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Dietary profiles, organic food consumption, and urinary glyphosate levels in a cohort of postmenopausal women in Orange County, California

Author
Huang, Wei-Lin

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Dietary profiles, organic food consumption, and urinary glyphosate levels in a cohort of postmenopausal women in Orange County, California

THESIS

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MASTER OF SCIENCE

in Biomedical and Translational Science

by

Wei-Lin Huang

Thesis Committee:
Assistant Professor Hannah Lui Park, Ph.D., Chair
Professor Sheldon Greenfield, M.D.
Assistant Professor Andrew Odegaard, Ph.D.

2019

DEDICATION

To

My Loved Family and Friends

and

Science

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ABSTRACT OF THE THESIS

Dietary profiles, organic food consumption, and urinary glyphosate levels in a cohort of postmenopausal women in Orange County, California

By

Wei-Lin Huang

Master of Science in Biomedical and Translational Science

University of California, Irvine, 2019

Assistant Professor Hannah Lui Park, Ph.D., Chair

Background: Health concerns surrounding the herbicide, glyphosate, have increased recently due to its widespread use in agriculture and lack of regular surveillance programs in the food supply. However, studying the potential association between organic food consumption, which should decrease one's exposure to glyphosate, and human health may be complicated due to confounding by the profiles of organic food consumers. Thus, understanding organic food consumer profiles is crucial. In addition, it is unknown whether self-reported organic food consumption frequency is indicative of one's actual exposure to glyphosate.

Objective: To identify demographic, health-related, and diet-related factors associated with organic food consumption and to determine the association between self-reported organic food consumption frequency and urinary glyphosate levels.

Method: 375 women in Orange County were ranked into three groups based on their self-reported frequency of organic food consumption. Factors associated with frequency of organic food consumption, and urinary glyphosate levels across groups were analyzed.

Results: Self-reported organic food consumption frequency was associated with education, BMI, and healthy diet. We found highest urinary glyphosate levels in the sometime organic food consumers among the three groups, but it was not statistically different. However, grain intake was positively associated with glyphosate levels in infrequent organic food consumers.

Conclusion: The organic food consumer characteristics we observed were consistent with previous findings, and must be considered as confounders when studying the potential health effects of organic food consumption. We observed associations between urinary glyphosate levels and the diet, but our study findings should be confirmed in a larger population.

INTRODUCTION

The Rising Popularity of Organic Food

The United States became the most significant agricultural product exporter in the world due to advancements in mechanization along with chemical and pharmaceutical agents. This mechanization enlarged the scale of production and decreased the workforce that was needed. Use of pesticides and fertilizers dramatically increased the yield of crops. The use of pesticides on crops increased by 143 % from 1964 to 1976[1].

As evidence accumulated that using fertilizers and pesticides may cause adverse effects on the ecosystem and human health[2], organic farming was introduced to the market in many parts of the world, aimed at producing crops and livestock without depending on synthetic fertilizers and pesticides[3]. The criteria for organic food farming varies from country to country. In the United States, the standards for organic food production and handling are made and regulated by the United States Department of Agriculture (USDA), including no use of most agrochemicals (synthetic pesticides, soluble fertilizers, and growth regulators) or genetically modified organisms, and limited use of veterinary medicine and pesticides. Only certain substances are approved for organic farming, such as some naturally occurring ingredients and some synthetic substances used for equipment cleaning[4].

The scale of organic food production ranges from small local farms to large high-technology enterprises located in over 178 countries in 2017. Worldwide, the area of land used for organic agriculture has steadily grown. From 1999 to 2017, the organic agriculture land increased six-fold. Australia, Argentina and China have the largest agricultural area for organic crops, which account for 43.06% of organic agriculture land worldwide. 90% of organic product sales are in North America and Europe. According to the latest survey in 2017, the United States has the most abundant organic food market in the world, which was valued at 43 billion US dollars, 4 times more than Germany, which is ranked second[5].

The Motivation and Profiles of Organic Food Consumers

Several factors have been shown to drive people to choose organic foods including health, taste, and animal welfare[6] as well as environmental concerns[7]. For example, studies have shown differences in nutrient composition between organic and conventional products. 12 of 15 reviews or meta-analyses showed that organic foods have higher concentrations of vitamin C, total antioxidants and total omega-3 fatty acids[8]. The belief in healthfulness of eating organic food is the most frequent motivation. This result was not only found in developed countries, including Poland[9], Australia[10], Sweden[11], Denmark[12], and the United States[13,14] but also found in developing countries, such as India[15] and China[16].

Several studies conducted in Europe have shown that organic food consumption is associated with healthier dietary habits and lifestyles. People who consume organic food tended to be physically active, non-smokers and consume less alcohol[17–19]. Studies from France[17], Germany[19], Denmark[18], and Norway[20] showed that organic food consumers tended to consume more plant-based foods and less sweets and meat products than conventional food consumers. The Nutrinet-santé Cohort Study in France also demonstrated that organic food consumers exhibited a dietary pattern that more closely adheres to dietary guidelines compared to conventional food consumers[17].

Food safety is also a very important factor that drives consumers to choose organic food[11,21]. Several systematic reviews have confirmed that organic foods are less likely to have pesticide residues compared with conventional foods,[22,23] and diet intervention studies have also shown that consumption of organic food results in lower urinary organophosphorus pesticide levels compared to conventional foods[24–28].

Although differences in composition and pesticide residues between organic and conventional food have been demonstrated, the evidence supporting a potential health effect of consuming organic food in humans is greatly insufficient[29]. Few intervention and observational studies have focused on the association between health and organic food consumption. The biggest challenge for observational studies to address this question is the residual confounding. For example, organic food intake has been shown to be confounded by health behaviors and socioeconomic factors. Organic food consumers have been shown to have healthier lifestyles, dietary intake, and socioeconomic advantage[17–20]. Thus, in order to determine if organic food intake is associated with health, the first critical step is to understand the profiles of organic food consumers so that factors associated with organic food consumption can be adjusted for in the analysis.

The Safety of Using Pesticides

Besides the nutrition advantage of organic food, low risk of pesticide exposure is another important advantage that people value in organic food. Many studies have shown the association between use of some types of pesticides and health issues, including negative effects on the skin and in the digestive, nervous, hepatic, circulatory, respiratory, reproductive and endocrine systems as well as on genetic integrity[30–34]. Some pesticides were abandoned due to their severe effects on human health and the environment. For example, dichlorodiphenyltrichloroethane (DDT), an organochlorine pesticide, the most commonly used pesticide from 1940 to 1960, was banned in 1972 by the United States Environmental Protection Agency (EPA)[35] due to its persistent negative effect on the environment and human health[36–38]. Organophosphates, another type of pesticide, are considered more environmentally-friendly than organochlorines, but research has shown that several serious health effects are associated with organophosphate exposure, including cardiovascular disease[31], male reproductive dysfunction[39], neuropsychological disorder[40], dementia[41] and non-Hodgkin's lymphoma[42].

The Safety Concerns of Glyphosate

The safety of glyphosate is controversial. Glyphosate, which is a broad-spectrum systemic herbicide, is the most heavily used herbicide in the United States[43]. When it was first introduced to the market in 1974, glyphosate was only used to kill off weeds before planting crops, which would have likely only resulted in very low exposure in humans through ingestion. Based on the observations that 1) no significant toxicity has occurred in acute, sub-chronic, and chronic studies; 2) dermal absorption of glyphosate and its plant metabolite, Aminomethylphosphonic acid (AMPA), is low; 3) Glyphosate and AMPA are eliminated from humans unmetabolized; and 4) the accumulation rates of glyphosate and AMPA in any animal tissue are low, regulatory agencies concluded that glyphosate has low toxicity in humans[44]. However, because glyphosate is now also used as a desiccant on pre-harvest crops, and, because of the introduction of genetically modified glyphosate-tolerant crops (genetically modified organisms [GMOs]), its use has greatly expanded. Glyphosate-tolerant crops survive against glyphosate exposure because they can metabolize glyphosate, and the primary metabolite is AMPA. Glyphosate is now widely used on 70 fruit and vegetable items, including maize, soybean, canola wheat, barley and edible beans[45]. The use of glyphosate has increased nearly 100-fold since 1974[46], which has resulted in high levels of residues of glyphosate and AMPA on many crops as well as in processed foods[47]. A report from the U.S. Department of Agriculture (USDA) in 2011 revealed that glyphosate residues were found in 90.3% of 300 soybean samples, and AMPA was found in 95.7% of samples[47]. Bohn et al. also showed that glyphosate-tolerant soybeans contain high levels of glyphosate residues[48]. Other studies have demonstrated that glyphosate residues can be found in different popular U.S. food products[49,50] , farm animals[51,52], baby and children's food, and even tap water[53]. The urinary levels of glyphosate that have been observed in humans also reflect these results. Mills et al. found that urinary glyphosate levels in older adults increased from 1993 to 2006. The prevalence rate of finding urinary glyphosate levels above the limit of detection (LOD) also increased[54].

However, the glyphosate residue levels in and on raw food, including fruits, vegetables and grains, are not regularly monitored by USDA, which conducts the national-wide pesticide surveillance program, resulting in a severely limited capacity to estimate individuals' glyphosate exposure through their diet.

Research Gap and Research Questions

Although several studies have indicated the differences in composition and pesticide residues between organic and conventional food, there is limited evidence on the potential health effect of organic food consumption in humans. One of the biggest challenges is that organic food consumption may be confounded by a healthy lifestyle, including a healthy diet, and socioeconomic status. For example, European studies have demonstrated that higher organic food consumption is associated with healthier dietary habits, including less meat, less sweets and alcohol, and more vegetable and fruit[55]. However, there is limited research comparing the dietary profiles between organic food consumers and conventional food consumers in the U.S.[13,14], which has the biggest organic food market in the world. Since there are differences in health behaviors[56] and lifestyles between Americans and Europeans, results from the European studies may not be suitable for generalizing to the U.S. population. Therefore, a critical first step to studying the potential health effects of organic food consumption is to understand the overall profiles of organic food consumers in the U.S. in order to identify potential confounders.

Another challenge is that assessment of one's glyphosate exposure can only currently be done via biochemical analysis of urine samples. This would necessitate the availability of urine samples or collection of new samples in a cohort in order to study the potential association between glyphosate exposure and health outcomes. Several studies have demonstrated that there is an association between organic food consumption and several pesticide exposures in humans[24–27,57], but the evidence on glyphosate exposure is limited. A better understanding of the relationship between organic food consumption and glyphosate exposure is needed.

Hypothesis

Based on the evidence from European studies that organic food consumers have a healthier lifestyle, namely, lower BMI, higher physical activity, non-smoker, and higher diet quality, I hypothesize that the organic food consumers in Orange County, CA, U.S., will also have a healthier lifestyle. Moreover, based on the evidence that exposure of humans to pesticides in a non-agricultural setting is mainly through the diet, I hypothesize that frequency of organic food consumption will be negatively associated with urinary glyphosate levels. Based on the fact that glyphosate is commonly used in plant cultivation, I also hypothesize that the intake of non-organic fruit, vegetable and grains will be associated with increased urinary glyphosate levels.

Research Objectives

To test these hypotheses, I will collect data from 400 women in Orange County, CA. This will include demographic and dietary information, including socioeconomic status, eating habits, dietary intake, and organic food consumption frequency. Glyphosate exposure will be measured using urine samples. First, I will identify the demographic characteristics associated with self-reported frequency of organic food consumption and examine whether eating habits, dietary intake and diet quality are associated with self-reported frequency of organic food consumption. Second, I will determine if there is an association between urinary glyphosate levels and self-reported organic food consumption. Then, I will examine the relationship between urinary glyphosate levels and fruit, grain and vegetable intake, stratified by self-reported frequency of organic food consumption. Finally, the association between urinary glyphosate levels and diet quality will be examined.

Chapter 1 – Background

1-1. The advantages of consuming organic food

Two factors are important for belief in the healthfulness of organic food: that there is an inherent nutritional advantage of organic food, and that consuming organic food avoids chemical exposures, such as pesticides. Systematic reviews and meta-analyses which compared the nutrient composition between organic and conventional crops varied in their scope, inclusion criteria and statistical methods, but there is generally an agreement between them[22,23,58,59]. Lower total nitrate and higher phosphorus in organic crops compared to conventional crops have been consistently found in these systematic reviews[23,59]. Level of vitamin C, which has been focused on most, is higher in organic crops compared with conventional crops[22,23]. Systematic reviews and meta-analyses have also demonstrated the increase of polyphenols, which play an important role in preventing diseases including cardiovascular disease (CVD), neurodegeneration and cancer, in organic crops compared to conventional crops[22,23,58]. In terms of cadmium and other toxic metals, Smith-Spangler et al.[22] and Barański et al.[23] showed conflicting results. Smith-Spangler's research did not show a significant difference in cadmium level, while Barański's result indicated the reduction of cadmium in organic crops compared to conventional crops. Both studies agreed that there are no differences in the levels of lead, mercury and arsenic between organic and conventional crops[22,23]. For animal-based foods, fatty acids have received the most attention. Most of the groups focused on the composition of omega-3 fatty acids, which have been considered an important factor for human health[60]. A meta-analysis reported a higher content of total omega-3 fatty acids in organic cow's milk than conventional milk[61], which was consistently observed in other studies[22,62]. There is no available systematic review comparing the fatty acid difference between organic and conventional eggs. A meta-analysis conducted by Średnicka-Tober's group indicated the significantly higher total PUFA and omega-3 PUFA in organic meats compared to conventional meats[63].

Regarding chemical exposures, pesticides are the most common chemicals that consumers will be exposed to when consuming plant foods from conventional farming. In contrast, organic farming, which is done without using synthetic fertilizers and pesticides, gives consumers a lower chance of exposure to pesticides. Reports from both European[64] and U.S[65] pesticide surveillance programs have shown lower pesticide contamination in organic crops than conventional crops. Besides, systematic reviews have consistently found reduced pesticide levels in organic crops[22,23,66]. Intervention studies also showed a similar conclusion. Controlled feeding studies in children and adults have indicated that the consumption of organic foods resulted in lower urinary pesticide levels compared to the groups that consumed conventional foods[24–27]. Research from Curl's group, which estimated exposure to organophosphate pesticides through dietary intake, also showed that there is an association between dietary pesticide exposure and organic food consumption[57].

1-2. Dietary profiles, lifestyle factors and socioeconomic status between organic food and conventional food consumers – what is currently known

Likely due to the popular belief that organic food is healthier than conventional food, most of the research has shown that organic food consumers tend to have healthier diets, more awareness on nutrition, higher education and income, lower body mass index, and are more physically active than people who seldom or never consume organic food. The Nutrinet-

Santé Cohort Study from France is probably the leading group that has focused on the characteristics of organic food consumers. Their studies have shown that regular organic food consumers are more educated and physically active[17]. Regular organic food consumers were more aware of nutrition guidelines recommending plant-based foods rather than animal-based foods and had better diet quality[67]. In addition, regular organic food consumers consumed more plant-based foods and less sweets, alcoholic beverages, processed meat, and milk. Their nutrient intake profiles were also healthier than non-organic food consumers[17,55]. Research from other European countries, such as Germany[19], Denmark[18], and Norway[20,68] also showed similar results. However, some research showed that the pattern of organic food consumers was more complicated and not easy to generalize. Subgroup analysis showed the two-sided trend that both young adult (<25 years) and older adult (> 40 years) age groups were most likely to consume organic food, which does not follow the typical age gradient[14,68]. Households with children were more likely to consume organic food than ones without children[21]. Race and ethnicity may also be associated with organic food consumption. Dettmann's study showed that African American families were less likely to buy organic food than white families. However, among African Americans who did purchase organic food, they spent the greater proportion of their vegetable budget on organic vegetable[13], which is similar to Zepeda's result[69].

However, Torjusen et al. showed an interesting result that frequency of organic food consumption was positively associated with prevalence of smoking and use of alcohol in pregnant women in Norway, which are not considered to be healthy lifestyle factors[68]. This suggests that organic food consumers may not lead a universally healthy lifestyle, particularly in subgroups such as pregnant women[68]. A Finnish group tried to understand the association between a combination of health indicators and good health, and found it difficult. Although the health indicators were associated with good health, the combination of health indicators were quite diverse in participants[70]. For example, smoking has been considered as a main factor on health, but the majority of smokers had either no or one additional unhealthy habit[70]. In addition, diets may change based on the health conditions. For example, Alfano et al. found that there were dietary changes made after diagnosis with cancer or having cancer-related symptoms in breast cancer survivors[71]. Overall, these results demonstrate the complexity of profiling organic food consumers, which may be affected by culture, age, education, race/ethnicity, household status, income, health status, and life experience.

1-3. The health impact of consuming organic food

Studies examining the association between organic food consumption and health outcomes are limited. Issues in methodology may be one of the reasons why. There have only been a few long-term interventional studies, focused on the association between organic food consumption and health, presumably largely because of the high cost. Recent intervention studies were only based on small populations and had short follow-up time, which results in limited statistical power and inability to determine the potential long-term effects of organic food consumption. Six intervention studies included in Smith-Spangler's meta-analysis showed no difference in plasma or urinary carotenoids, polyphenols, vitamins E and C levels, low-density lipoprotein cholesterol, antioxidant activity ability to protect against DNA damage, or immune system markers between organic and conventional food consumers[22].

There are some challenges to observational studies of organic food consumption, including the lack of a suitable biomarker and measurement error when evaluating the intake of organic food. The absence of a biomarker increases the difficulty of assessing chemical exposure. The intake of organic food can only rely on self-reported questionnaires, which makes the measurement prone to have measurement error. Moreover, the profiles of organic food consumers may confound results of observational studies. Organic food consumers have been found to have healthier lifestyles, higher nutrient intake, and higher socioeconomic status. All of these factors are also associated with reduction in risk and mortality for various diseases. Therefore, observational studies focused on the association between organic food intake and health outcomes have to carefully adjust for variables such as demographics and lifestyle, including dietary intake and dietary quality variables.

Several observational studies in children showed a lower prevalence of allergy and atopic diseases in families who prefer to consume organic food[72–78]. After excluding the children who consumed organic dairy products in utero and infancy, the result still showed a significantly lower risk of eczema at age 2 years. Another study, the MOBA birth cohort study, has demonstrated a reduced risk of pre-eclampsia in pregnant women following an organic diet[79]. Research from Denmark[80] and Norway[81] showed negative associations between organic food consumption and the prevalence of hypospadias in boys.

In adults, the Nutrinet-Santé Cohort Study investigated weight change across individuals who reported different frequencies of organic food consumption. Their results indicated that the BMI of frequent organic food consumers increased slower than non-organic food consumers. A significantly reduced risk of obesity was also found in organic food consumers compared to conventional food consumers[82]. Research on sperm quality have also indicated positive results associated with organic food consumption. Two of these four studies examined Danish farmers and showed higher sperm density and quality in organic food farmers[83,84]. One of the studies focusing on the Danish general population demonstrated lower concentrations of morphologically normal spermatozoa in non-organic food consumers[85]; however, Larsen's research which had a similar population setting showed no difference between organic and conventional food consumers[86].

In terms of chronic diseases, including cancer, which have high prevalence rates in developed countries, the evidence is scant. Organic food consumers have a lower incidence of hypertension, type 2 diabetes, hypercholesterolemia, and cardiovascular problems in the Nutrinet-Santé Cohort Study[67]. However, the frequency of a declared history of cancer was higher in organic food consumers. Because of the cross-sectional design, the possibility of reverse causation cannot be excluded. For example, the diagnosis of cancer can change one's diet and exercise[71]. The Nutrinet-Santé Cohort Study from France and the Million Women study from the United Kingdom are the only two prospective cohort studies conducted in adults focusing on the association between organic food consumption and cancer incidence. Both of them recruited over 60,000 participants. The Nutrinet-Santé Cohort Study found that the overall risk of getting diagnosed with cancer was lower among the organic food consumers compared to non-organic food consumers[87]. However, the Million Women study did not show reduced overall risk of cancer, but only reduced risk of non-Hodgkin lymphoma in participants who often consumed organic foods[88].

1-4. Health concerns of using glyphosate and risks associated with glyphosate exposure

Although the assessment of glyphosate safety has been done several times by the U.S. Environmental Protection Agency (EPA), the assessments may be insufficient to comprehensively address the potential health impacts of glyphosate[89]. Most of the studies that were used to evaluate the risk of glyphosate exposure were conducted more than 30 years ago, which did not consider the usage of glyphosate which has since diversified and expanded dramatically. Besides, classical toxicity assessment only focuses on the acute toxic effects of high dosages of glyphosate administration but not the chronic effects under low dosages, which is crucial for assessing the safety of exposure to chemicals such as endocrine disruptors, of which glyphosate is one[90,91].

Recent studies have shown that glyphosate may cause adverse effects in animals and humans with long-term and low-dose exposure, including damage of the liver and kidney[92–99], interruption of the endocrine system[100–102], congenital malformations[51], and changes in gut microbiome species[103–105]. Several groups studied the safety of glyphosate-based herbicides with the dosage is now generally considered as “safe” for humans and found the potential ability to induce hepatorenal damage[93,95,98,99]. In the examination of the effect on the endocrine system, research has shown disruption of the reproductive development in male rat offspring[100] as well as the ovarian function in zebrafish[101]. Research on human cell lines have also revealed that the glyphosate-based herbicides are toxic and inhibits the conversion of androgen into estrogen in human cell lines[102]. One study found the ability of glyphosate to cause malformation of the piglets[51]. The effect of glyphosate on microbiomes has also been studied. Relationships between microbiomes and human health have been shown and received much attention in recent years[106]. Clair et al. showed that a glyphosate-based herbicide can inhibit the growth of three food microorganisms, *Geotrichum candidum*, *Lactococcus lactis subsp. cremoris* and *Lactobacillus delbrueckii subsp. Bulgaricus*, which are widely used in traditional and industrial dairy technologies[104]. Examining the potential effect of glyphosate on gut bacteria, Shehata et al. revealed that the beneficial bacteria were moderately to highly susceptible to glyphosate, while the pathogenic bacteria were highly resistant to the glyphosate[103]. This result indicated that glyphosate has the ability to change the composition of the gut microbiome. Krüger's group also demonstrated that glyphosate can suppress the antagonistic effect of *Enterococcus spp.* on *Clostridium botulinum*, which may result in the increased prevalence rate of C. *botulinum*-related diseases in cattle[105].

Several studies in human cell lines and human epidemiology studies have also shown the potential effect of glyphosate on cancer[107–111]. Thongprakaisang et al. showed that glyphosate induces the growth of human breast cancer cells via estrogen receptor[111] and Wiatkowska et al. revealed that glyphosate can cause DNA damage and DNA hypomethylation, which can lead to activation of oncogenes in human peripheral blood cells[107]. Epidemiologic studies have also shown an association between glyphosate exposure and lymphoma[108–110].

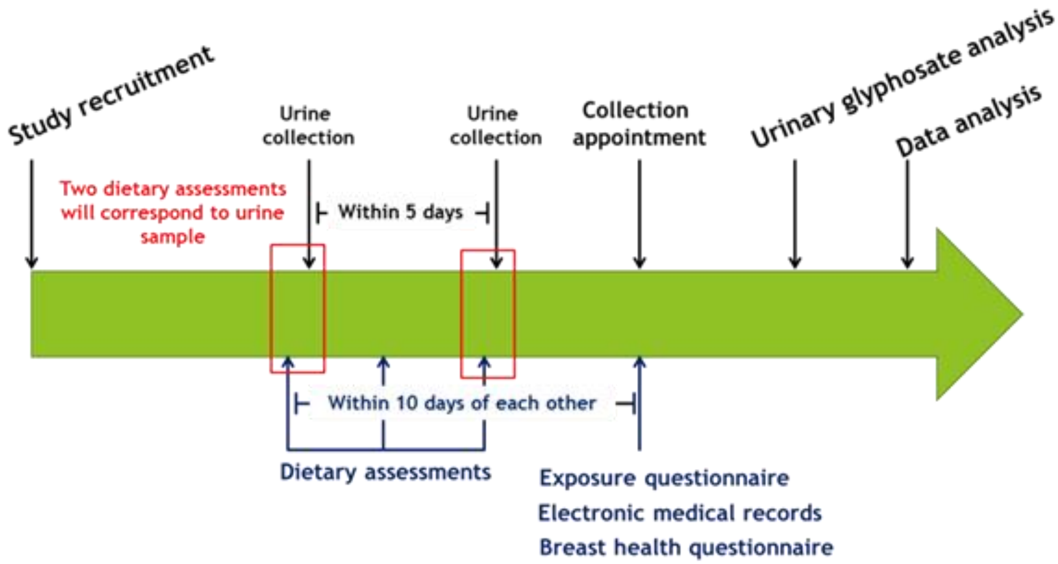
Although there is only limited evidence in humans for non-Hodgkin lymphoma and the few animal and cell line studies which showed the potential of glyphosate on cancer development, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans" in 2015. Subsequently, concern about the carcinogenic potential of glyphosate has increased. In August 2018, for the first time, a San Francisco jury ordered Monsanto, the maker of glyphosate, to pay $289 million to a school groundskeeper who was diagnosed with terminal non-Hodgkin lymphoma after using Roundup, a commercial pesticide with glyphosate. After eight months, in Edwin Hardeman's case, the jury reached a similar decision again in which Roundup was a substantial factor in causing their cancer. It was the first time that such a case was tried in federal court. Now, thousands of similar cases are pending at either the federal or state levels in accusation of Monsanto.

Chapter 2 – Methods

2-1. Study design

This study has a cross-sectional design. Two first void urine samples, five questionnaires and access to electronic medical records were collected from each study participant (Fig 1). Kissel et al. showed that first-void urine is a good predictor of the overall daily exposure for pesticides such as organophosphate[112]. To deal with the variation in diet from day to day, two individual first-void urine samples were self-collected, each requested the night before on random days within 5 days of each other via text message from the study team. Five questionnaires including three 24-hour dietary recalls, a pesticide exposure questionnaire, and a breast health questionnaire, were completed within 10 days of each other. Of the three ASA24 dietary recalls, two were paired with a urine collection to enable the dietary recall and corresponding urine specimen to reflect the same day's intake. The pesticide exposure questionnaire and breast health questionnaire were completed by participants on the collection appointment day. For a subset of participants who were recruited from the UCI Athena Breast Health Network, the breast health questionnaire had been previously completed at their most recent mammogram appointment.

Figure 1. Overall study design



2-2. Study population and recruitment

375 postmenopausal women age 45 to 65 with no personal history of breast cancer were recruited from the UC Irvine Athena Breast Health Network, an ongoing cohort of women undergoing breast screening at UCI Health, and from the UCI and surrounding communities in Orange County, California. Study recruitment began in 2017. Potential participants who met the recruitment criteria were recruited by mail, email, and telephone communication. After the verbal or emailed consent was received, the collection appointment was made based on the participant's preference for the date, time and location. In addition to verbal instructions on how to collect and store urine samples by the study coordinator, a paper copy of the detailed instructions along with two urine specimen collection cups were mailed out to the participants prior to the collection appointment. Lastly, the study coordinator guided the participants in completing the three dietary recalls. All study-related materials were

available in both English and Spanish, and the study coordinator was fluent in both languages.

2-3. Individual characteristics

The breast health questionnaire was used to collect the data on demographic and lifestyle factors, including age, race/ethnicity, education, smoking status, alcohol consumption, physical activity and body mass index (BMI). The pesticide exposure questionnaire was collected and managed using REDCap electronic data capture tools hosted at the University of California, Irvine[113]. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. The pesticide exposure questionnaire provided the information about participant's organic food consumption, dining preferences and their potential exposure to pesticides from other sources. "Organic" food was defined as being either labeled "USDA Organic," purchased from a local organic farm, grown without pesticides in a home garden, or raised on organic feed without hormones and without antibiotics. The organic food consumption questions, which were adapted from Curl et al.[57], included one general question ("Do you eat organic food?") and six specific questions regarding different food groups ("If you eat [food group], how often is that food organic?"). The food groups included fruits, vegetables, grains, meats, eggs and dairy products. Each of these questions had the answer options "Seldom or never", "Sometimes", "Often and always", and "Do not know". For the six food groups' questions, there was an additional option "I do not eat the food". The dining preferences questions provided information about the frequency of having a meal in fast food restaurants or other food establishments, deli or grocery stores, and home-cooked food. The exposure questions included pesticide exposure at home or workplace, and history of living on a farm.

2-4. Dietary data from Automated Self-Administered Recall System (ASA24)

Automated Self-Administered Recall System (ASA24) is a well-established and validated web-based system dietary recall system supported by the National Cancer Institute[114–116]. Based on USDA's Food and Nutrient Database for Dietary Surveys (FNDDS) food codes, ASA24 generates total intakes of 65 nutrients, including macronutrients and micronutrients, and 37 foods groups.

Participants recorded all foods, beverages and supplements consumed at breakfast, lunch, dinner and any other occasion from midnight to midnight (24 hours) for the day before. Participants first entered the basic information of their meal, including occasion name, time and location. Then, participants chose each food they had from the food browser and provided the condition of the food, including cooking method, sauce, oil and any additional items that were added. The amount of the food was also estimated by portion size images linked to portions in FNDDS. Upon the completion of ASA24 dietary recalls, the data was exported as an Excel spreadsheet, including the total intake of every macronutrient, micronutrient, food group, and supplement.

2-5. Biospecimen collection, processing and storage

Participants were sent text requests on two random evenings within the week before their blood collection appointment to self-collect their first-void urine samples the following mornings and place them in a freezer (-20C) until their study appointment. The 4-oz (118 ml) collection cups labeled with their study ID were individually wrapped, made of high-density polyethylene plastic and have a spill-proof screw-on sealing cap to provide leak resistance. A security seal on the cap prevented any tampering. The refrigerated or frozen urine samples were collected by the study coordinator at the time of the blood collection appointment and were temporarily stored in a cooler with ice while the coordinator was in the field. Upon arrival at the lab, the urine samples were aliquoted into 2 x 7-ml aliquot (for glyphosate and other pesticides levels analysis), 1 x 25-ml aliquot (for future analysis), and the remaining in 25-ml aliquots. Aliquots will be stored at -80°C until they are sent out to the analytic lab.

2-6. Urinary glyphosate levels analysis

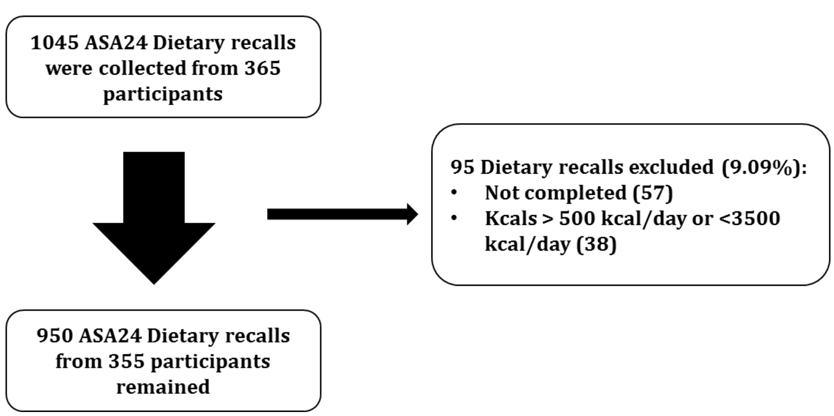
7 ml aliquots of urine samples were shipped with dry ice to the Translational Genomics Research Institute (TGen), which is an affiliate of City of Hope, for the urinary glyphosate and AMPA levels analysis, which were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS, Thermo Vanquish UPLC coupled with a Thermo TSQ-Altis triple quadrupole mass spectrometer) using the method described by Jensen et al.[117]. Thermo XCalibur Quan Browser software was used to perform quantitative analysis. Quality control was assessed based on recommendations by the U.S. Food Drug and Administration (FDA) on Bioanalytical Method Validation Guidance for Industry[118]. The limit of detection (LOD) for glyphosate was 0.044 ng/ml and for AMPA was 0.03 ng/ml. The glyphosate and AMPA levels which were lower than the LOD were replaced by ½ LOD, based on the guideline from the EPA used to address samples that have a value lower than the LOD[119]. The creatinine concentration was quantified by Arbor Assays' DetectX Urinary Creatinine Detection Kit. Standards and samples were prepared in triplicates and then read at 490 nm

on Biotek Multiplate reader. The urinary glyphosate and AMPA levels were normalized by urinary creatinine concentration to adjust for the influence of water intake on glyphosate concentration.

2-7. Dietary data cleaning and processing

1045 ASA24 dietary recalls were collected. 365 participants had at least one dietary recall (97.33%). The process of cleaning the dietary recalls is shown in Figure 2. 57 dietary recalls which were not considered "complete" (meaning that quantities consumed of the reported foods were not recorded) were removed (5.45%), and 988 dietary recalls from 358 participants remained in the dataset. Dietary recalls which had total Kcals lower than 500 kcal/day and higher than 3,500 kcal/day were removed, which followed the recommended method for excluding outliers used in epidemiologic studies involving diet analysis[120]. Thus, an additional 38 dietary recalls were removed and 950 dietary recalls from 355 (94.4%) participants remained in the dataset. 324 (86.1%) participants had at least two dietary recalls, and 249 (66.2%) participants had three dietary recalls.

Figure 2. ASA24 dietary recall exclusion criteria and number of valid ASA24 dietary recalls

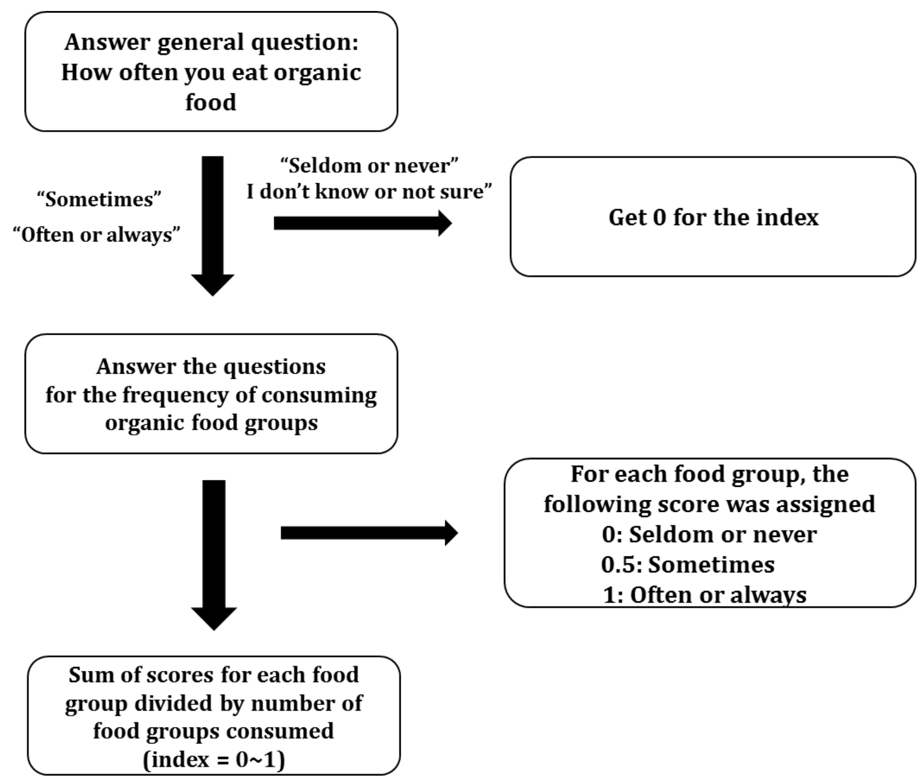


2-8. Organic food consumption index development

We generated an organic food consumption index to represent the ratio of organic eating to their overall diet, which was adapted from Nutrinet-Santé Cohort Study[87]. The organic food consumption index was calculated and processed from answers to questions about the frequency of organic food group consumption (Figure 3).

Participants were first asked the general question, "Do you eat organic food?" with answer options being "Seldom or Never," "Sometimes," "Often or Always," and "Do not know/Not sure" (adapted from Curl et al, 2015[57]). Participants who answered "Seldom or Never" or "Do not know/Not sure" were assigned an index of 0 for the organic food consumption index, while those who answered "Sometimes" or "Often or Always" to the general question above were further asked about their frequencies of consumption of six groups of food: "If you eat [food group], how often is that food organic?" The food groups included fresh fruits/fruit juice, vegetables/vegetable juice, grains, meat, eggs, and dairy. These questions shared the same answer options with the general question except with the addition of "I do not eat the food." Answers to these questions were assigned points (0: "Do not know/Not sure" or "Seldom and never;" 0.5: "Sometimes;" 1: "Often and always"). Then, the scores from these questions were summed up and divided by the number of food groups the participant reported consuming. That is, to adjust for differences in participants' general consumption of different types of food (for example, no meat), the score was calculated after excluding the food groups that participant answered "I do not eat the food" from the denominator. The minimum index score was 0 while the maximum index score was 1.0. Based on the index and the percentile ranks, all participants were grouped into three groups for different types of organic food consumers ("Infrequent," "Sometime," and "Frequent" organic food consumers). The cut-off to differentiate between "Infrequent" and "Sometime" was 0 (33rd percentile rank), while the cut-off to differentiate between "Sometime" and "Frequent" was 0.5 (67th percentile rank). 131 (35.03%) participants were in the "Infrequent" group, 122 (32.62%) participants were in the "Sometime" group, and 121 (32.35%) participants were in the "Frequent" group.

Figure 3. Organic food consumption index development



2-9. Determination of dietary intake and healthy eating index (HEI-2015) scores

The daily intake for each participant was measured, and values are shown in average with standard deviation. Daily amounts of energy (kcal), fruit, vegetable, grains, meat, dairy, sodium, fiber, protein, total fat, saturated fat and added sugars were calculated and adjusted for energy, which is a common method ("Nutrient density" approach) in epidemiologic studies focusing on dietary intake[121]. For food groups, daily intake was expressed as intake (cup equiv. or oz. equiv.) per 1000 kcal, while for macronutrients, including protein, carbohydrate, fat, and sugar, daily intake was expressed as % kcal.

The Healthy Eating Index-2015 (HEI-2015) was generated and released by the USDA Center for Nutrition Policy and Promotion, which was designed to evaluate diet quality according to the 2015-2020 Dietary Guidelines for Americans (DGA)[122]. HEI-2015 has been evaluated and validated, and consists of 13 components, which includes 9 adequacy components and 4 moderation components (Table 1). The total maximum score is 100 points. Each component is scored on a density basis out of 1000 calories, except the fatty acids, which is scored by a ratio of unsaturated to saturated fatty acids. For the 9 adequacy components, which include 9 types of healthy food groups, the closer the dietary intake of the food group reaches the values set in the guidelines, the higher the score. For the 4 moderation components, which include 4 unhealthy food groups, the lower the dietary intake of the food group, the higher the score. A higher HEI-2015 score represents higher diet quality and adheres more closely to the DGA. In this study, the HEI-2015 score per participant was calculated instead of per day per participants by using a component in the ASA24 output. The HEI score per participants was calculated from all the ASA24 recalls collected from a certain participant, regardless of how many 24-hours recalls participants had completed. The SAS code was provided by the Epidemiology and Genomics Research Program in the Division of Cancer Control and Population Sciences (DCCPS)[123]. The total food group intake over days was divided by the total energy summed over days, where:

$$\frac{\sum\ total\ food\ group\ intake\ over\ days}{\sum\ total\ energy\ over\ days}$$

Then, the ratio was compared with the HEI scoring standards on an amount of food group intake per 1000 kcal (Table 1).

Table 1. HEI-2015 components and scoring standards

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
Adequacy:			
Total Fruits	5	≥0.8 cup equiv. per 1,000 kcal	No Fruit
Whole Fruits	5	≥0.4 cup equiv. per 1,000 kcal	No Whole Fruit
Total Vegetables	5	≥1.1 cup equiv. per 1,000 kcal	No Vegetables
Greens and Beans	5	≥0.2 cup equiv. per 1,000 kcal	No Dark Green Vegetables or Legumes
Whole Grains	10	≥1.5 oz equiv. per 1,000 kcal	No Whole Grains
Dairy	10	≥1.3 cup equiv. per 1,000 kcal	No Dairy
Total Protein Foods	5	≥2.5 oz equiv. per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins	5	≥0.8 oz equiv. per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids*	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 oz equiv. per 1,000 kcal	≥4.3 oz equiv. per 1,000 kcal
Sodium	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	10	≤6.5% of energy	≥26% of energy
Saturated Fats	10	≤8% of energy	≥16% of energy

* PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; SFA, saturated fatty acids

2-10.Statistical methods

SAS software (SAS Studio 3.8, SAS Institute Inc., Cary, NC, USA) was used for data analysis. The value of each variable was expressed as the mean and standard deviation or as the number and percentage in the group. All statistical tests were 2-sided, and the p-value <0.05 was considered as statistically significant.

For the first part of the thesis, profile of organic food consumers, chi-square and Analysis of variance (ANOVA) tests were used to analyze whether the frequency of organic food consumption was associated with different variables, including demographic variables, lifestyles variables, weight, environmental exposure variables, dietary habits, dietary intake of macronutrient and food groups, and dietary quality. For the continuous variables, after the data were confirmed to be normally distributed, ANOVA was performed to detect significant differences between different levels of organic food consumption. For the categorical variables, the chi-square test was used to test for significantly different distributions across organic food consumption groups.

In the second part, the association between diet and glyphosate exposure was examined. To compare urinary glyphosate exposure across different levels organic food consumption, a linear regression model was generated in which the independent variable was three levels of organic food consumption and the dependent variable was urinary glyphosate levels. The model was further adjusted for age, race/ethnicity, education, BMI, and alcohol consumption, which were considered as the factors affecting urinary glyphosate levels other than the diet. Then Analysis of covariance (ANCOVA), which can adjust for covariance, was used to test whether the food groups intake was associated with urinary glyphosate levels. It was also adjusted for age, race/ethnicity, education, BMI, smoking status and alcohol consumption. Results are shown as adjusted means with standard error. Finally, the association between diet quality (Health Eating Index-2015) and urinary glyphosate levels stratified by frequency of organic food consumption was also examined using ANCOVA.

Chapter 3 – Results

3-1. Participant characteristics

Demographics, lifestyle factors, BMI and environmental exposures were analyzed across the three groups with different frequencies of organic food consumption (Table 2). The mean age of participants was 56.8 years. Most participants (66.3%) were White followed by Hispanic (16.8%) and Asian (11.0%). Neither age nor race/ethnicity were significantly associated with frequency of organic food consumption. White and Hispanic participants were evenly distributed across three groups, while more Asian participants were infrequent organic food consumers, but this was not statistically different. The distribution of education level was different across the three groups (p=0.0098). The majority of participants with a high school education or less did not frequently consume organic food, while for participants who had at least some college, the proportions were nearly equal in different levels of organic food consumption. BMI was significantly associated with levels of organic food consumption (p= 0.0046). Frequent organic food consumers had lower BMI compared to infrequent organic food consumers. Among the lifestyle variables examined, organic food consumption did not vary with smoking status, alcohol consumption or weekly physical activity. Environmental exposures were also examined. Organic food consumption was not associated with the length of time living on a farm or the starting age of living on a farm.

Table 2. Cohort characteristics

	Organic food consumption frequency			*p*-value
	Infrequent	Sometime	Frequent	
N (%)	131 (35.0%)	122 (32.6%)	121 (32.4%)	
Age, years, mean (SD)	56.21 (4.83)	57.34 (4.09)	56.98 (4.62)	0.13
Race/Ethnicity, N (%)				0.43
White	81 (32.7%)	80 (32.3%)	87 (35.1%)	
Hispanic	23 (36.5%)	19 (30.2%)	21 (33.3%)	
Asian	18 (43.9%)	16 (39.0%)	7 (17.1%)	
Other/unknown	9 (40.1%)	7 (31.8%)	6 (27.3%)	
Education, N (%)				0.0098
High school graduate or less	19 (65.5%)	5 (17.2%)	5 (17.2%)	
Some college or technical school	26 (31.3%)	27 (32.5%)	30 (36.1%)	
College graduate or more	84 (32.6%)	89 (34.5%)	85 (33.0%)	
Smoking status, N (%)				0.86
Current smoker	7 (46.7%)	4 (26.7%)	4 (26.7%)	
Former smoker	26 (31.7%)	30 (36.6%)	26 (31.7%)	
Non-smoker	88 (35.1%)	85 (33.9%)	78 (31.9%)	
Alcohol consumption, N (%)				0.52
Never	34 (36.2%)	35 (37.2%)	25 (26.6%)	
Less than 2 drinks per week	53 (34.6%)	52 (34.0%)	48 (31.4%)	
2-7 drinks per week	26 (37.1%)	16 (22.9%)	28 (40.0%)	
More than 7 per week	17 (34.0%)	16 (32.0%)	17 (34.0%)	
Weekly physical activity that meets the physical activity guidelines, N (%)				0.16
No	87 (38.3%)	74 (32.6%)	66 (29.1%)	
Yes	44 (29.9%)	48 (32.7%)	55 (37.4%)	
BMI, kg/m^2, mean (SD)	28.22 (6.71)	26.22 (5.55)	25.66 (6.99)	0.0046
Environmental exposures				
History of living on the farm, N (%)				0.51
> 10 years	4 (20.0%)	7 (35.0%)	9 (45.0%)	
≤ 10 years	13 (43.3%)	9 (30.0%)	8 (26.7%)	
None	114 (35.2%)	106 (32.7%)	104 (32.1%)	
Age when started living on a farm, mean (SD)	5.88 (4.72)	6.81 (6.96)	11.65 (13.54)	0.28

3-2. Dietary habits

Eating outside the home has been considered a health risk and is associated with less healthy food choices[124,125]. In our study, fast food was defined as a meal or snack from a place such as McDonald's, In-N-out, Subway, Burger King and other chain restaurants. Home-cooked food was defined as food cooked at home with basic simple ingredients. Results for the dietary habits across different organic food consumption groups are presented in Table 3. Infrequent organic food consumers had more fast food (p=0.0002). There was a trend that infrequent organic food consumers had less home-cooked food, but it was not statistically different (p=0.07). There was no difference in the frequency of eating in other (non-fast food) eating establishments or from a deli or grocery store.

Table 3. Dietary habits across different levels of organic food consumption

	Organic food consumption frequency			*p*-value
	Infrequent	**Sometime**	**Frequent**	
Eating fast food				0.0002
≥ 1 time/week	39 (51.32%)	26 (34.21%)	11 (14.47%)	
< 1 time/week	92 (30.87%)	96 (32.21%)	110 (36.91%)	
Eating in other eating establishments				0.10
≥ 1 time/week	81 (33.47%)	74 (30.58%)	87 (35.95%)	
< 1 time/week	50 (38.17%)	48 (36.64%)	33 (25.19%)	
Having prepared food in deli or grocery store				0.74
≥ 1 time/week	20 (31.75%)	23 (36.51%)	20 (31.75%)	
< 1 time/week	111 (35.69%)	99 (31.83%)	101 (32.48%)	
Having home-cooked food				0.07
< 1 time/day	75 (40.76%)	55 (29.89%)	54 (29.35%)	
≥ 1 time/day	56 (29.47%)	67 (35.26%)	67 (35.26%)	

3-3. Dietary intake profiles

Dietary intake profiles were analyzed and shown in mean values across groups (Table 4). Overall, the mean caloric intake was 1,685 kcal, which was close to 1,600 kcal, the recommended energy intake for women over 50. The average intakes of fruits, vegetables, and grains were each lower than the recommended values. Table 4 shows the mean daily intakes of food groups and macronutrients across different levels of organic food consumption. The intake of vegetables, including dark green, red and orange, starchy and other vegetables, and legumes, was significantly higher in organic food consumers ($p=0.0034$). Frequent organic food consumers consumed fewer grains overall, especially refined grains ($p=0.0067$ and 0.0057). There was no difference in the intake of whole grains between groups. However, frequent organic food consumption was associated with higher intake of fiber ($p=0.0012$).

Table 4. Energy-adjusted dietary intake per day across different levels of organic food consumption

	Organic food consumption frequency			***p*-value**
	Infrequent	**Sometime**	**Frequent**	
Total energy intake, kcal	1733.55 (487.76)	1686.38 (477.81)	1633.39 (419.82)	0.25
Total Fruit, cup-equiv. /1,000 kcal	0.61 (0.95)	0.76 (0.72)	0.70 (0.59)	0.33
Total Vegetable, cup-equiv. /1,000 kcal	1.12 (0.72)	1.30 (0.82)	1.50 (1.02)	0.0034
Total Grains, cup-equiv. /1,000 kcal	2.70 (1.16)	2.66 (1.15)	2.27 (1.10)	0.0067
Whole Grains, cup-equiv. /1,000 kcal	0.53 (0.58)	0.66 (0.66)	0.56 (0.53)	0.21
Refined Grains, cup-equiv. /1,000 kcal	2.17 (1.13)	2.00 (1.14)	1.72 (1.00)	0.0057
Total meat, ounces- equiv. /1,000 kcal	2.63 (1.67)	2.42 (1.65)	2.37 (1.84)	0.47
Total dairy, cup-equiv. /1,000 kcal	0.75 (0.51)	0.71 (0.47)	0.78 (0.56)	0.64
Sodium, g per 1,000 kcal	1.78 (0.49)	1.73 (0.50)	1.71 (0.42)	0.54
Fiber, % total calories	4.10 (1.83)	4.95 (1.95)	4.80 (1.94)	0.0012
Total Protein, % total calories	17.85 (4.81)	17.51 (5.06)	17.97 (5.25)	0.78
Carbohydrate, % total calories	43.90 (10.37)	44.74 (10.55)	41.51 (10.10)	0.05
Total fat, % total calories	36.84 (8.06)	36.22 (8.54)	38.13 (9.60)	0.24
Saturated fat, % total calories	11.44 (3.46)	10.70 (3.25)	11.52 (3.86)	0.15
Added Sugars, % of total calories	8.84 (6.30)	8.41 (5.83)	8.50 (5.60)	0.84

3-4. Diet quality

The overall diet quality and diet quality across three groups were examined (Table 5) Overall, the mean HEI score was 63.7, which was higher than in the U.S. population, which was 59 in 2011-2012 according to data from the National Health and Nutrition Examination Survey[126]. Table 5 shows the mean values of the HEI scores, including total and individual component scores. Frequent organic food consumers had significantly higher total HEI-2015 scores compared to infrequent organic food consumers (p=0.0003). High levels of organic food consumption were positively associated with HEI score in components including total vegetables, greens and beans, total fruits, whole fruits, seafood and plant proteins, and refined grains. Scores in other components, including whole grains, dairy, total protein foods, fatty acids, sodium, added sugars and saturated fats, were not significantly different across different levels of organic food consumption.

Table 5. Diet quality across different levels of organic food consumption

	Organic food consumption frequency			*p*-value
	Infrequent	**Sometime**	**Frequent**	
Total HEI-2015	60.37 (11.85)	65.40 (11.95)	66.04 (11.59)	0.0003
Adequacy Components (The higher intake, the higher score)				
Total Vegetables	3.83 (1.40)	4.07 (1.38)	4.31 (1.32)	0.02
Greens and Beans	3.23 (2.20)	3.77 (1.95)	4.06 (1.72)	0.0055
Total Fruits	2.46 (1.94)	3.18 (1.89)	3.17 (1.72)	0.0029
Whole Fruits	2.91 (2.15)	3.57 (1.89)	3.81 (1.79)	0.002
Whole Grains	3.30 (3.27)	3.85 (3.18)	3.52 (3.22)	0.42
Dairy	5.30 (3.01)	5.09 (2.83)	5.38 (3.01)	0.74
Total Protein Foods	4.68 (0.88)	4.66 (0.88)	4.69 (0.87)	0.95
Seafood & Plant Proteins	3.86 (1.79)	4.09 (1.62)	4.40 (1.39)	0.04
Fatty Acids	5.85 (3.46)	6.63 (3.32)	6.20 (3.50)	0.21
Moderation Components (The lower intake, the higher score)				
Sodium	3.33 (3.26)	3.87 (3.33)	3.92 (3.28)	0.30
Refined Grains	7.62 (3.04)	7.87 (2.88)	8.57 (2.25)	0.01
Added Sugars	8.41 (2.18)	8.39 (2.4)	8.40 (2.23)	0.99
Saturated Fats	5.60 (3.32)	6.37 (3.27)	5.61 (3.35)	0.13

3-5. Urinary glyphosate levels across different levels of organic food consumption

Basic characteristics of the participants whose urine samples were analyzed (N = 201) were first examined to check whether the distribution of the basic characteristics was similar to the overall sample population (Table 6). Similar to the results from Table 2, only education and BMI were significantly different between different levels of organic food consumption.

Glyphosate and AMPA levels in urine were measured. The values from two urine samples were first normalized by creatinine levels and then averaged for each participant (Table 7). Results showed there was no association between organic food consumption and urinary glyphosate levels (p=0.96). Interestingly, sometime organic food consumers had the highest glyphosate levels compared to frequent and infrequent organic food consumers. However, the AMPA results showed a significant difference between groups and presented a gradient pattern in which frequent organic food consumers had the highest AMPA levels followed by sometime organic food consumers and infrequent organic food consumers (p=0.01). After adjusting for age, race/ethnicity, education, BMI, alcohol consumption and smoking status, urinary AMPA level remained significantly associated with organic food consumption.

Table 6. Basic distribution of participants who were included in the urinary analysis

	Organic food consumption frequency			*p*-value
	Infrequent	**Sometime**	**Frequent**	
N	70 (34.8%)	63 (31.3%)	68 (33.8%)	
Age, years, mean (SD)	56.59 (4.7)	57.14 (3.76)	56.21 (4.18)	0.45
Race/Ethnicity, N (%)				0.63
White	46 (33.3%)	43 (31.2%)	49 (35.5%)	
Hispanic	6 (28.6%)	6 (28.6%)	9 (42.9%)	
Asian	13 (46.4%)	10 (35.7%)	5 (17.9%)	
Other/unknown	5 (35.7%)	4 (28.6%)	5 (35.7%)	
Education, N (%)				0.047
High school graduate or less	9 (69.2%)	3 (23.9%)	1 (7.7%)	
Some college or technical school	9 (23.7%)	14 (36.8%)	15 (39.5%)	
College graduate or more	51 (34.2%)	46 (30.9%)	52 (34.9%)	
Smoking status, N (%)				0.81
Current smoker	4 (50.0%)	2 (25.0%)	2 (25.0%)	
Former smoker	9 (27.3%)	13 (39.4%)	11 (33.3%)	
Non-smoker	47 (35.1%)	45 (33.6%)	42 (31.3%)	
Alcohol consumption, N (%)				0.84
Never	20 (35.1%)	20 (35.1%)	17 (29.8%)	
Less than 2 drinks per week	31 (39.2%)	24 (30.4%)	24 (30.4%)	
2-7 drinks per week	10 (32.3%)	8 (25.8%)	13 (41.9%)	
More than 7 per week	8 (28.6%)	9 (32.1%)	11 (39.3%)	
Weekly physical activity that meets the physical activity guidelines, N (%)				0.15
No	46 (40.4%)	34 (29.8%)	34 (29.8%)	
Yes	24 (27.6%)	29 (33.3%)	34 (39.1%)	
BMI, kg/m², mean (SD)	28.31 (6.71)	24.75 (5.07)	25.88 (8.14)	0.0097
Environmental exposures				
History of living on the farm, N (%)				0.08
> 10 years	0 (0%)	6 (50.0%)	6 (50.0%)	
≤ 10 years	9 (47.4%)	6 (31.6%)	4 (21.1%)	
None	61 (35.9%)	51 (30.0%)	58 (34.1%)	
Age when started living on a farm, mean (SD)	6.11 (4.48)	7.42 (7.83)	11.5 (15.26)	0.49

Table 7. Urinary glyphosate levels by levels of organic good consumption

	N	Glyphosate levels (μg/g creatinine)			AMPA levels (μg/g creatinine)		
		mean (SD)	***p*-value**	**Adjusted *p*-value***	**mean (SD)**	***p*-value**	**Adjusted *p*-value***
Organic food consumption index	201	0.39 (0.46)	0.68	0.60	0.16 (0.22)	0.01	0.02
Organic food consumption frequency			0.96	0.90		0.01	0.02
Infrequent	70	0.35 (0.37)			0.22 (0.32)		
Sometime	63	0.50 (0.57)			0.15 (0.15)		
Frequent	68	0.35 (0.41)			0.12 (0.13)		

*Models adjusted for age, race/ethnicity, education, BMI, alcohol consumption and smoking status

3-6. The association between food groups intake and urinary glyphosate levels

We examined whether food intake was associated with urinary glyphosate levels. The intake of fruits, vegetable, grains, meats and dairy product were shown as quantile to evaluate the pattern of glyphosate levels in four quantiles. The adjusted means and standard errors are shown in Table 8. The adjusted means were adjusted for age, race/ethnicity, education, alcohol consumption, smoking status and BMI, which were factors that may have affected the urinary glyphosate levels in the diet model. None of the food groups intake were associated with urinary glyphosate levels in overall participants. Then, the analysis was stratified by frequency of organic food consumption. In infrequent organic food consumers, grain intake was associated with urinary glyphosate levels (p=0.01). Quantile 4, which had the highest grains intake, had the highest glyphosate levels compared to quantile 1, which had the lowest grains intake. There was no association between any food group intake and urinary glyphosate levels in sometime and frequent organic food consumers (p=0.27 & p=0.45).

Table 8. The association between food groups intake and urinary glyphosate levels, stratified by the levels of organic food consumption.* Glyphosate levels are expressed as μg/g creatinine, mean (SE).

A. Fruits

	Fruits intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.4 (0.08)	0.44 (0.08)	0.41 (0.07)	0.36 (0.07)	0.91
Organic food consumption frequency					
Infrequent	0.48 (0.11)	0.5 (0.12)	0.24 (0.09)	0.25 (0.13)	0.23
Sometime	0.39 (0.17)	0.56 (0.16)	0.7 (0.16)	0.38 (0.13)	0.43
Frequent	0.19 (0.17)	0.25 (0.12)	0.47 (0.11)	0.48 (0.14)	0.38

B. Vegetables

	Vegetables intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.44 (0.08)	0.37 (0.07)	0.35 (0.08)	0.45 (0.07)	0.71
Organic food consumption frequency					
Infrequent	0.43 (0.1)	0.34 (0.1)	0.33 (0.15)	0.29 (0.12)	0.85
Sometime	0.46 (0.16)	0.45 (0.19)	0.58 (0.15)	0.47 (0.14)	0.93
Frequent	0.34 (0.23)	0.40 (0.12)	0.13 (0.11)	0.54 (0.11)	0.10

C. Grains

	Grains intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.26 (0.07)	0.43 (0.08)	0.42 (0.07)	0.49 (0.08)	0.17
Organic food consumption frequency					
Infrequent	0.27 (0.10)	0.25 (0.14)	0.15 (0.1)	0.60 (0.09)	0.01
Sometime	0.26 (0.14)	0.58 (0.18)	0.61 (0.13)	0.55 (0.15)	0.27
Frequent	0.27 (0.14)	0.42 (0.11)	0.45 (0.12)	0.16 (0.18)	0.45

D. Meats

	Meats intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.51 (0.07)	0.31 (0.08)	0.39 (0.08)	0.38 (0.08)	0.26
Organic food consumption frequency					
Infrequent	0.46 (0.12)	0.22 (0.15)	0.27 (0.10)	0.43 (0.11)	0.44
Sometime	0.58 (0.14)	0.36 (0.15)	0.71 (0.19)	0.39 (0.15)	0.44
Frequent	0.42 (0.11)	0.32 (0.12)	0.31 (0.17)	0.34 (0.17)	0.91

E. Dairy products

	Dairy products intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.41 (0.08)	0.54 (0.07)	0.38 (0.08)	0.29 (0.07)	0.08
Organic food consumption frequency					
Infrequent	0.27 (0.12)	0.48 (0.11)	0.4 (0.14)	0.31 (0.10)	0.58
Sometime	0.37 (0.13)	0.86 (0.14)	0.55 (0.17)	0.26 (0.14)	0.03
Frequent	0.58 (0.18)	0.38 (0.12)	0.28 (0.14)	0.28 (0.11)	0.50

*Adjusted for age, race/ethnicity, education, BMI, alcohol consumption and smoking status

3-7. The association between diet quality and urinary glyphosate levels

Then, we examined whether the diet quality is associated with urinary glyphosate levels. The HEI-2015 total scores were shown as quartiles. The urinary glyphosate and AMPA levels were shown as means and standard errors. Table 9 shows that there was no association between diet quality and urinary glyphosate levels. Then, the analysis was stratified by frequency of organic food consumption to assess whether organic food consumption frequency affects the association between diet quality and urinary glyphosate or AMPA levels. The result showed that there was no association between diet quality and urinary glyphosate or AMPA levels in three organic food consumption frequency groups.

Table 9. The association between diet quality and urinary glyphosate and AMPA levels, stratified by the levels of organic food consumption*

A. Urinary glyphosate levels (μg/g creatinine), mean (SE)

	HEI-2015 total scores				***p*-value**
	Q1	**Q2**	**Q3**	**Q4**	
Overall	0.42 (0.09)	0.41 (0.08)	0.41 (0.08)	0.39 (0.08)	0.99
Organic food consumption frequency					
Infrequent	0.31 (0.12)	0.46 (0.10)	0.26 (0.14)	0.32 (0.14)	0.65
Sometime	0.68 (0.17)	0.26 (0.22)	0.37 (0.13)	0.62 (0.14)	0.29
Frequent	0.41 (0.23)	0.32 (0.13)	0.46 (0.12)	0.27 (0.13)	0.68

B. Urinary AMPA levels (μg/g creatinine), mean (SE)

	HEI-2015 total scores				***p*-value**
	Q1	**Q2**	**Q3**	**Q4**	
Overall	0.23 (0.05)	0.16 (0.04)	0.18 (0.04)	0.13 (0.04)	0.41
Organic food consumption frequency					
Infrequent	0.28 (0.11)	0.17 (0.09)	0.37 (0.13)	0.17 (0.12)	0.60
Sometime	0.23 (0.06)	0.19 (0.07)	0.13 (0.04)	0.13 (0.05)	0.72
Frequent	0.10 (0.06)	0.13 (0.04)	0.15 (0.03)	0.11 (0.04)	0.74

*Adjusted for age, race/ethnicity, education, BMI, alcohol consumption and smoking status

Chapter 4 – Discussion

4-1. Significance

As organic food becomes more popular, a better understanding of the health effects of organic food is urgently needed. However, the association between organic food consumption and health outcomes is confounded by the profile of organic food consumers. Previous studies that focused on the profile of organic food consumption were conducted in Europe, but the United States has the largest organic food market in the world. The profile of organic food consumers may be distinct in the United States compared to European countries. In this study, we analyzed data and specimens from 375 women in Orange County, California. Basic demographics, diet intake, diet habit, diet quality, and other factors were analyzed. Although these participants are not fully representative of the general public in the United States, this is the first study to describe a profile of organic food consumers in a non-agricultural setting in the United States. While the results we found need to be confirmed using a larger population, our research provides an important first step towards understanding the profile of organic food consumers in the United States and gives a good foundation for future studies to focus on the health effects of organic food consumption.

Despite the growing evidence of the adverse health outcomes of glyphosate either on animals or humans, very few studies have evaluated overall human exposure. In a recent review[127], which collected 19 studies focusing on glyphosate exposure, 8 studies reported urinary glyphosate levels in occupationally and para-occupationally exposed subjects, while 14 studies collected the data from the general population. However, most of them focused on the spatial difference or temporal difference within a few years. Only one study examined the association between diet and urinary glyphosate levels in the general population setting.

Krüger et al. showed that conventional food consumers have higher urinary glyphosate levels compared to organic food consumers[128]. However, the detailed method of grouping participants and the characteristics of the participants were not revealed in the study, which decreases the impact of the research. For the first time, we measured urinary glyphosate levels and determined its association with dietary intake. Our results showed significantly lower AMPA levels in frequent organic food consumers compared to infrequent and sometime organic food consumers. Although glyphosate levels and organic food consumption were not statistically associated, we did observe a trend that glyphosate levels were higher in infrequent and sometime organic food consumers than in frequent organic food consumers.

Due to the lack of surveillance programs to regularly monitor glyphosate residues in food, it is hard to track the dietary exposure of glyphosate on humans. Although we did not analyze levels of glyphosate residues on/in food, we did find an association between grains intake and urinary glyphosate levels and thus propose that high glyphosate residues are found in grains. Although the urinary analysis results were based on a small population, our research provides a first glance at how diet affects glyphosate exposure in humans in the U.S. More research is needed to confirm the findings from this study.

4-2. Basic characteristics of organic food consumers

We found frequent organic food consumers had higher education and lower BMI across different levels of organic food consumers (Table 3). These results were consistent with findings from European and other U.S. studies. However, our result did not show a significant difference in age, race/ethnicity and lifestyle, including smoking status, alcohol consumption, and physical activity. The study was designed to only recruit women ages 45 to 65 years, which resulted in a narrow age range of our participants. This limited our ability to detect age differences in different levels of organic food consumers. For race/ethnicity, although it was not significantly different, we found that a higher proportion of Asian participants consumed low or no organic food, which is consistent with the result from Racheal et al.[13], whose study focused on organic food purchase preferences in the United States, and from the Hartman Group[129], which is the leading group in the industry that studies consumer behaviors on food and beverages in the United States. Both of them found that individuals of Asian race/ethnicity were less likely to purchase organic food compared to other race/ethnicity groups. Some European studies found that organic food consumers have healthier lifestyles than non-organic food consumers, including high physical activity, non-smoker, and low alcohol consumption. However, in our study, we did not find any differences in these variables. Only a small proportion of our study participants were current smokers or heavy alcohol consumers, which limited the power to detect differences in organic food consumption according to these lifestyle variables.

4-3. Dietary profile of organic food consumers

For the first time, we demonstrated that organic food consumption was inversely associated with the frequency of fast food consumption (Table 4). Fast foods have been considered as unhealthy, which is associated with increased risk of obesity[130,131], CVD[132], and type 2 diabetes[133]. Our finding suggests that organic food consumers were not only aware of the food they eat, but also have healthier eating habits compared with the participants who had less frequent organic food consumption.

In the dietary profiles analysis, we found that organic food consumers ate more vegetables, fewer grains, especially refined grains, and more fiber (Table 5). This result is consistent with the European conclusion that organic food consumers are more likely to consume plant-based food. Although frequent organic food consumers had lower grain intake, the intake of whole grains was not different than in infrequent organic food consumers, while the intake of refined grains was significantly lower than in infrequent organic food consumers. Compared to whole grains, refined grains are milled, which results in the removal of many nutrients. Unlike whole grains, which can reduce the risk of hypertension[134,135], obesity[136–138], type 2 diabetes[139], refined grains are not associated with reduced risk of these diseases[140,141]. Moreover, the Dietary Guidelines Advisory Committee (DGAC) also recommended replacing most refined grains with whole grains[142]. The report from the American Heart Association also supports this recommendation[143]. Although the intake of whole grains did not increase, the intake of refined grains was significantly reduced in frequent organic food consumers, which can be considered closer to a healthy diet than in infrequent organic food consumers.

The pattern of diet quality was also consistent with the result from other European studies showing that frequent organic food consumers exhibit an overall better diet quality than infrequent organic food consumers[17,55,67]. If breaking down the total score into individual components, the significant differences appeared in specific components, including vegetables, greens and beans, total fruits, whole fruits and refined grains. This agreed with the result from the NutriNet-Santé study, which found that regular organic food consumers are more aware of the nutrition guidelines recommending eating plant-based foods rather than animal-based foods[67].

4-4. Association between organic food consumption frequency and urinary glyphosate levels

Interestingly, in the urinary glyphosate analysis, we found a different pattern for glyphosate than for AMPA. Sometime organic food consumers had the highest glyphosate levels compared to both infrequent organic food consumers and frequent organic food consumers, while AMPA levels showed the gradient from high organic food consumption to low organic food consumption. Compared to infrequent and frequent organic food consumers, sometime organic food consumers have higher uncertainty. Their degree of how their diet adheres to organic food as well as their motivation about organic food is not clear. By comparing the profile of different levels of organic food consumption, we found that sometime organic food consumers were closer to organic food consumers for some variables, while they were the opposite for other variables, which indicates uncertainty within these participants. In addition, the days of urine collection were randomly chosen. Although participants reported the foods they ate on a certain day, participants did not reveal the percentages of organic food consumed on that day, which may have also increased the variability of the analysis.

Based on the different nature of glyphosate and AMPA, another explanation is possible. One of the common strategies of generating glyphosate-tolerant crops is inserting a gene that can quickly transform glyphosate to AMPA, which becomes non-toxic to crops[144]. Since glyphosate remains unchanged in the human body and AMPA only occurs in glyphosate-tolerant crops, the AMPA in human urine may be due to the intake of glyphosate-tolerant crops (genetically modified [GM] crops). Based on this information and the results from our study, we can roughly hypothesize that participants who are sometime organic food consumers may be more concerned with GM crops more than other, non-GM organic foods. That is, they may have decreased intake of GM food, while simultaneously maintaining consumption of conventional foods, which resulted in higher glyphosate levels but lower AMPA levels compared to the other groups.

In our study, we found that only grains intake was associated with urinary glyphosate levels but not other plant-based foods. Although this result may not be consistent with findings which focused on other pesticides like organophosphates[57], which are also applied on crops, it aligns with the values of glyphosate residues in different foods that have been reported[49]. The Detox Project found that grain products, such as cereals, granolas and snack bars had high levels of glyphosate[49]. We also checked whether food groups intake was associated with urinary AMPA levels. No significant association was found when the stratification was applied (Supplement table 1). However, our result was based on about 70 samples; thus, more samples are needed to confirm this result.

In the adjusted model, age, race/ethnicity, BMI, education, alcohol consumption, and smoking status were chosen to be included in the model. Indeed, no epidemiologic studies to date have examined the association between dietary intake and urinary glyphosate levels, which made it difficult to select potential confounders. Thus, first we considered the variables that are commonly used in organic food consumption studies, which are age and race/ethnicity. BMI and education were also chosen based on our result that BMI and education were significantly different across the three levels of organic food consumption groups. Alcohol consumption and smoking status were included since the raw materials of wine, beer and tobacco may have been exposed to pesticides, and we did not address this when we generated the organic food consumption index.

4-5. Methodology advantage

To examine the profiles of organic food consumers, we generated an organic food consumption index, based on their answers to six organic food group consumption questions. This method was adapted from the NutriNet-Santé study, which gives scores to each answer and sums up the scores to get a total organic food consumption score. Unlike in the NutriNet-Santé study, the scores in our study were further adjusted for the possible food groups that the participant may consume. This adjustment makes the index more precise, since it avoids the overemphasis of organic food consumption in the food groups that the participant doesn't eat and also separates the participants who answered: "I am not sure or I don't know" and "I do not eat that food" in the food groups questions.

Urine is considered to be a good biospecimen for evaluating chemical exposures, such as pesticides[145]. Glyphosate is unchanged in the human body and is excreted mainly through urine[44], which makes the urine a suitable sample for examining glyphosate exposure. Kissel et al. also demonstrated that first-void urine is a good predictor of overall daily exposure for organophosphates (OP), another class of pesticide[112]. In addition to two urine samples, we also collected three 24-hour dietary recalls, two of which corresponded to the urine samples and reflected the dietary intake of the urine collection day. This design is different than the design from Curl et al[57], which estimated the urinary OP pesticide levels through a food frequency questionnaire reflecting the diet in the past year. Alison et al. has shown that there is a short biological half-life of glyphosate in humans, about $3.5 \sim 14.5$ hours[146]; thus, urine can only reflect short-term glyphosate exposure. Compared to the food frequency questionnaire, 24-hour dietary recalls can represent the short-term diet better. Thus, our design enabled us to track glyphosate exposure in the diet more precisely.

When recruiting, the study coordinator did not reveal the specific environmental exposure or potential associations we were focusing on, which may have reduced the possibility that participants changed their diet or other health behaviors when participating in the study. Additionally, participants were prompted to collect their urine the night before and to recall their diet over the previous 24 hours without prior notice. This limited the possibility that participants changed their diet to match our research hypothesis. Additional survey questions regarding the awareness of health may reduce this bias and can further be treated as a confounder in future analyses.

4-6. Limitations

Although this study presents several strengths, some limitations should be noted. First, the sample size is relatively small, with only 375 participants, and the study population consists of only postmenopausal women in Orange County, California. Compared to similar studies conducted in Europe, which have about 28,245 participants, our study has a significantly small sample size. Also, compared to other cohorts, such as the National Health and Nutrition Examination Survey (NHANES) cohort, our cohort is slightly more homogeneous in which all of our participants are women within a narrow age range coming from the UC Irvine Athena Breast Health Network and UC Irvine surrounding community. Moreover, our study also has volunteer bias[147]. That is, we were more likely to recruit health-conscious individuals, thus limiting the generalizability of our findings. Our participants may be more aware of their health and more willing to stay healthy.

Second, this study has some other bias and measurement error. The frequency of organic food consumption and 24-hour dietary intakes were self-reported, which are prone to have response bias[148], which is a common bias in behavioral and healthcare research. For example, in our questions, the definition of "Seldom or never," "Sometimes," and "Always" was not well defined, which may have caused measurement error. Another possible measurement error may have occurred in the development of the organic food consumption index. We only asked participants their organic food consumption frequencies for six types of food groups, namely, vegetables, fruits, grains, meats, eggs, and dairy products. However, there are substantial parts of food groups that were not included in the questionnaire, such as oil, coffee, tea, and wine. In the Nutrinet-santé Cohort Study, 16 types of food groups are included. Our instrument may not be able to give a comprehensive picture of organic food consumption in each participant, but only provide the information about organic food consumption in essential food groups[87].

Finally, the questionnaire for the frequency of organic food consumption and the organic food consumption index have not been validated. However, we have tested the index on the answer to the general question that asked participants about their frequency of eating organic food (without specifying which food groups). The results showed that the organic food consumption index was significantly associated with their answer to the general question (Supplemental Table 2). Participants who reported that they often or always consumed organic food had a higher organic food index followed by participants who sometimes and seldom consumed organic food. Moreover, our analysis of the profiles of organic food consumers indirectly validates the questionnaire. The participants who had a higher index had a profile similar to the profile of organic food consumers found in other research[17,67], which indicated that the organic food index is valid to represent the frequency of organic food consumption of the participant.

4-7. Future directions

In this study, we examined the profile of organic food consumers in Orange County, California. Since our cohort had a small sample size, 375 women, and homogenous characteristics, our findings need to be confirmed with a larger sample size and more generalized population, perhaps in other cohorts, such as Multi-Ethnic Study of Atherosclerosis (MESA), which is another cohort recruiting participants from six U.S. areas.

For the urinary glyphosate analysis, a larger sample size should also be applied. Currently, we only had 156 participants with urinary glyphosate levels available, who were evenly distributed in the three groups. These participants were the ones recruited in the first half of the study. It is possible that our results may change when the urinary analysis data from all the participants are available and analyzed.

Later, more items need to be added to the questionnaire in order to avoid measurement error from the one-item-scale questionnaire. More behavior, motivation, and nutrient knowledge questions would also be very important when considering confounders in a study focusing on the association between organic food consumption and health. In addition, the food groups should be considered thoroughly and expanded in the questionnaire to include seafood, oil, wine, coffee and so on, since more and more research has found variable pesticide residue levels in different types of raw and processed foods. The questionnaire should also be validated and tested. Validation with a different biomarker, such as a different chemical in the urine or serum, may help validate the questionnaire.

Finally, unfortunately, we only had about two years study time, which is not enough to generate any conclusions about the health effects of organic food consumption. We will follow up with current participants on their health and other environmental exposures, and in the meantime, we will enlarge our sample size.

Bibliography

1. Lin B-H, Padgitt M, Bull L, Delvo H, Shank D, Taylor H. *Pesticide and Fertilizer Use and Trends in U. S. Agriculture*.; 1995.
2. Longnecker MP, Rogan WJ, Lucier G. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health. *Annu Rev Public Health*. 1997;18(1):211-244. doi:10.1146/annurev.publhealth.18.1.211
3. Lotter DW. Organic Agriculture. *J Sustain Agric*. 2003;21(4):59-128. doi:10.1300/J064v21n04_06
4. National Archives and Records Administration. Electronic Code of Federal Regulations, Title 7: Agriculture, Part 205—National Organic Program.
5. FiBL&IFOAM. *The World of Organic Agriculture: Statistics and Emerging Trends 2019*.; 2019.
6. Harper GC, Makatouni A. Consumer perception of organic food production and farm animal welfare. *Br Food J*. 2002;104(3/4/5):287-299. doi:10.1108/00070700210425723
7. Williams PRD, Hammitt JK. A Comparison of Organic and Conventional Fresh Produce Buyers in the Boston Area. *Risk Anal*. 2000;20(5):735-746. doi:10.1111/0272-4332.205066
8. Reganold JP, Wachter JM. Organic agriculture in the twenty-first century. *Nat Plants*. 2016;2(2):15221. doi:10.1038/nplants.2015.221
9. Bryła P. Organic food consumption in Poland: Motives and barriers. *Appetite*. 2016;105:737-746. doi:10.1016/J.APPET.2016.07.012
10. Lockie S, Lyons K, Lawrence G, Mummery K. Eating "Green": Motivations behind organic food consumption in Australia. *Sociol Ruralis*. 2002;42(1):23-40. doi:10.1111/1467-9523.00200
11. Magnusson MK, Arvola A, Hursti U-KK, Åberg L, Sjödén P-O. Choice of organic foods is related to perceived consequences for human health and to environmentally friendly behaviour. *Appetite*. 2003;40(2):109-117. doi:10.1016/S0195-6663(03)00002-3
12. Hjelmar U. Consumers' purchase of organic food products. A matter of convenience

and reflexive practices. *Appetite*. 2011;56(2):336-344. doi:10.1016/J.APPET.2010.12.019

13. Dettmann RL, Dimitri C. Who's Buying Organic Vegetables? Demographic Characteristics of U.S. Consumers. *J Food Prod Mark*. 2009;16(1):79-91. doi:10.1080/10454440903415709
14. Nie C, Zepeda L. Lifestyle segmentation of US food shoppers to examine organic and local food consumption. *Appetite*. 2011;57(1):28-37. doi:10.1016/J.APPET.2011.03.012
15. Yadav R, Pathak GS. Intention to purchase organic food among young consumers: Evidences from a developing nation. *Appetite*. 2016;96:122-128. doi:10.1016/J.APPET.2015.09.017
16. Thøgersen J, Zhou Y. Chinese consumers' adoption of a 'green' innovation – The case of organic food. *J Mark Manag*. 2012;28(3-4):313-333. doi:10.1080/0267257X.2012.658834
17. Kesse-Guyot E, Péneau S, Méjean C, et al. Profiles of Organic Food Consumers in a Large Sample of French Adults: Results from the Nutrinet-Santé Cohort Study. Alquier T, ed. *PLoS One*. 2013;8(10):e76998. doi:10.1371/journal.pone.0076998
18. Petersen SB, Rasmussen MA, Strøm M, Halldorsson TI, Olsen SF. Sociodemographic characteristics and food habits of organic consumers – a study from the Danish National Birth Cohort. *Public Health Nutr*. 2013;16(10):1810-1819. doi:10.1017/S1368980012004119
19. Eisinger-Watzl M, Wittig F, Heuer T, Hoffmann I. Customers Purchasing Organic Food - Do They Live Healthier? Results of the German National Nutrition Survey II. *EJNFS*. 2015;5(1):59-71. doi:10.9734/EJNFS/2015/12734
20. Torjusen H, Lieblein G, Næs T, Haugen M, Meltzer HM, Brantsæter AL. Food patterns and dietary quality associated with organic food consumption during pregnancy; data from a large cohort of pregnant women in Norway. *BMC Public Health*. 2012;12(1):612. doi:10.1186/1471-2458-12-612
21. Hughner RS, McDonagh P, Prothero A, Shultz CJ, Stanton J. Who are organic food consumers? A compilation and review of why people purchase organic food. *J Consum Behav*. 2007;6(2-3):94-110. doi:10.1002/cb.210

22. Smith-Spangler C, Brandeau ML, Hunter GE, et al. Are Organic Foods Safer or Healthier Than Conventional Alternatives? *Ann Intern Med*. 2012;157(5):348. doi:10.7326/0003-4819-157-5-201209040-00007
23. Barański M, Średnicka-Tober D, Volakakis N, et al. Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *Br J Nutr*. 2014;112(05):794-811. doi:10.1017/S0007114514001366
24. Bradman A, Quirós-Alcalá L, Castorina R, et al. Effect of Organic Diet Intervention on Pesticide Exposures in Young Children Living in Low-Income Urban and Agricultural Communities. *Environ Health Perspect*. 2015;123(10):1086-1093. doi:10.1289/ehp.1408660
25. Curl CL, Fenske RA, Elgethun K. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. *Environ Health Perspect*. 2003;111(3):377-382. doi:10.1289/ehp.5754
26. Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. Organic Diets Significantly Lower Children's Dietary Exposure to Organophosphorus Pesticides. *Environ Health Perspect*. 2006;114(2):260-263. doi:10.1289/ehp.8418
27. Oates L, Cohen M, Braun L, Schembri A, Taskova R. Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. *Environ Res*. 2014;132:105-111. doi:10.1016/J.ENVRES.2014.03.021
28. Baudry J, Debrauwer • Laurent, Durand • Gaël, et al. Urinary pesticide concentrations in French adults with low and high organic food consumption: results from the general population-based NutriNet-Santé. *J Expo Sci Environ Epidemiol*. 2018. doi:10.1038/s41370-018-0062-9
29. Brantsæter AL, Ydersbond TA, Hoppin JA, Haugen M, Meltzer HM. Organic Food in the Diet: Exposure and Health Implications. *Annu Rev Public Health*. 2017;38(1):295-313. doi:10.1146/annurev-publhealth-031816-044437
30. Sanborn M, Kerr KJ, Sanin LH, Cole DC, Bassil KL, Vakil C. Non-cancer health effects of pesticides: systematic review and implications for family doctors. *Can Fam Physician*. 2007;53(10):1712-1720.
31. Hung D-Z, Yang H-J, Li Y-F, et al. The Long-Term Effects of Organophosphates

Poisoning as a Risk Factor of CVDs: A Nationwide Population-Based Cohort Study. 2015. doi:10.1371/journal.pone.0137632

32. Samsel A, Seneff S, Samsel A, Seneff S. Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases. *Entropy*. 2013;15(12):1416-1463. doi:10.3390/e15041416

33. Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O, Roig B. Effect of Endocrine Disruptor Pesticides: A Review. *Int J Environ Res Public Health*. 2011;8(6):2265. doi:10.3390/IJERPH8062265

34. Salameh P, Waked M, Baldi I, Brochard P, Saleh BA. Respiratory diseases and pesticide exposure: a case-control study in Lebanon. *J Epidemiol Community Health*. 2006;60(3):256-261. doi:10.1136/jech.2005.039677

35. EPA. Suspended, canceled, and restricted use pesticides, EPA/20T-1002. 1990.

36. Thuy TT. Effects of DDT on environment and human health. *J Educ Soc Sci*. 2.

37. Turusov V, Rakitsky V, Tomatis L. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. *Environ Health Perspect*. 2002;110(2):125-128. doi:10.1289/ehp.02110125

38. Pirsaheb M, Limoee M, Namdari F, Khamutian R. Organochlorine pesticides residue in breast milk: a systematic review. *Med J Islam Repub Iran*. 2015;29:228.

39. Miranda-Contreras L, Gómez-Pérez R, Rojas G, et al. Occupational exposure to organophosphate and carbamate pesticides affects sperm chromatin integrity and reproductive hormone levels among Venezuelan farm workers. *J Occup Health*. 2013;55(3):195-203.

40. Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K. Chronic central nervous system effects of acute organophosphate pesticide intoxication. *Lancet*. 1991;338(8761):223-227. doi:10.1016/0140-6736(91)90356-T

41. Lin J-N, Lin C-L, Lin M-C, et al. Increased Risk of Dementia in Patients With Acute Organophosphate and Carbamate Poisoning. *Medicine (Baltimore)*. 2015;94(29):e1187. doi:10.1097/MD.0000000000001187

42. Waddell BL, Zahm SH, Baris D, et al. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Cancer Causes Control*. 2001;12(6):509-517.

http://www.ncbi.nlm.nih.gov/pubmed/11519759. Accessed April 11, 2019.

43. Coupe RH, Capel PD. Trends in pesticide use on soybean, corn and cotton since the introduction of major genetically modified crops in the United States. *Pest Manag Sci*. 2016;72(5):1013-1022. doi:10.1002/ps.4082

44. Williams GM, Kroes R, Munro IC. Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. *Regul Toxicol Pharmacol*. 2000;31(2):117-165. doi:10.1006/RTPH.1999.1371

45. Environmental Protection Agency. Glyphosate case screening level usage analysis.

46. Myers JP, Antoniou MN, Blumberg B, et al. Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environ Heal A Glob Access Sci Source*. 2016;15(1). doi:10.1186/s12940-016-0117-0

47. Agricultural Marketing service. *Pesticide Data Program Annual Summary, Program Year 2011.* Washington, D.C.; 2013. www.ams.usda.gov. Accessed November 29, 2018.

48. Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. *Food Chem*. 2014;153:207-215. doi:10.1016/J.FOODCHEM.2013.12.054

49. The Detox Project. *Glyphosate: Unsafe on Any Plate*. http://apps.who.int/iris/bitstream/10665/43624/1/9241665203_eng.pdf. Accessed November 29, 2018.

50. Emily Guo FR, Guo E, kamp L. Survey of Glyphosate Residues in Honey, Corn and Soy Products. *J Environ Anal Toxicol*. 2014;05(01):1-8. doi:10.4172/2161-0525.1000249

51. Krüger M, Schrödl W, Pedersen I, Pedersen I, Shehata AA. Detection of Glyphosate in Malformed Piglets. *J Environ Anal Toxicol*. 2014;04(05):1-2. doi:10.4172/2161-0525.1000230

52. Wieland Schrodl MK, Schrödl W, Neuhaus J, Shehata AA. Field Investigations of Glyphosate in Urine of Danish Dairy Cows. *J Environ Anal Toxicol*. 2013;03(05):1-7. doi:10.4172/2161-0525.1000186

53. Moms Across America. *Glyphosate Testing Full Report: Findings in American Mothers' Breast Milk, Urine and Water.*; 2014.

54. Mills PJ, Kania-Korwel I, Fagan J, McEvoy LK, Laughlin GA, Barrett-Connor E. Excretion of the Herbicide Glyphosate in Older Adults Between 1993 and 2016. *JAMA*. 2017;318(16):1610. doi:10.1001/jama.2017.11726
55. Baudry J, Allès B, Péneau S, et al. Dietary intakes and diet quality according to levels of organic food consumption by French adults: cross-sectional findings from the NutriNet-Santé Cohort Study. *Public Health Nutr*. 2017;20(04):638-648. doi:10.1017/S1368980016002718
56. Michaud P-C, Goldman D, Lakdawalla D, Gailey A, Zheng Y. Differences in health between Americans and Western Europeans: Effects on longevity and public finance. *Soc Sci Med*. 2011;73(2):254-263. doi:10.1016/J.SOCSCIMED.2011.05.027
57. Curl CL, Beresford SAA, Fenske RA, et al. Estimating pesticide exposure from dietary intake and organic food choices: the Multi-Ethnic Study of Atherosclerosis (MESA). *Environ Health Perspect*. 2015;123(5):475-483. doi:10.1289/ehp.1408197
58. Brandt K, Leifert C, Sanderson R, Seal CJ. Agroecosystem Management and Nutritional Quality of Plant Foods: The Case of Organic Fruits and Vegetables. *CRC Crit Rev Plant Sci*. 2011;30(1-2):177-197. doi:10.1080/07352689.2011.554417
59. Dangour AD, Dodhia SK, Hayter A, Allen E, Lock K, Uauy R. Nutritional quality of organic foods: a systematic review. *Am J Clin Nutr*. 2009;90(3):680-685. doi:10.3945/ajcn.2009.28041
60. Shahidi F, Ambigaipalan P. Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. *Annu Rev Food Sci Technol*. 2018;9(1):345-381. doi:10.1146/annurev-food-111317-095850
61. Średnicka-Tober D, Barański M, Seal CJ, et al. Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: a systematic literature review and meta- and redundancy analyses. *Br J Nutr*. 2016;115(06):1043-1060. doi:10.1017/S0007114516000349
62. Palupi E, Jayanegara A, Ploeger A, Kahl J. Comparison of nutritional quality between conventional and organic dairy products: a meta-analysis. *J Sci Food Agric*. 2012;92(14):2774-2781. doi:10.1002/jsfa.5639
63. Średnicka-Tober D, Barański M, Seal C, et al. Composition differences between organic and conventional meat: a systematic literature review and meta-analysis. *Br J*

Nutr. 2016;115(06):994-1011. doi:10.1017/S0007114515005073

64. The 2013 European Union report on pesticide residues in food. *EFSA J*. 2015;13(3):4038. doi:10.2903/j.efsa.2015.4038

65. Bakery BP, Benbrook CM, Groth III E, Lutz Benbrook K. Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets. doi:10.1080/0265203011011379

66. Pussemier L, Larondelle Y, Van Peteghem C, Huyghebaert A. Chemical safety of conventionally and organically produced foodstuffs: A tentative comparison under Belgian conditions. *Food Control*. 2006;17(1):14-21. doi:10.1016/J.FOODCONT.2004.08.003

67. Baudry J, Méjean C, Péneau S, et al. Health and dietary traits of organic food consumers: results from the NutriNet-Santé study. *Br J Nutr*. 2015;114(12):2064-2073. doi:10.1017/S0007114515003761

68. Torjusen H, Brantsæter AL, Haugen M, et al. Characteristics associated with organic food consumption during pregnancy; data from a large cohort of pregnant women in Norway. *BMC Public Health*. 2010;10(1):775. doi:10.1186/1471-2458-10-775

69. Zepeda L, Chang H-S, Leviten-Reid C. Organic Food Demand: A Focus Group Study Involving Caucasian and African-American Shoppers. *Agric Human Values*. 2006;23(3):385-394. doi:10.1007/s10460-006-9001-9

70. Luoto R, Prättälä R, Uutela A, Puska P. Impact of Unhealthy Behaviors on Cardiovascular Mortality in Finland, 1978–1993. *Prev Med (Baltim)*. 1998;27(1):93-100. doi:10.1006/PMED.1997.0249

71. Alfano CM, Day JM, Katz ML, et al. Exercise and dietary change after diagnosis and cancer-related symptoms in long-term survivors of breast cancer: CALGB 79804. *Psychooncology*. 2009;18(2):128-133. doi:10.1002/pon.1378

72. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle - the PARSIFAL study. *Allergy*. 2006;61(4):414-421. doi:10.1111/j.1398-9995.2005.00939.x

73. Kummeling I, Thijs C, Huber M, et al. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. *Br J Nutr*. 2008;99(3):598-

605. doi:10.1017/S0007114507815844

74. Rist L, Mueller A, Barthel C, et al. Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. *Br J Nutr*. 2007;97(04):735. doi:10.1017/S0007114507433074

75. Stenius F, Swartz J, Lilja G, et al. Lifestyle factors and sensitization in children - the ALADDIN birth cohort. *Allergy*. 2011;66(10):1330-1338. doi:10.1111/j.1398-9995.2011.02662.x

76. Fagerstedt S, Hesla HM, Ekhager E, et al. Anthroposophic lifestyle is associated with a lower incidence of food allergen sensitization in early childhood. *J Allergy Clin Immunol*. 2016;137(4):1253-1256.e3. doi:10.1016/j.jaci.2015.11.009

77. Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. *Lancet*. 1999;353(9163):1485-1488. doi:10.1016/S0140-6736(98)09344-1

78. Flöistrup H, Swartz J, Bergström A, et al. Allergic disease and sensitization in Steiner school children. *J Allergy Clin Immunol*. 2006;117(1):59-66. doi:10.1016/J.JACI.2005.09.039

79. Torjusen H, Brantsæter AL, Haugen M, et al. Reduced risk of pre-eclampsia with organic vegetable consumption: results from the prospective Norwegian Mother and Child Cohort Study. *BMJ Open*. 2014;4(9):e006143. doi:10.1136/bmjopen-2014-006143

80. Christensen JS, Asklund C, Skakkebæk NE, et al. Association Between Organic Dietary Choice During Pregnancy and Hypospadias in Offspring: A Study of Mothers of 306 Boys Operated on for Hypospadias. *J Urol*. 2013;189(3):1077-1082. doi:10.1016/j.juro.2012.09.116

81. Brantsæter AL, Torjusen H, Meltzer HM, et al. Organic Food Consumption during Pregnancy and Hypospadias and Cryptorchidism at Birth: The Norwegian Mother and Child Cohort Study (MoBa). *Environ Health Perspect*. 2016;124(3):357-364. doi:10.1289/ehp.1409518

82. Kesse-Guyot E, Baudry J, Assmann KE, Galan P, Hercberg S, Lairon D. Prospective association between consumption frequency of organic food and body weight change, risk of overweight or obesity: results from the NutriNet-Santé Study. *Br J*

Nutr. 2017;117(02):325-334. doi:10.1017/S0007114517000058

83. Abell A, Ernst E, Bonde JP. High sperm density among members of organic farmers' association. *Lancet (London, England)*. 1994;343(8911):1498. doi:10.1016/S0140-6736(94)92605-0

84. Jensen TK, Giwercman A, Carlsen E, Scheike T, Skakkebaek N. Semen quality among members of organic food associations in Zealand, Denmark. *Lancet*. 1996;347(9018):1844. doi:10.5555/URI:PII:S0140673696916694

85. Juhler RK, Larsen SB, Meyer O, et al. Human semen quality in relation to dietary pesticide exposure and organic diet. *Arch Environ Contam Toxicol*. 1999;37(3):415-423.

86. Larsen SB, Spanò M, Giwercman A, Bonde JP. Semen quality and sex hormones among organic and traditional Danish farmers. ASCLEPIOS Study Group. *Occup Environ Med*. 1999;56(2):139-144. http://www.ncbi.nlm.nih.gov/pubmed/10448320. Accessed April 15, 2019.

87. Baudry J, Assmann KE, Touvier M, et al. Association of Frequency of Organic Food Consumption With Cancer Risk. *JAMA Intern Med*. October 2018. doi:10.1001/jamainternmed.2018.4357

88. Bradbury KE, Balkwill A, Spencer EA, et al. Organic food consumption and the incidence of cancer in a large prospective study of women in the United Kingdom. *Br J Cancer*. 2014;110(9):2321-2326. doi:10.1038/bjc.2014.148

89. Vandenberg LN, Blumberg B, Antoniou MN, et al. Is it time to reassess current safety standards for glyphosate-based herbicides? *J Epidemiol Community Health*. 2017;71(6):613-618. doi:10.1136/jech-2016-208463

90. Bempah CK, Agyekum AA, Akuamoa F, Frimpong S, Buah-Kwofie A. Dietary exposure to chlorinated pesticide residues in fruits and vegetables from Ghanaian markets. *J Food Compos Anal*. 2016;46. doi:10.1016/j.jfca.2015.12.001

91. Zoeller RT, Brown TR, Doan LL, et al. Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society. *Endocrinology*. 2012;153(9):4097-4110. doi:10.1210/en.2012-1422

92. Mesnage R, Renney G, Séralini G-E, Ward M, Antoniou MN. Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of

Roundup herbicide. *Sci Rep*. 2017;7(1):39328. doi:10.1038/srep39328

93. Séralini G-E, Clair E, Mesnage R, et al. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerantgenetically modified maize. *Environ Sci Eur*. 2014;26(1):14. doi:10.1186/s12302-014-0014-5

94. Plhalova L, Stepanova S, Praskova E, et al. The Effects of Subchronic Exposure to Metribuzin on *Danio rerio*. *Sci World J*. 2012;2012:1-6. doi:10.1100/2012/728189

95. Mesnage R, Arno M, Costanzo M, Malatesta M, Séralini G-E, Antoniou MN. Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. *Environ Heal*. 2015;14(1):70. doi:10.1186/s12940-015-0056-1

96. Robinson C, Habib M, Habib M, et al. Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence. *J Environ Anal Toxicol*. 2012;01(S4). doi:10.4172/2161-0525.S4-006

97. Mesnage R, Defarge N, Spiroux De J, Eralini GES. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food Chem Toxicol*. 2015;84:133-153. doi:10.1016/j.fct.2015.08.012

98. Benedetti AL, Vituri C de L, Trentin AG, Domingues MAC, Alvarez-Silva M. The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb®. *Toxicol Lett*. 2004;153(2):227-232. doi:10.1016/J.TOXLET.2004.04.008

99. Larsen K, Najle R, Lifschitz A, Maté ML, Lanusse C, Virkel GL. Effects of Sublethal Exposure to a Glyphosate-Based Herbicide Formulation on Metabolic Activities of Different Xenobiotic-Metabolizing Enzymes in Rats. *Int J Toxicol*. 2014;33(4):307-318. doi:10.1177/1091581814540481

100. Romano MA, Romano RM, Santos LD, et al. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. *Arch Toxicol*. 2012;86(4):663-673. doi:10.1007/s00204-011-0788-9

101. Armiliato N, Ammar D, Nezzi L, Straliotto M, Muller YMR, Nazari EM. Changes in Ultrastructure and Expression of Steroidogenic Factor-1 in Ovaries of Zebrafish *Danio rerio* Exposed to Glyphosate. *J Toxicol Environ Heal Part A*. 2014;77(7):405-414. doi:10.1080/15287394.2014.880393

102. Gasnier C, Dumont C, Benachour N, Clair E, Chagnon M-C, Séralini G-E. Glyphosate-

based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*. 2009;262(3):184-191. doi:10.1016/J.TOX.2009.06.006

103. Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. The Effect of Glyphosate on Potential Pathogens and Beneficial Members of Poultry Microbiota In Vitro. *Curr Microbiol*. 2013;66(4):350-358. doi:10.1007/s00284-012-0277-2

104. Clair E, Linn L, Travert C, Amiel C, Séralini G-E, Panoff J-M. Effects of Roundup® and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. *Curr Microbiol*. 2012;64(5):486-491. doi:10.1007/s00284-012-0098-3

105. Krüger M, Shehata AA, Schrödl W, Rodloff A. Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum. *Anaerobe*. 2013;20:74-78. doi:10.1016/J.ANAEROBE.2013.01.005

106. Hoffmann AR, Proctor LM, Surette MG, Suchodolski JS. The Microbiome: The Trillions of Microorganisms That Maintain Health and Cause Disease in Humans and Companion Animals. *Vet Pathol*. 2016;53(1):10-21. doi:10.1177/0300985815595517

107. Kwiatkowska M, Reszka E, Woźniak K, Jabłońska E, Michałowicz J, Bukowska B. DNA damage and methylation induced by glyphosate in human peripheral blood mononuclear cells (in vitro study). *Food Chem Toxicol*. 2017;105:93-98. doi:10.1016/J.FCT.2017.03.051

108. Eriksson M, Hardell L, Carlberg M, Åkerman M. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer*. 2008;123(7):1657-1663. doi:10.1002/ijc.23589

109. De Roos AJ, Zahm SH, Cantor KP, et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med*. 2003;60(9):E11. doi:10.1136/OEM.60.9.E11

110. McDuffie HH, Pahwa P, McLaughlin JR, et al. Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men: Cross-Canada Study of Pesticides and Health. *Cancer Epidemiol Biomarkers Prev*. 2001;5(1):11-16.

111. Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J. Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food*

Chem Toxicol. 2013;59:129-136. doi:10.1016/J.FCT.2013.05.057

112. Kissel JC, Curl CL, Kedan G, et al. Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington State. *J Expo Sci Environ Epidemiol*. 2005;15(2):164-171. doi:10.1038/sj.jea.7500384

113. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

114. Thompson FE, Dixit-Joshi S, Potischman N, et al. Comparison of Interviewer-Administered and Automated Self-Administered 24-Hour Dietary Recalls in 3 Diverse Integrated Health Systems. *Am J Epidemiol*. 2015;181(12):970-978. doi:10.1093/aje/kwu467

115. Park Y, Dodd KW, Kipnis V, et al. Comparison of self-reported dietary intakes from the Automated Self-Administered 24-h recall, 4-d food records, and food-frequency questionnaires against recovery biomarkers. *Am J Clin Nutr*. 2018;107(1):80-93. doi:10.1093/ajcn/nqx002

116. Kirkpatrick SI, Potischman N, Dodd KW, et al. The Use of Digital Images in 24-Hour Recalls May Lead to Less Misestimation of Portion Size Compared with Traditional Interviewer-Administered Recalls. *J Nutr*. 2016;146(12):2567-2573. doi:10.3945/jn.116.237271

117. Jensen PK, Wujcik CE, McGuire MK, McGuire MA. Validation of reliable and selective methods for direct determination of glyphosate and aminomethylphosphonic acid in milk and urine using LC-MS/MS. *J Environ Sci Health B*. 2016;51(4):254-259. doi:10.1080/03601234.2015.1120619

118. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). *Bioanalytical Method Validation Guidance for Industry*.; 2018.

119. EPA. *ASSIGNING VALUES TO NON-DETECTED/NON-QUANTIFIED PESTICIDE RESIDUES IN HUMAN HEALTH FOOD EXPOSURE ASSESSMENTS*.; 2000.

120. Rhee JJ, Sampson L, Cho E, Hughes MD, Hu FB, Willett WC. Comparison of Methods to

Account for Implausible Reporting of Energy Intake in Epidemiologic Studies. *Am J Epidemiol*. 2015;181(4):225. doi:10.1093/AJE/KWU308

121. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr*. 1997;65(4):1220S-1228S. doi:10.1093/ajcn/65.4.1220S

122. DCCPS. Evaluating the Healthy Eating Index. https://epi.grants.cancer.gov/hei/evaluation-validation.html.

123. DCCPS. Sample SAS code for calculating Healthy eating index. https://epi.grants.cancer.gov/hei/sas-code.html.

124. Ziauddeen N, Almiron-Roig E, Penney TL, Nicholson S, Kirk SFL, Page P. Eating at Food Outlets and "On the Go" Is Associated with Less Healthy Food Choices in Adults: Cross-Sectional Data from the UK National Diet and Nutrition Survey Rolling Programme (2008-2014). *Nutrients*. 2017;9(12). doi:10.3390/nu9121315

125. Cohen DA, Story M. Mitigating the health risks of dining out: the need for standardized portion sizes in restaurants. *Am J Public Health*. 2014;104(4):586-590. doi:10.2105/AJPH.2013.301692

126. Wilson MM, Reedy J, Krebs-Smith SM. American Diet Quality: Where It Is, Where It Is Heading, and What It Could Be. *J Acad Nutr Diet*. 2016;116(2):302-310.e1. doi:10.1016/J.JAND.2015.09.020

127. Gillezeau C, van Gerwen M, Shaffer RM, et al. The evidence of human exposure to glyphosate: a review. *Environ Heal*. 2019;18(1):2. doi:10.1186/s12940-018-0435-5

128. Philipp Schledorn MK, Schledorn P, Schrödl W, Hoppe H-W, Lutz W, Shehata AA. Detection of Glyphosate Residues in Animals and Humans. *J Environ Anal Toxicol*. 2014;04(02):1-5. doi:10.4172/2161-0525.1000210

129. Hartman Group. *Organic & Natural 2016*.; 2016.

130. McCrory MA, Fuss PJ, Hays NP, Vinken AG, Greenberg AS, Roberts SB. Overeating in America: association between restaurant food consumption and body fatness in healthy adult men and women ages 19 to 80. *Obes Res*. 1999;7(6):564-571.

131. Bowman SA, Vinyard BT. Fast food consumption of U.S. adults: impact on energy and nutrient intakes and overweight status. *J Am Coll Nutr*. 2004;23(2):163-168.

132. Bahadoran Z, Mirmiran P, Golzarand M, Hosseini-Esfahani F, Azizi F. Fast food consumption in Iranian adults; dietary intake and cardiovascular risk factors: Tehran

Lipid and Glucose Study. *Arch Iran Med*. 2012;15(6):346-351. doi:012156/AIM.006

133. Krishnan S, Coogan PF, Boggs DA, Rosenberg L, Palmer JR. Consumption of restaurant foods and incidence of type 2 diabetes in African American women. *Am J Clin Nutr*. 2010;91(2):465-471. doi:10.3945/ajcn.2009.28682

134. Flint AJ, Hu FB, Glynn RJ, et al. Whole grains and incident hypertension in men. *Am J Clin Nutr*. 2009;90(3):493-498. doi:10.3945/ajcn.2009.27460

135. Wang L, Gaziano JM, Liu S, Manson JE, Buring JE, Sesso HD. Whole- and refined-grain intakes and the risk of hypertension in women. *Am J Clin Nutr*. 2007;86(2):472-479. doi:10.1093/ajcn/86.2.472

136. Bazzano LA, Song Y, Bubes V, Good CK, Manson JE, Liu S. Dietary Intake of Whole and Refined Grain Breakfast Cereals and Weight Gain in Men. *Obes Res*. 2005;13(11):1952-1960. doi:10.1038/oby.2005.240

137. Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr*. 2003;78(5):920-927. doi:10.1093/ajcn/78.5.920

138. Steffen LM, Jacobs DR, Murtaugh MA, et al. Whole grain intake is associated with lower body mass and greater insulin sensitivity among adolescents. *Am J Epidemiol*. 2003;158(3):243-250.

139. Aune D, Norat T, Romundstad P, Vatten LJ. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies. *Eur J Epidemiol*. 2013;28(11):845-858. doi:10.1007/s10654-013-9852-5

140. Steffen LM, Jacobs DR, Stevens J, Shahar E, Carithers T, Folsom AR. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr*. 2003;78(3):383-390. doi:10.1093/ajcn/78.3.383

141. Chen J, Huang Q, Shi W, Yang L, Chen J, Lan Q. Meta-Analysis of the Association Between Whole and Refined Grain Consumption and Stroke Risk Based on Prospective Cohort Studies. *Asia Pacific J Public Heal*. 2016;28(7):563-575.

doi:10.1177/1010539516650722

142. Office of Disease Prevention and Health Promotion. *Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines of Americans 2015 to the Secretary of Agriculture and the Secretary of Health and Human Services*. https://health.gov/dietaryguidelines/2015-scientific-report/.
143. Van Horn L, Carson JAS, Appel LJ, et al. Recommended Dietary Pattern to Achieve Adherence to the American Heart Association/American College of Cardiology (AHA/ACC) Guidelines: A Scientific Statement From the American Heart Association. *Circulation*. 2016;134(22):e505-e529. doi:10.1161/CIR.0000000000000462
144. Duke SO. Glyphosate Degradation in Glyphosate-Resistant and-Susceptible Crops and Weeds. *J Agric Food Chem*. 2011;59:5835-5841. doi:10.1021/jf102704x
145. Wessels D, Barr DB, Mendola P. Use of biomarkers to indicate exposure of children to organophosphate pesticides: implications for a longitudinal study of children's environmental health. *Environ Health Perspect*. 2003;111(16):1939-1946. doi:10.1289/ehp.6179
146. Connolly A, Jones K, Basinas I, et al. Exploring the half-life of glyphosate in human urine samples. *Int J Hyg Environ Health*. 2019;222(2):205-210. doi:10.1016/j.ijheh.2018.09.004
147. Simundić A-M. Bias in research. *Biochem medica*. 2013;23(1):12-15. doi:10.11613/BM.2013.003
148. Rosenman R, Tennekoon V, Hill LG. Measuring bias in self-reported data. *Int J Behav Healthc Res*. 2011;2(4):320-332. doi:10.1504/IJBHR.2011.043414

Appendices

Supplemental table 1. The association between food groups intake and urinary AMPA levels, stratified by the level of organic food consumption.* AMPA levels are expressed as µg/g creatinine, mean (SE).

A. Fruits

	Fruits intake				*p*-value
	Q1	**Q2**	**Q3**	**Q4**	
Overall	0.25 (0.04)	0.16 (0.04)	0.12 (0.04)	0.17 (0.04)	0.20
Organic food consumption frequency					
Infrequent	0.29 (0.1)	0.19 (0.12)	0.13 (0.09)	0.34 (0.12)	0.45
Sometime	0.25 (0.05)	0.16 (0.05)	0.09 (0.05)	0.13 (0.04)	0.17
Frequent	0.13 (0.05)	0.11 (0.03)	0.15 (0.03)	0.09 (0.04)	0.60

B. Vegetables

	Vegetables intake				*p*-value
	Q1	**Q2**	**Q3**	**Q4**	
Overall	0.25 (0.04)	0.18 (0.04)	0.13 (0.04)	0.13 (0.04)	0.15
Organic food consumption frequency					
Infrequent	0.31 (0.09)	0.24 (0.09)	0.16 (0.14)	0.14 (0.11)	0.67
Sometime	0.22 (0.05)	0.12 (0.06)	0.16 (0.05)	0.11 (0.04)	0.38
Frequent	0.09 (0.06)	0.13 (0.03)	0.09 (0.03)	0.15 (0.03)	0.55

C. Grains

	Grains intake				*p*-value
	Q1	**Q2**	**Q3**	**Q4**	
Overall	0.17 (0.04)	0.2 (0.04)	0.16 (0.04)	0.16 (0.04)	0.91
Organic food consumption frequency					
Infrequent	0.28 (0.10)	0.47 (0.13)	0.11 (0.10)	0.17 (0.09)	0.18
Sometime	0.11 (0.04)	0.13 (0.06)	0.16 (0.04)	0.21 (0.05)	0.50
Frequent	0.14 (0.04)	0.09 (0.03)	0.17 (0.03)	0.10 (0.05)	0.31

D. Meats

	Meats intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.2 (0.04)	0.14 (0.04)	0.22 (0.04)	0.12 (0.04)	0.21
Organic food consumption frequency					
Infrequent	0.37 (0.11)	0.18 (0.14)	0.22 (0.09)	0.15 (0.10)	0.55
Sometime	0.14 (0.04)	0.16 (0.05)	0.27 (0.06)	0.09 (0.04)	0.13
Frequent	0.13 (0.03)	0.11 (0.03)	0.13 (0.04)	0.14 (0.05)	0.91

E. Dairy products

	Dairy products intake				*p*-value
	Q1	Q2	Q3	Q4	
Overall	0.16 (0.04)	0.26 (0.04)	0.15 (0.04)	0.11 (0.03)	0.03
Organic food consumption frequency					
Infrequent	0.16 (0.10)	0.46 (0.10)	0.22 (0.12)	0.12 (0.08)	0.07
Sometime	0.14 (0.04)	0.23 (0.05)	0.16 (0.06)	0.09 (0.05)	0.25
Frequent	0.14 (0.05)	0.14 (0.03)	0.14 (0.04)	0.08 (0.03)	0.42

*Adjusted for age, race/ethnicity, education, BMI, alcohol consumption and smoking status

Supplemental table 2. Association between organic food consumption index and the level of organic food consumption assessed by general organic food consumption question

	Organic food consumer type according to one general question			p-value
	Seldom / never	Sometimes	Often / always	
N, (%)	120 (32.09%)	106 (28.34%)	148 (39.57%)	
Organic food consumption index	0 (0)	0.35 (0.22)	0.67 (0.25)	<0.0001