Pesticide Residues in Fruits and Vegetables: Assessment and Their Associations With Reproductive Health Outcomes

Pesticide Residues in Fruits and Vegetables:

Assessment and their Associations with Reproductive Health Outcomes

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ABSTRACT

According to the Dietary Guideline, consumption of fruits and vegetables (FVs) are recommended throughout the lifespan, including during pregnancy. FVs, on the other hand, can serve as a vehicle of exposure to pesticide residues. In the US, Environmental Protection Agency (EPA) is responsible for regulating pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act and the Food Quality Protection Act. While majority of the produce sampled through the US Department of Agriculture had residues below the EPA limits, there is a growing concern whether chronic exposure to these pesticide residues may have adverse health effects, especially among susceptible populations such as pregnant women. Yet, such research is scarce. This dissertation focuses on the assessment of pesticide residues in FVs and evaluates their associations with pregnancy outcomes.

We previously have developed the Pesticide Residue Burden Score (PRBS) based on self-reported diet and national surveillance data on food pesticide residues to characterize dietary exposure over the past year. In Chapter 1, we evaluated the association of the PRBS with urinary pesticide metabolites in the Environment and Reproductive Health (EARTH) Study. We found intake of high pesticide residues FVs was positively associated with urinary concentrations of pesticide biomarkers, suggesting that PRBS can characterize dietary exposure to select pesticides.

In Chapter 2, we assessed the relation between preconception intake of high and low FVs and assisted reproductive technology outcomes in EARTH study. We found that intake of high pesticide residues FVs was associated with lower probability of clinical pregnancy and live birth, while intake of

low pesticide residue FVs had the opposite relations among women undergoing infertility treatment. This is the first report of such relation in humans.

In Chapter 3, we examine the association between maternal intake of high and low pesticide residue FVs with birth outcomes in a pre-birth cohort. We found that maternal intake of high pesticide residue FVs during the first trimester was associated with higher risks of small-for-gestational-age among white women, while these exposures was associated with large-for-gestational-age among non-white women.

In conclusion, this work demonstrated the usefulness of PRBS in assessing pesticide residue intake through FVs. Using this method, these studies suggest exposure to pesticide residues may adversely affect pregnancy and birth outcomes.

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Introduction

Pesticide exposure is ubiquitous. More than 90% of the United States population has detectable concentrations of pesticides or their metabolites in their urine or blood samples.(Centers for Disease Control and Prevention 2015) While pesticide exposure occurs through a variety of routes, diet—especially intake of fruits and vegetables—is the primary route of pesticide exposure in the general population.(Lu et al. 2008) According to the U.S. Food and Drug Administration's Pesticide Monitoring Program, fruits and vegetables have a considerably higher percentage of detectable pesticide residues and higher percentage of samples with residue exceeding the tolerance levels than any other food group.(US Food and Drug Administration 2012) Others have shown that substituting conventionally grown produce with organic produce dramatically decreases the select urinary concentrations of pesticide metabolites. (Lu et al. 2006, Bradman et al. 2015, Oates et al. 2014)

In the United States, Environmental Protection Agency (EPA) is responsible for regulating pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA). EPA sets the limits on the amount of pesticide residues that are allowed to remain in or on each food, which is called tolerance. Tolerance levels are established based on risk assessment process, including the information on toxicity of pesticides. Nonetheless, intake of pesticide residues below tolerance levels does not imply safety or lack of adverse health outcomes. For example, toxicology studies typically test a limited number of adverse endpoints. Even when data are available, most studies covered only a portion of the possible range of the dose-response relationships in animals, where EPA has to extrapolate the data to determine safe levels for human. While these extrapolation uses uncertainty factors to acknowledge differences between humans and animals, and variation in vulnerability between people, these uncertainty factors are informed guesses and not quantitatively based calculations(Vandenberg et al. 2012). Thus, it is still unclear whether intake of pesticide residues

has any adverse health effects in humans, especially for susceptible populations such as pregnant women and their fetuses (Vandenberg et al. 2012, Hayes et al. 2002). As pesticide metabolites can be detected in amniotic fluid as early as 15-18 weeks gestation(Bradman et al. 2003), the fetus, due to its rapid growth, immaturity of metabolic pathways (e.g., lower detoxifying ability), and development of vital organ systems(Berkowitz et al. 2004), may exhibit greater susceptibility to the effects of pesticide residues than adults.

With an aim to investigate the potential adverse health effects of dietary pesticide intake, we leveraged data from U.S. Department of Agriculture Pesticide Data Program, a nation-wide surveillance program that provides statistically representative data on pesticide residues in the U.S. food supply. Integrating surveillance data from the Pesticide Data Program with food frequency questionnaires, I developed a novel approach— the Pesticide Residue Burden Score (PRBS)— to classifying fruits and vegetables into high versus low pesticide residue groups. In Chapter 1, I evaluated the ability of the PRBS to characterize individuals' exposure by comparing it to traditional biomarkers of pesticide exposure. In Chapters 2 and 3, I applied PRBS to investigate the potential adverse health effects of high pesticide residue fruit and vegetable intake among susceptible populations – women who attempted and who achieved pregnancy.

In rodent models, ingestion of pesticide mixtures in early pregnancy at environmentally relevant concentrations, increased the percentage of apoptosis in embryos and decreased the number of live pups born (Greenlee, Ellis, and Berg 2004, Cavieres, Jaeger, and Porter 2002). However, this relationship has never been examined in a human study. In Chapter 2, I investigated the associations between preconceptional intake of high and low pesticide residue fruit and vegetable intake and assisted reproductive technology outcomes among women presenting to a fertility clinic.

In Chapter 3, I further investigated the associations between maternal intake of high and low pesticide residue fruits and vegetables and birth outcomes. Because susceptibility to pesticides might

differ across race/ethnicity(Chen et al. 2003), I examined the association of high and low pesticide fruit and vegetable intake with fetal growth and gestational age at delivery stratified by race/ethnicity in a pre-birth cohort in Eastern Massachusetts.

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CHAPTER 1

Comparison of questionnaire-based estimation of pesticide residue intake from fruits and vegetables with urinary concentrations of pesticide metabolites

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Abstract

Background: We developed a pesticide residue burden score (PRBS) based on self-reported diet and surveillance data on food pesticide residues to characterize dietary exposure over the past year.

Objectives: To evaluate the association of the PRBS with urinary concentrations of pesticide metabolites.

Methods: Fruit and vegetable (FV) intake, assessed by a validated diet questionnaire, was classified as high (PRBS]≥4) or low (PRBS<4) in pesticide residues for 90 men from the ongoing EARTH study (2007-2015). Two urine samples per man were analyzed for seven biomarkers of organophosphate and pyrethroid insecticides, and the herbicide 2,4-dichlorophenoxyacetic acid. We used generalized estimating equations to analyze the association of the PRBS with urinary concentrations of pesticide metabolites.

Results: Men had median (interquartile range) intake of 1.2 (0.8, 1.8) servings/day of high pesticide FVs, and 2.5 (1.6, 3.2) servings/day of low pesticide FVs. Urinary concentrations of pesticide metabolites were positively related to high pesticide FV intake but inversely related to low pesticide FV intake. The specific gravity-adjusted molar sum of urinary concentrations of pesticide metabolites was 21% (95%CI: 2%, 44%) higher for each one serving/day increase in high pesticide FV intake, and 10% (95%CI: 1%, 18%) lower for each one serving/day increase in low pesticide FV intake. Furthermore, intake of high pesticide FVs also positively related to most individual urinary metabolites.

Conclusions: The association between PRBS and urinary concentrations of pesticide metabolites supports the usefulness of the PRBS approach to characterize dietary exposure to select pesticides.

Introduction

Human exposure to pesticides is ubiquitous. More than 90% of the U.S. population has detectable concentrations of pesticides or pesticide metabolites in their urine or blood (Centers for Disease Control and Prevention 2015).While pesticide exposure occurs through a variety of routes, diet - especially intake of fruits and vegetables- is the major exposure pathway to these chemicals in the general population. According to the U.S. Pesticide Monitoring Program, fruits and vegetables have a considerably higher percentage of detectable pesticide residues and higher percentage of samples with residue exceeding the tolerance level than any other foods (US Food and Drug Administration 2012). Others have shown that intake of vegetables, but not other food groups, was positively related to urinary concentrations of metabolites of pyrethroid insecticides (Fortes et al. 2013), and that substituting conventionally grown produce with organic produce dramatically decreases the urinary concentrations of select pesticide metabolites (Lu et al. 2006);(Bradman et al. 2015, Oates et al. 2014).

Urinary biomarkers are used as the gold standard for contemporary, non-persistent pesticide exposure assessment, but short half-lives, the episodic nature of exposure (Wielgomas 2013, Bradman et al. 2013, Sudakin and Stone 2011, Spaan et al. 2015), and high analytic costs may limit the biomarkers use in large-scale studies. We previously developed a low-cost, questionnaire-based method –the dietary pesticide residue burden score (PRBS)– to estimate exposure to pesticide residues from foods in epidemiologic studies (Chiu et al. 2015, Chiu et al. 2016). This approach can make it possible to explore hypotheses regarding the potential health effects of these chemicals quickly and economically. Nonetheless, the usefulness of this approach and the ability to extend its use relies on the extent that the PRBS can adequately characterize individuals' exposure when compared to traditional biomarkers of pesticide exposure.

We previously showed using data from the National Health and Nutrition Examination Study (NHANES) that the PRBS can reasonably rank individuals' pesticide exposure through diet when

compared against urinary concentrations of non-specific metabolites of organophosphate insecticides (Hu et al. 2016). However, pesticide exposure in that study was based on the concentrations of pesticide biomarkers in a single spot urine sample, which may result in exposure missclassification given the high within-person variability in urinary concentrations of the biomarkers (Spaan et al. 2015, Sudakin and Stone 2011, Wielgomas 2013, Bradman et al. 2013). In addition, the relationship with urinary pyrethroid metabolites has not been evaluated, which is particularly important as the use of pyrethroids is gaining popularity because they have become an available alternative to organophosphate insecticides (ATSDR 2003).

The present study aimed to validate the PRBS in a well-established longitudinal cohort by using two urine samples per participant to characterize exposures to commonly used pesticides, including organophosphate and pyrethroid pesticides, two of the most commonly used classes of insecticides, and 2,4-dichlorophenoxyacetic acid, an herbicide currently in use for broadleaf weed control in agricultural and non-agricultural settings (Environmental Protection Agency 2016a).

Materials and Methods

Study population

The study population comprised men participating in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort study evaluating the relationship of environmental and nutritional factors with fertility among couples presenting to the Massachusetts General Hospital Fertility Center (Boston, MA). In April 2007, a food frequency questionnaire (FFQ) was introduced into the study to assess diet. Of 164 men who had completed a FFQ and provided at least two urine samples between April 2007 and July 2015, we selected 90 men, whose urine samples were collected within 9 months before or after FFQ completion, to have their stored urine samples analyzed for urinary

pesticide metabolites. Of the 180 samples, three (from three men) had record errors, leaving 177 samples available for analysis.

Upon study entry, men underwent an anthropometric assessment and completed a nurse-administered questionnaire in which basic demographic data were collected. Participants also completed a detailed take-home questionnaire, which contained questions on various lifestyle factors including pesticide exposure history, organic fruit and vegetable consumption frequency, and physical activity. Specifically, men were asked if their homes had been treated with pesticides in the past 5 years, if their lawns had been treated with pesticides in the past year, if they had used pesticide products personally or on pets to repel or kill pests in the past year, and if anyone in their household had been treated for head lice in the past year. Men were considered to have a history of recent residential pesticide exposure if they replied "yes" to any of these questions. Participants were also asked how often they consumed any organic fruits and vegetables during the past 3 months. Men were considered as organic fruit and vegetable consumers if they consumed organic FVs ≥ 3 times per week; men with a lower intake of organic fruits and vegetables were considered as conventional fruit and vegetable consumers. We calculated total physical activity (hrs/week) according to participants' time spent in physical activities at enrollment using a validated questionnaire (Wolf et al. 1994). The study was approved by the Human Subjects Committees of the Harvard T.H. Chan School of Public Health and the Massachusetts General Hospital, and the Centers for Disease Control and Prevention (CDC). Informed consent was obtained from all participants.

Dietary Assessment

Diet was assessed using a previously validated 131-item FFQ (Rimm et al. 1992). Men were asked to report how often, on average, they had consumed specified amounts of each food, beverage, and supplement in the questionnaire over the past year. The serving sizes for fruits and vegetables were described specifically for each item in the FFQ using standard portion sizes (e.g., one apple, ½ avocado)

or volumes (e.g., ½ cup of broccoli). In a validation study (Feskanich et al. 1993) the de-attenuated correlation (i.e., corrected for random within-person variability) between two, one-week diet records and FFQ reports ranged from 0.27 for spinach to 0.95 for banana.

Pesticide Residue Assessment

We assessed pesticide residues in fruits and vegetables using data from US Department of Agriculture's Pesticide Data Program, a national program started in 1991 that annually tests agricultural commodities in the USA for the presence of ~450 different pesticide residues (USDA 2006-2015). To best represent the pesticide residues in the food supply, the Pesticide Data Program collects samples from 10 or more participating States comprising 50% of the nation's population. Before testing, the produce is either washed or peeled to mimic consumer practices, allowing for realistic estimates of exposure. To determine the average pesticide residue status of fruits and vegetables, we developed the PRBS using the Pesticide Data Program annual reports corresponding to the periods in which the diet history of the participants was captured by the FFQ (USDA 2006-2015). Briefly, we defined PRBS (Chiu et al. 2015) according to three contamination measures from the Pesticide Data Program: 1) the percentage of samples tested with any detectable pesticides; 2) the percentage of samples tested with pesticides exceeding tolerance levels; and 3) the percentage of samples with three or more individual detectable pesticides. We ranked the 36 FVs included in the FFQ according to each of the three contamination measures, divided them into tertiles for each of these three measures, and assigned each food a score of 0, 1, and 2 corresponding to the bottom, middle, and top tertile, respectively. The final PRBS for each food was the sum of tertile scores across the three PDP contamination measures (Table S1.1). We classified foods with a PRBS ≥4 as high pesticide residue foods and those with a PRBS<4 as low pesticide residue foods (Chiu et al. 2015). To derive a PRBS specific to a class of pesticides, we used a similar algorithm (i.e., three contamination measures) but restricted Pesticide Data Program data to organophosphates and pyrethroids only for calculating organophosphate-PRBS and pyrethroid-PRBS,

respectively. In sensitivity analyses, we also considered an alternate measure, PRBS-weighted fruit and vegetable intake, calculated as the product of each food's PRBS score (on a scale of 0 to 6) and its intake frequency.

Urine pesticide metabolite measurements

Men collected spot urine samples at the baseline and follow-up clinic visits in sterile polypropylene cups. Specific gravity was measured using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA). The urine was aliquoted and stored at -80°C. Samples were shipped on dry ice overnight to the CDC (Atlanta, GA, USA) where they were analyzed for seven pesticide biomarkers: three organophosphate metabolites: 3,5,6-trichloro-2-pyridinol (TCPY), a metabolite of chlorpyrifos and chlorpyrifos-methyl; 2-isopropyl-4-methyl-6-hydroxy- pyrimidine (IMPY), a metabolite of diazinon; and *para*-nitrophenol (PNP), a metabolite of parathion and methyl parathion; three metabolites of pyrethroids: 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), a metabolite of cyfluthrin; trans-3- (2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA), a metabolite of permethrin, cypermethrin, and cyfluthrin; and 3-phenoxybenzoic acid (3-PBA), a non-specific metabolite of cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, permethrin, and tralomethrin; and one chlorophenoxy herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D) (Table S2). Solid phase extraction and high-performance liquid chromatography-isotope dilution tandem mass spectrometry was used to quantify the concentrations of these metabolites. Procedure details and quality control procedures are described elsewhere (Davis et al. 2013). Due to presence of interfering compounds, IMPY concentrations in 19 urine samples could not be quantified. The limit of detection for each metabolite is shown in Table 2.

Statistical analysis

We adjusted metabolite concentrations for using dilution using the formula Pc = P[(1.015 - 1)/specific gravity - 1], where Pc is the specific gravity-adjusted pesticide metabolite concentration (μg/L), P is the measured pesticide metabolite concentration (μg/L), and 1.015 is the mean specific gravity concentration in the study population (Smith et al. 2012). Non-detectable pesticide metabolite concentrations were replaced with a value equal to the limit of detection divided by square root of 2 prior to specific gravity adjustment (Hornung and Reed 1990). To quantify variability in urinary pesticide metabolites, we calculated the intraclass correlation coefficient (Pastore, Hertz-Picciotto, and Beaumont) based on the estimates of within- and between-subject variance obtained from the repeated measures in mixed effect models. Due to low detection rates for 4F-3-PBA and *trans*-DCCA concentrations, these two metabolites were not considered in the following analyses.

To estimate the total pesticide burden based on urinary concentrations of pesticide metabolites, we calculated the molar sum of the metabolites (in μmol/L) by dividing each metabolite concentration by its molecular weight and then summing all concentrations across metabolites. The molar sum was also calculated separately for each class of pesticides. We also ranked the participants according to each urinary pesticide concentration, and summed the ranks across the urinary metabolites for each participant. Of note, for the summary measures of pyrethroid insecticide metabolites, we only used the data from 3-PBA, which is a non-specific metabolite of a wide class of pyrethroids (Starr et al. 2008). Intake of fruits and vegetables (i.e., high pesticide fruit and vegetable intake, low pesticide fruit and vegetable intake, and PRBS-weighted fruit and vegetable intake) was modeled as continuous variables as well as in quartiles. We used linear regression with generalized estimating equations to evaluate the relation of fruit and vegetable intake (modeled as independent variables) with specific gravity-adjusted individual pesticide metabolites as well as the overall molar sum (modeled as dependent variables),

while accounting for within-person correlations in repeated samples of the same individual. Specific gravity-adjusted urinary pesticide metabolites were log-transformed to meet normality assumptions of linear regression. Resulting coefficients were back transformed to improve interpretability. Models were adjusted for age (years), body mass index (BMI) (kg/m^2), total physical activity (hr/week), race (white or non white), smoking status (ever or never), education levels (some college or lower, or college graduate), organic fruit and vegetable consumption (<3 times per week, or ≥ 3 times per week), years and season (spring, summer, fall or winter) of urine sample collection, and recent residential pesticide exposure history (yes or no) with the goal of decreasing extraneous variation in urinary metabolite concentrations (Willett 1987). Models for high pesticide residue fruit and vegetable intake were additionally adjusted for low pesticide fruit and vegetable intake, and vice versa, as intake of high pesticide fruits and vegetables and low pesticide fruits and vegetables may confound each other. Robust estimators of variance were used to compute 95% confidence intervals (CIs). Population marginal means were utilized to present population averages adjusted for the covariates (Searle, Speed, and Milliken 1980) at their average level for continuous covariates and reference level for categorical variables. Tests for linear trend were performed using median intake of fruits and vegetables in each quartile as a continuous variable. In addition, we calculated the de-attenuated Spearman correlation (i.e., observed correlation corrected for within-person variability) between high/low pesticide fruit and vegetable intake and molar sum of urinary pesticide metabolites (Rosner and Glynn 2007).

We also conducted additional sensitivity analyses in which we excluded the urine samples provided more than 6 months before or after FFQ completion. In addition, effect modification by organic food consumption (< 3 times per week vs. ≥ 3 times per week), and recent residential pesticide exposure history (yes vs. no) was tested using cross-product terms in the multivariable model. Statistical analyses were performed with SAS v9.4 (SAS Institute, Cary, N.C.). Two-sided P values <0.05 were considered significant.

Results

Most of the 90 men were white (89%), nonsmokers (68%), overweight or obese (68%), and their median age was 36.1 years (Table 1). The median (25^{th}, 75^{th} percentile) intake of fruits and vegetables was 3.6 (2.6, 5.1) servings/day. Seventy-three men (81%) reported a history of residential pesticide exposure in the past year. Approximately one fourth of the participants reported consuming organic fruits and vegetables three times or more per week. Organic fruit and vegetable consumers had higher intakes of both high pesticide (mean: 2.1 vs. 1.2 servings/day) and low pesticide (mean: 3.2 vs. 2.5 servings/day) fruits and vegetables than conventional fruit and vegetable consumers. The molar sum of urinary pesticide metabolites was the same among organic and conventional fruit and vegetable consumers (mean: 17 µmol/L).

All pesticide metabolites were detected in over 50% of samples, except for 4F-3PBA and trans-DCCA which were detected only in 8.5% and 17% of the samples, respectively (Table 2). The SG-adjusted geometric mean urinary concentrations for the 177 samples from 90 men were 0.69 (TCPY), 0.84 (PNP), 0.57 (IMPY), 0.38 (3PBA) and 0.35 (2,4-D) µg/L (Table 1.2). These urinary pesticide metabolites had low reproducibility with ICCs ranging from 0.03 (TCPY) to 0.37 (3PBA) (Table S1.2).

The PRBS for high residue fruits and vegetables was positively related to urinary pesticide metabolite concentrations (de-attenuated r=0.55). In the unadjusted analysis, the molar sum of urinary pesticide metabolites was 20% (95%CI: -1%, 44%) higher for each one serving/day increase in high pesticide fruit and vegetable intake. Multivariable adjustment slightly strengthened the association, with the molar sum of urinary pesticide metabolites increasing by 21% (95%CI: 2%, 44%) per one serving increase in high pesticide fruit and vegetable intake. Results were similar when consumption of high pesticide fruit and vegetable was modeled in quartiles of intake. Specifically, adjusted molar sum of urinary pesticide metabolites were 18, 18, 23 and 28 µmol/L for men in increasing quartiles of high

pesticide fruit and vegetable intake (p, trend=0.03; Figure 1.1, Panel A). When each urinary pesticide was assessed individually, positive trends for high pesticide fruit and vegetable intake and urinary pesticide metabolites were observed for most individual pesticides except for TCPY (Table 1.3). These associations were stronger when the analysis was restricted to urine samples collected within 6 months of FFQ completion (Table S1.3). On the other hand, PRBS for low residue fruits and vegetables were negatively associated with urinary pesticide metabolites concentrations (de-attenuated r= −0.36). The unadjusted and adjusted molar sum of urinary pesticide metabolites were 9% (95%CI: 1%, 19%) and 10% (95%CI: 1%, 18%) lower, respectively, for each one serving/day increase in low pesticide fruit and vegetable intake. The PRBS for low residue fruits and vegetables, when modeled as quartile variable, was also inversely related to the molar sum of urinary pesticide metabolites (p, trend=0.05; Figure 1.1, Panel A) but unrelated to any of the individual urinary metabolites (Table 1.3). Furthermore, the PRBS-weighted fruit and vegetable intake was unrelated to urinary pesticide metabolites (Figure 1.1, Panel A). These results were similar when the sum of ranks of pesticide metabolites was used as comparison (Figure 1.1, Panel B) and when we excluded three men without a second sample available for analysis.

In analyses within class of pesticide, the PRBS based only on organophosphates for high pesticide fruits and vegetables were associated with higher urinary concentrations of organophosphate pesticides metabolites (Tables 1.4). Specifically, men in the highest quartile of organophosphate-PRBS for high pesticide fruit and vegetable intake had 56% (95%CI: 8%, 125%) higher molar sum of organophosphate metabolites than men in the lowest quartile (p, trend= 0.02). Results were similar when the organophosphate-PRBS was compared against the sum of ranks of urinary organophosphate metabolites (Table 1.4). On the other hand, pyrethroid-PRBS for high pesticide fruit and vegetable intake were also positively related to the molar concentrations of 3-PBA and rank of the urinary 3-PBA concentration, albeit the association was weaker for molar concentration (Table 1.5).

Lastly, there was no statistical evidence of heterogeneity in the association between high pesticide fruit and vegetable intake and molar sum of urinary pesticide metabolites according to organic fruit and vegetable consumption frequency, or recent residential pesticide exposure history (P, interaction >0.10 in all cases).

Discussion

To evaluate the usefulness of a food frequency questionnaire method to estimate dietary intake of pesticides in epidemiologic studies, we compared the PRBS against urinary pesticide metabolites. We found that intake of high pesticide fruit and vegetable was positively associated with urinary concentrations of pesticide biomarkers suggesting that this low-cost questionnaire-based method could be used as a tool to evaluate exposure in studies on health effects of pesticides before making the significant financial investment entailed in collecting and generating exposure biomonitoring data.

As objective measures reflecting aggregate exposure and internal dose, urinary biomarkers have been widely used to assess contemporary pesticide exposure in many studies (Harley et al. 2016, Bradman et al. 2015, Lu et al. 2009, Marks et al. 2010). These biomarkers of exposure, however, are well known for having short half-lives, being sensitive to the episodic nature of exposure (reflected as low ICCs in the present study) (Bradman et al. 2013, Wielgomas 2013), relatively poor time integration and high analytic costs, limiting their use for long-term exposure assessment in epidemiologic studies when repeated measurements over years can not been obtained. On the other hand, the PRBS leverages the features of FFQ data, which reflect a longer period of dietary intake (i.e., a year) and is not as costly. Therefore, the PRBS approach can be useful in studies where the goal is to assess the effect of dietary pesticide exposure on chronic diseases, especially suitable for in cohorts with repeated FFQ measurements across years. In fact, the underlying principle of coupling a dietary questionnaire and national surveillance data, in the form of nutrient composition tables, has been widely implemented in nutritional epidemiology and used as a biologically meaningful measure of intake (Willett 1987). Foods

are vehicles for nutrients as well as non-nutritive constituent chemicals including pesticide residues. The present study shows that we may extend the coupling method to screen hypotheses regarding the potential health effects of pesticide residues as well.

The study findings complement the previous research from our group (Hu et al. 2016) showing that PRBS had value as a surrogate for dietary organophosphate pesticide exposure. Hu et al. found that there was a dose response relationship between dietary pesticide exposure estimated by PRBS and urinary dialkylphosphate metabolites (non-specific organophosphate biomarkers) in 1918 adult participants from the 2003-2004 US National Health and Nutrition Examination Survey (Hu et al. 2016). In the present study conducted in a well-characterized longitudinal cohort, we further targeted three commonly used organophosphate pesticides, including chlorpyrifos, parathion, and diazinon, using two urine samples from each participant. Notably, these pesticides have been banned for indoor residential use since early 2000 (Centers for Disease Control and Prevention 2009), suggesting that at present the major source of these chemicals is likely from diet, assuming that these pesticides were all used legally. In partial agreement with this hypothesis, we found positive trends of high pesticide fruit and vegetable intake with urinary concentrations of IMPY and PNP, and, to a lesser extent, TCPY. The weaker association with TCPY could be related to its high within-person variability (reflected in a low ICC), or to exposure to chlorpyrifos in public spaces such as golf-courses, turf, green houses, and wood treatment, that were not affected by the residential use ban(Environmental Protection Agency 2016b); such uses could make this metabolite less specific to exposure via diet than the other two organophosphate metabolites studied.

In addition to evaluating specific metabolites of organophosphate pesticides, we added to the previous study (Hu et al. 2016) by evaluating the association of pyrethroid-PRBS derived high pesticide fruit and vegetable intake with urinary concentrations of pyrethroid metabolites. There was a suggestive positive trend between high pesticide fruit and vegetable intake and molar sum of pyrethroid

biomarkers. Interestingly, the distinguishability of PRBS was stronger for organophosphate than pyrethroid pesticides. This finding was not surprising as pyrethroids are increasingly used in households as a replacement for organophosphates (ATSDR 2003), which may in turn, reduce the predictability of the urinary biomarkers to dietary exposure. Moreover, we found a significantly positive association between high pesticide fruit and vegetable intake and 2,4-D urinary concentrations. These findings are consistent with those of an organic diet intervention study among young children (Bradman et al. 2015) in which the investigators observed lower, albeit not significantly, 3-PBA urinary concentrations ($P=0.16$), and significantly lower 2,4-D concentrations ($P<0.01$) during the organic diet phase compared to the conventional phase. Lastly, and unexpectedly, we found that organic produce consumers and conventional produce consumers had similar pesticide metabolite urinary concentrations.
One likely explanation was that organic produce consumers had higher fruit and vegetable intake and not all the consumed produce was organic. Alternatively, misclassification of organic fruit and vegetables intake cannot be ruled out as an online survey showed that among representative sample of 1005 U.S adult consumers, half of consumers think "natural" labeling means no pesticide (Natural Marketing Institue 2015), suggesting that assessing only the overall intake frequency of organic fruits and vegetables may be insufficient to characterize exposure to pesticides through diet. Taken together, our findings show that PRBS may serve a useful tool for assessment of long-term dietary exposure to selected pesticide residues, namely organophosphate insecticides, pyrethroid insecticides, and the herbicide 2,4-D.

A similar method was developed by Curl et al. to assess dietary organophosphate pesticide exposure in the Multi-Ethnic Study of Atherosclerosis (MESA) (Curl et al. 2015). Briefly, Curl et al. estimated the organophosphate pesticide exposure in units of nanomoles per day for each individual by summing the product of average daily intake of each fruit and vegetable, concentration of organophosphate pesticides in each fruit and vegetable, and molecular weight of each organophosphate

pesticide. Consistent with our findings, Curl et al. found that increasing tertiles of estimated exposure to dietary organophosphate pesticide were associated with higher urinary concentrations of dialkylphosphates. In comparison to the MESA score, our PRBS first identified produce with high versus low pesticide residue contamination, and then summed the intake of the fruits and vegetables with high and low pesticide residue, respectively. In spite of using different algorithms, both approaches correlate well with urinary pesticide exposure biomarkers. Nonetheless, it is worth highlighting that de-linking the potentially deleterious effect of pesticide residues from the beneficial components in fruits and vegetables remains a significant challenge in studies evaluating pesticide residue intake and associated health risks. While Curl et al.'s approach provided a quantitative measure of organophosphate pesticide exposure, our PRBS method created a "control" group—low pesticide fruit and vegetable intake, allowing us to compare the effect of high versus low pesticide residue on outcomes of interest in parallel while simultaneously accounting for overall intake of fruits and vegetables. In fact, a suggestive inverse association between low pesticide fruit and vegetable intake and urinary pesticide exposure as well as beneficial effect of low pesticide fruit and vegetable intake on semen quality shown in a previous study (Chiu et al. 2015) suggest that separating fruits and vegetables into high versus low pesticide residue content may be a viable approach to disentangle the health effects of fruits and vegetables from those of pesticide contamination of these foods.

Although the PRBS overcomes some of the shortcomings of urinary biomarkers including relatively high cost, high variability and lack of time integration, the method is not without limitations. First, pesticide exposure may occur through other routes including inhalation and dermal contact, but the PRBS captures exposure only through dietary ingestion. Nonetheless, pharmacodynamic studies suggest that dermal and inhalation exposure to organophosphate and pyrethroid pesticides in the general population is likely to be relatively low due to poor dermal absorption [~1% excreted in urine (Garfitt et al. 2002, Woollen et al. 1992, Nolan et al. 1984)] and reduced volatility (Poet et al. 2014).

Second, our estimates of pesticide residues in foods were based on surveillance data rather than actual pesticide residues in the food consumed by the participants. Nevertheless, the Pesticide Data Program includes selection at random of the food samples to be tested from supermarkets across the nation, and monthly sampling of foods over each two-year cycle to allow measurement of seasonal and year-to-year variation in pesticide residue concentrations. Therefore, this design helps ensure that pesticide contamination values assigned to specific foods are reasonable estimates of the actual exposure concentration of any one person consuming foods sold in the United States. Third, PRBS does not take potency of toxicity of individual chemicals into account. Another limitation is that the findings may not be generalizable to the US population because the participants were recruited through a fertility clinic, who have a higher social economic status and higher intake of total fruit and vegetable (median: 3.6 servings/day) compared to the median intake in the US population (median 2.0 servings/day) (Moore et al. 2015). Finally, comparison of the PRBS to urinary biomarkers was not expected to demonstrate strong correlation for several reasons including: 1) differences in time-integration of exposure between FFQ and urinary biomarkers (i.e., FFQ captures dietary exposure in the past year while urinary biomarkers reflect exposure over past few hours or days; mismatch of time at FFQ and urinary sample assessment), 2) non-specificity of PRBS (i.e., PRBS captured overall pesticide exposure instead of targeting a certain pesticide metabolite), 3) non-dietary sources of exposure (i.e., PRBS captured dietary pesticide exposure while urinary biomarkers capture both dietary and non-dietary sources of exposure), 4) measurement error in the FFQ, and 5) high within-person variability in urinary pesticide metabolite concentrations, which could, individually and collectively, attenuate the observed association of dietary pesticide residue intake measured by PRBS and the urinary pesticide metabolite concentrations. Nonetheless, we used two urine samples to account for within-person variability in the concentrations of these chemicals, collected detailed information including residential exposure history, organic

produce consumption frequency and years as well as season of urine sample collection, which allowed to remove extraneous variation attributed to these factors.

Conclusions

The PRBS scoring system is a useful tool for dietary pesticide assessment in epidemiological studies aimed at evaluating hypotheses regarding the health effects of long-term exposure to pesticides through diet.

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Table 1.1. Baseline Characteristics of the 90 men contributing 177 urine samples from the Environment and Reproductive Health (EARTH) Study

Characteristic of men	Median (25^{th}, 75^{th}) or N (%)
Number of men	90
Demographics	
Age, years	36.1 (33.8, 40.4)
Body mass index (BMI), kg/m^2	27.0 (23.7, 28.9)
Total physical activity, hours/week	6.0 (2.9, 10.5)
Never smokers, n (%)	61 (68)
Race, n (%)	
White	80 (89)
Black/African Americans	1 (1)
Asian	6 (7)
Others	3 (3)
College graduates or higher, n (%)	72 (80)
Consumed organic FVs ≥ 3 times/week, n (%)	24 (27)
Residential pesticide exposure, n (%)	66 (73)
Diet	
High pesticide FV intake, servings/day	1.2 (0.8, 1.8)
Low pesticide FV intake, servings/day	2.5 (1.6, 3.2)
Total energy intake, kcal/day	2045 (1592, 2470)
Characteristics of urine samples	
Number of urine samples	177
Year of urine sample collection	2010 (2009, 2011)
Time from FFQ completion to urine sample collection, days	78 (-14, 165)
Season of urine sample collection, n (%)	
Spring	40 (23)
Summer	39 (22)
Fall	44 (25)
Winter	54 (31)

Table 1.2. Distributions of pesticide metabolite urinary concentrations (µg/L) in 177 urine samples from 90 men from the EARTH Study

	Class	N[2]	Detection frequency[3]	Geometric[4] mean	Percentile							LOD	NHANES GM[5]
					min	25th	50th	75th	90th	95th	max		
TCPY	OP	177	77%	0.61 (0.07)	<LOD	0.29	0.68	1.4	2.23	2.66	5.93	0.1	0.865
SG-adjusted TCPY				0.69 (0.07)	<LOD	0.31	0.83	1.26	1.97	2.91	6.62		0.77[6]
PNP	OP	177	100%	0.74 (0.06)	0.18	0.41	0.74	1.24	1.73	2.09	12.09	0.1	0.52
SG-adjusted PNP				0.84 (0.05)	0.21	0.58	0.79	1.09	1.67	2.51	9.54		0.47[6]
IMPY	OP	158	53%	0.15 (0.01)	<LOD	<LOD	0.12	0.27	0.39	0.53	2.75	0.1	.
SG-adjusted IMPY				0.17 (0.01)	<LOD	0.11	0.18	0.25	0.37	0.43	0.9		.
3-PBA	PYR	177	88%	0.50 (0.06)	<LOD	0.24	0.39	0.92	2.54	4.32	13.77	0.1	0.42
SG-adjusted 3-PBA				0.57 (0.06)	<LOD	0.26	0.45	1.03	2.42	3.26	12.81		0.38[6]
4F-3PBA	PYR	177	9%	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	0.13	0.27	0.1	.
SG-adjusted 4F 3-PBA				<LOD	<LOD	<LOD	<LOD	0.13	0.2	0.24	0.56		.
trans-DCCA	PYR	177	17%	< LOD	<LOD	<LOD	<LOD	0.86	2.01	4.55	17.63	0.6	.
SG-adjusted t-DCCA				< LOD	<LOD	<LOD	0.63	1.27	2.14	3.5	16.4		.
2,4-D	H	177	78%	0.31 (0.02)	<LOD	0.19	0.30	0.47	0.7	0.91	1.57	0.15	0.347
SG-adjusted 2,4-D				0.35 (0.02)	<LOD	0.24	0.36	0.5	0.69	0.96	1.95		0.309[6]

Table 1.2 (Continued). Distributions of pesticide metabolite urinary concentrations (µg/L) in 177 urine samples from 90 men from the EARTH Study

Abbreviations: <LOD, below limit of detection; GM, geometric mean; max, maximum; min, minimum; H, herbicide; IMPY, 2-isopropyl-4-methyl-6-hydroxy- pyrimidine; N, number of urine samples; NHANES, National Health and Nutrition Examination Survey; OP, organophosphate pesticides; PNP, *para*-nitrophenol; PYR, pyrethroids; *trans*-DCCA, trans-3- (2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; SG-adjusted, specific gravity adjusted; TCPY, 3,5,6-trichloro-2-pyridinol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 3-PBA, 3-phenoxybenzoic acid; 4-F-3PBA, 4-fluoro-3-phenoxybenzoic acid.

[2] N=number of urine samples

[3] Percent of metabolite concentrations above the LOD of the urine samples.

[4]All concentrations <LOD were assigned a value equal to the LOD divided by √2 to calculate the geometric means of urinary pesticide metabolite.

[5] Geometric mean for US male population from NHANES 2009-2010.

[6] Creatinine was used to correct for urine dilution in NHANES. Therefore these values (µg/creatinine) represent creatinine-adjusted concentrations.

Table 1.3. Individual concentrations of SG-adjusted urinary pesticide metabolites according to quartile of high/low pesticide residue and PRBS-weighted fruit and vegetable intake among 90 men in the EARTH Study

	Adjusted[1] mean (95%CIs) of individual pesticide metabolites (µg/L)				
	Organophosphate pesticide metabolites			Pyrethroid pesticide metabolites	Chlorophenoxy herbicide
	TCPY	IMPY	PNP	3PBA	2,4-D
Quartile (range) of high pesticide fruit and vegetable intake derived by PRBS					
Q1 (0.18, 0.79)	1.19 (0.73, 1.93)	0.16 (0.12, 0.22)	0.60 (0.48, 0.77)	0.57 (0.31, 1.06)	0.34 (0.26, 0.43)
Q2 (0.81, 1.23)	0.74 (0.48, 1.13)	0.21 (0.15, 0.30)	0.56 (0.44, 0.70)	0.58 (0.34, 1.00)	0.37 (0.26, 0.51)
Q3 (1.23, 1.80)	0.93 (0.57, 1.51)	0.25 (0.18, 0.35)*	0.82 (0.61, 1.10)	0.70 (0.41, 1.19)	0.44 (0.34, 0.56)
Q4 (1.92, 4.34)	1.56 (0.88, 2.76)	0.24 (0.16, 0.35)	0.85 (0.61, 1.20)*	0.94 (0.56, 1.58)	0.50 (0.36, 0.69)*
P, trend[2]	0.16	0.24	0.04	0.17	0.03
Quartile (range) of low pesticide fruit and vegetable intake derived by PRBS					
Q1 (0.36, 1.59)	0.89 (0.53, 1.47)	0.27 (0.19, 0.38)	0.76 (0.59, 0.99)	0.60 (0.36, 1.01)	0.43 (0.34, 0.55)
Q2 (1.63, 2.52)	1.34 (0.77, 2.33)	0.25 (0.17, 0.35)	0.86 (0.60, 1.23)	1.09 (0.57, 2.06)	0.43 (0.30, 0.63)
Q3 (2.56, 3.18)	1.08 (0.67, 1.74)	0.17 (0.12, 0.25)	0.67 (0.54, 0.83)	0.58 (0.36, 0.93)	0.37 (0.30, 0.47)
Q4 (3.25, 9.31)	0.99 (0.64, 1.52)	0.18 (0.13, 0.24)	0.54 (0.39, 0.74)	0.58 (0.32, 1.06)	0.38 (0.30, 0.49)
P, trend[2]	0.91	0.11	0.13	0.59	0.48
Quartile of PRBS-weighted fruit and vegetable intake					
Q1	1.02 (0.65, 1.60)	0.20 (0.15, 0.27)	0.60 (0.50, 0.72)	0.55 (0.33, 0.92)	0.34 (0.27, 0.41)
Q2	0.92 (0.58, 1.47)	0.24 (0.17, 0.35)	0.63 (0.48, 0.83)	0.67 (0.36, 1.25)	0.42 (0.30, 0.58)
Q3	0.83 (0.52, 1.35)	0.21 (0.15, 0.29)	0.76 (0.58, 1.00)	0.57 (0.33, 0.98)	0.41 (0.33, 0.52)
Q4	1.33 (0.80, 2.23)	0.19 (0.13, 0.28)	0.60 (0.45, 0.78)	0.75 (0.47, 1.21)	0.44 (0.33, 0.58)
P, trend[2]	0.33	0.69	0.81	0.40	0.10

Table 1.3 (Continued). Individual concentrations of SG-adjusted urinary pesticide metabolites according to quartile of high/low pesticide residue and PRBS-weighted fruit and vegetable intake among 90 men in the EARTH Study

Abbreviations: IMPY, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine; OP, organophosphate pesticides; PNP, *para*-nitrophenol; PRBS, pesticide residue burden score; PYR, pyrethroids; SG-adjusted, specific gravity adjusted; TCPY, 3,5,6-trichloro-2-pyridinol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 3-PBA, 3-phenoxybenzoic acid.

[1] Adjusting for age, race, BMI, total physical activity, smoking status, education, organic fruit and vegetable consumption, years and season of urine sample collections, and residential pesticide use history.

[2] Estimated using median intake in each quartile as a continuous variable.

*P-value <0.05 compared to men in the lowest quartile of intake.

Table 1.4. Adjusted[1] mean of SG-adjusted urinary organophosphate pesticide metabolites according to organophosphate-PRBS derived high/low pesticide fruit and vegetable intake among 90 men in the EARTH Study

	Urinary organophosphate metabolites	
Quartile (range) of high pesticide fruit and vegetable intake derived by organophosphate-PRBS	**Adjusted[1] mean (95%CI) in molar sum[2] (μmol/L)**	**Adjusted[1] mean (95%CI) in sum[2] of ranks**
Q1 (0.08, 0.53)	11 (9, 14)	108 (92, 126)
Q2 (0.54, 0.89)	10 (8, 12)	107 (92, 125)
Q3 (0.90, 1.33)	14 (11, 17)	131 (115, 150)*
Q4 (1.35, 3.21)	17 (12, 24)*	142 (116, 174)*
P, trend[3]	0.02	0.006
Quartile (range) of low pesticide fruit and vegetable intake derived by organophosphate-PRBS		
Q1 (0.46, 1.93)	14 (10, 18)	126 (105, 153)
Q2 (1.95, 2.74)	17 (13, 22)	129 (110, 152)
Q3 (2.76, 3.62)	11 (9, 14)	122 (105, 143)
Q4 (3.69, 10.4)	10 (8, 12)	107 (93, 124)
P, trend[3]	0.03	0.08

[1] Adjusting for age, race, BMI, total physical activity, smoking status, education, organic fruit and vegetable consumption frequency, years and season of urine sample collections, and residential pesticide use history.

[2] Including 3,5,6-trichloro-2-pyridinol, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine , and *para*-nitrophenol. Due to presence of interfering compounds in 19 samples for IMPY, only 158 samples (from 88 men) were available for molar sum organophosphate metabolite analysis.

[3] Estimated using median intake in each quartile as a continuous variable.

*P-value <0.05 compared to men in the lowest quartile of intake.

Table 1.5. Adjusted mean of SG-adjusted urinary pyrethroid pesticide metabolites according to pyrethroid-PRBS derived high/low pesticide fruit and vegetable intake among 90 men in in the EARTH Study

	Urinary 3-phenoxybenzoic acid (3-PBA)	
Quartile (range) of high pesticide fruit and vegetable intake derived by pyrethroid-PRBS	**Adjusted mean (95%CI) in molar concentration[2] (µmol/L)**	**Adjusted mean (95%CI) in rank[2]**
Q1 (0.16, 0.78)	2.3 (1.2, 4.1)	25 (15, 42)
Q2 (0.83, 1.37)	2.8 (1.6, 4.9)	34 (23, 52)
Q3 (1.39, 2.00)	2.9 (1.7, 5.1)	43 (29, 66)*
Q4 (2.06, 5.78)	4.0 (2.4, 6.5)	61 (43, 87)*
P, trend[3]	0.15	0.01
Quartile (range) of low pesticide fruit and vegetable intake derived by pyrethroid-PRBS		
Q1 (0.32, 1.64)	2.9 (1.7, 5.1)	45 (30, 69)
Q2 (1.64, 2.30)	3.4 (2.0, 5.8)	45 (31, 64)
Q3 (2.31, 3.13)	3.1 (1.9, 5.0)	38 (26, 54)
Q4 (3.21, 7.87)	1.9 (1.4, 4.4)	30 (19, 49)
P, trend[3]	0.48	0.13

Abbreviations: PRBS, pesticide residue burden score; SG-adjusted, specific gravity adjusted.

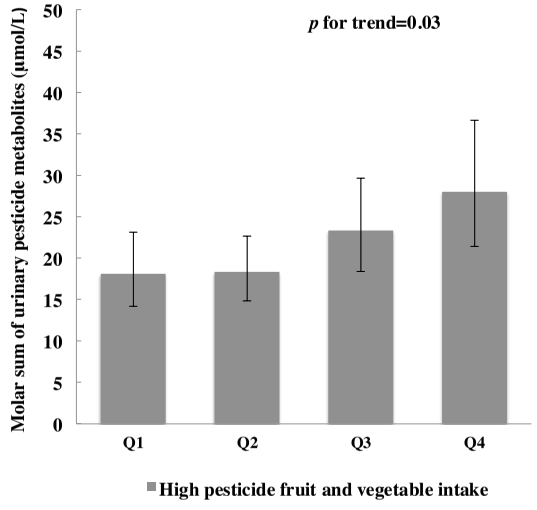
[1] Adjusting for age, race, BMI, total physical activity, smoking status, education, organic fruit and vegetable consumption, years and season of urine sample collections, and residential pesticide use history.

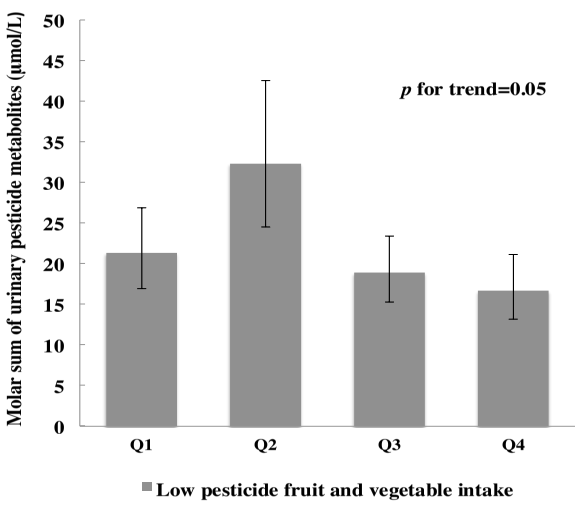
[2] Uses 3-phenoxybenzoic acid as a biomarker of exposure to pyrethroids

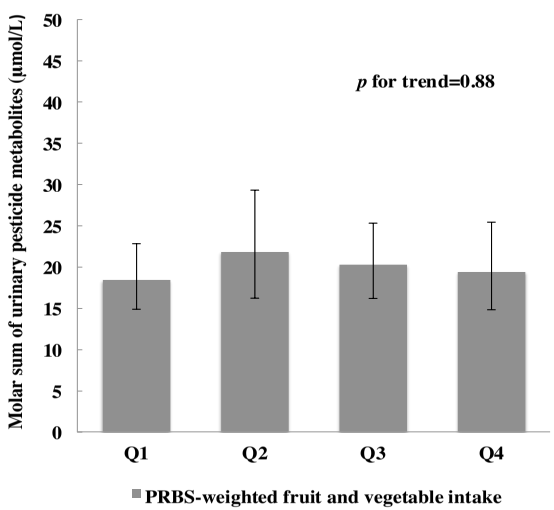
[3] Estimated using median intake in each quartile as a continuous variable.

*P-value <0.05 compared to men in the lowest quartile of intake.

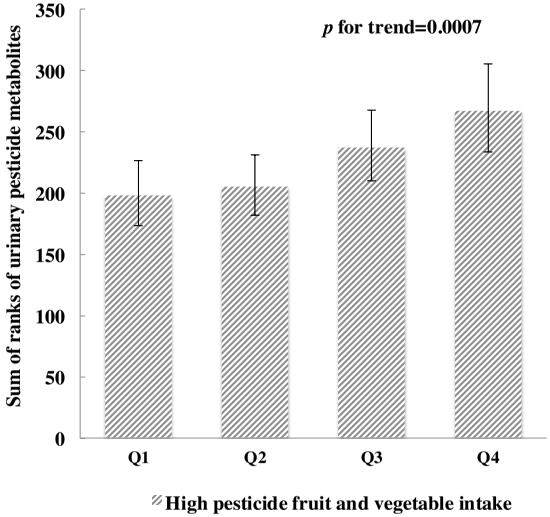
A)

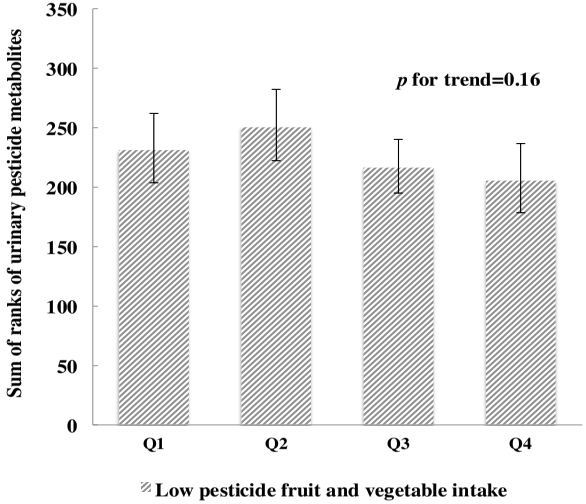

Molar sum of urinary pesticide metabolites (µmol/L)
p for trend=0.03
Q1
Q2
Q3
Q4
High pesticide fruit and vegetable intake


Molar sum of urinary pesticide metabolites (µmol/L)
p for trend=0.05
Q1
Q2
Q3
Q4
Low pesticide fruit and vegetable intake


Molar sum of urinary pesticide metabolites (µmol/L)
p for trend=0.88
Q1
Q2
Q3
Q4
PRBS-weighted fruit and vegetable intake

B)


Sum of ranks of urinary pesticide metabolites
p for trend=0.0007
Q1
Q2
Q3
Q4
High pesticide fruit and vegetable intake


Sum of ranks of urinary pesticide metabolites
p for trend=0.16
Q1
Q2
Q3
Q4
Low pesticide fruit and vegetable intake

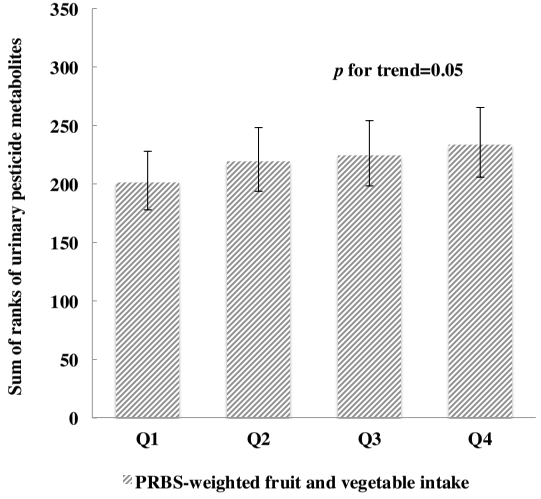

Sum of ranks of urinary pesticide metabolites
p for trend=0.05
Q1
Q2
Q3
Q4
PRBS-weighted fruit and vegetable intake

Figure 1.1 (Continued). Sum of SG-adjusted urinary pesticide metabolites according to quartile of high pesticide residue, low pesticide residue, and PRBS-weighted fruit and vegetable intake among 88 men (158 samples) in the EARTH Study. Data are presented as predicted mean (95%CI) in each quartile adjusted for age, race, BMI, total physical activity, smoking status, education, organic fruit and vegetable consumption, years and season of urine sample collections, and residential pesticide use history. Summed pesticide metabolites included TCPY, IMPY, PNP, 3-PBA, and 2,4-D. Panel A: molar sum of the urinary pesticide metabolites (µmol/L). Panel B: sum of ranks of the urinary pesticide metabolites.

Table S1.1. Fruit and vegetable items in the food frequency questionnaire and pesticide data program, and corresponding scores for 1st, 2nd and 3rd measure, and PRBS

Definition of measure contamination		**1st**	**2nd**	**3rd**	**PRBS**
Items in FFQ	Items in PDP	**score**	**score**	**score**	
peas or lima beans (FFC)	sweet pea, fz	0	0	0	0
dried plums or prunes	dried plum	0	0	0	0
onions	onions	0	0	0	0
beans or lentils	beans	0	0	0	0
avocado	avocado	0	0	0	0
corn (FFC)	corn, fz	0	0	0	0
cabbage or cole slaw	cabbage	0	0	0	0
orange juice, regular or calcium fortified	orange juice	0	0	0	0
tomato sauce	tomato paste	0	0	0	0
apple juice or cider	apple juice	0	0	1	1
cauliflower	cauliflower	1	0	0	1
grapefruit	grapefruit	1	0	0	1
cantaloupe	cantaloupe	0	1	1	2
tofu	soybeans	2	0	0	2
bananas	bananas	1	1	1	3
eggplant, summer squash, zucchini	eggplant, summer squash (0.5: 0.5)[a]	0	2	1	3
yam or sweet potatoes	sweet potatoes	1	2	0	3
oranges	oranges	2	0	1	3
broccoli	broccoli	1	1	1	3
carrots	carrots	1	0	2	3
head lettuce, leaf lettuce	lettuce	1	0	2	3
celery	celery	1	0	2	3
tomatoes	tomatoes	1	2	1	4
apple sauce	apple sauce	2	0	2	4
blueberry (FFC)	blueberry, Fs, Fz (0.5:0.5)[a]	2	0	2	4
kale, mustard, chard greens	kale	1	2	1	4
winter squash	winter squash	1	2	1	4
fresh apple or pear	apple, pear (0.7:0.3)[a]	2	1	2	5
string beans	green beans	1	2	2	5
grape or raisin	grape, raisin (0.6: 0.4)[a]	2	1	2	5
potatoes	potatoes	2	2	1	5
spinach, cooked	spinach, frozen	1	2	2	5
peach or plum	peach, plum (0.7: 0.3)[a]	2	2	2	6
strawberries (FFC)	strawberries, fresh	2	2	2	6
spinach, raw	spinach, fresh	2	2	2	6
green/yellow/red peppers	sweet peppers	2	2	2	6

Table S1.1. Fruit and vegetable items in the food frequency questionnaire and pesticide data program, and corresponding scores for 1st, 2nd and 3rd measure, and PRBS

Abbreviations: FFC, fresh, frozen, or canned; Fs, fresh; Fz, frozen; PDP, pesticide data program; PRBS, pesticide residue burden score.

[a] Ratio weighted for pesticide residue for each produce according to the ratio of consumption of each produce from the USDA repor

Table S1.2. Selective urinary pesticide metabolites and their corresponding parent compounds measured in the in the EARTH Study

Metabolites Abbreviation	Metabolite	Parent compound(s)	Chemical class	Intra-class correlations (95%CI)
TCPY	3,5,6-trichloro-2-pyridinol	chlorpyrifos, chlorpyrifos-methyl	Organophosphate	0.03 (0.00, 0.97)
PNP	*para*-Nitrophenol	parathion; methyl parathion	Organophosphate	0.26 (0.12, 0.49)
IMPY	2-isopropyl-4-methyl-6-hydroxypyrimidine	diazinon	Organophosphate	0.26 (0.10, 0.53)
3-PBA	3-phenoxybenzoic acid	cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, permethrin, tralomethrin	Pyrethroid	0.37 (0.21, 0.56)
4-F-3-PBA	4-fluoro-3-phenoxybenzoic acid	cyfluthrin	Pyrethroid	NA[1]
trans-DCCA	*trans*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid	permethrin; cypermethrin; cyfluthrin	Pyrethroid	NA[1]
2,4-D	2,4-dichlorophenoxyacetic acid	2,4-Denoxyacetic acid (and its esters)	chlorophenoxy herbicide	0.21 (0.08, 0.47)

Abbreviations: CI, confidence interval; NA, not available.

[1] Due to low detection frequency, we did not calculate intra-class correlations for 4-F-3PBA and *trans*-DCCA.

Table S1.3. Molar sum and individual concentrations of SG-adjusted urinary concentrations of pesticide metabolites according to quartile of high pesticide residue fruit and vegetable intake, restricting the analysis where urine sample provided within 6 months to FFQ completion (84 men, 144 urine samples)

Quartile of high pesticide fruit and vegetable intake	**Q1**	**Q2**	**Q3**	**Q4**	**P, trend**
Range of intake (serving/day)	0.2, 0.8	0.8, 1.2	1.2, 1.8	1.9, 4.3	
N	19	22	21	22	
Number of urine samples	31	38	37	38	
Adjusted[1] mean(95% CI) for summed[2] values of urinary pesticide metabolites					
molar sum of the urinary pesticide metabolites (μmol/L)	17 (14, 22)	19 (15, 25)	23 (17, 31)	28 (21, 38)*	0.02
sum of ranks of the urinary pesticide metabolites	192 (170, 218)	204 (178, 234)	235 (204, 270)	270 (234, 312)*	0.0002
Adjusted[1] mean (95%CI) for individual urinary pesticide metabolites (μg/L)					
TCPY concentrations	1.00 (0.60, 1.68)	0.72 (0.44, 1.17)	1.02 (0.61, 1.71)	1.49 (0.82, 2.70)	0.09
IMPY concentrations	0.16 (0.11, 0.23)	0.20 (0.14, 0.30)	0.24 (0.16, 0.35)	0.25 (0.17, 0.38)*	0.11
PNP concentrations	0.56 (0.42, 0.75)	0.51 (0.38, 0.69)	0.73 (0.51, 1.05)	0.85 (0.60, 1.19)*	0.04
3-PBA concentrations	0.52 (0.27, 0.98)	0.63 (0.33, 1.18)	0.76 (0.42, 1.38)	0.92 (0.54, 1.57)	0.15
2,4-D concentrations	0.31 (0.24, 0.41)	0.37 (0.24, 0.56)	0.43 (0.32, 0.57)	0.51 (0.36, 0.70)*	0.01

Abbreviations: IMPY, 2-isopropyl-4-methyl-6-hydroxy-pyrimidine; PNP, *para*-nitrophenol; PRBS, pesticide residue burden score; SG-adjusted, specific gravity adjusted; TCPY, 3,5,6-trichloro-2-pyridinol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 3-PBA, 3-phenoxybenzoic acid.

[1]Adjusting for age, race, BMI, total physical activity, smoking status, education, organic fruit and vegetable consumption, years and season of urine sample collections, and residential pesticide use history.

[2] Including TCPY, IMPY, PNP, 3-PBA, and 2,4-D

*P-value <0.05 compared to men in the lowest quartile of intake

CHAPTER 2

Pesticide residues intake from consumption of fruits and vegetables and pregnancy outcomes among women undergoing assisted reproductive technology: a prospective cohort study

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ABSTRACT

OBJECTIVE: To examine the association of preconception intake of pesticide residues in fruits and vegetables (FV) with outcomes of assisted reproductive technologies (ART).

DESIGN: Prospective cohort study.

SETTINGS: Women attending the Massachusetts General Hospital Fertility Center.

PARTICIPANTS: 325 women who completed a validated food frequency questionnaire and subsequently underwent 541 ART cycles in the Environment and Reproductive Health (EARTH) Study (2007-2016).

EXPOSURE MEASURE: We categorized FVs as having high or low pesticide residues using a validated method based on surveillance data from the US Department of Agriculture.

MAIN OUTCOME MEASURE: Unadjusted and adjusted probabilities of clinical pregnancy, pregnancy loss, and live birth per initiated cycle using generalized linear mixed models.

RESULTS: Mean (standard deviation) intakes of high and low pesticide residue FVs were 1.7 (1.0) and 2.8 (1.6) servings/day, respectively. Greater intake of high-pesticide residue FVs was associated with a lower probability of clinical pregnancy and live birth. Compared with women in the lowest quartile of high pesticide FV intake (<1.0 servings/day), women in the highest quartile (≥2.3 servings/day) had 15% (95% confidence interval (CI): 4%, 26%) lower probability of clinical pregnancy and 25% (95%CI: 14%, 35%) lower probability of live birth (P, trends=0.04 and 0.01, respectively). The impact on live birth was primarily attributable to a higher probability of pregnancy loss for women with high pesticide residue FV intake [39% (95%CI: 23%, 58%) in quartile 4 vs. 11% (95%CI: 5%,20%) in quartile 1]. Intake of low pesticide residue FVs was unrelated to ART outcomes.

CONCLUSIONS: Women who consumed more high pesticide residue FVs had lower probability of pregnancy and live birth following ART. Our results suggest that dietary pesticide exposure within the range of typical human exposure may have adverse reproductive consequences.

Introduction

Approximately 1.1 billion pounds of pesticides are used in the U.S annually, and the agricultural market sector accounted for 80% of the use of these chemicals(1). Due to widespread use of pesticides, more than 90% of the United States population has detectable concentrations of pesticides or their metabolites in their urine or blood samples(2). While pesticide exposure occurs through a variety of routes, the primary route of pesticide exposure in the general population is through diet -- especially intake of fruits and vegetables (FVs)(3). The U.S. Food and Drug Administration's Pesticide Monitoring Program showed that FVs have a considerably higher percentage of detectable pesticide residues and higher percentage of samples with residue exceeding the tolerance level than any other food group (4). Others have shown that intake of vegetables, but not other food groups, was positively related to urinary metabolite concentrations of pyrethroid pesticides (5), and that substituting organic produce for conventionally grown produce dramatically decreases the urinary concentrations of pesticide metabolites by 25% to 89% (6-8).

In the United States, pesticides are regulated and evaluated by the US Environmental Protection Agency to ensure the safety of the food supply for human consumption. Nonetheless, there has been a growing concern that permitted levels of pesticide residues in food defined by traditional toxicological testing may be too high, especially for susceptible populations such as pregnant women or infants (9, 10). In rodent models, ingestion of pesticide mixtures in early pregnancy at a concentration assumed to be without adverse health effects for humans increased the percentage of apoptosis (cell death) in embryos and decreased the number of live pups born (11, 12). Evidence from human studies, however, is scarce. Women occupationally exposed to pesticides, as well as women living in or near agricultural areas, may have increased risk of infertility and adverse pregnancy outcomes such as spontaneous abortion, stillbirth, and congenital anomalies (13-25). However, whether exposure within the range of typical human exposure, such as through diet, has any impact on reproductive outcomes in humans is unknown.

We previously developed and validated a low-cost, questionnaire-based method – the Pesticide Residue Burden Score (PRBS) – to estimate exposure to pesticide residues from foods in epidemiologic studies (26, 27). In the present study, we aimed to investigate the association of high and low pesticide residue FV intake, based on their PRBS, with outcomes of assisted reproductive technologies (ART) in a prospective cohort of women undergoing infertility treatment.

Methods

Study population

Women in this study were participants in the Environment and Reproductive Health Study (EARTH) Study, an ongoing prospective cohort established in 2006 to identify determinants of fertility among couples presenting to the Massachusetts General Hospital Fertility Center (Boston, MA)(28). Women were eligible to participate if they were between 18 and 45 years and planned to use their own gametes for infertility treatment. Among women referred by physicians, approximately 60% of those approached by the research nurses enrolled in the study. A food frequency questionnaire (FFQ) was introduced to the study in April 2007. The current analysis includes 325 women (contributing 541 ART cycles) who completed an FFQ and contributed at least one subsequent ART cycle between April 2007 and August 2016. Women who did not complete an FFQ (n=113 women) or whose ART cycles started prior to FFQ completion (n=7 women) were excluded from the present analysis. Compared to women included in the analysis, women who were excluded were slightly older, less likely to report residential pesticide exposure history, and had a higher prevalence of diminished ovarian reserve and endometriosis. Other baseline characteristics did not differ between included and excluded women (data not shown). The study was approved by the Human Studies Institutional Review Boards of the Massachusetts General Hospital, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (29). All participants signed an informed consent after the study procedures were explained by a trained study staff.

Covariates assessment

Upon entry, height and weight were measured by a trained study staff to calculate body mass index (BMI). Study staff also administered a brief questionnaire to collect data on demographics, medical history and lifestyle factors. Participants completed a detailed take-home questionnaire with additional questions on reproductive history and lifestyle factors, including history of residential pesticide exposure and organic food consumption frequency. Specifically, women were asked if their homes had been treated with pesticides in the past 5 years, if their lawns had been treated with pesticides in the past year, if they had used pesticide products personally or on pets to repel or kill pests in the past year, and if anyone in their household had been treated for head lice in the past year. We considered women to have a history of residential pesticide exposure if they replied "yes" to any of these questions. On this take-home questionnaire, participants were also asked how often they consumed organic FVs during the past 3 months. We considered women as organic FV consumers if they consumed organic fruits and vegetables ≥ 3 times per week (median); women with lower intake of organic FVs (<3 times per week) were considered as conventional FV consumers.

Outcomes assessment

Clinical information was abstracted by trained study staff from the patient's electronic medical record. We have previously described details of patient clinical management elsewhere (28). Briefly, on the day 3 of induced menses after taking a cycle of oral contraceptives, women underwent one of three ovarian stimulation protocols as clinically indicated: 1) luteal phase GnRH-agonist protocol, 2) follicular phase GnRH-agonist/Flare protocol, or 3) GnRH-antagonist protocol. Clinical staff monitored patients during gonadotropin stimulation for serum estradiol, follicle size and counts, and endometrial thickness for 2 days before oocyte retrieval, and administered human chorionic gonadotropin (β-hCG) to induce ovulation approximately 36 hours before the scheduled oocyte retrieval procedure. Couples underwent ART with either conventional in vitro fertilization or intracytoplasmic sperm injection as clinically

indicated. Embryologists classified oocytes as germinal vesicle, metaphase I, metaphase II (MII) or degenerated, and determined fertilization rate as the number of oocytes with two pronuclei divided by the number of MII oocytes at 17 to 20 hours after insemination. Cell cleavage rates of embryos were considered as normal with a division of 2-4 cells on day 2 and 4-8 cells on day 3 of culture. A division below 2 cells on day 2 and 6 cells on day 3 was considered as slow whereas a division of 4+ cells on day 2 and 8+cells on day 3 was designated accelerated. The morphology quality of embryos was graded from 1 (best) to 5 (worst) on days 2 and day 3. We defined embryos as best quality if they had four cells on day 2, eight cells on day 3, and a morphologic quality score of 1 or 2 on days 2 and day 3. For this study, early ART endpoints were referred to any endpoints prior to implantation, including markers of ovarian responses to stimulation (peak estradiol levels, endometrial thickness, MII and total oocytes), fertilization rate, and embryo quality.

In the primary analysis, clinical outcomes were assessed per initiated cycle, including implantation (defined as a serum β-hCG level > 6 mIU/mL typically measured around 17 days after oocyte retrieval), clinical pregnancy (defined as presence of an intrauterine gestational sac(s) on an ultrasound at 6 weeks), and live birth (as the birth of a neonate on or after 24 weeks of gestation).

In secondary analyses, we defined total pregnancy loss as any loss after a positive serum β-hCG. We also categorized total pregnancy loss into 1) early pregnancy loss, as a positive urine β-hCG followed by the absence of signs of clinical pregnancy, including chemical pregnancy loss and ectopic pregnancy; and 2) clinical pregnancy loss, as an intrauterine pregnancy demise after a clinical pregnancy, including spontaneous abortion (fetal loss occurring before 20 completed weeks of gestation), stillbirth (fetal loss after 20 weeks of gestation) and therapeutic abortion (n=3, terminated pregnancy due to brain malformation, conjoined twins, and Apert syndrome). No molar pregnancies occurred in this cohort. Of note that, total pregnancy loss and early pregnancy loss was assessed among women who had a positive β-hCG, while clinical pregnancy loss was assessed among those who had achieved clinical pregnancy.

Dietary Assessment

Diet was assessed using a self-administered FFQ (30). Women were asked to report how often, on average, they consumed specified amounts of each food, beverage, and supplement over the past year. The questionnaire described serving sizes for FVs using standard portion sizes (e.g., one apple, ½ avocado) or volumes (e.g., ½ cup of broccoli). In a validation study in a different cohort, the de-attenuated correlation coefficient (i.e., observed correlation corrected for random within-person variability) between two, one-week diet records(31) and FFQ reports ranged from 0.27 for spinach to 0.95 for bananas. Two data-derived dietary pattern scores, the Prudent and Western pattern (32), were used to summarize overall food choices. Data on whether individual food items consumed were organic or conventional was not collected on the FFQ.

Pesticide Residue Assessment

We used the annual reports from the US Department of Agriculture Pesticide Data Program (PDP) to classify FVs according to their average pesticide residue status in the US food supply (33). Details of the PRBS methods have been described elsewhere (26, 34). We considered three measures of contamination from the PDP to classify FVs: 1) the percentage of samples tested with any detectable pesticides; 2) the percentage of samples tested with pesticides exceeding the tolerance level; and 3) the percentage of samples with three or more individual detectable pesticides. The pesticide residue data in FVs were averaged by annual PDP reports from 2006-2015, which corresponds to the periods when the diet history of the participants was captured by the FFQ.

Next, we categorized foods according to tertiles for each of the three measurements of contamination and assigned a score of 0 to FVs in the bottom tertile, 1 to FVs in the middle tertile, and 2 for FVs in the top tertile. The PRBS for each food was the sum of scores across the three PDP contamination measures. We considered FVs with the PRBS ≥4 on a scale of 0-6 (ie., at least one of three

measurements was in the highest tertile) as a high pesticide residue food while FVs with the PRBS <4 as a low pesticide residue food. Based on these criteria, 14 FVs were categorized as high pesticide residue produce, and 24 as low pesticide produce (Supplemental Table 1). The de-attenuated correlations between the PRBS and sum of urinary pesticide metabolites from two urine samples were 0.53 for high pesticide FV intake, and -0.45 for low pesticide FV intake (under review).

Statistical analysis

Women were classified according to quartiles of total FV intake, high pesticide residue FV intake, and low pesticide residue FV intake. We conducted Kruskal-Wallis tests (for continuous variables) and Fisher's exact tests (for categorical variables) to compare demographic, dietary and baseline reproductive characteristics across quartiles of FV intake. To evaluate the relationship of FV intake with ART outcomes, generalized linear mixed models were used with random intercepts to account for within-person correlations between repeated cycles within the same individual. A normal distribution and identity link were specified for peak estradiol and endometrial thickness; the Poisson distribution and log link function were specified for oocyte counts; and a binomial distribution and logit link function were specified for fertilization, embryo quality, total pregnancy loss, and clinical outcomes. Robust estimators of variance were used to compute 95% confidence intervals (CI) for parameter estimates. Population marginal means were utilized to present population averages adjusted for the covariates at their average levels for continuous variables and weighted average levels of categorical variables in the model (35). Tests for linear trend were performed using the median intake of FVs in each quartile as a continuous variable. In a post-hoc analysis, we fit multinomial logistic regression models to assess whether the associations of FV intake with early pregnancy loss significantly differed from that with clinical pregnancy loss.

Confounding was evaluated using directed acyclic graphs based on prior knowledge.

Specifically, variables previously reported to be associated with live birth/pregnancy loss as well as associated with FV intake were considered as potential confounders (36-39). In addition, we included dietary pattern scores to distinguish relations between FV intake from those of overall food choices. The final multivariable models were adjusted for age (years), BMI (kg/m^2), smoking status (current/former and never), race (white and nonwhite), supplemental folate (μg/day), organic FV consumption frequency (<3 times/week and ≥ 3 times/week), residential pesticide exposure history (yes and no), Prudent and Western dietary patterns, total energy intake (kcal/day), and infertility diagnosis (male factor, female factor, and unexplained). The model for high pesticide residue FV intake was additionally adjusted for low pesticide FV intake, and vice versa, since they may confound each other. To minimize residual confounding, we performed separate sensitivity analyses restricting to the first ART cycle per woman, women < 40 years, and cycles initiated within one year of the FFQ completion. Because infertility diagnosis can be an intermediate variable and/or a confounder, we also conducted a sensitivity analysis excluding infertility diagnosis as a covariate from the final model. We also estimated the effect of substituting 1 serving/day of low pesticide residue FVs for high pesticide residue FVs on clinical outcomes. We calculated the difference between their regression coefficients, and utilized this difference along with its corresponding variance to estimate the odds ratios (ORs) and 95%CI of the substitution. All statistical analyses were performed with SAS v9.4 (SAS Institute, Cary, N.C.).

Results

Figure 1 summarizes participant enrollment and follow-up including the number of cycles at each endpoint. Overall, 325 women underwent 541 ART cycles, 228 of which (42%) resulted in a live birth. The mean (standard deviation) intake of total FVs was 4.4 (2.4) servings/day. Women had an average intake of 1.7 (1.0) servings/day of high pesticide residue FVs and 2.8 (1.6) servings/day of low pesticide residue FVs. Intakes of high and low pesticide residue FVs were positively correlated with each other ($r_{spearman}$=0.57). Women who consumed more high pesticide FVs were more likely to report regular organic FV

consumption, had higher total calorie intake, had higher adherence to the Prudent dietary pattern, and had a slightly higher prevalence of diminished ovarian reserve. Similar trends were observed for women who consumed more low pesticide FVs except that no difference in prevalence of diminished ovarian reserve was observed. In addition, women in the lowest and highest quartiles of low pesticide FV intake were more likely to have ever smoked than women in the 2nd and 3rd quartiles. All other demographic and reproductive characteristics were similar across quartiles of high or low pesticide FV intake (Table 1).

Total FV intake was unrelated to probability of implantation, clinical pregnancy and live birth (Table 2). However, when FVs were classified as having high or low pesticide residues, divergent patterns of associations with clinical pregnancy and live birth emerged (Table 2). Specifically, high pesticide residue FV intake was inversely associated with probability of clinical pregnancy and live birth per initiated cycle (P, trend=0.05 and 0.02, respectively). Compared with women in the lowest quartile of high pesticide residue FV intake (<1 serving/day), women in the highest quartile (≥2.3 servings/day) had 15% (95% CI: 4%, 26%) lower probability of clinical pregnancy and 25% (95% CI: 14%, 35%) lower probability of live birth. On the other hand, low pesticide residue FV intake was associated with higher, albeit non-significant, probability of clinical pregnancy and live birth (P, trend=0.06 and 0.11, respectively). Neither high nor low pesticide residue FV intake was associated with probability of implantation per initiated cycle. We found no associations between intake of high or low pesticide residue FVs with markers of response to ovarian stimulation (Supplemental Table 2), fertilization rate, or markers of embryo quality (Supplemental Table 3).

The association of high pesticide residue FV intake with probability of clinical pregnancy and live birth persisted in sensitivity analyses restricted to women < 40 years, or to the first treatment cycle per woman (Supplemental Table 4). The results were consistent when not adjusting for infertility diagnosis (Supplemental Table 4).The associations became slightly stronger when the analysis was restricted to cycles initiated within 1 year after diet assessment (Supplemental Table 4).

We conducted a secondary analysis to investigate the extent to which the observed associations of high pesticide FV intake with live birth was driven by an increased frequency of pregnancy loss. Among the 316 cycles with a positive β-hCG, 85 (27%) ended in a loss. Of these, 35 (11%) were early pregnancy losses and 50 (16%) were clinical pregnancy losses. A dose-response relationship was observed between high pesticide residue FV intake and total pregnancy loss (P, trend=0.03; Figure 2). Specifically, the adjusted probabilities of total pregnancy loss were 11% (5%, 20%), 28% (19%, 40%), 31% (21%, 43%), and 39% (23%, 58%) for women in increasing quartiles of high pesticide FV intake. When total pregnancy loss was divided into early pregnancy loss and clinical loss, the relationships with high pesticide FV intake were not significantly different from each other (Figure 2).

Lastly, we estimated the effect of replacing high pesticide residue FVs with low pesticide residue FVs on the probability of clinical pregnancy, live birth and pregnancy loss (Figure 3). Consuming 1 serving/day of low pesticide residue FVs in lieu of 1 serving/day of high pesticide residue FVs was associated with 91% (95%CI: 23%, 195%) higher odds of live birth and 58% (95%CI: 19%, 78%) lower odds of total pregnancy loss.

Discussion

We evaluated the association between self-reported intake of FVs, considering their pesticide residue status based on surveillance data from the USDA, with ART outcomes among women undergoing infertility treatment. We observed that higher intake of high pesticide FVs in the year prior to infertility treatment was associated with lower probability of clinical pregnancy and live birth per initiated cycle. The observed association with live births was mainly driven by the higher risk of pregnancy loss in increasing quartiles of high pesticide FV intake. On the other hand, there was a suggestive increasing trend between low pesticide FV intake and probability of live birth.

While FVs are an important part of a healthy diet (40), they also serve as the primary vehicle for pesticide residues exposure in the general population(4). Earlier epidemiological studies have shown that many pesticide chemicals used in agriculture have deleterious effects on reproductive health outcomes, such as decreased fertility, spontaneous abortion, stillbirth, preterm birth or developmental abnormalities(13-25), while a few others reported no associations(41, 42). Of note, in one of these studies, among 684 participants (73 cases, 611 controls) from 10 agricultural counties of California, Bell *at al*. found that the adjusted odds ratio of fetal death for those exposed to three ore more pesticide classes was 2.6 (95%CI: 1.3, 5.3), where as those exposed to one or two pesticide classes had an odds ratio of 1.1 (95%CI: 0.6, 2.1) (20). In another study of women living on Ontario farms, Arbuckle *et al*. showed that exposure to both fungicides and herbicides before conception doubled the risk of spontaneous abortion as compared to women exposed only to fungicides (19), suggesting that pesticide mixtures may confer a greater risk of fetal loss. Nonetheless, the majority of these studies have focused on occupational workers or women living in or near agricultural areas. The influence of exposure to pesticide residues primarily through foods on pregnancy outcomes in the general population (i.e. not exposed occupationally or residentially) remains unknown.

To the best of our knowledge, this is the first prospective study evaluating dietary pesticide exposure with reproductive success in humans. The most closely-related study to ours is a prospective study of 28,192 Norwegian women, which found that women choosing organically grown vegetables during pregnancy had reduced risk of pre-eclampsia (43), and this association persisted after adjusting for various healthy food scores. One possible explanation was that organic vegetables may reduce exposure to pesticides. It is postulated that some forms of miscarriage and preeclampsia are related, representing a continuum whose origin is an oxidative stress-induced placental dysfunction (44, 45). Therefore, pesticide-induced placental dysfunction (44, 46, 47) may link the relationship of lower pregnancy loss associated with lower intake of high pesticides FVs in the present study, as well as lower prevalence of preeclampsia

associated with organic vegetable consumption in the earlier study (43). However, given the paucity of the data, future studies are warranted to replicate these findings.

Our results are in agreement with animal data suggesting that low doses of multiple pesticide ingestion during early pregnancy may have fetal toxicity. For example, Cavieres *et al.* showed that pregnant mice exposed to a pesticide mixture (e.g.,2,4-D, atrazine, metolachlor, dicamba) at a levels lower than drinking water standards during a period spanning preimplantation and organogenesis produced a significant decrease in implantation sites and number of live pups born(11). In another animal study, Greenlee *et al.* showed that a mixture of agricultural chemicals, including herbicides (decamba, 2,4-D, and atrazine), insecticides (chlorpyrifos, terbufos, and permethrin), or fungicides (chlororthalonil, mancozeb, diquat) at 1 reference dose (i.e., an estimate of daily oral exposure that is likely to be without an appreciable risk of deleterious effects during life time) increased blastomere apoptosis and suppressed cell proliferation of two-cell embryos and morulae (12), which may result in embryonic demise or pregnancy loss. Nonetheless, our study did not find differences in embryo quality or day of embryo transfer across categories of high FV intake. It is possible that pesticides may impair pregnancy maintenance by affecting early embryo development after implantation, during which is also known as a period of heightened susceptibility to malformations (48).

The present study offers preliminary evidence that high pesticide FV intake may potentially have a negative impact on sustaining a pregnancy to live birth. Nonetheless, these results should be interpreted with caution due to certain limitations. First, exposure to pesticides was not directly assessed but was rather estimated from self-reported FV intake paired with pesticide residue surveillance data. However, our previous work has shown that higher intake of high pesticide residue FVs were significantly associated with higher urinary pesticide metabolites, supporting the notion that the PRBS adequately characterizes exposure to pesticides through diet (27). Second, we were not able to identify specific pesticides and it is

possible that some may not elicit adverse reproductive effects. Further confirmation studies, preferably accounting for common chemical mixtures used in agriculture by biomarkers, are needed. Third, as in all observational studies, we cannot rule out the possibility that women with higher intake of high pesticide FVs have unknown lifestyles or fertility factors that are confounding the associations with clinical pregnancy and live birth. However, women with greater high pesticide FV intake and those of greater low pesticide FV intake had similar patterns of baseline characteristics suggesting that the observed associations are due to intake of FVs rather than to residual confounding. Furthermore, results were consistent after accounting for many factors (by adjustment or sensitivity analyses) that could potentially affect the risk of pregnancy loss. In addition, there were no differences in number, day, and quality of embryos transferred by high pesticide FV intake, suggesting that the positive association with pregnancy loss cannot be explained by differences in pre-selection of embryos to transfer. An additional limitation is that findings may not be generalizable to the general population because participants were recruited through a fertility clinic and intake of FVs in our cohort was double the median intake in the US population (median: 2 servings/day)(49), However, the infertility cohort allowed us to examine the effects of dietary pesticide exposure on many pregnancy outcomes that are not observable among couples becoming pregnant on their own such as very early pregnancy losses. In addition, demographic characteristics of the study participants were comparable to those of women seeking fertility treatment in the United States (50), suggesting that results may be generalizable to women seeking infertility treatment. Additional strengths of the study include its prospective study design and well-documented outcome measures, eliminating the possibility of the early pregnancy losses that would have been otherwise gone undetected, and permitting us to examine subtypes of losses. In addition, we used a previously validated dietary pesticide measurement (27), which directly addresses public health concerns while being an inexpensive way to explore the effect of multiple pesticide-containing foods on pregnancy outcomes.

In conclusion, intake of high pesticide residue FVs in the year prior to infertility treatment was associated with lower probability of clinical pregnancy and live birth, while intake of low pesticide residue FVs had the opposite relations among women undergoing infertility treatment. Our findings are consistent with studies in mice showing that low dose pesticide ingestion may exert an adverse impact on sustaining pregnancy(11). Since, to our knowledge, this is the first report of this relationship in humans, confirmation of these findings is warranted.

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Table 2.1. Demographic, dietary and reproductive characteristics of the study population according to quartiles of high and low pesticide fruit and vegetable intake among 325 women in the EARTH study

Characteristic	Overall	High pesticide residue FV intake			Low pesticide residue FV intake		
		Q1	Q4	P, value [1]	Q1	Q4	P, value [1]
Number of women	325	81	81		82	81	
Median, serving/day	-	0.5	2.2		1.4	4.6	
Range (min-max)	-	0.3-1.0	2.3-6.8		0.5-1.7	3.6-11.5	
Data presented as mean (SD) or n (%)							
Demographics							
Age, years	35.1 (4.0)	35.3 (4.2)	35.3 (3.8)	0.08	35.1 (3.8)	34.7 (4.2)	0.28
BMI, kg/m	24.1 (4.3)	23.1 (2.7)	24.3 (5.2)	0.51	23.8 (3.3)	24.2 (4.8)	0.86
White, n%	272 (83.7)	71 (88)	61 (75)	0.16	71 (88)	63 (78)	0.25
Education, n%				0.12			0.52
< College graduate	29 (9.2)	5 (6.3)	8 (10.0)		8 (10.4)	11 (13.9)	
College graduate	98 (31.1)	22 (27.8)	21 (26.3)		27 (35.1)	21 (26.6)	
Graduate degree	188 (59.7)	52 (65.8)	51 (63.8)		42 (54.6)	47 (59.5)	
Never smokers, n%	235 (72.3)	55 (68)	61 (75)	0.35	54 (67)	52 (64)	0.05
Residential pesticide use, n%	244 (75.1)	61 (75)	56 (69)	0.32	64 (79)	57 (70)	0.62
Diet							
Organic FV consumers, n%	118 (36.3)	11 (14)	45 (56)	<.0001	17 (21)	47 (58)	<.0001
Alcohol, g/day	8.9 (10.7)	8.6 (12.8)	9.9 (11.2)	0.44	8.2 (11.7)	11.1 (12.1)	0.14
Caffeine, g/day	126.6 (107.7)	131.6 (122.6)	130 (103.7)	0.48	132.8 (112.2)	138.7 (106)	0.11
Supplemental Folate , ug/day	630 (401)	602 (354)	641 (394)	0.91	627 (415)	644 (371)	0.63
Use of multivitamin, n %	283 (87.9)	72 (90)	70 (88)	0.81	71 (88)	73 (91)	0.31
Total energy intake, kcal/day	1800 (584)	1472 (438)	2077 (625)	<.0001	1792 (899)	1939 (732)	<.0001
Prudent pattern score	0.0 (1)	-0.9 (0.4)	1.0 (1.1)	<.0001	-0.8 (0.5)	1.1 (1.1)	<.0001
Western pattern score	0.0 (0.9)	-0.1 (0.7)	-0.2 (1.1)	0.12	-0.1 (0.8)	0.0 (1.2)	0.68
Baseline reproductive characteristics							
Prior miscarriage history, n%	69 (21.2)	15 (19)	19 (23)	0.84	12 (15)	21 (26)	0.29

Table 2.1. (Continued). Demographic, dietary and reproductive characteristics of the study population according to quartiles of high and low pesticide fruit and vegetable intake among 325 women in the EARTH study

	Overall	**High pesticide residue FV intake**			**Low pesticide residue FV intake**		
		Q1	Q4	P, value [1]	Q1	Q4	P, value [1]
Infertility diagnosis, n (%)				0.03			0.24
Male factor	106 (32.6)	18 (22)	21 (26)		21 (26)	23 (28)	
Female factor	95 (29.2)	25 (31)	30 (37)		27 (33)	27 (33)	
DOR	26 (8)	7 (8.6)	11 (13.6)		6 (7.4)	7 (8.6)	
Ovulatory	26 (8)	5 (6.2)	7 (8.6)		9 (11.1)	7 (8.6)	
Tubal	23 (7.1)	7 (8.6)	9 (11.1)		9 (11.1)	8 (9.9)	
Uterine	5 (1.5)	3 (3.7)	0 (0)		2 (2.5)	0 (0)	
Endometriosis	15 (4.6)	3 (3.7)	3 (3.7)		1 (1.2)	5 (6.2)	
Unexplained	124 (38.2)	38 (47)	30 (37)		33 (40)	31 (38)	
Initial treatment protocol, n (%)				0.34			0.84
Antagonist	37 (11.4)	9 (11)	12 (15)		9 (11)	11 (14)	
Follicular phase GnRH-agonist/Flare protocol	35 (10.8)	5 (6)	7 (9)		11 (14)	6 (7)	
Luteal phase GnRH-agonist protocol	253 (77.9)	67 (83)	62 (77)		61 (75)	64 (79)	
Embryo transfer day, n%				0.42			0.44
No embryos transferred	31 (9.6)	11 (13.6)	6 (7.5)		10 (12.4)	5 (6.2)	
Day 2	16 (4.9)	3 (3.7)	5 (6.3)		5 (6.2)	5 (6.2)	
Day 3	132 (40.7)	28 (34.6)	35 (43.8)		33 (40.7)	34 (42)	
Day 5	121 (37.4)	31 (38.3)	28 (35)		29 (35.8)	33 (40.7)	
Egg donor or cryo cycle	24 (7.4)	8 (9.9)	6 (7.5)		4 (4.9)	4 (4.9)	
Number of embryo transferred, n%				0.24			0.29
No embryos transferred	31 (9.6)	11 (13.6)	6 (7.5)		10 (12.4)	5 (6.2)	
1 embryo	64 (19.8)	9 (11.1)	20 (25)		11 (13.6)	18 (22.2)	
2 embryos	157 (48.5)	44 (54.3)	36 (45)		42 (51.9)	43 (53.1)	
3+ embryos	48 (14.8)	9 (11.1)	12 (15)		14 (17.3)	11 (13.6)	
Egg donor or cryo cycle	24 (7.4)	8 (9.9)	6 (7.5)		4 (4.9)	4 (4.9)	

Table 2.1 (Continued). Demographic, dietary and reproductive characteristics of the study population according to quartiles of high and low pesticide fruit and vegetable intake among 325 women in the EARTH study

Abbreviations: EARTH, Environment and Reproductive Health Study; N, number; IVF, in vitro fertilization, ICSI, intracytoplasmic sperm injection; BMI, body mass index; DOR, diminished ovarian reserve; FV, fruits and vegetables; Q1, quartile 1; Q4, quartile 4;

[1] For continuous variables, a Kruskal-Wallis test was used to compare characteristics across quartiles of fruit and vegetable intake. For categorical variables, a Fisher's exact test was used to compare characteristics across quartiles of fruit and vegetable intake.

Table 2.2. Clinical outcomes according to fruit and vegetable intake, considering pesticide residue status, in 325 women (541 cycles) from the EARTH study

	Probability of Implantation (95% CI)		Probability of Clinical Pregnancy (95%CI)		Probability of Live birth (95%CI)	
	Unadjusted	**Adjusted[1,2]**	**Unadjusted**	**Adjusted[1,2]**	**Unadjusted**	**Adjusted[1,2]**
Quartile (range) of Total Fruit and Vegetable Intake						
Q1 (0.7, 2.7)	0.62 (0.54, 0.70)	0.62 (0.50, 0.73)	0.53 (0.45, 0.61)	0.52 (0.41, 0.64)	0.42 (0.34, 0.50)	0.41 (0.30, 0.53)
Q2 (2.7, 3.8)	0.56 (0.47, 0.64)	0.56 (0.47, 0.65)	0.49 (0.41, 0.57)	0.49 (0.40, 0.58)	0.40 (0.32, 0.48)	0.39 (0.31, 0.48)
Q3 (3.9, 5.3)	0.61 (0.52, 0.69)	0.62 (0.53, 0.71)	0.56 (0.47, 0.64)	0.57 (0.48, 0.66)	0.48 (0.40, 0.56)	0.51 (0.42, 0.60)
Q4 (5.3, 14.9)	0.55 (0.46, 0.64)	0.56 (0.42, 0.69)	0.50 (0.41, 0.58)	0.50 (0.36, 0.64)	0.39 (0.31, 0.48)	0.37 (0.24, 0.51)
P, trend [5]	0.45	0.77	0.77	0.94	0.85	0.97
Quartile(range) of High Pesticide Fruit and Vegetable intake [3] (servings/day)						
Q1 (0.3, 1.0)	0.61 (0.51, 0.70)	0.61 (0.50, 0.72)	0.57 (0.47, 0.66)	0.57 (0.46, 0.68)	0.54 (0.44, 0.64)	0.56 (0.44, 0.67)
Q2 (1.0, 1.6)	0.65 (0.57, 0.73)	0.66 (0.56, 0.74)	0.61 (0.52, 0.69)	0.60 (0.51, 0.69)	0.46 (0.38, 0.55)	0.45 (0.36, 0.54)
Q3 (1.6, 2.2)	0.59 (0.50, 0.67)	0.59 (0.50, 0.68)	0.50 (0.42, 0.59)	0.51 (0.42, 0.59)	0.39 (0.31, 0.47)*	0.39 (0.31, 0.48)
Q4 (2.3, 6.8)	0.50 (0.40, 0.59)	0.51 (0.39, 0.63)	0.40 (0.31, 0.50)*	0.42 (0.31, 0.53)	0.31 (0.23, 0.40)*	0.31 (0.21, 0.42)*
P, trend [5]	0.05	0.15	0.007	0.05	0.002	0.02
Quartile (range) of Low Pesticide Fruit and Vegetable intake[4] (servings/day)						
Q1 (0.5, 1.7)	0.54 (0.45, 0.64)	0.56 (0.46, 0.66)	0.43 (0.34, 0.52)	0.43 (0.34, 0.54)	0.30 (0.22, 0.38)	0.31 (0.22, 0.41)
Q2 (1.7, 2.5)	0.59 (0.50, 0.68)	0.58 (0.48, 0.67)	0.52 (0.44, 0.61)	0.51 (0.42, 0.6)	0.43 (0.35, 0.52)*	0.43 (0.34, 0.52)
Q3 (2.5, 3.5)	0.58 (0.49, 0.67)	0.58 (0.49, 0.67)	0.54 (0.45, 0.62)	0.54 (0.45, 0.63)	0.48 (0.39, 0.56)*	0.48 (0.39, 0.57)
Q4 (3.6, 11.5)	0.63 (0.53, 0.72)	0.65 (0.52, 0.76)	0.59 (0.50, 0.69)*	0.61 (0.48, 0.72)	0.50 (0.40, 0.60)*	0.47 (0.35, 0.60)
P, trend [5]	0.23	0.32	0.02	0.06	0.01	0.11

[1] Model was adjusted for age, BMI, smoking status, race, folate supplement, organic fruit and vegetable consumption frequency, residential pesticide exposure history, total energy intake, Western and Prudent dietary pattern scores, and infertility diagnosis.

[2] Adjusted proportions were calculated at mean levels for continuous covariates and weighted average over categorical covariates.

[3] Model additionally adjusted for low pesticide FV intake.

[4] Model additionally adjusted for high pesticide FV intake

[5] Tests for trend were performed using the median intake in each quartile as a continuous variable in the model.

*P <0.05 compared to the lowest quartile

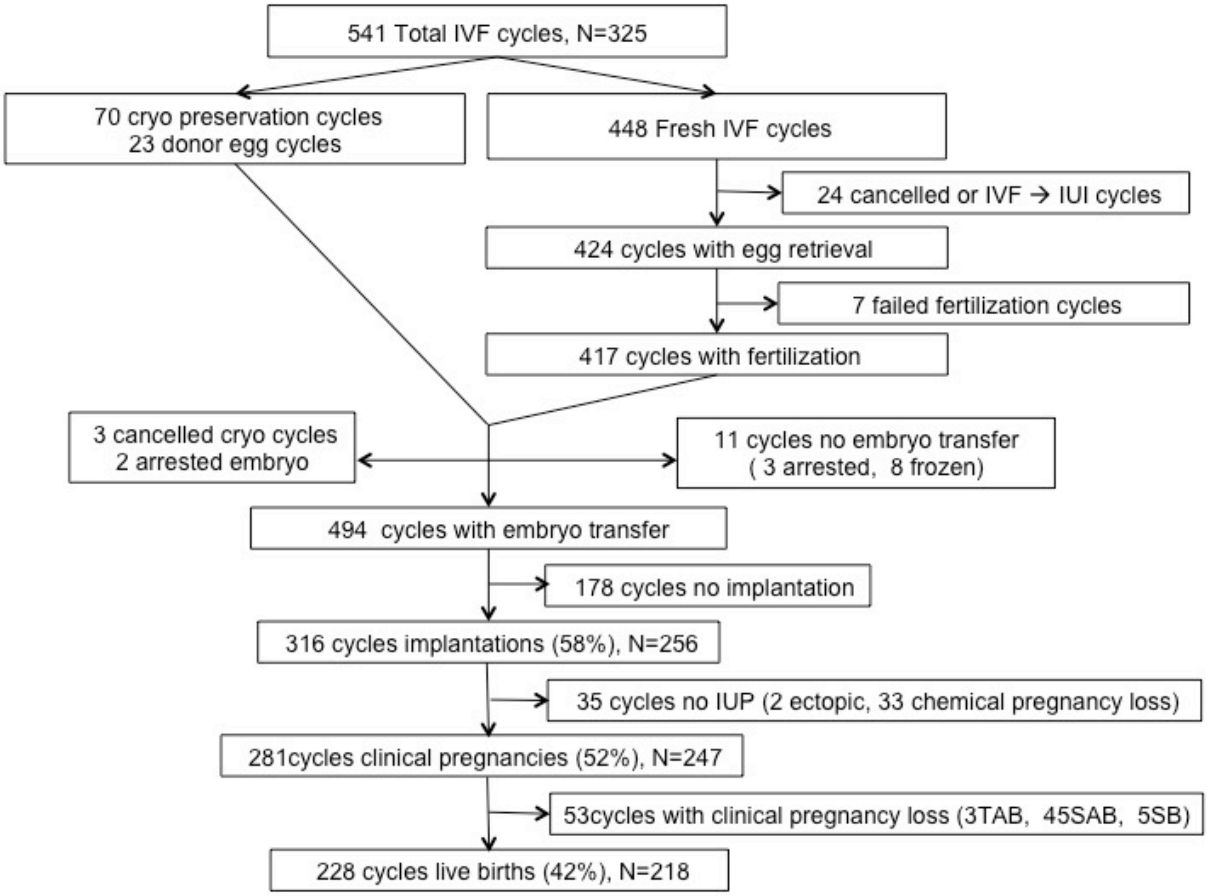

Figure 2.1. Overview of 541 initiated cycles in the EARTH study between April 2007 and August 2016. Abbreviations: N, number of women; IUP, intrauterine pregnancy; IVF, in vitro fertilization; IUI, intrauterine insemination; SAB, spontaneous abortion; SB, stillbirth; TAB, therapeutic abortion; EARTH, Environment and Reproductive Health Study.

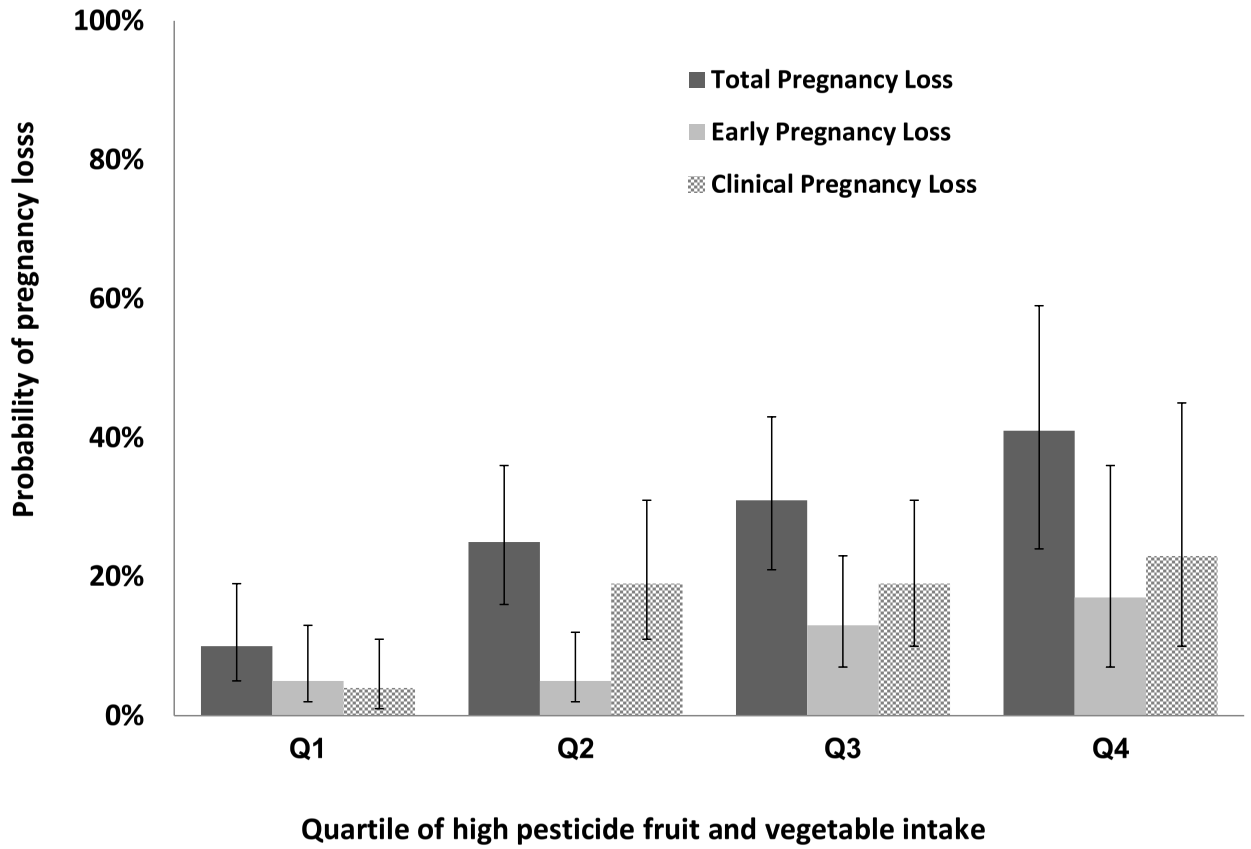

Figure 2.2. Total pregnancy loss, early pregnancy loss and clinical fetal loss according to high pesticide fruit and vegetable intake in 256 women who had successful implantation (316 cycles) from EARTH study. Data are presented as predicted mean (95%CI) in each quartile adjusting for age, BMI, smoking status, race, folate supplement, organic fruit and vegetable consumption frequency, residential pesticide exposure history total energy intake, Western and Prudent dietary pattern scores, and infertility diagnosis.

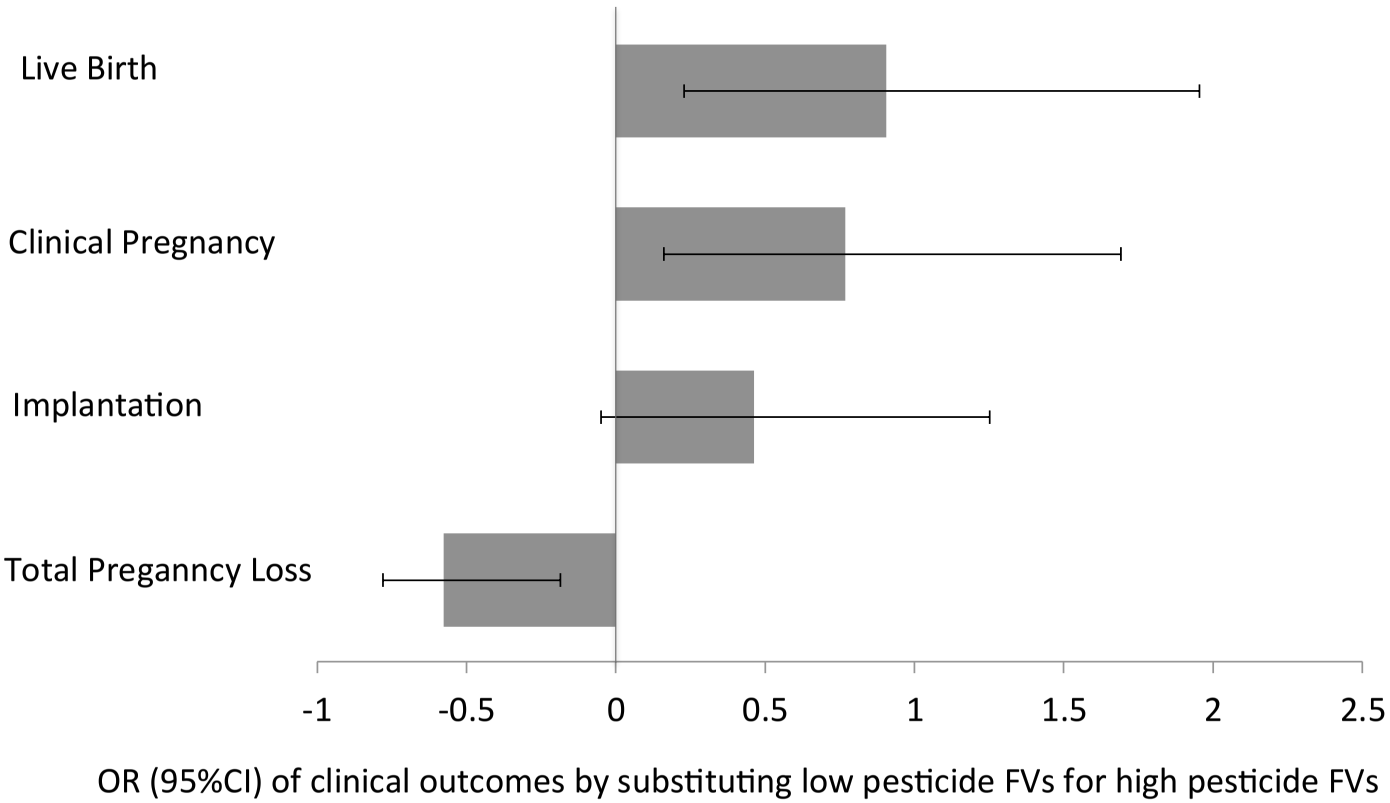

Figure 2.3. Adjusted odds ratio (95%CI) of clinical outcomes by substituting 1 serving/day of low pesticide residue fruit and vegetable for high pesticide residue fruits and vegetables. Data were adjusted for for age, BMI, smoking status, race, folate supplements, organic fruit and vegetable consumption frequency, residential pesticide exposure history, total energy intake, Western and Prudent dietary pattern scores, and infertility diagnosis. Abbreviations: OR, odds ratio; FVs, fruits and vegetables

Table S2.1. Fruit and vegetable items in the food frequency questionnaire and pesticide data program, and corresponding scores for 1st, 2nd and 3rd measure, and PRBS.

Definition of measure contamination		1st	2nd	3rd	PRBS
Items in FFQ	**Items in PDP**	**score**	**score**	**score**	
peas or lima beans (FFC)	sweet pea, fz	0	0	0	0
dried plums or prunes	dried plum	0	0	0	0
onions	onions	0	0	0	0
beans or lentils	beans	0	0	0	0
avocado	avocado	0	0	0	0
corn (FFC)	corn, fz	0	0	0	0
cabbage or cole slaw	cabbage	0	0	0	0
Orange juice, regular or calcium fortified	orange juice	0	0	0	0
tomato sauce	tomato paste	0	0	0	0
apple juice or cider	apple juice	0	0	1	1
cauliflower	cauliflower	1	0	0	1
grapefruit	grapefruit	1	0	0	1
cantaloupe	cantaloupe	0	1	1	2
tofu	soybeans	2	0	0	2
bananas	bananas	1	1	1	3
eggplant, summer squash, zucchini	eggplant, summer squash (0.5: 0.5)[a]	0	2	1	3
yam or sweet potatoes	sweet potatoes	1	2	0	3
oranges	oranges	2	0	1	3
broccoli	broccoli	1	1	1	3
carrots	carrots	1	0	2	3
head lettuce, leaf lettuce	lettuce	1	0	2	3
celery	celery	1	0	2	3
tomatoes	tomatoes	1	2	1	4
apple sauce	apple sauce	2	0	2	4
blueberry (FFC)	blueberry, Fs, Fz (0.5:0.5)[a]	2	0	2	4
kale, mustard, chard greens	kale	1	2	1	4
winter squash	winter squash	1	2	1	4
fresh apple or pear	apple, pear (0.7:0.3)[a]	2	1	2	5
string beans	green beans	1	2	2	5
grape or raisin	grape, raisin (0.6: 0.4)[a]	2	1	2	5
potatoes	potatoes	2	2	1	5
spinach, cooked	spinach, frozen	1	2	2	5
peach or plum	peach, plum (0.7: 0.3)[a]	2	2	2	6
strawberries (FFC)	strawberries, fresh	2	2	2	6
spinach, raw	spinach, fresh	2	2	2	6
green/yellow/red peppers	sweet peppers	2	2	2	6

Table S2.1 (Continued). Fruit and vegetable items in the food frequency questionnaire and pesticide data program, and corresponding scores for 1st, 2nd and 3rd measure, and PRBS.

Abbreviations: FFC, fresh, frozen, or canned; Fs, fresh; Fz, frozen; PDP, pesticide data program; PRBS, pesticide residue burden score.

[a] Ratio weighted for pesticide residue for each produce according to the ratio of consumption of each produce from the USDA report

Table S2.2. Ovarian biomarker and ovarian stimulation outcomes according to quartile of fruit and vegetable intake, considering pesticide residue status, among 325 women (541 fresh cycles).

	Adjusted[1,2] mean (95%CI)				
	Day 3 FSH, IU/L	**Peak E2 trigger results, pmol/L**	**Endometrial thickness, mm**	**Total oocyte yield, count[6]**	**MII Oocyte, count[6]**
Quartile (range) of High Pesticide Fruit and Vegetable Intake[3] (servings/day)					
Q1 (0.3, 1.0)	7.0 (6.3, 7.7)	2454 (2102, 2807)	10.7 (10.1, 11.2)	12.4 (10.9, 14.2)	10.7 (9.4, 12.2)
Q2 (1.0, 1.6)	7.9 (7.2, 8.6)	2271 (1995, 2547)	9.9 (9.4, 10.4)	11.0 (9.9, 12.3)	8.9 (8.0, 9.9)
Q3 (1.6, 2.2)	7.2 (6.5, 7.9)	2245 (1976, 2515)	10.2 (9.7, 10.6)	10.2 (9.2, 11.4)	8.7 (7.8, 9.7)
Q4 (2.3, 6.8)	7.3 (6.6, 8.1)	1967 (1588, 2347)	10.3 (9.6, 10.9)	11.2 (9.8, 12.8)	9.3 (8.1, 10.6)
P, trend [5]	0.95	0.18	0.30	0.33	0.24
Quartile (range) of Low Pesticide Fruit and Vegetable intake[4] (servings/day)					
Q1 (0.5, 1.7)	7.1 (6.4, 7.8)	2321 (1991, 2650)	9.6 (9.1, 10.2)	10.9 (9.6, 12.3)	9.2 (8.1, 10.4)
Q2 (1.7, 2.5)	7.5 (6.8, 8.2)	2396 (2105, 2687)	10.5 (10.0, 10.9)	12.3 (11, 13.7)	10.3 (9.2, 11.5)
Q3 (2.5, 3.5)	7.6 (6.9, 8.3)	2110 (1830, 2390)	10.4 (9.9, 10.9)	10.3 (9.2, 11.6)	8.3 (7.4, 9.3)
Q4 (3.6, 11.5)	7.2 (6.4, 8.0)	2111 (1715, 2507)	10.5 (9.8, 11.2)	11.4 (10, 13.1)	9.8 (8.5, 11.2)
P, trend [5]	0.84	0.54	0.73	0.91	0.94

Abbreviations: E2, estradiol; FSH: follicle-stimulating hormone; MII, metaphase II.

[1] Model was adjusted for age, BMI, smoking status, race, folate supplement, organic fruit and vegetable consumption frequency, residential pesticide exposure history, total energy intake, Western pattern score, Prudent pattern score, and infertility diagnosis.

[2] Adjusted mean was calculated at mean levels for continuous covariates and weighted average over categorical covariates.

[3] Model additionally adjusted for low pesticide FV intake.

[4] Model additionally adjusted for high pesticide FV intake

[5] Tests for trend were performed using the median intake in each quartile as a continuous variable in the model.

[6] N=305, 424 cycles

Table S2.3. Fertilization and embryo quality according to fruit and vegetable intake, considering pesticide residue status, in 305 women (424 fresh cycles) from EARTH study.

	Adjusted[1,2] proportion (95%CI)				
	Fertilization	**Accelerated embryo cleavage**	**Slow embryo cleavage**	**Poor quality embryo**	**≥ 1 best quality embryos on day 2 and day 3**
Quartile (range) of High Pesticide Fruit and Vegetable intake [3] (servings/day)					
Q1 (0.3, 1.0)	0.70 (0.64, 0.75)	0.10 (0.06, 0.15)	0.25 (0.19, 0.33)	0.12 (0.08, 0.19)	0.63 (0.49, 0.75)
Q2 (1.0, 1.6)	0.74 (0.7, 0.79)	0.12 (0.08, 0.17)	0.21 (0.16, 0.26)	0.13 (0.09, 0.18)	0.58 (0.47, 0.69)
Q3 (1.6, 2.2)	0.72 (0.67, 0.76)	0.10 (0.07, 0.14)	0.24 (0.19, 0.30)	0.15 (0.11, 0.20)	0.59 (0.48, 0.69)
Q4 (2.3, 6.8)	0.73 (0.67, 0.78)	0.07 (0.04, 0.12)	0.31 (0.23, 0.40)	0.15 (0.10, 0.23)	0.55 (0.41, 0.69)
P, trend [5]	0.83	0.30	0.26	0.53	0.49
Quartile (range) of Low Pesticide Fruit and Vegetable intake [4] (servings/day)					
Q1 (0.5, 1.7)	0.74 (0.68, 0.78)	0.12 (0.08, 0.18)	0.20 (0.15, 0.27)	0.11 (0.07, 0.17)	0.61 (0.48, 0.72)
Q2 (1.7, 2.5)	0.71 (0.66, 0.75)	0.13 (0.09, 0.19)	0.24 (0.18, 0.30)	0.15 (0.11, 0.21)	0.66 (0.54, 0.76)
Q3 (2.5, 3.5)	0.71 (0.66, 0.76)	0.08 (0.05, 0.11)	0.25 (0.19, 0.32)	0.15 (0.10, 0.20)	0.49 (0.38, 0.60)
Q4 (3.6, 11.5)	0.74 (0.68, 0.79)	0.06 (0.04, 0.11)	0.31 (0.23, 0.41)	0.15 (0.10, 0.23)	0.59 (0.44, 0.72)
P, trend [5]	0.76	0.08	0.13	0.49	0.53

[1] Model was adjusted for age, BMI, smoking status, race, folate supplement, organic fruit and vegetable consumption frequency, residential pesticide exposure history, total energy intake, Western pattern score, Prudent pattern score, and infertility diagnosis.

[2] Adjusted proportions were calculated at mean levels for continuous covariates and weighted average over categorical covariates.

[3] Model additionally adjusted for low pesticide FV intake.

[4] Model additionally adjusted for high pesticide FV intake

[5] Tests for trend were performed using the median intake in each quartile as a continuous variable in the model.

Table S2.4. Adjusted probability of clinical pregnancy and live birth according to high pesticide fruit and vegetable intake, restricting for different characteristics

	Adjusted [1,2] probability of clinical pregnancy (%)				
	Main analysis (541 cycles)	**Without adjusting infertility diagnosis**	**Restricting to women <40 year (441 cycles)**	**Restricting to women without prior miscarriage history (424 cycles)**	**Restricting to cycles initiated within 1 year after FFQ completion (383 cycles)**
Quartile of high pesticide fruit and vegetable intake					
Q1	0.57 (0.46, 0.68)	0.57 (0.46, 0.67)	0.58 (0.46, 0.70)	0.58 (0.45, 0.70)	0.60 (0.47, 0.73)
Q2	0.60 (0.51, 0.69)	0.61 (0.52, 0.69)	0.62 (0.52, 0.70)	0.61 (0.51, 0.70)	0.58 (0.47, 0.68)
Q3	0.51 (0.42, 0.59)	0.51 (0.42, 0.6)	0.53 (0.43, 0.63)	0.51 (0.40, 0.61)	0.53 (0.42, 0.64)
Q4	0.42 (0.31, 0.53)	0.41 (0.3, 0.53)	0.41 (0.29, 0.55)	0.39 (0.27, 0.53)	0.38 (0.26, 0.52)*
P, trend [3]	0.05	0.04	0.06	0.04	0.02
	Adjusted [1,2] probability of live birth (%)				
Quartile of high pesticide fruit and vegetable intake					
Q1	0.56 (0.44, 0.67)	0.55 (0.44, 0.66)	0.61 (0.48, 0.73)	0.57 (0.44, 0.69)	0.61 (0.46, 0.74)
Q2	0.45 (0.36, 0.54)	0.46 (0.37, 0.55)	0.46 (0.37, 0.56)	0.47 (0.37, 0.57)	0.41 (0.30, 0.52)*
Q3	0.39 (0.31, 0.48)	0.39 (0.31, 0.48)*	0.40 (0.30, 0.50)	0.39 (0.30, 0.50)	0.40 (0.28, 0.52)*
Q4	0.31 (0.21, 0.42)*	0.3 (0.21, 0.42)*	0.29 (0.19, 0.42)*	0.30 (0.19, 0.44)*	0.27 (0.17, 0.41)*
P, trend [3]	0.02	0.01	0.007	0.02	0.02

[1] Model was adjusted for age, BMI, smoking status, race, folate supplement, organic fruit and vegetable consumption frequency, residential pesticide exposure history, total energy intake, Western pattern score, Prudent pattern score, and infertility diagnosis.

[2] Adjusted mean was calculated at mean levels for continuous covariates and weighted average over categorical covariates.

[3] Tests for trend were performed using the median intake in each quartile as a continuous variable in the model.

CHAPTER 3

Maternal intake of fruit and vegetable, pesticide residues, in relation to fetal growth

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ABSTRACT

IMPORTANCE: Little is known about the potential adverse effects on fetal growth of in utero exposure to pesticide residues in food, particularly through maternal intake of fruits and vegetables (FVs), which are an important source of exposure in the general population. Race/ethnicity, as a marker of the underlying frequency of polymorphisms involved in pesticide metabolism, could modify this association.

OBJECTIVE: To examine the associations of maternal intake of FVs, considering pesticide residue status, with fetal growth among white and minority mother-child pairs.

DESIGN: Project Viva, a prospective pre-birth cohort that enrolled pregnant women at their initial prenatal visit between 1999 and 2002.

SETTING: A multi-specialty urban and suburban group practice in Massachusetts.

PARTICIPANTS: 1777 mother-child pairs (1275 white and 502 nonwhite).

EXPOSURES: We first categorized FVs as having high or low pesticide residues based on surveillance data from the US Department of Agriculture. We summed the intakes of high and low pesticide residues FVs, separately, using a validated food frequency questionnaire in the 1^{st} and 2^{nd} trimester of pregnancy.

MAIN OUTCOMES AND MEASURES: Adjusted odds ratio (OR) of small-for-gestational-age (SGA; lowest 10^{th} percentile in birth-weight-for-gestational-age), large-for-gestational-age (LGA; upper 10^{th} percentile in birth-weight-for-gestational-age) and preterm birth (gestational age <37 weeks).

RESULTS: Of 1777 newborns, 5.3% were SGA, 13.7% were LGA, and 7.3% were born preterm. Among white women, first trimester intake of high pesticide residue FVs was positively associated with SGA (adjusted OR (95%CI)= 2.81 (1.05, 7.53) for highest quartile vs. lowest quartile). In contrast, among non-white women, first trimester intake of high pesticide FVs was associated with higher risk of LGA (adjusted OR=2.37 (0.70, 8.04) for highest quartile vs. lowest quartile). Low pesticide residue FV intake, regardless of race/ethnicity, was unrelated to risks of SGA, LGA, or preterm birth. Second trimester intake

of FV, regardless of pesticide residue status and race/ethnicity, was not associated with these outcomes. **CONCLUSIONS:** Maternal intake of high pesticide FVs during first trimester was associated with greater risks of SGA among white mothers while these intakes were associated with greater risks of LGA among non-white mothers.

Introduction

Fruits and vegetables (FVs) are important sources of many nutrients such as vitamins, minerals, fiber, folate, and polyphenols, and are considered as essential components of a healthy diet(Millen, Abrams, Adams-Campbell, Anderson, Brenna, Campbell, Clinton, Hu, Nelson, and Neuhouser 2016). According to the Dietary Guidelines for Americans 2015-2020, consumption of a variety of FVs are recommended throughout the lifespan including during pregnancy(Millen, Abrams, Adams-Campbell, Anderson, Brenna, Campbell, Clinton, Hu, Nelson, Neuhouser, et al. 2016). Nonetheless, FVs can also serve as a source of exposure to pesticide residues in the general population. According to US Department of Agriculture (USDA) Pesticide Data Program, in 2015, 85% of the sampled foods (97% FVs) in the U.S. markets had detectable pesticide residues, and 56% had three or more individual pesticides(USDA 2015). In the United States, Environmental Protection Agency (EPA) is responsible for regulating pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Food Quality Protection Act (FQPA). While majority of the foods had residues around or well below EPA's the regulatory limits(USDA 2015), even at low residue levels there is growing concern that chronic exposure to these pesticide residues may have adverse health effects(Vandenberg et al. 2012), especially among susceptible populations and life stages such as fetal life and infants. Pesticide metabolites can be detected in amniotic fluid as early as 15-18 weeks gestation(Bradman et al. 2003). The fetus, due to its rapid growth, immaturity of metabolic pathways, and development of vital organ systems(Berkowitz et al. 2004), may exhibit greater susceptibility to the effects of pesticide residues than adults.

A limited number of human studies have prospectively examined maternal urinary concentrations of organophosphate pesticide metabolites in relation to fetal growth and gestational age(Eskenazi et al. 2004, Harley et al. 2011, Whyatt et al. 2004, Rauch et al. 2012, Naksen et al. 2015), showing that certain populations may be more susceptible to the potential hazards of pesticide

exposure than the others. No studies have directly evaluated the impact of dietary pesticide exposure on fetal growth. Paraoxonase 1 (PON1) is an enzyme involved in detoxification of organophosphate pesticide. Genetic polymorphisms of PON1 affecting its function differ across racial groups. There are two common polymorphisms in *PON1* gene: a Glutamine (Q)/Arginine (R) substitution at position 192 in the PON1 coding sequence that affect the catalytic efficiency of enzymes toward different substrates; and a Thymine(T)/Cytosine(C) substitution in the promoter at position -108 that affect levels of PON1 expression. Earlier studies have shown that $PON1_{Q192}$ has much less efficiency at hydrolyzing chlorpyrifo-oxon and paraoxon (the metabolites of two commonly used organophosphate pesticides), and $PON1_{-108T}$ has lower plasma PON1 levels(Costa et al. 2013). As Caucasians have a higher frequency of $PON1_{Q192}$ (73% vs. 37%) and PON_{-108T} (51-52% vs. 0-20%) than African Americans (Draganov and La Du 2004, Chen et al. 2003), infants born to white mothers may be more susceptible to certain pesticides. Based on this difference, we hypothesized that maternal intake of high pesticide residue FVs would be associated with reduced fetal growth among white women but not among non-white women. We examined this hypothesis in a cohort of pregnant women from Massachusetts.

Materials and Methods

Study population

This study included participants in Project Viva, a prospective cohort that recruited women carrying a singleton pregnancy during their initial obstetric care visit (median: 9.9 weeks gestation) between 1999 and 2002 at a multi-site/multi-specialty practice in Eastern Massachusetts. Details of the cohort have been described previously(Oken et al. 2015). Briefly, research assistants obtained informed consent and collected demographic, diet and health history information by conducting an interview. The research assistants asked women “Which of the following best describes your race or ethnicity?” The participants had a choice of ≥1 the following racial/ethnic groups: Hispanic or Latina, white or Caucasian,

black or African American, Asian or Pacific Islander, American Indian or Alaskan Native, and other (please specify). If the participants identified herself as more than 1 racial/ethnic groups (e.g, white and Hispanic), her race/ethnicity will be classified as others. For participants who chose the "other" race/ethnicity, we compared the specified responses to US census definitions for other 5 race ethnicities and reclassified them where appropriate. For this analysis, we collapsed participants who reported multiple race/ethnicity into "other". We also provided a take-home questionnaire at the first (median: ~9 weeks of gestation) and second study visits (~26-28 weeks of gestation). Of 2128 women who delivered a live born infant, 1777 completed the first trimester food frequency questionnaire (FFQ) and 1543 completed a second trimester FFQ. Compared to women who completed the first trimester FFQ, women who did not complete first trimester FFQ were younger, were more likely to be black, had a higher pre-pregnancy BMI and less college graduates. Institutional review boards of Harvard Pilgrim Health Care, Brigham and Women's Hospital, and Beth Israel Deaconess Medical Center approved the study protocols and all mothers provided written informed consent.

Dietary Assessment

We assessed diet using a 140-item, self-administered food frequency questionnaire (FFQ) based on a well-validated FFQ used in other cohorts(Farvid et al. 2016) and adapted for use among pregnant women(Fawzi et al. 2004). The first trimester FFQ assessed diet intake since the last menstrual period and was completed by participants at enrollment. The second trimester FFQ assessed diet "during the past 3 months" at 26-28 weeks of gestation. In a previous calibration study among early pregnant women, association of α-carotene between the FFQ and plasma levels was stronger for white than African American(Fawzi et al. 2004). In another a validation study among non-pregnant women, the de-attenuated correlation (i.e. observed correlation corrected for random with-person variability) between two, one-week diet records and reported intakes of FVs in the FFQ ranged from 0.27 for spinach to 0.95

for bananas (Feskanich et al. 1993). We also used two dietary pattern scores (Prudent dietary pattern and Western dietary pattern scores) derived by principle component factor analysis to summarize overall food choices (Lange et al. 2010). The factor scores were standardized to having a mean of 0 and standard deviation of 1, with higher score indicating higher adherence to Prudent or Western dietary patterns. We estimated Nutrient intakes using a nutrient database derived from the USDA with additional information obtained from manufacturers (Gebhardt et al. 2008).

Pesticide Residue Assessment

We have described the pesticide residue assessment method elsewhere (Chiu et al. 2015). Briefly, we developed the Pesticide Residue Burden Score (PRBS) to assess pesticide residue status in FVs using national surveillance data from US Department of Agriculture Pesticide Data Program (USDA 2013). We defined the PRBS according to three contamination measures from the Pesticide Data Program: 1) the percentage of samples tested with any detectable pesticides; 2) the percentage of samples tested with pesticides exceeding the tolerance levels; and 3) the percentage of samples with three or more types of detectable pesticides. We ranked the 36 FVs included in the FFQ according to each of the three contamination measures, divided them into tertiles for each of these three measures, and assigned each food a score of 0, 1, and 2 corresponding to the bottom, middle, and top tertile, respectively. The final PRBS for each food was the sum of tertile scores across the three contamination measures on the scale of 0 to 6. We classified FVs with a PRBS ≥4 as high pesticide residue foods and those with a PRBS <4 as low pesticide residue foods (Table S3.1). In a previous study, the de-attenuated correlations between the PRBS and sum of urinary pesticide metabolites from two urine samples were 0.53 for high pesticide FV intake, and -0.45 for low pesticide FV intake (Chapter 1).

Ascertainment of outcomes

We obtained infant birth weight in grams from hospital medical records. We calculated gestational age at delivery in weeks by subtracting the date of the last menstrual period (LMP) from the date of delivery. For women whose prenatal ultrasound (performed at 16-20 weeks of gestation) estimate differed from the LMP estimate by > 10 days (~9%), we calculated gestational age at delivery based on the ultrasound results. We defined preterm birth as birth <37 completed weeks of gestation. We used sex-specific birth-weight-for-gestational age (BW/GA) Z scores (an index as fetal growth) from 1999-2000 US national reference data(Oken et al. 2003). We defined small-for-gestational-age (SGA) and large-for-gestational-age (LGA) as BW/GA Z score below the 10^{th} percentile and those greater than the 90^{th} percentile, respectively.

Statistical analysis

We classified women according to quartiles of high pesticide residue and low pesticide residue FV intake, respectively. We used Kruskal-Wallis (for continuous variable) and Fisher exact tests (for categorical variable) to evaluate differences in participant characteristics according to quartiles of high and low pesticide FV intake. We modeled high and low FV intake in quartiles and also as a continuous variable. To evaluate the associations of high and low pesticide FV intake with birth outcomes, we fit multivariable quantile regressions for BW/GA Z score and gestational age at delivery, multivariable logistic regression models for preterm birth, and multinomial logistic regression models for SGA and LGA using appropriate-for-gestational-age as the reference group. As we hypothesized a priori that the association between intake of pesticide residues through FVs and perinatal outcomes would be stronger in white women than nonwhite women, we stratified all analyses by race/ethnicity.

We selected covariates based on prior knowledge through the use of directed acyclic graphs. All models were adjusted for maternal age (years), pre-pregnancy body mass index (BMI, kg/m^2), height (<1.6m, 1.6 to < 1.7m, ≥ 1.7m), smoking during pregnancy (current, past, or never), education level (some college degree or lower versus college graduate or higher), annual household income (<$70,000, >$70,000), marital status (yes or no), total energy intake (kcal/day), Prudent and Western dietary pattern scores, and paternal height (<1.7m, 1.7 to <1.8m, ≥ 1.8m). As high and low pesticide FV intake may confound each other, we additionally adjusted for low pesticide FV intake in the models of high pesticide FV intake, and vice versa. We conducted tests for trend using the median intake of FV in each quartile as a continuous variable in the regression model.

Approximately 8% of participants had missing data on one (n=140) or two covariates (n=5). We employed multiple imputations to impute the missing values using 50 imputed datasets. We combined the estimates of multivariable modeling results using Proc MI ANALYZE. We performed statistical analyses with SAS v9.4 (SAS Institute, Cary, N.C.).

Results

The baseline characteristics of 1777 mother-child pairs are shown in Table 1. Most participants were white or Caucasian (72%), followed by Black or African American (12%), Hispanic or Latino (7%) and Asian or Alaskan Native (6%) (Table 3.1). Of 1777 live born infants, 5.3% were SGA, 13.7% were LGA, and 7.1% were born preterm. Infants born to minority mothers were more often born preterm (11.4% vs. 5.7%) and SGA (9.2% vs. 4.1%), but less often born LGA (9.4% vs. 15.4%) than infants born to white mothers. Among 1543 with both first and second trimester FFQs, intakes of FVs in the first and second trimester were correlated with each other ($r_{spearman}$=0.69 for high pesticide FVs, and $r_{spearman}$=0.60 for low pesticide FVs). High pesticide and low pesticide residue FV intakes were also positively correlated with each other ($r_{spearman}$=0.54 for the first trimester and 0.44 for the second trimester). On average,

consumption of high pesticide residue FVs was similar between white and nonwhite mothers (mean(SD): 2.4 (1.3) vs. 2.3 (1.7) servings/day for white vs. non-white), while consumption of low pesticide residues FVs was lower for white mothers compared to non-white mothers (mean(SD): 3.0 (1.5) vs. 3.4 (2.1) servings/day for white vs. non-white).

Among white women, those who consumed more high pesticide residue FVs tended to be older, were more likely to be college graduates, and were less likely to smoke during pregnancy, while there were no differences in baseline demographics characteristics across quartiles of low pesticide FV intake. Similarly, among non-white women, those who consumed more high pesticide residue FVs tended to be older, be college graduates, and be married/cohabitating. Educational attainment also differed according to low pesticide residue FV intake (college graduates: 63% in Q1 vs. 70% in Q4).

Linear regression model showed that maternal intake of high pesticide FVs during 1st trimester was inversely associated with BW/GA Z scores among white women while positively associated with BW/GA Z scores among non-white women. Quantile regressions further suggested that the effect of high pesticide FV intake during first trimester on BW/GA were larger on the tails of the distribution (Table 3.2). Specifically, among white mothers, a significant decrease in BW/GA was observed at the 10th percentile, with BW/GA decreased by 0.26 (95%CI: 0.04, 0.48) standard deviations comparing women in the highest versus lowest quartiles of high pesticide FV intake. In contrast, among non-white mothers, there was a positive increase in BW/GA at the 90th percentile, with BW/GA increased by 0.77 (95%CI: 0.43, 1.11) standard deviations comparing women in the highest versus lowest quartiles of high pesticide FV intake. These associations were similar when fetal growth was modeled as dichotomous outcomes (Table 3.4). Specifically, among white women, the adjusted OR (95%CI) of delivering a SGA infant in increasing quartiles of first trimester high pesticide residue FV intake was 1, 1.44 (0.61, 3.36),

1.15 (0.43, 3.07) and 2.81 (1.05, 7.53), respectively. Among non-white women, the adjusted OR (95%CI) of delivering a LGA infant in increasing quartiles of first trimester high pesticide residue FV intake was 1, 1.93 (0.73, 5.08), 3.71 (1.23, 11.2) and 2.37 (0.70, 8.04), respectively. On the other hand, first trimester intake of low pesticide residue FVs was not associated with BW/GA Z score or risks of SGA, LGA in either race/ethnicity groups (Table 3.4).

First trimester intake of high pesticide FVs was also associated with longer gestational age at 90th percentile among white women but not among non-white women (Table 3.3). Specifically, among white women, the 90th percentile of gestational age at delivery for women in the highest quartile of low pesticide residue FV intake was 0.32 (95%CI: 0.08, 0.57) weeks longer than women in the lowest quartile. On the other hand, neither high or low pesticide FV intake during first trimester was associated with preterm birth in either race/ethnicity groups (Table 3.5).

We also examined the association of FV intake during second trimester with birth outcomes (Table S3.2-3.4). There were no associations between intake of high or low pesticide residue FVs with fetal growth, SGA, LGA, or preterm birth in either race/ethnicity group.

Discussion

We evaluated the associations of high and low pesticide residue FV intake during pregnancy with fetal growth and preterm delivery in a pre-birth cohort in Massachusetts. Among white mothers, intake of high pesticide residue FVs during first trimester was associated with greater risk of SGA. On the other hand, among non-white mothers, intake of high pesticide residue FVs during first trimester was associated with greater risk of LGA. In addition, first trimester intake of low pesticide residue FVs was associated with longer gestational length at 90th percentile (but only by 2 days between highest and lowest quartile) among white but not among non-white women. Risks of preterm birth were unrelated to high or low pesticide FV intake in either race/ethnicity groups.

To the best of our knowledge, this is the first study to examine prenatal exposure to dietary pesticide residues on fetal growth. Possible mechanisms to explain the association of early pregnancy FV intake and higher rates of SGA among white women are that pesticides may interfere with placental transport (Saulsbury et al. 2008, Burton and Jauniaux 2004, Brantsaeter et al. 2016, Bretveld et al. 2006, Souza et al. 2005) or alter the activity of the adenylyl cyclase signaling cascade(Eskenazi, Bradman, and Castorina 1999), which could reduce fetal growth by disrupting cell development. As race/ethnicity is a marker of the underlying frequency of polymorphisms involved in pesticide metabolism, we hypothesized that we might observe associations between high pesticide FV intake with greater risk of SGA or preterm birth among white, who are more likely to carry vulnerable $PON1_{-108TT}$ and $PON1_{192QQ}$ genotypes, but not among non-white women, who might be more resistant to the effects of pesticide residues due to their improved efficiency at detoxifying certain organophosphate pesticides. In partial agreement with this hypothesis, we observed reduced fetal growth (also higher risk of SGA) among infants born to white mothers in the present study. Notably, while intakes of high pesticide FVs were similar between first and second trimester ($r_{spearman}$=0.69, 89 % of perfect or adjacent agreement of quartile classification between first and second trimesters), we observed associations only for first trimester intake. One possible explanation is early pregnancy may be the critical period of heightened susceptibility of low dose effect (i.e., effect observed at doses below those used for traditional toxicological studies). Although no previous studies evaluated the association of pesticide exposure with fetal growth at different time points across pregnancy, studies of other environmental exposures such as polycyclic aromatic hydrocarbons cadmium, and particulate matter ≤2.5 μm have shown that exposure during the first trimester exerts greater effect on reduced fetal growth than exposures later in pregnancy(Choi et al. 2012, Cheng et al. 2017, Kumar 2016).

Unexpectedly, however, we found high pesticide FV intake was associated with higher birth weight (driven by higher prevalence of LGA) among non-white women. While we did not anticipate this association, this finding was consistent with CHARMACO study (n=470) in Mexican-American women. In that study, Harley et al. reported that among infants with the non-susceptible genotype ($PON1_{192RR}$), each ten-fold increase in prenatal urinary organophosphate pesticides (average of two measurement at the first and the second trimesters) was associated with a 258.8 g (95%CI: 23.9, 493.6) increase in birth weight(Harley et al. 2011). One possible explanation for this finding was that although non-white women are more likely to carry functional PON1 genotypes ($PON1_{-108CC,}$ $PON1_{192RR}$) for detoxifying organophosphate pesticides, these genotypes ($PON1_{192RR}$), on the other hand, are also known to be less efficient at metabolizing oxidized high-density lipoprotein (HDL) or low-density lipoprotein (LDL) than $PON1_{192QQ}$(Costa et al. 2005). It is possible that pesticides may kick in in different oxidative-stress pathways according to individuals' genotypes. Intriguingly, in a crossover 5-week of dietary intervention study in Finland (n=37), Kleemola et al. showed that a diet high in vegetable, berries and fruits reduced PON1 activity among women with $PON1_{192RR}$ but not $PON1_{192QQ}$ (Rantala et al. 2002). The authors hypothesized that some unknown factors in vegetables might inhibit PON1 activity (a risk factor for coronary heart disease) although the pathways are unclear. We speculated that pesticide residues in in vegetables might link the relationship of high pesticide FV intake with higher risks of LGA among non-white women in the present study, as well as high vegetable intakes and reduced PON1 activity among women with $PON1_{RR}$ in the earlier crossover study(Kleemola et al. 2002). Nonetheless, to the best of our knowledge, no studies have investigated the relationship between pesticides, PON1 genotypes or PON1 activity, and metabolic disorders altogether.

Several earlier studies have evaluated the effects of the interaction of PON1 polymorphisms with prenatal pesticide exposure on fetal growth, and the results were inconsistent. For example, a

recent pooled analysis (total sample size~1100) of 4 cohorts (CHAMACOS, HOME, Columbia, and Mount Sinai birth cohorts) showed that prenatal urinary dimethyl organophosphate pesticide concentration had a borderline inverse association with birth weight (β=-66.78 g; 95%CI: -137.46, 3.90) in non-Hispanic black women (non-susceptible populations) but no evidence of smaller birth weight among whites (susceptible populations). However, a pilot study of 52 mothers and children in Thailand found that newborn birth weight and gestational age were inversely associated with maternal dialkylphosphate urinary concentrations (collected multiple times during pregnancy) among mothers with low PON1 activity(Naksen et al. 2015). These disparate results between these studies and our study may be attributed to diverse racial/ethnic composition, social-economic status, and differences in timing, routes, levels and classes of pesticide exposure across studies. Furthermore, some associations in earlier studies might be masked if the effect of pesticides on fetal growth only acts on the tails of the distributions but authors only examined the changes in birth weight at mean level.

It is important to consider our study's strengths and limitations. First, our method for pesticide residue exposure relied on national pesticide surveillance data rather than individual-level of biomarkers. Second, we assumed all FVs were conventionally grown due to lack of data on organic food consumption. However, these limitations are likely to result in non-differential misclassification, which would tend to attenuate the observed associations. In addition, we previously have shown that the PRBS predicts urinary pesticide metabolites and allows adequate characterization of pesticide exposure through diet. Nonetheless, PRBS captured overall pesticide exposure instead of targeting a certain pesticide metabolite. Therefore, we cannot identify the pesticides that are associated with fetal growth. Another important limitation was lack of information of PON enzyme activity or PON1 genotype data, a more direct marker for pesticide susceptibility than race/ethnicity. In addition, we don't have sufficient powers to stratify the analysis for minority race/ethnic groups (African Americans, Hispanic, Asian, and

multiple race/ethnicity). Nonetheless, the proportions of racial/ethnic minorities in Project Viva were higher than in Massachusetts as a whole. The strengths of the study include prospective study design, a relatively large sample size, and detailed covariate information on many maternal factors that have previously been associated with fetal growth. In addition, the availability of full length FFQ during first trimester and second trimester of pregnancy allows us to capture the most susceptible time period in which an exposure may have feto- or embyrotoxic effect (Sadler 2011, Rozman and Klaassen 2007).

In conclusion, we found that greater intake of high pesticide residue FVs was associated with reduced fetal growth among infants born to white mothers, while greater intake of high pesticide FVs was associated with higher birth weight among non-white women. These effects were stronger for high pesticide FV intake in the first trimester than in second trimester. On the other hand, risks of preterm birth were unrelated to high or low pesticide FV intake in either race/ethnicity groups. Given that diet represents a major route of exposure to pesticides and pesticide residues in the general population, the results suggest the needs for further investigation of the impact of prenatal dietary pesticide on birth and later childhood outcomes.

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Table 3.1. Baseline characteristics among 1777 singleton pregnant women in Project Viva

Baseline characteristics	**Total**	**White**	**Non-white**
High pesticide FV intake, servings/d	2.4 (1.4)	2.4 (1.3)	2.3 (1.7)
Low pesticide FV intake, servings/d	3.1 (1.7)	3 (1.5)	3.4 (2.1)
Maternal age, years	32.2 (4.9)	32.9 (4.3)	30.5 (5.9)
Gestational age at enrollment, wks	10.4 (2.5)	10.4 (2.4)	10.6 (2.8)
Prepregnancy BMI, kg/m^2	24.6 (5.3)	24.2 (4.9)	25.6 (6.0)
Total energy intake, kcal/day	2061 (673)	2058 (592)	2066 (845)
Prudent dietary pattern	0.0 (1.0)	-0.1 (0.8)	0.2 (1.3)
Western dietary pattern	0.0 (1.0)	0 (0.9)	0 (1.1)
Race/ethnicity, N(%)			
. Non-Hispanic black	219 (12.3)	-	-
. Hispanic or Latino	115 (6.5)	-	-
. Asian or Alaskan Native	103 (5.8)	-	-
. Non-Hispanic White	1275 (71.8)	-	-
. Other	65 (3.7)	-	-
Married or cohabitating, N(%)	1657 (93.3)	1239 (97)	418 (83)
College graduate or higher, N(%)	1233 (69.4)	975 (76)	258 (51)
Annual household income >$70,000/y, N(%)	1059 (64.1)	869 (71)	190 (44)
Nulliparous, N(%)	873 (49.1)	643 (50)	230 (46)
Smoking status, N(%)			
. Never	1194 (67.4)	811 (64)	383 (77)
. Former	382 (21.6)	330 (26)	52 (10)
. During pregnancy	195 (11)	131 (10)	64 (13)
Maternal height, N(%)			
. <1.6 m	311 (17.5)	188 (15)	123 (25)
. 1.6 - <1.7 m	947 (53.3)	671 (53)	276 (55)
. ≥1.7 m	519 (29.2)	416 (33)	103 (21)
Infant sex female, N(%)	884 (49.8)	636 (50)	248 (49)
Paternal height, N(%)			
. <1.7 m	160 (9.1)	68 (5)	92 (19)
. 1.7 - <1.8 m	713 (40.5)	510 (40)	203 (41)
. ≥1.8 m	889 (50.5)	692 (54)	197 (40)

Data are presented as mean (standard deviation) or N(%)

Table 3.2. Association of high and low pesticide fruit and vegetable intake during first trimester with fetal growth in 1275 white and 502 non-white mother-child pairs participating in Project Viva.

	Change in BW/GA Z score					
	10th (95%CI)	**50th (95%CI)**	**90th (95%CI)**	**10th (95%CI)**	**50th (95%CI)**	**90th (95%CI)**
White (n=1275)						
High pesticide residue FV intake in first trimester				**Low pesticide residue FV intake in first trimester**		
Q1 (0.1, 1.4)	Reference	Reference	Reference	Q1 (0.3, 1.9)	Reference	Reference	Reference
Q2 (1.4, 2.1)	-0.14 (-0.33, 0.05)	0.02 (-0.16, 0.19)	0.12 (-0.10, 0.35)	Q2 (1.9, 2.8)	-0.03 (-0.20, 0.15)	0.07 (-0.10, 0.23)	-0.13 (-0.34, 0.09)
Q3 (2.2, 3.1)	-0.23 (-0.43, -0.03)	-0.08 (-0.27, 0.1)	0.05 (-0.19, 0.28)	Q3 (2.8, 4.0)	0.08 (-0.11, 0.27)	0.03 (-0.15, 0.21)	-0.08 (-0.31, 0.15)
Q4 (3.1, 13.2)	-0.26 (-0.48, -0.04)	-0.13 (-0.34, 0.07)	0.00 (-0.27, 0.27)	Q4 (4.0, 13.4)	0.04 (-0.18, 0.26)	-0.01 (-0.21, 0.2)	-0.17 (-0.43, 0.09)
P, trend [2]	0.01	0.09	0.76	P, trend [2]	0.74	0.82	0.47
Continuous[3]	-0.07 (-0.13, 0.00)	-0.04 (-0.1, 0.02)	-0.01 (-0.09, 0.07)	Continuous[3]	0.03 (-0.03, 0.08)	0 (-0.05, 0.06)	-0.03 (-0.09, 0.04)
Minority (N=502)						
High pesticide residue FV intake in first trimester				**Low pesticide residue FV intake in first trimester**		
Q1 (0.1, 1.4)	Reference	Reference	Reference	Q1 (0.3, 1.9)	Reference	Reference	Reference
Q2 (1.4, 2.1)	0.13 (-0.17, 0.44)	0.08 (-0.18, 0.33)	0.49 (0.23, 0.76)	Q2 (1.9, 2.8)	0.20 (-0.13, 0.52)	-0.04 (-0.31, 0.23)	0.28 (0.00, 0.56)
Q3 (2.2, 3.1)	0.11 (-0.26, 0.47)	0.20 (-0.10, 0.49)	0.59 (0.29, 0.88)	Q3 (2.8, 4.0)	-0.16 (-0.50, 0.17)	-0.15 (-0.44, 0.14)	-0.01 (-0.34, 0.31)
Q4 (3.1, 13.2)	0.24 (-0.15, 0.63)	0.25 (-0.09, 0.58)	0.77 (0.43, 1.11)	Q4 (4.0, 13.4)	0.03 (-0.34, 0.41)	-0.15 (-0.47, 0.17)	0.13 (-0.21, 0.47)
P, trend [2]	0.20	0.17	0.05	P, trend [2]	0.95	0.39	0.34
Continuous[3]	0.01 (-0.09, 0.12)	0.06 (-0.02, 0.14)	0.14 (0.03, 0.25)	Continuous[3]	0.03 (-0.05, 0.12)	-0.02 (-0.08, 0.05)	-0.05 (-0.13, 0.04)

Abbreviations: FV, fruit and vegetable; BW/GA, birth weight adjusting for gestational age

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide fruit and vegetable intake was additionally adjusted for low pesticide fruit and vegetable intake, and vise versa.

[2] P, trend was performed using median intake in each quartile as continuous variable in the model.

[3] Change in BW/GA Z scores for one serving increase in high or low pesticide fruit and vegetable intake

Table 3.3. Association of high and low pesticide fruit and vegetable intake during first trimester with gestational age at delivery in 1275 white and 502 non-white mother-child pairs participating in Project Viva.

	Change in gestational age at delivery (weeks)					
	10th (95%CI)	**50th (95%CI)**	**90th (95%CI)**	**10th (95%CI)**	**50th (95%CI)**	**90th (95%CI)**
			White (n=1275)			
High pesticide residue FV intake in first trimester				**Low pesticide residue FV intake in first trimester**		
Q1 (0.1, 1.4)	Reference	Reference	Reference	Q1 (0.3, 1.9)	Reference	Reference	Reference
Q2 (1.4, 2.1)	0.00 (-0.47, 0.47)	0.10 (-0.16, 0.35)	-0.02 (-0.23, 0.20)	Q2 (1.9, 2.8)	0.32 (-0.12, 0.76)	0.20 (-0.04, 0.44)	0.24 (0.04, 0.45)
Q3 (2.2, 3.1)	-0.12 (-0.61, 0.37)	-0.05 (-0.32, 0.21)	0.00 (-0.22, 0.23)	Q3 (2.8, 4.0)	0.40 (-0.07, 0.88)	0.30 (0.04, 0.55)	0.26 (0.05, 0.48)
Q4 (3.1, 13.2)	0.18 (-0.37, 0.73)	-0.09 (-0.39, 0.20)	-0.05 (-0.30, 0.20)	Q4 (4.0, 13.4)	0.22 (-0.32, 0.76)	0.30 (0.01, 0.60)	0.32 (0.08, 0.57)
P, trend [2]	0.22	0.44	0.71	P, trend [2]	0.53	0.06	0.02
Continuous[3]	0.13 (-0.05, 0.31)	0.04 (-0.04, 0.13)	0.03 (-0.05, 0.12)	Continuous[3]	0.03 (-0.12, 0.18)	0.03 (-0.05, 0.1)	0.07 (0, 0.14)
			Minority (N=502)			
High pesticide residue FV intake in first trimester				**Low pesticide residue FV intake in first trimester**		
Q1 (0.1, 1.4)	Reference	Reference	Reference	Q1 (0.3, 1.9)	Reference	Reference	Reference
Q2 (1.4, 2.1)	-0.36 (-1.34, 0.63)	-0.05 (-0.41, 0.32)	-0.02 (-0.34, 0.30)	Q2 (1.9, 2.8)	0.03 (-0.98, 1.04)	0.05 (-0.33, 0.43)	0.13 (-0.21, 0.47)
Q3 (2.2, 3.1)	0.76 (-0.30, 1.81)	0.41 (-0.01, 0.82)	0.17 (-0.18, 0.53)	Q3 (2.8, 4.0)	0.28 (-0.80, 1.35)	0.01 (-0.40, 0.41)	0.29 (-0.06, 0.64)
Q4 (3.1, 13.2)	0.53 (-0.67, 1.74)	0.33 (-0.14, 0.80)	-0.18 (-0.57, 0.21)	Q4 (4.0, 13.4)	0.46 (-0.68, 1.60)	-0.08 (-0.53, 0.37)	0.03 (-0.38, 0.44)
P, trend [2]	0.34	0.07	0.37	P, trend [2]	0.60	0.74	0.41
Continuous[3]	0.19 (-0.14, 0.51)	0.03 (-0.08, 0.14)	-0.03 (-0.13, 0.06)	Continuous[3]	-0.01 (-0.27, 0.24)	0.01 (-0.07, 0.1)	0.03 (-0.04, 0.11)

Abbreviations: FV, fruit and vegetable

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide FV intake was additionally adjusted for low pesticide FV intake, and vise versa.

[2] P, trend was performed using median intake in each quartile as continuous variable in the model.

[3] Change in gestational age at delivery for one serving increase in high or low pesticide fruit and vegetable intake

Table 3.4. Association of high and low pesticide fruit and vegetable intake during first trimester with SGA and LGA in 1275 white and 502 non-white mother-child pairs participating in Project Viva.

	N	SGA Adjusted[1] OR (95%CI)	N	LGA Adjusted[1] OR (95%CI)		N	SGA Adjusted[1] OR (95%CI)	N	LGA Adjusted[1] OR (95%CI)
White (n=1275)									
High pesticide residue FV intake in first trimester					**Low pesticide residue FV intake in first trimester**				
Q1 (0.1, 1.4)	13	1.0 (ref)	43	1.0 (ref)	Q1 (0.3, 1.9)	15	1.0 (ref)	41	1.0 (ref)
Q2 (1.4, 2.1)	12	1.44 (0.61, 3.36)	45	1.01 (0.63, 1.62)	Q2 (1.9, 2.8)	16	1.00 (0.47, 2.13)	46	0.92 (0.58, 1.45)
Q3 (2.2, 3.1)	10	1.15 (0.43, 3.07)	48	0.92 (0.56, 1.52)	Q3 (2.8, 4.0)	10	0.82 (0.33, 2.00)	53	1.11 (0.69, 1.79)
Q4 (3.1, 13.2)	16	2.81 (1.05, 7.53)	47	1.01 (0.58, 1.75)	Q4 (4.0, 13.4)	10	1.01 (0.37, 2.76)	43	1.00 (0.58, 1.73)
P, trend		0.04		1.00	P, trend		0.87		0.87
Continuous[2]		1.37 (1.04, 1.81)		0.94 (0.80, 1.11)	Continuous[2]		0.90 (0.70, 1.17)		1.01 (0.88, 1.16)
Minority (N=502)									
High pesticide residue FV intake in first trimester					**Low pesticide residue FV intake in first trimester**				
Q1 (0.1, 1.4)	12	1.0 (ref)	10	1.0 (ref)	Q1 (0.3, 1.9)	10	1.0 (ref)	10	1.0 (ref)
Q2 (1.4, 2.1)	7	0.77 (0.31, 1.94)	10	1.93 (0.73, 5.08)	Q2 (1.9, 2.8)	7	0.72 (0.26, 1.99)	9	1.00 (0.36, 2.78)
Q3 (2.2, 3.1)	10	1.03 (0.38, 2.77)	9	3.71 (1.23, 11.2)	Q3 (2.8, 4.0)	6	1.21 (0.45, 3.23)	8	1.12 (0.40, 3.15)
Q4 (3.1, 13.2)	7	0.37 (0.10, 1.34)	8	2.37 (0.70, 8.04)	Q4 (4.0, 13.4)	13	1.19 (0.39, 3.63)	10	0.66 (0.21, 2.09)
P, trend		0.18		0.17	P, trend		0.54		0.58
Continuous[2]		0.83 (0.59, 1.15)		1.13 (0.85, 1.50)	Continuous[2]		0.97 (0.76, 1.23)		0.89 (0.70, 1.13)

Abbreviations: FV, fruit and vegetable; LGA, large-for-gestational-age; N, number of cases; SGA, small-for-gestational-age; OR, odd ratios; ref, reference

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide residue FV intake was additionally adjusted for low pesticide residue FV intake, and vise versa.

[2] Risk of outcome for one serving increase in high/low pesticide fruit and vegetable intake

Table 3.5. Association of high and low pesticide fruit and vegetable intake during first trimester with preterm birth in 1275 white and 502 non-white mother-child pairs participating in Project Viva.

	Preterm birth			
	N	**Adjusted[1] OR (95%CI)**	**N**	**Adjusted[1] OR (95%CI)**
		White (n=1275)		
High pesticide residue FV intake in first trimester		**Low pesticide residue FV intake in first trimester**		
Q1 (0.1, 1.4)	11	1.0 (ref)	Q1 (0.3, 1.9)	19	1.0 (ref)
Q2 (1.4, 2.1)	13	1.26 (0.54, 2.94)	Q2 (1.9, 2.8)	13	0.55 (0.26, 1.15)
Q3 (2.2, 3.1)	24	2.06 (0.90, 4.73)	Q3 (2.8, 4.0)	19	0.68 (0.33, 1.39)
Q4 (3.1, 13.2)	21	1.71 (0.69, 4.26)	Q4 (4.0, 13.4)	18	0.68 (0.30, 1.51)
P, trend		0.27	P, trend		0.62
Continuous[2]		1.04 (0.82, 1.31)	Continuous[2]		0.94 (0.78, 1.15)
P for non-linearity		0.25	P for non-linearity		0.26
		Minority (N=502)		
High pesticide residue FV intake in first trimester		**Low pesticide residue FV intake in first trimester**		
Q1 (0.1, 1.4)	17	1.0 (ref)	Q1 (0.3, 1.9)	14	1.0 (ref)
Q2 (1.4, 2.1)	14	1.44 (0.68, 3.08)	Q2 (1.9, 2.8)	12	1.08 (0.48, 2.43)
Q3 (2.2, 3.1)	7	0.73 (0.27, 1.98)	Q3 (2.8, 4.0)	9	0.99 (0.39, 2.47)
Q4 (3.1, 13.2)	9	0.81 (0.27, 2.47)	Q4 (4.0, 13.4)	12	1.03 (0.36, 2.91)
P, trend		0.52	P, trend		0.99
Continuous[2]		0.82 (0.60, 1.13)	Continuous[2]		0.99 (0.78, 1.25)
P for non-linearity		0.92	P for non-linearity		0.57

Abbreviations: FV, fruit and vegetable; LGA, large-for-gestational-age; N, number of cases; SGA, small-for-gestational-age; OR, odd ratios.

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide fruit and vegetable intake was additionally adjusted for low pesticide fruit and vegetable intake, and vise versa.

[2] Risk of outcome for one serving increase in high/low pesticide fruit and vegetable intake

Table S3.1. Fruit and vegetable items in the food frequency questionnaire and pesticide data program, and corresponding scores for 1st, 2nd and 3rd measure, and PRBS.

Definition of measure contamination		**1st score**	**2nd score**	**3rd score**	**PRBS**
Items in FFQ	Items in PDP				
Orange juice, regular or calcium fortified	orange juice	0	0	0	0
Onions	onions	0	0	0	0
beans or lentils	beans	0	0	0	0
Avocado	avocado	0	0	0	0
cabbage or cole slaw	cabbage	0	0	0	0
corn (FFC)	corn, fz	0	0	0	0
dried plums or prunes	dried plum	0	0	0	0
peas or lima beans (FFC)	sweet pea, fz	0	0	0	0
tomato sauce	tomato paste	0	0	0	0
apple juice or cider	apple juice	0	0	1	1
Cauliflower	cauliflower	1	0	0	1
Tofu	soybeans	0	1	0	1
head lettuce, leaf lettuce	lettuce	0	1	1	2
Broccoli	broccoli	0	1	1	2
Grapefruit	grapefruit	1	0	1	2
Oranges	oranges	2	0	1	3
yam or sweet potatoes	sweet potatoes	1	2	0	3
Tomatoes	tomatoes	1	1	1	3
apple sauce	apple sauce	2	0	2	4
blueberry (FFC)	blueberry, Fs, Fz (0.5:0.5)[a]	2	0	2	4
Cantaloupe	cantaloupe	1	2	1	4
Carrots	carrots	2	1	1	4
grape or raisin	grape, raisin (0.6: 0.4)[a]	1	1	2	4
bananas	bananas	1	2	1	4
eggplant, summer squash, zucchini	eggplant, summer squash (0.5: 0.5)[a]	1	2	1	4
winter squash	winter squash	1	2	1	4
fresh apple or pear	apple, pear (0.7:0.3)[a]	2	1	2	5
Celery	celery	2	1	2	5
string beans	green beans	1	2	2	5
kale, mustard, chard greens	kale	1	2	2	5
Potatoes	potatoes	2	2	1	5
green/yellow/red peppers	sweet peppers	2	1	2	5
spinach, cooked	spinach, frozen	2	2	2	6
spinach, raw	spinach, fresh	2	2	2	6
peach or plum	peach, plum (0.7: 0.3)[a]	2	2	2	6
strawberries (FFC)	strawberries, fresh	2	2	2	6

Table S3.1.(Continued). Fruit and vegetable items in the food frequency questionnaire and pesticide data program, and corresponding scores for 1st, 2nd and 3rd measure, and PRBS.

Abbreviations: FFC, fresh, frozen, or canned; Fs, fresh; Fz, frozen; PDP, Pesticide Data Program; PRBS, pesticide residue burden score.

[a] Ratio weighted for pesticide residue for each produce according to the ratio of consumption of each produce from the USDA report

Table S3.2. Association of high and low pesticide fruit and vegetable intake during second trimester with fetal growth in 1152 white and 391 non-white mother-child pairs participating in Project Viva.

	Change in BW/GA Z scores					
	10th (95%CI)	**50th (95%CI)**	**90th (95%CI)**	**10th (95%CI)**	**50th (95%CI)**	**90th (95%CI)**
White (n=1275)						
High pesticide residue FV intake in 1st trimester			**Low pesticide residue FV intake in 1st trimester**			
Q1 (0.1, 1.4)	Reference	Reference	Reference	Q1 (0.3, 1.9)	Reference	Reference	Reference
Q2 (1.4, 2.1)	-0.08 (-0.27, 0.12)	0.010 (-0.18, 0.20)	-0.04 (-0.29, 0.20)	Q2 (1.9, 2.8)	-0.11 (-0.29, 0.07)	0.23 (0.05, 0.41)	0.43 (0.21, 0.65)
Q3 (2.2, 3.1)	-0.09 (-0.29, 0.11)	-0.12 (-0.31, 0.08)	-0.16 (-0.40, 0.08)	Q3 (2.8, 4.0)	-0.06 (-0.25, 0.13)	0.22 (0.03, 0.40)	0.21 (-0.02, 0.45)
Q4 (3.1, 13.2)	-0.04 (-0.26, 0.17)	0.09 (-0.13, 0.30)	-0.04 (-0.31, 0.22)	Q4 (4.0, 13.4)	0.06 (-0.16, 0.27)	0.07 (-0.14, 0.28)	0.21 (-0.06, 0.48)
P, trend [2]	0.35	0.10	0.30	P, trend [2]	0.71	0.90	0.95
Continuous[3]	-0.03 (-0.1, 0.05)	0.02 (-0.04, 0.09)	-0.05 (-0.13, 0.03)	Continuous[3]	0.02 (-0.04, 0.08)	0.01 (-0.04, 0.06)	0.01 (-0.06, 0.08)
Minority (N=502)						
High pesticide residue FV intake in 1st trimester			**Low pesticide residue FV intake in 1st trimester**			
Q1 (0.1, 1.4)	Reference	Reference	Reference	Q1 (0.3, 1.9)	Reference	Reference	Reference
Q2 (1.4, 2.1)	-0.23 (-0.53, 0.06)	0.08 (-0.21, 0.36)	0.19 (-0.10, 0.47)	Q2 (1.9, 2.8)	-0.36 (-0.67, -0.05)	0.01 (-0.31, 0.32)	0.19 (-0.12, 0.50)
Q3 (2.2, 3.1)	0.12 (-0.21, 0.46)	0.06 (-0.28, 0.39)	-0.03 (-0.35, 0.30)	Q3 (2.8, 4.0)	-0.59 (-0.91, -0.28)	-0.17 (-0.48, 0.14)	0.23 (-0.08, 0.54)
Q4 (3.1, 13.2)	-0.16 (-0.54, 0.22)	0.26 (-0.12, 0.64)	0.44 (0.06, 0.83)	Q4 (4.0, 13.4)	-0.56 (-0.93, -0.19)	-0.33 (-0.69, 0.04)	-0.01 (-0.37, 0.36)
P, trend [2]	0.50	0.14	0.59	P, trend [2]	0.07	0.07	0.36
Continuous[3]	0.03 (-0.08, 0.13)	0.07 (-0.03, 0.16)	0.04 (-0.1, 0.19)	Continuous[3]	0.00 (-0.09, 0.10)	-0.01 (-0.08, 0.07)	0.00 (-0.13, 0.13)

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide fruit and vegetable intake was additionally adjusted for low pesticide fruit and vegetable intake, and vise versa.

[2] P, trend was performed using median intake in each quartile as continuous variable in the model.

[3] Risk of outcome for one serving increase in high/low pesticide fruit and vegetable intake.

Table S3.3. Association of high and low pesticide fruit and vegetable intake during second trimester with SGA and LGA in 1152 white and 391 non-white mother-child pairs participating in Project Viva.

	N	SGA Adjusted[1] OR (95%CI)	N	LGA Adjusted[1] OR (95%CI)		N	SGA Adjusted[1] OR (95%CI)	N	LGA Adjusted[1] OR (95%CI)
White (N=1152)									
High pesticide residue FV intake in 2nd trimester					**Low pesticide residue FV intake in 2nd trimester**				
Q1 (0.1, 1.3)	6	1.0 (ref)	35	1.0 (ref)	Q1 (0.1, 2.0)	11	1.0 (ref)	26	1.0 (ref)
Q2 (1.4, 2.1)	13	1.57 (0.55, 4.49)	45	0.86 (0.51, 1.44)	Q2 (2.0, 2.9)	12	0.94 (0.39, 2.30)	55	2.21 (1.29, 3.79)
Q3 (2.1, 3.1)	14	2.10 (0.70, 6.31)	46	0.75 (0.43, 1.31)	Q3 (2.9, 4.1)	14	0.93 (0.36, 2.40)	47	2.17 (1.21, 3.89)
Q4 (3.1, 13.5)	11	1.68 (0.50, 5.62)	41	0.85 (0.44, 1.64)	Q4 (4.1, 15.5)	7	0.57 (0.18, 1.82)	39	1.67 (0.84, 3.32)
P, trend		0.47		0.39	P, trend		0.35		0.79
Continuous[2]		1.29 (0.96, 1.74)		0.93 (0.79, 1.1)	Continuous[2]		0.83 (0.63, 1.09)		1.01 (0.88, 1.16)
Minority (N=391)									
High pesticide residue FV intake in 2nd trimester[4]					**Low pesticide residue FV intake in 2nd trimester[4]**				
Q1 (0.1, 1.3)	11	1	11	1	Q1 (0.1, 2.0)	7	1	8	1
Q2 (1.4, 2.1)	10	1.09 (0.41, 2.94)	6	0.86 (0.29, 2.59)	Q2 (2.0, 2.9)	3	1.04 (0.28, 3.84)	5	1.11 (0.33, 3.76)
Q3 (2.1, 3.1)	3	0.60 (0.16, 2.27)	3	0.51 (0.11, 2.30)	Q3 (2.9, 4.1)	13	3.55 (1.24, 10.2)	6	1.35 (0.40, 4.55)
Q4 (3.1, 13.5)	8	0.85 (0.24, 2.97)	7	0.62 (0.14, 2.74)	Q4 (4.1, 15.5)	9	1.68 (0.43, 6.49)	8	0.65 (0.15, 2.77)
P, trend		0.62		0.41	P, trend		0.29		0.58
Continuous[2]		0.92 (0.65, 1.29)		0.89 (0.61, 1.29)	Continuous[2]		1.01 (0.79, 1.29)		0.88 (0.63, 1.21)

Abbreviations: FV, fruit and vegetable; LGA, large-for-gestational-age; N, number of cases; SGA, small-for-gestational-age; OR, odd ratios.

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide fruit and vegetable intake was additionally adjusted for low pesticide fruit and vegetable intake, and vise versa.

[2] Risk of outcome for one serving increase in high/low pesticide fruit and vegetable intake.

Table S3.4. Association of high and low pesticide fruit and vegetable intake during second trimester with preterm birth in 1152 white and 391 non-white mother-child pairs participating in Project Viva.

Quartile (min, max)	N	Preterm birth Adjusted[1] OR (95%CI)		N	Preterm birth Adjusted[1] OR (95%CI)
			White (N=1152)		
High pesticide residue FV intake in 2nd trimester			**Low pesticide residue FV intake in 2nd trimester**		
Q1 (0.1, 1.3)	14	1	Q1 (0.1, 2.0)	19	1
Q2 (1.4, 2.1)	14	0.82 (0.36, 1.87)	Q2 (2.0, 2.9)	9	0.37 (0.17, 0.82)
Q3 (2.1, 3.1)	17	1.09 (0.48, 2.48)	Q3 (2.9, 4.1)	13	0.43 (0.19, 0.96)
Q4 (3.1, 13.5)	14	0.76 (0.30, 1.90)	Q4 (4.1, 15.5)	18	0.64 (0.27, 1.51)
P, trend		0.49	P, trend		0.70
Continuous[2]		0.88 (0.68, 1.14)	Continuous[2]		0.97 (0.78, 1.20)
			Minority (N=391)		
High pesticide residue FV intake in 2nd trimester[4]			**Low pesticide residue FV intake in 2nd trimester[4]**		
Q1 (0.1, 1.3)	13	1	Q1 (0.1, 2.0)	10	1
Q2 (1.4, 2.1)	6	0.64 (0.24, 1.75)	Q2 (2.0, 2.9)	5	1.39 (0.48, 4.05)
Q3 (2.1, 3.1)	4	0.48 (0.14, 1.59)	Q3 (2.9, 4.1)	4	1.05 (0.33, 3.31)
Q4 (3.1, 13.5)	5	0.37 (0.09, 1.52)	Q4 (4.1, 15.5)	9	2.34 (0.66, 8.32)
P, trend		0.16	P, trend		0.25
Continuous[2]		0.86 (0.58, 1.27)	Continuous[2]		1.05 (0.79, 1.39)

Abbreviations: FV, fruit and vegetable; LGA, large-for-gestational-age; N, number of cases; SGA, small-for-gestational-age; OR, odd ratios.

[1] The models were adjusted for maternal age, pre-pregnancy BMI, smoked during pregnancy, marital status, parity, household income, education, dietary patterns, and total energy intake. High pesticide fruit and vegetable intake was additionally adjusted for low pesticide fruit and vegetable intake, and vise versa.

[2] Risk of outcome for one serving increase in high/low pesticide fruit and vegetable intake.

Concluding Remarks

Approximately 1.1 billion pounds of pesticides are used by agriculture, industry, commercial, government, home and garden in the U.S. annually, and the agricultural market sector accounted for 80% of the use of these chemicals(Grube et al. 2011). The widespread use of pesticides makes exposure to these chemicals inevitable for most people, with nearly 100% of US pregnant women has detectable concentrations of pesticides and in their urine or blood samples. (Castorina et al. 2010, Woodruff, Zota, and Schwartz 2011) While a number of randomized controlled trials have shown that substituting conventionally grown produce with organic produce substantially reduces specific urinary concentrations of pesticide metabolites (Lu et al. 2006, Bradman et al. 2015, Oates et al. 2014), a large knowledge gap remains in our understanding of the clinical relevance of exposures to dietary pesticide residues. The work we present here provides researchers with an inexpensive tool to assess dietary pesticide residues and health outcomes across different cohorts. In addition, these papers provide preliminary evidence on potential adverse effects of intake of pesticide residues from fruits and vegetables among susceptible populations.

In Chapter 1, we found that intake of high pesticide residue fruits and vegetables (derived by the Pesticide Residue Burden Score (PRBS)) was positively associated with higher urinary concentrations of pesticide biomarkers, including organophosphate insecticides, pyrethroid insecticides, and the herbicide 2,4-D (EARTH study). The findings complement the previous research findings in our group, showing that high pesticide fruit and vegetable intake derived by PRBS was associated with higher serum organochlorine and higher urinary organophosphate pesticides in National Health and Nutrition Examination Survey (NHANES)(Hu et al. 2016). The results of Chapter1 suggest that PRBS scoring system is a useful tool for dietary pesticide assessment, potentially allowing researchers to explore the potential health effects of long-term exposure to pesticides through diet quickly and economically.

In Chapter 2, we found intake of high pesticide residue fruits and vegetables among women in the year prior to infertility treatment was associated with lower probability of live birth, primarily owing to higher probability of pregnancy loss, while intake of low pesticide residue fruits and vegetables had opposite relations. This is the first human study to show a link between dietary pesticide residue intake and pregnancy outcomes, which is consistent with an experimental study showing that ingestion of pesticide mixture at a reference dose concentration decreased the number of live pups born in mice.(Cavieres, Jaeger, and Porter 2002) Our results provide preliminary evidence of safety concerns on pesticide residues in food, and raise the possibility for improving pesticide regulations in fruits and vegetables. Future studies are needed to replicate and substantiate these findings by objectively measured biomarkers.

In Chapter 3,we found that greater intake of high pesticide residue fruits and vegetables was associated with reduced fetal growth among infants born to white mothers, whereas greater intake of high pesticide fruits and vegetables was associated with higher birth-weight-for-gestational-age among non-white women. Paraoxonase 1 (PON1) is an enzyme involved in detoxification of certain pesticides and also metabolizing oxidized high-density and low-density lipoproteins. Genetic polymorphisms of PON1 affecting its function differ across racial groups, which could possibly explain the divergent findings of high pesticide fruit and vegetable intake and fetal growth between white and non-white groups. Further studies, with information on these polymorphisms, for which race/ethnicity is a proxy, are clearly needed to evaluate this hypothesized gene-diet interaction.

As we found that intake of high pesticide fruits and vegetables was associated with higher total pregnancy loss in EARTH study (Chapter 2), we have to be cautious of potential survival bias when evaluating associations of high pesticide fruit and vegetable intake with birth outcomes (Chapter 3) due to the presence of unmeasured common causes of pregnancy loss and birth outcomes. Nonetheless, we found high pesticide fruit and vegetable intake was not associated with pregnancy loss in Project Viva,

obviating the need to use survivor average causal effect to account for potential survival bias.(Tchetgen Tchetgen, Phiri, and Shapiro 2015) While this finding might seem to contradict what we have observed in Chapter 2, it is worth highlighting that most participants in Project Viva were recruited at their initial obstetric care visit at median gestational age of 9.9 weeks, which is beyond the most common window of pregnancy loss (≤ 9 weeks)(Mumford et al. 2016). As Project Viva is not a cohort that was designed to evaluate the associations of prenatal exposures with pregnancy loss, potential survival bias may still exist but we don't have necessary data to account for those could have been enrolled had they not had early pregnancy loss. Future prospective cohort studies that enroll couples preconceptionally are needed to further confirm the relationship between high pesticide fruit and vegetable intake and risks of pregnancy loss.

Taken together, this work was one of the first studies to demonstrate the potential adverse health effect of high pesticide residue fruit and vegetable intake. Given that diet represents a major route of exposure to pesticides and pesticide residues in the general population, the results suggest the needs for further investigation of the impact of dietary pesticide residues on pregnancy, birth and other health outcomes.

As Rachel Carson once put in the *Silent Spring*, "*If we are going to live so intimately with these chemicals eating and drinking them, taking them into the very marrow of our bones - we had better know something about their nature and their power.*" We hope this dissertation will gather more public's attention and our methods can stimulate further research and more solid evidence into these topics. Ultimately, we hope these may lead to changes in pesticide use in agriculture and improve population health outcomes.

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