

Study Protocol
Measurement of Agricultural and Dietary Glyphosate
Exposure among Pregnant Women
NCT04155463
June 30, 2022

TABLE OF CONTENTS

1. INTRODUCTION.....	PAGE 4
1.1 Organization of this Document	
1.2 General Information for Lab Members	
1.3 Terminology	
1.4 New Lab Member Training Requirements	
1.5 Scope	
2. RECRUITMENT.....	PAGE 8
2.1 Summary of Methods	
2.2 Materials*	
2.3 Procedures	
3. ENROLLMENT & INITIALCONTACTLESS MEETING.....	PAGE 11
3.1 Summary of Methods	
3.2 Materials*	
3.3 Procedures	
4. WEEKLY URINE SAMPLE COLLECTION.....	PAGE 15
4.1 Summary of Methods	
4.2 Materials*	
4.3 Procedures	

5. WEEKLY URINE SAMPLE ANALYSIS& STORAGEPAGE 18

- 5.1 Summary of Methods
- 5.2 Materials*
- 5.3 Procedures
- 5.4 Weekly Urine Sample Labeling System

6. DIETARY INTERVENTION.....PAGE 21

- 6.1 Summary of Methods
- 6.2 Schedule
- 6.3 Materials*
- 6.4 Procedures

7. DAILY URINE SAMPLE COLLECTION.....PAGE 27

- 7.1 Summary of Methods
- 7.2 Materials*
- 7.3 Procedures

8. DAILY URINE SAMPLE ANALYSIS& STORAGE...PAGE 30

- 8.1 Summary of Methods
- 8.2 Materials*
- 8.3 Procedures
- 8.4 Daily Urine Sample Labeling System

9. URINE SAMPLE SHIPMENT & GLYPHOSATE ANALYSIS.... PAGE 35

9.1 Summary of Methods

9.2 Materials

9.3 Procedures

*Materials Available Upon Request

1. INTRODUCTION

1.1 Organization of this Document

The standard operating procedures and associated materials for this study are included in this compilation, outlining all of the fieldwork and laboratory activities to be carried out by the Curl Agricultural Health Lab as part of the study *Measurement of Agricultural and Dietary Glyphosate Exposure among Pregnant Women*. The documents are arranged in nine categories, each with the standard operating procedures for the following aspects of the study: 1) Introduction; 2) Recruitment; 3) Enrollment & Initial Contactless Meeting; 4) Weekly Urine Sample Collection; 5) Weekly Urine Sample Analysis & Storage; 6) Dietary Intervention; 7) Daily Urine Sample Collection; 8) Daily Urine Sample Analysis & Storage; and 9) Urine Sample Shipment & Glyphosate Analysis.

1.2 General Information for Lab Members

The procedures presented in these standard operating procedures are intended to ensure the collection of high-quality data, and lab members should follow them at all times.

To ensure no COVID-19-specific risks, no more than two lab members will be in the lab at the same time. Such interaction will comply with all Boise State University policies, will only occur when required and will follow all standard COVID-19 safety guidelines, including face masks, social distancing, and frequent hand washing. Masks will be worn in the lab and in the Environmental Research Building (ERB) at all times, and social distancing protocols will be followed. Before returning to the Boise State University campus after scheduled breaks or campus closures, all lab members will follow university guidelines for COVID-19 testing and isolation, if required. All lab members will participate in a weekly or bi-weekly virtual meeting to remain up-to-date and aligned on tasks. There will be no face-to-face interactions between lab members and potential or enrolled study participants.

All communication with participants and any potential participants will be done via their preferred method of communication (call or text) and in their preferred language. In most instances in this protocol it is assumed that text is the preferred method of communication for participants once enrolled in this

study. In addition to collecting and testing urine samples, as well as managing food ordering and delivery activities, lab members will also be responsible for keeping clear and thorough records of these activities. Accurate, detailed documentation is a vital part of the data collection process. The following guidelines will help lab members in completing the forms included in this document and logging activities:

- Be compulsive in record keeping. It is better to record too much information than not enough. After the data are collected, these records will be the only way of knowing what occurred during sample collection and in the lab.
- Always use pen (no pencils).
- If a record on a form needs to be changed, draw a single line through the entry that needs to be changed, write in the correct information, and then write the date of the change and the initials of the lab member making the change.
- If a mistake is made or there are deviations from protocol, indicate this on the associated form. This is very important in helping the data analysts identify factors that may affect data quality.
- Always use 24 hour (i.e. military) time.

1.3 Terminology

The following abbreviations are used throughout this document:

IRB IRB stands for Institutional Review Board. This is a committee that protects the rights and welfare of human subjects recruited to participate in research conducted through Boise State University.

ERB ERB stands for Environmental Research Building. This building is located on the Boise State University main campus, and houses the Curl Agricultural Health Lab.

CAHL	CAHL stands for Curl Agricultural Health Lab. The CAHL is located in the ERB, room 3106, at Boise State University.
WIC Clinic	WIC stands for Women, Infant, and Children. Recruitment for this study will be completed in cooperation with WIC clinics in the Southwest, Central Southwest, Central, and South Central Health Districts of Idaho. This includes clinics in Caldwell and Nampa (Southwest); Boise, Meridian, and Garden City (Central); and Twin Falls, Shoshone, Gooding, Jerome, and Heyburn (South Central).
SG	SG stands for specific gravity. CAHL lab members will be testing the specific gravity of the participants' urine samples.
DI	DI stands for deionized water. The refractometer used for measuring specific gravity will be cleaned using DI water.
CDC	CDC stands for the Centers for Disease Control and Prevention. This entity will be responsible for analyzing all urine samples collected in this study. At the conclusion of the urine sampling component of this study, all samples will be shipped to a laboratory at the CDC for glyphosate analysis.
K01	K01 refers to the Mentored Research Scientist Development Award, the funding for this project.

1.4 New Lab Member Training Requirements

IRB Training Requirements

In order to interact with human subjects, new lab members must complete the CITI training, the IRB Additional Personnel Form, and the IRB Modification Form.

Lab Training Requirements

Lab Training

All new lab members will receive general lab training prior to involvement in the study, although the manner in which this training occurs will depend on the current rules regarding COVID-19 safety.

CAHL Shared Research Drive and K01 Database

All electronic documentation will be stored in a protected Microsoft Access document entitled “K01 Database” located on a shared research drive operated through Boise State University. In order to access the shared research drive, all new lab members will email Ms. Spivak and ask her to request security access.

CAHL Calendar and Google Sheets

All scheduling will be electronically documented in the password protected CAHL Calendar, and all cryovial storage locations will be electronically documented in the password protected CAHL Google Sheets.

1.5 Scope

Glyphosate is the most commonly used herbicide in the world. This increase has been driven, in large part, by the introduction of glyphosate-resistant crops such as “Roundup Ready” corn and soybeans. In Idaho, nearly a quarter million acres of Roundup Ready sugarbeets are cultivated each year.

Human exposure to glyphosate and its formulations may occur through dietary, agricultural and residential pathways. Despite its extensive use, frequent presence in foodstuffs and environmental media, and potential toxicity, current exposure levels in human populations are not well documented. However, several studies have suggested that in utero exposure to glyphosate and glyphosate formulations may have reproductive effects and adversely impact developing fetuses. This study therefore aims to quantify pathways of glyphosate exposure among pregnant women.

2. RECRUITMENT

2.1 Summary of Methods

This study will involve recruiting 40 pregnant women in their first trimester to measure residential and dietary glyphosate exposure throughout their pregnancies. This will be done in cooperation with local WIC clinics.

2.2 Materials

WIC Clinic Collaboration

- Introduction to Study and Recruitment Video for WIC Staff (<https://youtu.be/pFH3MIELXNU>)
- Video for Potential Participants (English: <https://youtu.be/sOG0lppKzUw> or Spanish: <https://youtu.be/p75mIj0hwrc>)
- Recruitment Flyer*
- Index Card Flyer*
- Slip for Potential Participant Contact Information*

Contacting Potential Participants

- Completed Slip for Potential Participant Contact Information
- Recruitment Scripts*
- Eligibility Questionnaire*
- Cell phone with One Talk App
- CAHL Calendar
- K01 Database

2.3 Procedures

WIC Clinic Collaboration

WIC Clinic Collaboration

The staff at the WIC clinics will watch the Introduction to Study and Recruitment Video for WIC Staff to provide them with general information about the study and the virtual study recruitment process. During appointments with clients, the staff will show the Video for Potential Participants to women who are thought to meet eligibility requirements based on available information, or this video may be emailed to these women from the WIC staff to watch at their convenience. This video will be provided to potential participants in their preferred language, either English or Spanish. This video will instruct them to inform their WIC staff if they are interested in participating. The WIC staff will then complete a Slip for Potential Participant Contact Information with the potential participant's name and contact information in the participant's preferred language, English or Spanish. The WIC clinic staff will fax the completed Slip for Potential Participant Contact Information to Dr. Curl or will call Dr. Curl to relay potential participant information. The Recruitment Flyer and Index Card Flyer can be used by the WIC staff to aid in recruitment either by emailing these documents to potential participants or printing these documents for use in the clinics should face-to-face appointments at the WIC clinics take place. This process will continue until recruitment is complete.

Documentation

Dr. Curl will record all information contained in each Slip for Potential Participant Contact Information in the K01 Database. Dr. Curl will also perform an initial eligibility screening of each potential participant's address and due date. If a potential participant is eligible after the initial screening, a lab member will attempt to contact her.

Contacting Potential Participants

Contacting Potential Participants

A lab member will contact the potential participant during her preferred contact time and in her preferred language via her preferred contact method

no more than two days after receiving the Slip for Potential Participant Contact Information. If the potential participant is contacted via text message (using the Text Message Recruitment Script), a phone call will be arranged for that time (if available) or for a later date to cover the Phone Recruitment Script and Eligibility Questionnaire. If contacted via phone call but the potential participant does not answer, a voicemail will be left in accordance with the Voicemail Recruitment Script if the potential participant has a voicemail option set-up. If contacted via phone call and the potential participant does answer, the lab member will cover the Phone Recruitment Script and Eligibility Questionnaire. However, another call can be arranged if the potential participant is not available to talk at that time.

Determining Eligibility and Scheduling

Once the potential participant is contacted via a phone call, the lab member will recite the Phone Recruitment Script. If the potential participant is interested in the study at this point, the lab member will administer the Eligibility Questionnaire, following the instructions on the form and asking all the necessary questions. This questionnaire will be completed on paper for proper study documentation purposes. If the participant is eligible, the lab member will then arrange a date and time to have an initial meeting.

Documentation

Scheduled initial contact meetings will be documented in the CAHL Calendar. All correspondence with any potential participant (regardless of interest in study or eligibility) will be recorded in the Potential Participant Contact Form in the K01 Database. This also includes any attempts to reach a potential participant by text or phone call, and if a voicemail is left. If the participant is uninterested or ineligible, the reason will also be recorded in that form and an “XX” will be placed in “Participant ID” field. If the participant is interested and eligible, all paper documents containing contact information, eligibility questions, and/or notes will be placed in the “Pending” file in the locked filing cabinet located in CAHL. If the participant is uninterested or ineligible, all paper documents containing contact information, eligibility questions, and/or notes will be placed in the “Uninterested/Ineligible” file located in the same locked filing cabinet.

3. ENROLLMENT & INITIAL CONTACTLESS MEETING

3.1 Summary of Methods

After a woman has been determined eligible for the study and has indicated a willingness to participate, a lab member will schedule and attend an initial contactless meeting that will include the Informed Consent process, Demographics Questionnaire, Pesticide Exposure Questionnaire, collection of the first weekly urine sample, and an explanation of the process for the remaining weekly urine sample collections. A Urine Chain of Custody Form has been developed to track the movement of all urine samples from collection to final storage in the lab. Lab members will initial each step of sample transport on the log, and note any observations, deviations from sample handling protocols, and/or problems with each sample. Once back at CAHL the lab member will analyze and store the first weekly urine sample.

3.2 Materials

- Two Informed Consent Forms*
- Video for Informed Consent (English: <https://youtu.be/cI-wEKWKVso> or Spanish: <https://www.youtube.com/watch?v=sL3WqtcT7Zk>)
- Demographics Questionnaire*
- Pesticide Exposure Questionnaire*
- Video for Urine Collection Instructions (English: <https://youtu.be/Gf-xqsx3mLI> or Spanish: https://www.youtube.com/watch?v=WQ9MjRADA_o)
- Cell phone with One Talk App
- Supplies for first and second weekly urine sample collections:
 - a) Specimen cups with barcoded label for the first and second week's collections (week numbers 1 and 2, respectively) in separate sealable plastic bags

- b) Extra specimen cup
- c) Two pairs of nitrile gloves
- d) Two coolers
- e) Two ice packs
- f) Two Sharpies and a pen
- g) Urine Chain of Custody Form*
- h) \$10 Gift Card
- i) Urine Collection Instruction Form*
- j) Urine Collection Reminder Poster*

- CAHL Calendar
- K01 Database

3.3 Procedures

Supplies Preparation

A lab member will compile and set out the materials listed above. Hard copies of all necessary papers will be placed in a folder labeled with the potential participant's ID number. The lab member will take all supplies and this folder to the initial meeting.

Scheduling

All scheduling, including any scheduling changes, will be recorded in the CAHL Calendar and in the K01 Database. The lab member will call or text (based on the potential participant's preference) the potential participant one day prior to the scheduled meeting as a reminder.

Initial Contactless Meeting

A lab member will conduct the initial contactless meeting with the potential participant in her preferred language. For any exchange of materials, the participant should be inside her home with the door shut while the lab member drops off materials at her door while wearing a mask. Once the lab member is back inside the car, the participant may come out and pick up the materials. Only one lab member should be present for this meeting so that two people are not traveling in the car together. This lab member will be fluent in the potential participant's preferred language. At this meeting, the lab member will arrive and drop off the two copies of the Informed Consent Forms (in the preferred language) at the potential participant's door that have already been signed and dated by the lab member. The lab member will then return to the car and call the potential participant to introduce themselves, instruct her to pick up the Informed Consent Forms, and tell her that these forms will be explained via video. The lab member will then text the link for the Video for Informed Consent to the potential participant in her preferred language, either English or Spanish. After the potential participant watches the video, the lab member will review the document and answer questions (if necessary) over the phone. If the potential participant agrees to participate in the study, the lab member will then instruct the participant to sign and date both copies of the Informed Consent Form and keep one copy for herself and place one copy back outside for the lab member to collect and keep. The lab member will put this copy in the car to bring back to the lab. At this time, this woman is considered an official study participant, and will be assigned a participant ID number.

The lab member will then conduct the Demographics Questionnaire and Pesticide Exposure Questionnaire in the participant's preferred language over the phone, following the instructions provided at the top of these documents. After completion of both questionnaires, the lab member will inform the participant that she can now provide the first weekly urine sample and explain that the urine sample collection process will be explained via video. The lab member will then text the link for the Video for Urine Collection Instructions to the participant in her preferred language, English or Spanish. While the participant is watching this video, the lab member will drop off at the participant's door the following supplies: 1) specimen cup with barcoded label for the first week's collection (week number 1) in a sealed plastic bag; 2) a pair of nitrile gloves; and 3) a Sharpie. The video will instruct the participant

to call the lab member to discuss any questions or concerns, including if there is an accident and an extra specimen cup is needed.

After the participant provides the first urine sample, she will place the specimen cup outside her door in the sealed plastic bag. The lab member will then collect the sample, ensure the collection date and time have been provided and that the cup is labeled with the Participant ID number, and place it in the lab cooler with the lab ice pack to bring back to the lab. The lab member will also complete the appropriate sections of the Urine Chain of Custody Form. The lab member will then leave a \$10 gift card and the following supplies for the second week's collection (week number 2) at the participant's door: 1) specimen cup with barcoded label for the second week's collection in a sealed plastic bag; 2) a cooler for the participant to keep; 3) an ice pack for the participant to keep; 4) extra specimen cup to be used in case of an accident and only when barcoded cup is not available; and 5) the Urine Collection Instruction Form and Urine Collection Reminder Poster (both in the preferred language). Once back in the car, the lab member will call the participant and instruct her to pick up the supplies and will review the process for subsequent weekly urine sample collections as described in Section 4 of this document. The participant can use the Urine Collection Instruction Form as a reminder on how to complete the collection process, and the Urine Collection Reminder Poster as a reminder to collect samples weekly. The lab member will schedule subsequent weekly urine sample collections over the phone.

Documentation upon Returning to the Lab

After the initial contactless meeting, the lab member will return to the CAHL. There, the lab member will place the first weekly urine sample in the refrigerator and complete the appropriate sections of the Urine Chain of Custody Form. This sample will remain in the refrigerator for no more than 24 hours until analyzed. The cooler will be returned to the storage shelf and the ice pack will be placed back in the freezer.

The lab member will then document participant information in the K01 database and paper copies of materials will be placed in the locked filing cabinet. At this time the lab member should analyze and store the weekly urine sample as described in Section 5 of this document.

4. WEEKLY URINE SAMPLE COLLECTION

4.1 Summary of Methods

As with the initial urine sample, all subsequent weekly urine samples will be collected, analyzed, and stored by lab members in a completely contactless manner. Weekly urine samples will be numbered sequentially. Each week, each study participant will provide a first morning void sample on a pre-scheduled day, and will place the sample in her cooler with her ice pack outside her home. A lab member will then collect that week's sample, provide the participant with a specimen cup for the following week, and bring that week's sample back to CAHL in a lab cooler with a lab ice pack. Once back at CAHL, the lab member will analyze and store the weekly urine sample.

4.2 Materials

- Specimen cup with barcoded label
- Extra specimen cup
- Nitrile gloves
- Cooler
- Ice pack
- Urine Chain of Custody Form for the correct participant ID
- \$10 Gift Card
- One Sharpie and pen
- Cell phone with One Talk App
- CAHL Calendar
- K01 Database

4.3 Procedures

Supplies Preparation

A lab member will compile and set out the materials listed above. The Urine Chain of Custody Form will be taken from the folder in the locked filing cabinet labeled with the participant's ID number. The lab member will take all supplies to the urine collection.

Scheduling

For weekly urine sample collections, participants will be informed at the initial contactless meeting of a pre-established date and time for weekly sample pick ups, which will be decided *a priori* by lab staff based on CAHL staff availability to travel to certain locations on certain days and times. Scheduling changes will be considered should the pre-set day and time for weekly sample collection not work for a study participant. All scheduling will be recorded in the CAHL Calendar. The lab member will call or text the participant one day prior to the scheduled pick up as a reminder.

Weekly Urine Sample Collections

Each week on the pre-scheduled day, participants will be instructed to provide a first morning void in the specimen cup with the barcoded label for that week, which was provided to them at the prior week's collection. The participants will be instructed to use their previously provided Sharpie to record the date and time of collection directly on the specimen cup. The sample should be provided the morning of the collection day (first morning void), and then placed in the sealable plastic bag and into the previously provided cooler with the ice pack. This cooler will then be placed outside the participant's home. A lab member will then travel to each participant's home to collect the urine sample. If the lab member arrives at a participant's home and finds that the weekly urine sample has not been provided, the lab member will make reasonable attempts to contact the participant and collect the urine sample.

At collection, the lab member will ensure that the cup is labeled with the correct Participant ID number and correct week number. The cup will then be placed in the lab cooler with the lab ice pack to be taken back to CAHL. The lab member will leave in the participant's cooler: 1) specimen cup with barcoded label for the following week's collection in sealable plastic bag; 2)

extra specimen cup to be used in case of an accident and only when barcoded cup is not available; and 3) a \$10 gift card for the sample just provided. At collection time, the lab member will complete the appropriate sections of the Urine Chain of Custody Form. The lab member will ensure that the participant filled in the date and time of collection on the cup label, and if this did not occur this will be noted in the Urine Chain of Custody Form, along with any other problems or deviations from protocol.

After each sample collection, a text message will be sent to each participant in her preferred language.

If a participant does not provide a urine sample for a given week, the lab member will document the missed week on the Urine Chain of Custody Form. This will be considered a missed week and that week number will not be reused the following week, and instead the next sequential week number will be used.

Documentation upon Returning to the Lab

After collecting the sample, the lab member will return back to CAHL. There, the lab member will place the weekly urine sample in the refrigerator and complete the appropriate sections of the Urine Chain of Custody Form. This sample will remain in the refrigerator for no more than 24 hours until analyzed for color, clarity and specific gravity, and stored at -80°C. The lab member will then document all information in the K01 Database. The lab member will file the Urine Chain of Custody Form in the folder labeled with that participant's ID number in the locked filing cabinet. At this time, the lab member should analyze and store the weekly urine sample as described in Section 5 of this document.

Final Weekly Urine Sample Collection

When each participant is approximately 4 weeks away from her due date, a text message will be sent to the participant in her preferred language to remind her to let us know once she gives birth. Once the participant informs us that her baby has been born, no more weekly urine samples will be provided or collected. Each participant will receive a \$50 gift card for completion of the study.

Gift Cards

All gift card purchases and documentation will follow Boise State University rules and regulations.

5. WEEKLY URINE SAMPLE ANALYSIS & STORAGE

5.1 Summary of Methods

Weekly urine samples will be collected from participants and returned to CAHL in a lab cooler with a lab ice pack. Samples will be stored for no more than 24 hours under refrigeration at CAHL until analyzed and stored. During analysis, lab members will determine urine color, clarity, and specific gravity. Urine sample storage will occur immediately after urine sample analysis. Each sample will be aliquoted into four 4mL weekly cryovials for storage. These will be stored in a weekly cryobox in the CAHL -80°C freezer. At the conclusion of an individual's participation in the study (ideally when they give birth), one of the four 4 mL weekly cryovials from each week will be sent to the CDC for glyphosate analysis. The remaining three cryovials are duplicate samples that will be stored in the biorepository.

5.2 Materials

Weekly Urine Sample Analysis

- Absorbent pads
- Kimwipes
- Cup containing ethyl alcohol
- Squirt bottle containing DI water
- Nitrile gloves

- Pen
- Urine Sample Characteristics Log*
- Color and Clarity Guide*
- Transfer pipettes
- Atago Urine Specific Gravity Refractometer, PAL 10-S
- K01 Database

Weekly Urine Sample Storage

- Absorbent pads
- Nitrile gloves
- Eppendorf Plus Research Pipette and tip
- Four weekly cryovials with barcoded labels
- Weekly Cryobox
- Empty Cryobox at the lab station
- Google Drive Cryobox Document

5.3 Procedures

Weekly Urine Sample Analysis

Supplies Preparation

Prior to starting urine sample analysis, the lab member will set up a lab station by placing the following supplies on an absorbent pad: 1) Kimwipes; 2) cup containing ethyl alcohol; 3) squirt bottle containing DI water; 4) Atago Urine

Specific Gravity Refractometer, PAL 10-S; 5) transfer pipettes; and 6) the appropriate urine specimen cup from the refrigerator. The lab member will also have out the Color and Clarity Guide and a Urine Sample Characteristics Log. A pair of nitrile gloves will be worn when handling urinesamples.

Measurement of Color and Clarity

The color and clarity of each urine sample will be noted through comparison with the Color and Clarity Guide and recorded on the Urine Sample Characteristic Log.

Measurement of Specific Gravity

SG will be measured following the manufacturer's instructions that accompany the refractometer.

Documentation

The lab member will complete the Urine Sample Characteristics Log, Information from the Urine Sample Characteristics Log will be recorded in the K01 Database. The paper copy of the Urine Sample Characteristics Log will then be filed in the folder labeled with the participant's ID in the locked filing cabinet.

Weekly Urine Sample Storage

Supplies Preparation

Urine sample storage should occur immediately after analysis. Prior to beginning storage procedures, the lab member will set the following supplies out on a lab station on an absorbent pad (this can be the same absorbent pad used during analysis): 1) Eppendorf Plus Research Pipette (set at 4.0 mL) and tip; 2) four 4 mL weekly cryovials with the appropriate barcoded labels; 3) one cryobox at the lab station; and 4) the appropriate urine specimen cup. A pair of nitrile gloves will be worn when handling urine samples.

Weekly Urine Sample Storage

Four mL of the urine sample will be pipetted into four separate 4 mL weekly cryovials using the Eppendorf Plus Research pipette and tip. Once all four of

the weekly cryovials have been filled, the lab member will remove the weekly cryobox from the -80°C freezer. This weekly cryobox will be placed at the lab station, and the newly filled weekly cryovials will then be transferred from the cryobox at the lab station and into the freezer weekly cryobox. The weekly cryobox in the freezer will only be removed from the freezer briefly for daily cryovial transfer, and it will be immediately returned to the freezer as quickly as possible.

Documentation

The lab member will record the specific cryovial label and cell number where each of the four weekly cryovials were placed in the freezer weekly cryobox. This will be recorded in the CAHL shared Google Drive Cryobox Document.

6. DIETARY INTERVENTION

6.1 Summary of Methods

The dietary intervention will take place over the course of 17 days, from June 14 to June 30. Participants will receive one week of conventional groceries and one week of organic groceries, the order of which will be randomly assigned. Each week, each study participant will shop for up to \$150 worth of groceries using an online account at a local grocery store, and grocery orders will be delivered to participants. Participants will track their food intake each week using a Food Log. During the dietary intervention, participants will not provide weekly urine samples and will instead provide 14 daily urine samples.

6.2 Schedule

The dietary intervention will be divided into two weeks referred to as “Week One” and “Week Two.” Key dates during the dietary intervention, as well as key dates prior to starting the dietary intervention, are:

May 24-28: Dietary Intervention Reminder Flyers left in participant's coolers

June 3-June 9: Personalized Week One Grocery Flyer left in participant's coolers

June 7-11: Week One Food Logs left in participant's coolers

June 13: Week One grocery orders placed

June 14: Week One grocery orders delivered, participants begin to eat Week One groceries

June 15: Washout day

June 15-21: Participants complete Week One Food Log

June 16-22: Week One daily urine sample collections

June 17-June 18: Personalized Week Two Grocery Flyer left in participant's coolers

June 21: Week Two grocery orders placed, Week Two Food Logs left in participant's coolers

June 22: Week Two grocery orders delivered, participants stop eating Week One groceries and begin to eat Week Two groceries

June 23: Washout day

June 22-29: Participants complete Week Two Food Log

June 24-30: Week Two daily urine sample collections

June 24: Week One Food Logs collected

June 30: Week Two Food Logs collected

6.3 Materials

- Shared Research Drive
- Cell phone with One Talk App
- Dietary Intervention Reminder Flyer*
- Supplies for Week One:
 - a) Personalized Week One Grocery Flyer*
 - b) Week One Food Log*
- Supplies for Week Two:
 - a) Personalized Week Two Grocery Flyer*
 - b) Week Two Food Log*

6.4 Procedures

Supplies Preparation

Prior to beginning the dietary intervention, a lab member will create a new email account through Gmail for each participant. The lab member will then use this email to create an online account for each participant at the local grocery store.

Grocery Ordering and Delivery

During weekly urine sample collections from May 24-May 28, a lab member will leave the Dietary Intervention Reminder Flyer in the participants' coolers in their preferred language.

Participants will be provided their own local grocery store account username and password by a lab member. Participants will receive a personalized flyer (described in more detail below) with instructions regarding how to log into

their account and shop for up to \$150 worth of groceries (either conventional or organic, as randomly assigned) and select their preferred grocery delivery time.

Participants will also be instructed to call or text a lab member via the One Talk App once grocery shopping is complete. The lab member will then review the order to ensure that all items are available and correspond to the week in which the participant has been randomized (i.e., conventional or organic), and that no prohibited items have been added (e.g., no alcohol, non-food items, items from the “Personal Care & Health” department, cigarettes, or lottery tickets). The lab member will contact the participant if there are questions or concerns about the order. After the lab member has reviewed the order, he/she will contact the participant to confirm her delivery address and preferred delivery time. The participant or a member of her household must be home at the time of grocery delivery. The grocery order will be placed by the lab member.

Participants will also be given a brief food log for each week, in which they will be asked to note any food or drinks they consume from food outside of their grocery order, including any meals from restaurants. Participants will also be asked to complete a Likert scale question each day with the following options: “Everything I ate was from the study,” “Most of what I ate was from the study,” “About half what I ate was from the study,” “A little bit of what I ate was from the study,” or “None of what I ate was from the study.”

Week One

A lab member will leave a Personalized Week One Grocery Flyer in the participant’s cooler in her preferred language during weekly urine sample collections from June 3-June 9. Each Week One Grocery Flyer will be personalized for each participant. This flyer will clearly indicate: 1) the participant’s username and password for her online local grocery store account, 2) whether she should shop for conventional or organic groceries for this week, 3) how to complete her grocery shopping; 4) how to select her preferred grocery store location and delivery time frame, 5) the deadline for completing her grocery shopping, and 6) instructions to text or call lab members on the One Talk phone number once her grocery shopping is complete.

On the day before the participant is scheduled to complete her Week One grocery shopping, the lab member will send a text to the participant in her preferred language reminding her to complete her order by 5:00 PM the following day, if she has not done so yet.

Grocery orders for Week One will be delivered to participants on June 14. Participants will begin to eat their Week One groceries on June 14.

The Week One Food Log will be left in each participant's cooler in her preferred language by a lab member during urine sample collections on June 7-11. Participants will complete the Week One Food Log from June 15-June 21. Participants will be instructed to leave the completed Week One Food Log in their coolers along with their daily urine samples on June 24, and a lab member will collect it on June 24.

On June 14, after groceries have been delivered for Week One, the lab member will contact the participant in her preferred language to ensure the groceries were received, to remind her to complete the Week One Food Log, and to remind her that daily urine sample collection will begin on June 16.

Week Two

A lab member will leave the Personalized Week Two Grocery Flyer in the participant's cooler in her preferred language during daily urine sample collection on June 17 or June 18. This flyer will be similar to the Personalized Week One Grocery Flyer, but with appropriate changes made for Week Two.

On the day before the participant is scheduled to complete her Week Two grocery shopping, the lab member will send a text to the participant in her preferred language reminding her to complete her order by 5:00 PM the following day, if she has not done so yet.

Grocery orders for Week Two will be delivered to participants on June 22. Participants will begin to eat their Week Two groceries on June 22.

On the day before the participant is scheduled to receive her Week Two groceries, the lab member will send a text to the participant in her preferred language reminding her to collect her urine sample as a first morning void on the morning of June 22, prior to consuming any Week Two groceries.

The Week Two Food Log will be left in each participant's cooler in her preferred language by a lab member during urine sample collections on June 21. Participants will complete the Week Two Food Log from June 22-29. Participants will be instructed to leave the completed Week Two Food Log in their coolers along with their daily urine samples on June 30, and a lab member will collect it on June 30.

On June 22, after the groceries have been delivered for Week Two, the lab member will contact the participant in her preferred language to ensure the groceries were received, to remind her to stop eating Week One groceries and begin eating Week Two groceries, and to remind her that there will be no daily urine sample collection the following day, June 23, and daily urine sample collection will resume on June 24.

On June 23, the lab member will contact the participant in her preferred language to remind her that daily urine sample collection will resume the next day on June 24, to remind her to place her completed Week One Food Log in her cooler along with her daily urine sample, and to remind her to complete the Week Two Food Log.

On June 24, in addition to collecting the daily urine sample, the lab member will pick up the completed Week One Food Log.

On June 29, the lab member will contact the participant in her preferred language to remind her to place her completed Week Two Food Log in her cooler along with her final daily urine sample the following morning, and to remind her that the study will resume weekly urine sample collections the following week.

On June 30, in addition to collecting the final daily urine sample and picking up the completed Week Two Food Log, the lab member will leave the materials to resume weekly urine sampling the following week. The necessary weekly urine sample materials should be determined individually for each participant based on the week number last recorded prior to the dietary intervention.

7. DAILY URINE SAMPLE COLLECTION

7.1 Summary of Methods

Daily urine samples will be provided during the dietary intervention during Week One, from June 16-22, and during Week Two, from June 24-30. Participants will provide a total of 14 daily urine samples. Daily urine samples will be numbered sequentially. Each day, each study participant will provide a first morning void sample and will place the sample in her cooler with her ice pack outside her home. A lab member will then collect that day's sample, provide the participant with a specimen cup for the following day, and bring that week's sample back to CAHL in a lab cooler with a lab ice pack. Once back at CAHL, the lab member will analyze and store the daily urine sample.

Daily urine sample collections will follow the same protocol as described in Section 4 of this document, with the following major deviations:

- Lab members will collect and deliver daily urine sample cups, rather than weekly urine sample cups
- Lab members will complete a Daily Urine Chain of Custody Form, rather than the Urine Chain of Custody Form.
- For non-local collections two days of daily urine samples will be collected during a two-day trip. On the first day a lab member will drive to that area, collect the daily urine samples for that day, transport the daily urine samples in a lab cooler with a lab ice pack to a hotel, and store the daily urine samples in the study refrigerator at the hotel. The lab member will spend the night at the hotel, and then on the second day the lab member will collect the daily urine samples for that day and bring the urine samples for both days back to CAHL in a lab cooler with a lab ice pack
- Participants will receive one \$50 gift card at the end of the dietary intervention period if they provided all 14 daily urine samples, rather than receiving a \$10 gift card for each sample collected

7.2 Materials

- Specimen cup with barcoded label in a sealable plastic bag (day one)
- Specimen cup with barcoded label for the following day's collection in a sealable plastic bag
- Extra specimen cup
- Nitrile gloves
- Cooler
- Ice pack
- Daily Urine Chain of Custody Form for the correct participant ID*
- One sharpie and pen
- Cell phone with One Talk App
- CAHL Calendar
- K01 Database
- Study refrigerator
- Hotel confirmation number
- \$50 gift card (for the last day only)

7.3 Procedures

Supplies Preparation

The specimen cup with the barcoded label for the first day's collection (day one) in a sealable plastic bag will be left in each participant's cooler during June 7-11, the last weekly urine sample collection before starting the dietary intervention.

For daily urine sample collections, a lab member will compile and set out the materials listed above. The Daily Urine Chain of Custody Form will be taken from the folder in the locked filing cabinet labeled with the participant's ID number. The lab member will take all supplies to the urine collection.

Daily Urine Sample Collections

Each day, participants will be instructed to provide a first morning void in the specimen cup with the barcoded label for that day, which was provided to them at the prior day's collection. The participants will be instructed to use their previously provided Sharpie to record the date and time of collection directly on the specimen cup. The sample should be provided the morning of the collection day (first morning void), and then placed in the sealable plastic bag and into the previously provided cooler with the ice pack. This cooler will then be placed outside the participant's home.

Two lab members will collect local daily urine samples as described in Section 4 of this document. The two lab members will travel separately and divide the number of collections.

One lab member will collect non-local daily urine samples as described in section 4 of this document, with the exception that non-local daily urine sample collections will occur daily during a two-day trip. For each two-day trip, a lab member will drive on the first day (this will occur on June 16, 18, 20, 24, 26, and 28), collect the daily urine samples for that day, transport the daily urine samples in a lab cooler with a lab ice pack to the hotel, and store the samples in the study refrigerator at the hotel. The lab member will spend the night at the hotel, and then on the second day the lab member will collect the daily urine samples for that day (June 17, 19, 21, 25, 27, and 29). The lab member will then bring the urine samples for both days back to CAHL in a

lab cooler with a lab ice pack. Daily urine sample collected from the non-local area on June 22 and June 30 will be completed by one lab member in one day.

The lab member will text the participant one day prior to each daily pick up as a reminder. After each sample collection, a text message will be sent to each participant.

If a participant does not provide a urine sample for a given day, the lab member will document the missed day on the Daily Urine Chain of Custody Form. This will be considered a missed day and that day number will not be reused the following day; the next sequential day number will be used.

Documentation upon Returning to the Lab

After collecting samples, the lab member will return back to CAHL either 1) on the same day for local urine sample collections, or 2) on the second day of each two-day trip for non-local urine sample collections. There, the lab member will place the daily urine samples in the refrigerator and complete the appropriate sections of the Daily Urine Chain of Custody Form. The samples will remain in the refrigerator for no more than 24 hours until analyzed. Samples collected on the first day of each two-day trip must be prioritized for processing on the same day they return to the lab.

Gift Cards

A \$50 gift card will be given to participants who provide all 14 daily urine samples.

8. DAILY URINE SAMPLE ANALYSIS & STORAGE

8.1 Summary of Methods

Daily urine samples will be collected from participants and returned to CAHL in a lab cooler with a lab ice pack. Each day, one or more lab member(s) will be responsible for analyzing and storing all daily urine samples arriving in the lab that day. During analysis, the lab member will determine urine color, clarity, and specific gravity. Urine sample storage will occur immediately after urine sample analysis. Each sample will be aliquoted into two 4mL daily

cryovials for storage. These will be stored in the daily cryobox in the CAHL -80°C freezer. In addition, 0.6 mL of each urine sample will be aliquoted into two 4 mL composite cryovials created for each participant for each week. These will be stored in the composite cryobox in the CAHL -80°C freezer. At the end of the dietary intervention, each participant will have two 4 mL composite cryovials that contains 0.6 mL of each daily sample for each week, representing a weekly composite sample. At the conclusion of an individual's participation in the study (ideally when she gives birth), one of the two 4 mL composite cryovials from each week will be sent to the CDC for glyphosate analysis (up to 2 total composite cryovials per participant: one for week one and one for week two). The remaining composite cryovials, as well as the two daily 4 mL cryovials, will be stored in the biorepository.

8. 2 Materials

Daily Urine Sample Analysis

- Absorbent pads
- Kimwipes
- Cup containing ethyl alcohol
- Squirt bottle containing DI water
- Nitrile gloves
- Pen
- Urine Sample Characteristics Log*
- Color and Clarity Guide*
- Transfer pipettes
- Atago Urine Specific Gravity Refractometer, PAL 10-S
- K01 Database

Daily Urine Sample Storage

- Absorbent pads
- Nitrile gloves
- Eppendorf Plus Research Pipette and tip
- Four cryovials with barcoded labels (two daily cryovials and two composite cryovials)
- Daily Cryobox and Composite Cryobox
- Google Drive Cryobox Document

8.3 Procedures

Daily Urine Sample Analysis

Lab members will analyze the daily urine samples as described in Section 5.3 of this document.

Daily Urine Sample Storage

Supplies Preparation

Urine sample storage should occur immediately after analysis. Prior to beginning storage procedures, the lab member will set the following supplies out on a lab station on an absorbent pad (this can be the same absorbent pad used during analysis): 1) Eppendorf Plus Research Pipette (set at 4.0 mL and then changed to 0.6 mL) and tip; 2) two 4 mL daily cryovials with the appropriate barcoded labels and two 4 mL composite cryovials with the appropriate barcoded labels; 3) one cryobox at the lab station; and 4) the appropriate urine specimen cup. A pair of nitrile gloves will be worn when handling urine samples.

Daily Urine Sample Storage (Daily Cryovials)

Four mL of the urine sample will be pipetted into two separate 4 mL daily cryovials using the Eppendorf Plus Research pipette and tip. Once both of the daily cryovials have been filled, the lab member will remove the daily cryobox from the -80°C freezer. This daily cryobox will be placed at the lab station, and the newly filled daily cryovials will then be transferred from the cryobox at the lab station and into the freezer daily cryobox. The daily cryobox in the freezer will only be removed from the freezer briefly for daily cryovial transfer, and it will be immediately returned to the freezer as quickly as possible.

Daily Urine Sample Storage (Composite Cryovials)

For the first daily urine sample collection of each week, 0.6 mL of the urine sample will be pipetted into two separate 4 mL composite cryovials using the Eppendorf Plus Research pipette and tip. Once both of the composite cryovials have been filled, the lab member will transfer the composite cryovials from the cryobox at the lab station into the freezer composite cryobox. The composite cryobox will be immediately placed in the freezer as quickly as possible.

Each day when the next daily urine sample is collected from a participant, another 0.6 mL from that sample will be aliquoted into the two separate 4 mL composite cryovials, on top of the previously frozen sample. This process will be repeated until the composite cryovials contains 0.6 mL from each of the seven days, representing a weekly composite cryovial. The composite cryobox in the freezer will only be removed from the freezer briefly for aliquoting, and it will be immediately returned to the freezer as quickly as possible.

Documentation

The lab member will record the specific cryovial label and cell number where each of the two daily cryovials were placed in the freezer daily cryobox. This will be recorded in the CAHL shared Google Drive Cryobox Document. The lab member will also record the specific cryovial label and cell number where each of the two composite cryovials were placed in the

freezer composite cryobox. This will also be recorded in the CAHL shared Google Drive Cryobox Document.

9. URINE SAMPLE SHIPMENT & GLYPHOSATE ANALYSIS

9.1 Summary of Methods

Once all weekly and daily urine samples have been collected, analyzed, and stored for all 40 participants, two lab members will complete a quality assurance (QA) check of each cryovial to ensure that it is labeled with the correct barcoded label and that it is placed in the correct cryobox and correct cell, and that this information correlates with the information in the K01 Database, as well as with the Google Drive Cryobox Documents. The QA will be done thoroughly, but quickly, to prevent the thawing of the cryovials. Following the QA, the first of the four cryovials from every other week and the first composite cryovials from each week in the dietary intervention, along with a number of quality control vials and randomly selected cryovials, will be sent to the CDC for glyphosate analysis. These samples will be packaged by three lab members and then sent on dry ice to the CDC via Overnight Air Priority Shipping with tracking.

9.2 Materials

Quality Assurance

- Google Drive Cryobox Document
- K01 Database
- Nitrile gloves
- Frozen cryoboxes

Sample Shipment

- Twelve cryovial carrier boxes (labeled as box 1-12)

- Twenty-four small absorbent pads
- Twenty-four rubber bands
- Twelve biohazard bags
- Shipping cooler
- Shipping box
- Shipping stickers
- Shipping tape
- Nitrile gloves
- All weekly, composite, and daily cryoboxes in the -80°C freezer
- Four absorbent pads
- Five ice packs
- Five pounds of dry ice

9.3 Procedures

Quality Assurance

Weekly Urine Samples Electronic Documentation Check

The electronic database documents containing information on the stored weekly urine sample collections and cryovials will be exported from their original source. All changes to any of these documents will be done on the exported copies and will be tracked. No changes will be made the original data sources.

The lab member will cross-check the exported forms with the exported aliquot boxes document. The lab member will highlight any information on the

exported aliquot boxes document that does not match or that seems implausible. The lab member will briefly describe the issue in the margins of the exported aliquot boxes document. The lab member will refer to the Urine Chain of Custody Form as needed.

Weekly Urine Sample Cryovials Check

After completion of the Electronic Documentation Check, two lab members will cross-check the now highlighted exported aliquot boxes document with the frozen cryovials in order to 1) confirm that each cryovial is in the correct cell; 2) confirm that each cryovial is labeled correctly; and 3) clarify any typos or resolve other issues in the exported aliquot boxes document.

Documentation and Correction

After all the weekly cryoboxes have been checked and returned to the -80°C freezer, the lab members will review all the highlighting and notes in the exported aliquot boxes document. Typos will be corrected as appropriate.

Quality Assurance For Daily and Composite Urine Samples

QA for the daily and composite urine samples will follow the same procedures as QA for the weekly urine samples as described above.

Sample Shipment

Sample Selection

Dr. Curl will select 600 cryovials to send to the CDC. This selection will include the first of the four cryovials from every other weekly urine sample collected from each participant throughout the study. In addition, based on the weekly urine samples being sent to the CDC, Dr. Curl will randomly select participant IDs and week numbers to serve as weekly urine sample quality controls. For these urine samples the second of the four cryovials will also be sent to the CDC. Dr. Curl will also send the first of the two composite cryovials from each week of the dietary intervention from each participant to the CDC, as well as all 14 daily cryovials from one participant from each day of the dietary intervention to serve as composite sample quality control.

Supplies Preparation

Prior to the shipment of urine samples to the CDC, three lab members will set up a shipping station in the lab with the following supplies: 1) twelve cryovial carrier boxes; 2) twenty-four small absorbent pads; 3) twenty-four rubber bands; 4) twelve biohazard bags; 5) shipping cooler; 6) shipping box; 7) shipping tape; 8) shipping stickers; and 9) four absorbent pads. The lab members will also have five ice packs in the freezer and five pounds of dry ice in the -80°C freezer, and will have the Aliquot Boxes Spreadsheet available electronically.

Packaging and Shipping

Packaging will begin with weekly cryovials, then composite cryovials, and then daily cryovials. Cryoboxes will be pulled from the -80°C freezer one at a time and the selected cryovials from that cryobox will be transferred to a cryovial carrier box.

The lab members will place the five ice packs from the freezer at the bottom of the shipping cooler and place two absorbent pads on top. The lab members will then remove all twelve cryovial carrier boxes in biohazard bags from the -80°C freezer and place them into the shipping cooler along with five pounds of dry ice. Finally, two more absorbent pads will be placed on top and the lid to the shipping cooler will be taped closed with one single strip of shipping tape running horizontally across the lid. The shipping cooler will be placed into the shipping box and the shipping box will be taped closed with one single strip of shipping tape running horizontally across the box. No more tape should be used on the shipping cooler or shipping box as the dry ice in the shipment must not be completely sealed off from oxygen.

Appropriate shipping stickers will be placed on the shipping box, and the box will be transported for Overnight Air Priority Shipping with tracking with early morning arrival. The lab members will email the CDC to provide the tracking information, and will also request that the CDC confirm receipt of the samples and that all samples arrived frozen once the shipment arrives.