levels of both glyphosate and AMPA in both adults and children. As shown in Table 1, urinary levels of both glyphosate and AMPA consistently dropped following the transition from a conventional diet to an organic diet. Mean urinary glyphosate levels for all subjects decreased 70.93%, while mean AMPA levels decreased by 76.71%. Similar decreases were observed when data for adults were examined alone (71.59% and 83.53%) and when data for children were examined alone (70.85 % and 69.85 %). Confidence intervals for these measurements are presented in Table 1. In all cases, the p-values for diet-linked changes in urinary glyphosate and AMPA levels were less than 0.01.

An earlier pilot study examined urinary levels of glyphosate and AMPA in two subjects in Switzerland during an organic diet intervention (Göen et al., 2017), however, the starting levels of glyphosate were quite low, and a significant change was reported in only one of them. The lack of consistent change in glyphosate in this study may well be due to the low level of glyphosate reported in the conventional diet phase of the study. We observed much higher levels of glyphosate and AMPA during the conventional diet phase and consistent and substantial reductions in glyphosate and AMPA levels following transition to an organic diet.

Fig. 2 summarizes average urinary glyphosate and AMPA levels day-by-day during the course of the dietary intervention, showing that the transition from conventional to organic diet brings about a rapid drop in glyphosate and AMPA levels. The transition is essentially complete after the second day of shifting to the organic diet. Days 1 and 7 were considered wash out days and were not included in the analysis.

Fig. 3 presents the mean and 95th CI for urinary levels of glyphosate and AMPA among all participants, adults, and children. As shown in Fig. 4, the absolute levels of glyphosate differed for different subjects, both adults and children, as did the absolute magnitude of the change following transition to an organic diet. In all cases except one, the direction of change was consistent, showing a substantial drop in glyphosate following the transition to an organic diet. In the case of the exception, the overall trend during the organic phase was downward except for a spike in urinary glyphosate level on day 9, which may be due to consumption of non-organic food recorded in the subject's food diary.

3.5. Alignment with previous findings

The results presented here represent the second part of a two-part study. Our findings align with earlier results reported by Hyland et al. (2019) which found significant reductions in urinary levels of thirteen pesticide metabolites and parent compounds representing organophosphate, neonicotinoid, and pyrethroid insecticides and the herbicide 2, 4-D following the introduction of an organic diet. Together, these results provide the most comprehensive analysis to date of rapid and dramatic reduction in exposure to a wide range of the most commonly used pesticides in U.S. agriculture resulting from an organic diet intervention.

3.6. Comparison of children and adults

It is well-established that children are more vulnerable to pesticide exposure than adults (Landrigan and Goldman, 2011). We observed that urine glyphosate and AMPA levels were substantially higher in children than in their parents. This held true for both the conventional and the organic diet phases of the study. As shown in Table 1, the average level of glyphosate for children during the conventional phase was 1.03 ng/ml, and levels as high as 6.22 ng/ml were observed, while levels in adults averaged 0.26 ng/ml with a maximum of 0.82 ng/ml. During the organic diet period, the levels in children averaged 0.25 ng/ml with a maximum of 2.80 ng/ml, while the levels for adults averaged 0.04 ng/ml with a maximum of 0.91 ng/ml. In addition to higher levels of glyphosate and AMPA, variation from subject to subject was substantially greater among children than adults. The higher levels could conceivably be due to reduced dietary compliance, however, great care was taken in assuring that children would have negligible motivation or opportunity to consume conventional foods during the organic diet phase, and the food diaries kept by each participant reflected a high level of compliance with the organic diet. The higher levels of glyphosate and AMPA observed in children could also have been due to a non-dietary source of glyphosate exposure that children may be more likely to encounter than adults, such as environmental exposure on school grounds and in parks. Research indicates that children are less efficient at metabolizing certain organophosphate pesticides, raising (Huen et al., 2010) an important line of inquiry for future research on glyphosate.

Table 1
Urinary analyte concentrations (ng/mL) and percent change from conventional to organic diet.

Analyte	Conventional				Organic				Percent change	*p*-Value
	n	Mean	Median (IQR)	Max	n	Mean	Median (IQR)	Max	(95% CI)	
All (n = 16)										
Glyphosate	80	0.83	0.51 (0.19, 1.13)	6.22	78	0.30	0.12 (0.04, 0.36)	2.80	−70.93 (−77.96, −61.65)	<0.01
AMPA	80	0.59	0.43 (0.26, 0.85)	1.96	78	0.18	0.12 (0.03, 0.27)	1.03	−76.71 (−81.54, −70.62)	<0.01
Adults[a] (n = 7)										
Glyphosate	35	0.26	1.19 (0.09, 0.41)	0.82	33	0.09	0.04 (0.04, 0.09)	0.91	−71.59 (−82.87, −52.86)	<0.01
AMPA	35	0.32	0.27 (0.12, 0.40)	1.33	33	0.06	0.03 (0.03, 0.07)	0.28	−83.53 (−88.42, −76.56)	<0.01
Children[b] (n = 9)										
Glyphosate	45	1.27	1.03 (0.51, 1.71)	6.22	45	0.46	0.25 (0.12, 0.51)	2.80	−70.85 (−79.36, −67.15)	<0.01
AMPA	45	0.80	0.79 (0.40, 1.05)	1.96	45	0.27	0.20 (0.14, 0.37)	1.03	−69.85 (−77.56, −59.48)	<0.01

Abbreviations: CI, confidence interval; IQR, interquartile range.

[a] Adults in study 36–52 years old.

[b] Children in study 4–15 years old.

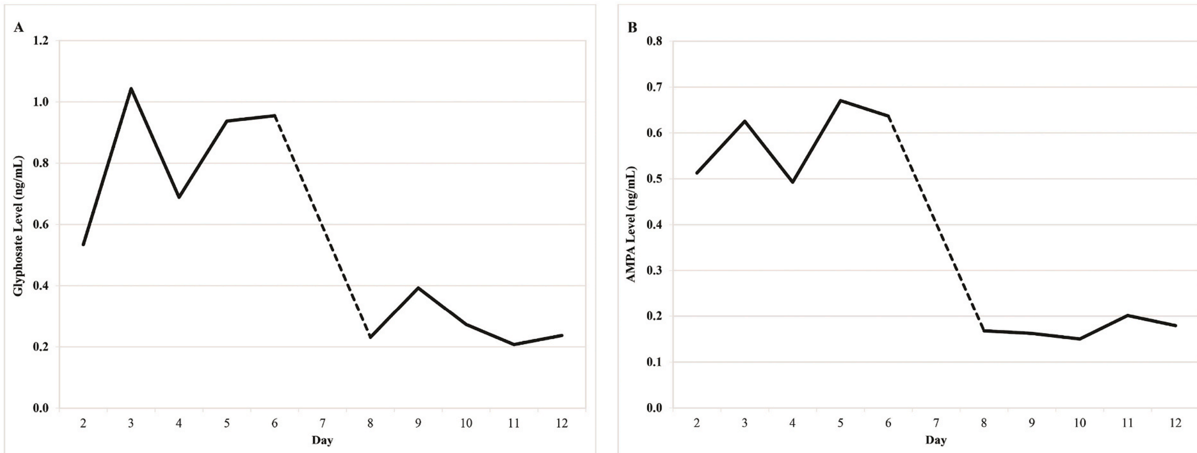

Fig. 2. Average urinary levels of glyphosate and AMPA by day. Average levels of glyphosate (A) and AMPA (B) for all subjects are plotted by day. Days 2 through 6 correspond to the period during which subjects consumed a diet of conventional food. Days 8 through 12 correspond to the period during which subjects consumed a diet of certified organic food. Days 1 and 7 were transition days and were not included in the analysis.

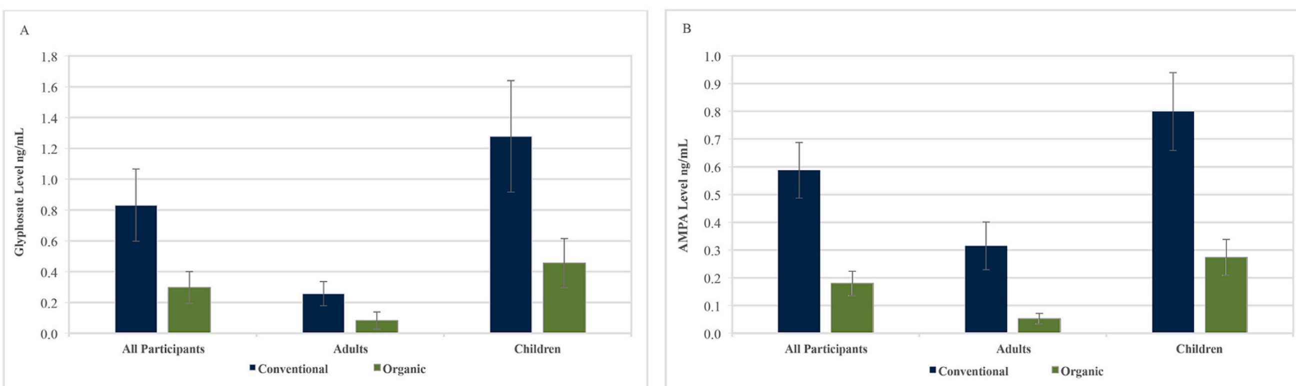

Fig. 3. Estimated mean and 95% CIs for urinary analytes during conventional and organic diet phase among all participants, adults, and children.

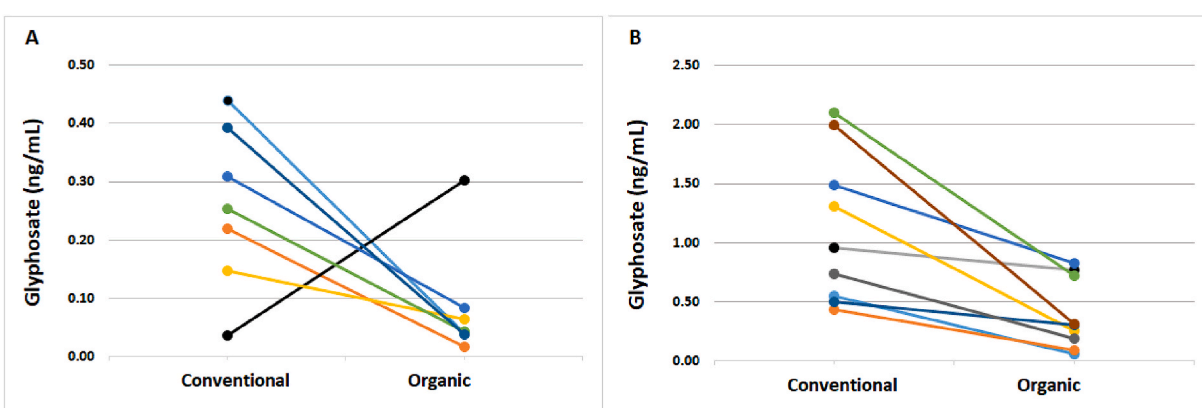

Fig. 4. Average urinary levels of glyphosate paired by subject during conventional and organic diet phase. Averaged levels of glyphosate for each subject over a 5-day period during which the subject consumed a conventional diet and a 5-day period during which the subject consumed a certified organic diet. Panel A is adults in study 36–52 years old. Panel B is children in study 4–15 years old.

3.7. 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 S4). We do not expect that minor changes in food choices between the diet phases confounded our results.

3.8. Limitations

While the study included only 16 participants, we had a sufficient number of samples (158) to draw statistically significant conclusions.

Because of the longitudinal design of the study, each participant served as their own control. These repeated measurements under both the control condition (conventional diet) and experimental condition (organic diet) made it possible to conclude with confidence that the organic diet resulted in statistically significant reductions in glyphosate and AMPA levels. Analysis of these samples also allowed us to gain a rough estimate of the rate at which urinary levels of glyphosate drop to a baseline when dietary input of glyphosate is stopped or drastically reduced by shifting to an organic diet. It is approximately 2–3 days, which is roughly consistent with the elimination half-life for glyphosate reported in rats (Anadón et al., 2009; Brewster et al., 1991). However, it would be necessary to test many more samples during that transition time to assess the kinetics of this reduction more accurately and answer questions such as whether the rate differs from individual to individual.

Sampling for the participating families took place at different times of the year between February and May. If the environment had been the primary source of glyphosate contamination, these seasonal variations could have been significant influences on urinary glyphosate levels. However, the participants lived in urban or suburban areas and, therefore, were not likely affected by seasonal agricultural use. Since the food system has become globalized it tends to deliver a relatively consistent product to the consumer, independent of the time of year. It should be pointed out, however, that participants did not have access to information about use of pesticides in work, school, or public settings they frequented during the study, therefore environmental exposure was not controlled.

4. Conclusion

This study builds on existing research by assessing the impact of an organic diet intervention on urinary levels of glyphosate and its main metabolite, AMPA. Glyphosate is the most widely used herbicide worldwide. This study is the first of its kind, although a 2-subject pilot analysis was reported previously (Göen et al., 2017).

Two new findings emerge from this work. First, in both children and adults, organic diet intervention led to a rapid decrease in urinary glyphosate and AMPA levels within three days. This demonstrates that diet is the primary source of glyphosate exposure for the general population and that controlling dietary input by shifting to an organic diet is a clear-cut approach to reducing exposure. Second, we found that levels of glyphosate and AMPA were higher in children than in adults. This is important because it reaffirms the importance of protecting young people from exposure to glyphosate as well as other pesticides.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.109898.

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