

National Human Exposure Assessment Survey (NHEXAS)

Arizona Study

Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

Cooperative Agreement CR 821560

Standard Operating Procedure

SOP-UA-F-20.1

Title: Collection, Storage, and Shipment of Urine Samples for Metals
and Pesticides Analysis

Source: The University of Arizona

U.S. Environmental Protection Agency
Office of Research and Development
Human Exposure & Atmospheric Sciences Division
Human Exposure Research Branch

Notice: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), partially funded and collaborated in the research described here. This protocol is part of the Quality Systems Implementation Plan (QSIP) that was reviewed by the EPA and approved for use in this demonstration/scoping study. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

Collection, Storage, and Shipment of Urine Sample for Metals and Pesticides Analysis

1.0 PURPOSE AND APPLICABILITY

The procedures described in this SOP are designed to guide the collection, storage, and shipment of urine samples collected for the NHEXAS Arizona project, AZ Border project, and other Health and Environment projects. This SOP provides a brief description of sample, collection, preservation, storage, shipping, and custody procedures.

2.0 DEFINITIONS

- 2.1 AZ BORDER = The US border region is defined as 100 km north of the border. In this study, we define the border as 40 km north of the border. The Arizona Border Study or "Border AZ" is an alias for "Total Human Exposure in Arizona: A Comparison of the Border Communities and the State" conducted in Arizona by the University of Arizona / Battelle / Illinois Institute of Technology Consortium.
- 2.2 CDC = Centers for Disease Control in Atlanta.
- 2.3 FIELD COORDINATOR = The employee of the research project who supervises field data collection and operations. The Field Coordinator collates individual data into HH packets, and upon completion of all visits, sampling and QA checks, forwards the packet to the Data Coordinator.
- 2.4 FIELD STAFF = The Field Coordinator, the Team Leader and the Team Members.
- 2.5 LAB SUPERVISOR = The employee of the research project who supervises laboratory analyses.
- 2.6 MATERIALS TECHNICIAN (Materials Tech) = The employee of the research project who is responsible for assembling and assigning field forms, questionnaires and equipment for field use.
- 2.7 NHEXAS Arizona = Acronym for National Exposure Assessment Survey, a research project conducted in Arizona by the University of Arizona / Battelle / Illinois Institute of Technology Consortium.
- 2.8 QUALITY ASSURANCE (QA)= All those planned and systematic actions necessary for ensuring the accuracy, validity, integrity, preservation and utility of collected data.
- 2.9 QUALITY CONTROL (QC) = Those quality assurance actions providing a means to control and measure the characteristics of a datum, processor the adherence to established parameters.

- 2.10 **RESPONDENT** = A person in the study population of NHEXAS Arizona project, AZ Border project, and other Health and Environment projects.. Each household is assigned an HHID. All the family respondents are assigned an Individual Respondent Number (IRN). Each respondent can be uniquely identified by an HHID, IRN combination number.
- 2.11 **SAMPLE IDENTIFICATION NUMBER** = A numeric code that uniquely identifies every sample. It is generated by the NHEXAS tracking system by the Materials Technician.
- 2.12 **TEAM LEADER** = The member of the field team who is primarily responsible for respondent contact, data collection, field form and questionnaire completion, and site QC checks of all data.
- 2.13 **TEAM MEMBER** = Member of a field team responsible for assisting the team leader in the collection of data and quality control checks in the field.
- 2.14 **TRACKING SYSTEM** = A database system containing information about the custody, transfer and storage of hard copy data, electronic data, field samples, and field sample aliquots.
- 2.15 **VISIT** = A scheduled appointment with participating respondents at their place of residence (HH) for the collection of samples, questionnaires and other data.

3.0 REFERENCES

- 3.1 Ashley, D. 1995. CDC Communication on Urine Sample Collection.
- 3.2 Lebowitz, M.D. 1993. Study Design (Revision of 31 Dec. 1993). EPA NHEXAS Cooperative Agreement.

4.0 DISCUSSION

- 4.1 This SOP outlines the correct procedure for the collection, storage, and transfer of urine samples at participating households according to the strategies outlined in the NHEXAS project, AZ border project and other Health and Environment projects. The HH respondent will collect a first morning void or first urination of the day. The sample will be collected while the respondent is participating in the duplicate food collection (UA-F-15.X). The Field Team Leader will collect the urine sample the day after the food diary has been completed. For example, if the respondent performs the 24 hour duplicate food collection on Monday, the first morning void must be collected Tuesday morning. The respondent will then be asked to place the sample container in a paper bag (provided) and store it in their freezer until the field crew can come and collect the sample.
- 4.2 After the urine sample has been collected from the HH, two aliquots will be made at CDC.

One aliquot will be analyzed for pesticide content and another for creatinine concentration. The volume of the aliquot analyzed is a function of the amount of urine provided by the respondent. Aliquots are taken only after sufficient sample has been obtained for metals analysis. These aliquots are made by personnel at CDC.

- 4.3 Bar-code sample-ID labels and a chain of custody record for each aliquot (creatinine and pesticides) will be sent from the U of A with the original frozen composite sample shipped to CDC on dry ice. The non-aliquotted portion of the original composite sample will be analyzed for metals concentration. Thus, there is no separate aliquot and chain of custody record for metals.

- 4.4 The relative timing of urine sample collection to other sampling types by stage is shown in Fig. 3.

5.0 RESPONSIBILITIES

5.1 Field Coordinator:

- (a) 100% QA check on all field forms.
- (b) Perform 10% QA in-field audit of collection, transportation and storage methods.

5.2 Materials Technician:

- (a) Prepare HH bucket with appropriate field forms and sample collection materials.
- (b) Record the assigned kits in the NHEXAS tracking system.
- (c) Ship the urine samples to CDC on dry ice, thus preserving sample integrity.
- (d) Prepare QA spikes and blanks and ship to CDC for analysis.

5.3 Team Leader:

- (a) Arrange HH visit.
- (b) Transport urine collection materials into Field.
- (c) Complete Chain of Custody Record/Field Data Collection Sheet.
- (d) Quality control checks in the field.

6.0 MATERIALS AND REAGENTS

- (a) Urine collection cup (5 oz., plastic, sterile).
- (b) Urine Sample Collection Instructions Hand-out (Fig. 4).
- (c) Ziploc freezer bags.
- (d) Small Brown Paper Bag.

7.0 PROCEDURE

7.1 Preparation

7.1.1 Containers

The urine collection containers are supplied by CDC. Containers are sterile upon receipt from CDC Labs and are maintained at room temperature pending field assignment. The plastic packaging surrounding the urine collection container is not removed until the respondent is given the container by the Team Leader.

7.1.2 Standards & Blanks

- (a) Standard and Blank reagents are prepared at CDC and shipped on blue ice to the project field office. Spike and blank solutions are stored at controlled temperatures until used.

QA Matrix Type	Pre-Field Temp. Conditions	Holding Period
Urine Metals Blank	-20°C	9 Months
Urine Metals Spike	-20°C	9 Months
Urine Pesticides Blank	-20°C	9 Months
Urine Pesticides Spike	-20°C	9 Months

- (b) QA & QC duplicate/replicate sampling will not be performed.
- (c) Blanks and spikes are prepared at the HRP facility by the Materials Technician. Ten percent of all samples shipped for analysis will be for QC/QA checks.

7.1.3 Standard and Blank Preparation

A: Urine Metals Blank

1. Obtain the purified water supplied by CDC from the Pre-Field Freezer. The vials are labeled URINE METALS BLANK.
2. Allow the QA matrix to thaw at room temperature.
3. Obtain a new sterile urine collection container. Label the container with the appropriate sample-ID in indelible pen and by affixing a barcode.
4. Transfer 10- 15 milliliters of the purified water from the vial to the urine cup.
5. Dispose of the vial in the Sharps Container or in the Broken Glass Container at the HRP Laboratory.
6. Transfer the urine cup to the - 20°C Post-Field Freezer to await shipment.
7. Ship to CDC for analysis with 'live' samples.

B: Urine Metals Spike

1. Obtain the spike matrix supplied by CDC from the Pre-Field Freezer. The vials are labeled URINE METALS SPIKE.
2. Allow the QA matrix to thaw at room temperature.
3. Obtain a new sterile urine collection container. Label the container with the appropriate sample-ID in indelible pen and by affixing a barcode.
4. Transfer the entire contents of the vial to the urine cup.
5. Dispose of the vial in the Sharps Biohazard Container
6. Transfer the urine cup to the - 20°C Post-Field Freezer.
7. Ship to CDC for analysis with 'live' samples.

C: Urine Pesticides Blank

1. Obtain the purified water supplied by CDC from the Pre-Field Freezer. The vials are labeled URINE PESTICIDES BLANK.
2. Allow the QA matrix to thaw at room temperature.
3. Obtain a new sterile urine collection container. Label the container with the appropriate sample-ID in indelible pen and by affixing a barcode.
4. Transfer 10- 15 milliliters of the purified water from the vial to the urine cup.
5. Dispose of the vial in the Sharps Container or in the Broken Glass Container at the HRP Laboratory.
6. Transfer the urine cup to the - 20°C Post-Field Freezer to await shipment.
7. Ship to CDC for analysis with 'live' samples.

D: Urine Pesticides Spike

1. Obtain the spike matrix supplied by CDC from the Pre-Field Freezer. The vials are labeled URINE PESTICIDES SPIKE.
2. Allow the QA matrix to thaw at room temperature.
3. Obtain a new sterile urine collection container. Label the container with the appropriate sample-ID in indelible pen and by affixing a barcode.
4. Transfer the entire contents of the vial to the urine cup.
5. Dispose of the vial in the Sharps Biohazard Container
6. Transfer the urine cup to the - 20°C Post-Field Freezer.
7. Ship to CDC for analysis with 'live' samples.

7.2 Sample Collection

7.2.1 Standards and Blanks Deployed.

Ten percent of all urine samples shipped for analysis at CDC are for QC\QA purposes. Urine blanks and spikes are not taken to the field, thus no FIELD BLANKS are collected.

All QA\QC samples are identified as LAB BLANKS

7.2.2 Sample Collection Instructions

- (a) The Team Leader will explain when the respondent needs to collect the urine sample. The Team Leader will give the respondent a urine collection container, a Ziploc Freezer and a brown paper bag, and a copy of the NHEXAS (or other project) **Urine Sample Collection Instructions** hand-out (Figure 4).
- (b) The Team Leader will thoroughly explain the following procedure and respond to any questions the participant may have. After providing the following instructions, the Team Leader will probe the respondent to determine whether the respondent understands the procedure.
 - (1) Collect the first urination of the day on the day following the 24 Hour diet collection.
 - (2) Keep the urine container in a place where the respondent won't forget to collect the sample at the scheduled time.
 - (3) Wash hands with soap and water and towel them dry before collecting the sample.
 - (4) Remove the collection cup with the cap in place from its plastic wrapping. Be careful not to dislodge the cap when removing the plastic wrapper. Do not touch the inside of the collection container or cap.
 - (5) The collection container should not be opened until just before the respondent begins to urinate.
 - (6) It is most important that the inside of the container and the cap not be touched or come into contact with any part of the respondent's body, clothing or other external surfaces.
 - (7) Collect a sample directly from the urine stream. The container should be two thirds to three quarters full (approximately 4 oz.) for a good specimen.
 - (8) Over filling the container (volume greater than 4.5 oz.) should be avoided since the sample will expand to occupy the remaining space in the container once it is frozen.
 - (9) Re-cap the container immediately and place it in the Ziploc bag. Be sure that the urine container lid is screwed on tight. Try not to touch the inside portions of the lid or container.
 - (10) Place the Ziploc bag inside the brown paper bag provided and ensure that the urine container remains upright to avoid leaks or spills.
 - (11) Immediately place the sample in the freezer. Put it up-right in a safe place where it cannot be spilled.
 - (12) Make a mental note of the time the urine was collected and write the time on the paper bag.
 - (13) Leave the sample in the freezer until the Field Team returns to collect the

sample.

- (c) When the Field Team returns to collect the sample the Team Leader will note the date and time the sample was expelled by the respondent in the *Collection Date* and *Collection Time* fields on the Urine Collection Data Sheet and Chain of Custody Record (Fig. 1).
- (d) The urine sample will be shipped back to the Field Office on blue-ice, logged-in to the Tracking System and immediately frozen to -20°C.
- (e) The urine sample will remain at -20 degrees °C until shipment to CDC Labs on Dry-Ice.

7.3 Sample Shipment

Materials needed per shipment

- 1. 1 Styrofoam bio-mailer
- 2. Dry ice - The appropriate DRY ICE Label must be completed before shipment.
- 3. Strapping Tape
- 4. Sheets of Bubble-Pack packing material
- 5. Pre-addressed **FEDERAL EXPRESS** label
- 6. Ziploc Freezer bag
- 7. One additional Aliquot Chain of Custody record and four copies of the bar-code legible sample-id for the **Pesticide Aliquot** to be made at CDC.
- 8. One additional Aliquot Chain of Custody record and four copies of the bar-code legible sample-id for the **Creatinine Aliquot** to be made at CDC.

7.3.1 Procedure for Packing:

- (a) Place each specimen inside a Ziploc Freezer bag and label with sample ID, HHID, collection date and Tech initials.
- (b) Pack the samples upright in the bottom of the shipper or Bio-Mailer. Ensure that specimens will remain up-right during transport. If necessary, use sheets of Bubble-Pack packing material to ensure the specimens vertical position. Put one layer of Bubble-Pack on top.
- (c) Fill the shipper with sufficient dry ice to maintain freezing temperatures during 24-hour delivery. Complete the appropriate DRY ICE WARNING LABEL (Figure 7).
- (d) Place more Bubble-Pack material or Styrofoam 'peanuts' to fill-up the remaining empty space in the bio-mailer and place the polyfoam lid on top of the shipper.
- (e) Include the original copy of Field Data Sheet/Chain of Custody Record (Figure 1). A photocopy of the Field Data Sheet is maintained for inclusion in the HH Packet.
- (f) Record the contents of the bio-mailer on the NHEXAS AZ (or other project) Shipment Log (Fig. 6). A photocopy of the Shipment Log is maintained by the

Materials Tech for QA and QC purposes.

- (g) Include the appropriate aliquot chain of custody records (Fig. 5) and aliquot sample-ids in the Ziploc Freezer bag for each sample.
- (h) Secure the outer cardboard lid on the shipper with filament tape.

7.3.2 Procedure for Shipping

- (a) Obliterate or remove previous address labels on all shippers.
- (b) Label each shipper with the following address:

Charles Dodson
Building 17, Loading Dock
Centers for Disease Control
4770 Buford Highway NE
Atlanta, GA 30341

- (c) The samples will be shipped via FEDERAL EXPRESS overnight in a pre-addressed biomailer with the original copy of the Chain of Custody and Field Data Sheet, plus additional Chain of Custody Records and sample-ids for aliquots.
- (d) Specimens should be received by CDC within 24 hours if possible. Telephone the laboratory at CDC the day the shipment is mailed at (404) 488-4305 and speak with Charles Dodson.
- (e) The samples should arrive at CDC on normal business day (Monday through Friday 8 to 5).

7.4 Analysis

7.4.1 Standards & Blanks - N/A

7.4.2 Samples - N/A

7.5 Calculations - N/A

7.6 Quality Control

7.6.1 QA and QC duplicate/replicate sampling will not be accomplished. Blank and spike samples will be forwarded to CDC for analysis with "live" samples as a QA check.

7.6.2 Ten percent of all samples shipped for analysis will be for QC\QA purposes

7.6.3 Tolerance Limits

Temperature and the lag-time between sample collection and sample analysis are critical

for urine sample integrity. Samples will be frozen in the respondents refrigerator immediately after collection. Samples will be returned to the Field Office on blue ice at 4°C and immediately frozen at -20°C. Samples are shipped to CDC on dry ice.

7.6.4 Detection Limits

- (a) 100% of all data collected is field verified for accuracy.
- (b) Sample integrity must be maintained and is checked at each node of sample transfer.

7.6.5 Corrective Actions

Apparent mis-labeling problems detected in the field may be corrected by the Team Members when appropriate in accordance with SOP #UA-C-2.X.

8.0 RECORDS

- 8.1 The Urine Collection Data Sheet and Chain of Custody Record (Figure 1).
- 8.2 The original chain of custody record/field data sheet remains with the sample at all times.
- 8.3 A copy of the custody record/field sheet is retained with the household sampling packet.

Figure 1. Urine Collection Data Sheet and Chain of Custody Record.

Urine Collection Data Sheet				
Project ID _____		Stage # _____		HHID _____ / _____ FS
Team Leader _____ Sample Retrieval by Field Team on ____/____/____ at ____:____ mo. day year				
Respondent's First Name:			IRN #	
Collection Date	Collection Time	Sample ID	# of Containers	Comments
____/____/____	____:____			
PESTICIDE ALIQUOT ---->			N/A	
CREATININE ALIQUOT---->			N/A	
Sample Frozen after Collection - Y or N			Comments:	
First Morning Void after 24 hr. Diet - Y or N			QC checks in field by [_____]	
Transported to Field Office at 4° C - Y or N				
History of Sample Handling and Custody				
Relinquished or Received	Signature	Date mo / day / yr	Time	Action
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	

Figure 2. Troubleshooting Guide

No field notes or Troubleshooting guides are currently on record for UA-F-20.X. Additions will be appended and the SOP reviewed and updated in accordance with UA-G-1.X as appropriate.

Figure 3. Relative Timing of Sample Collection (Page 1 of 3)

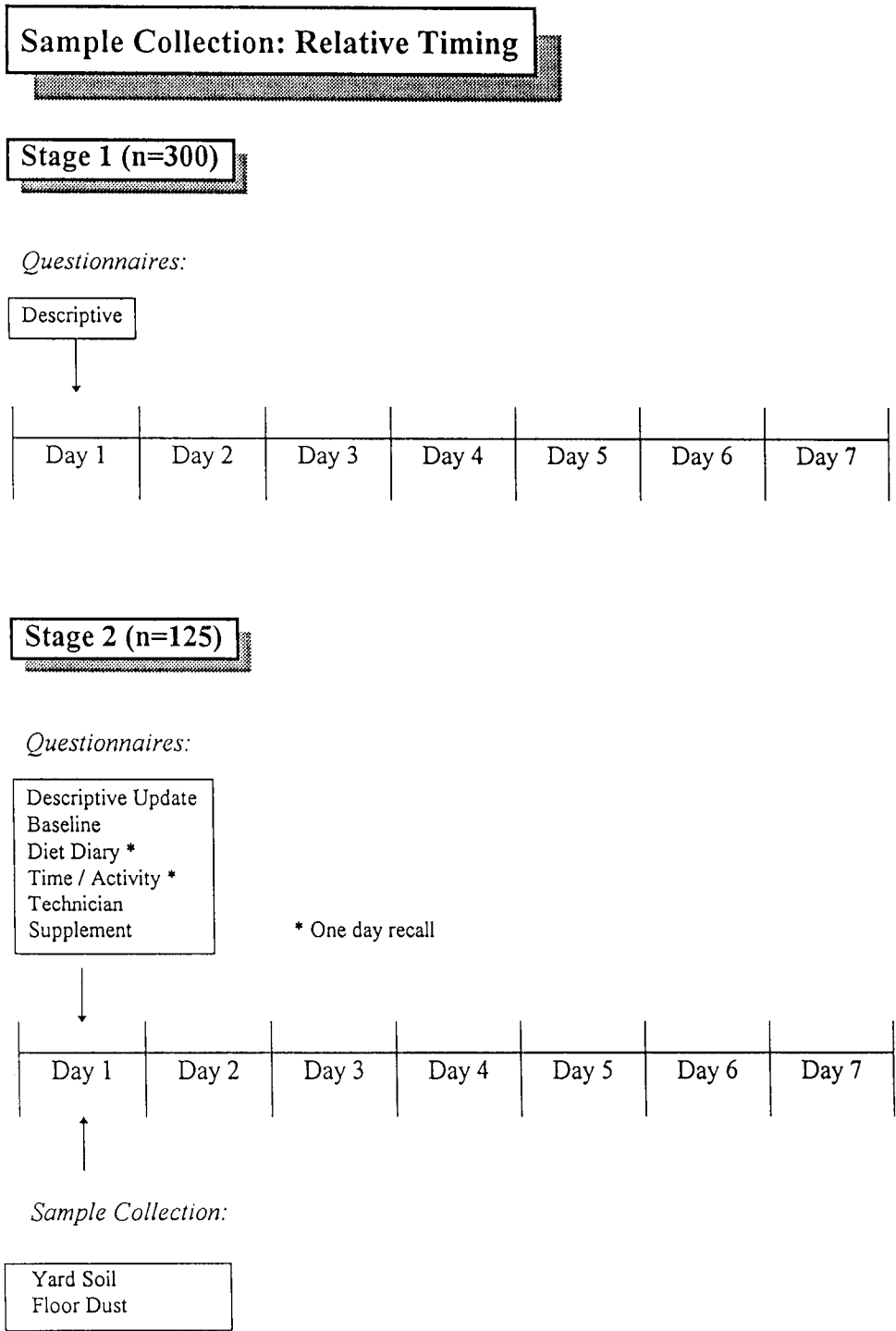
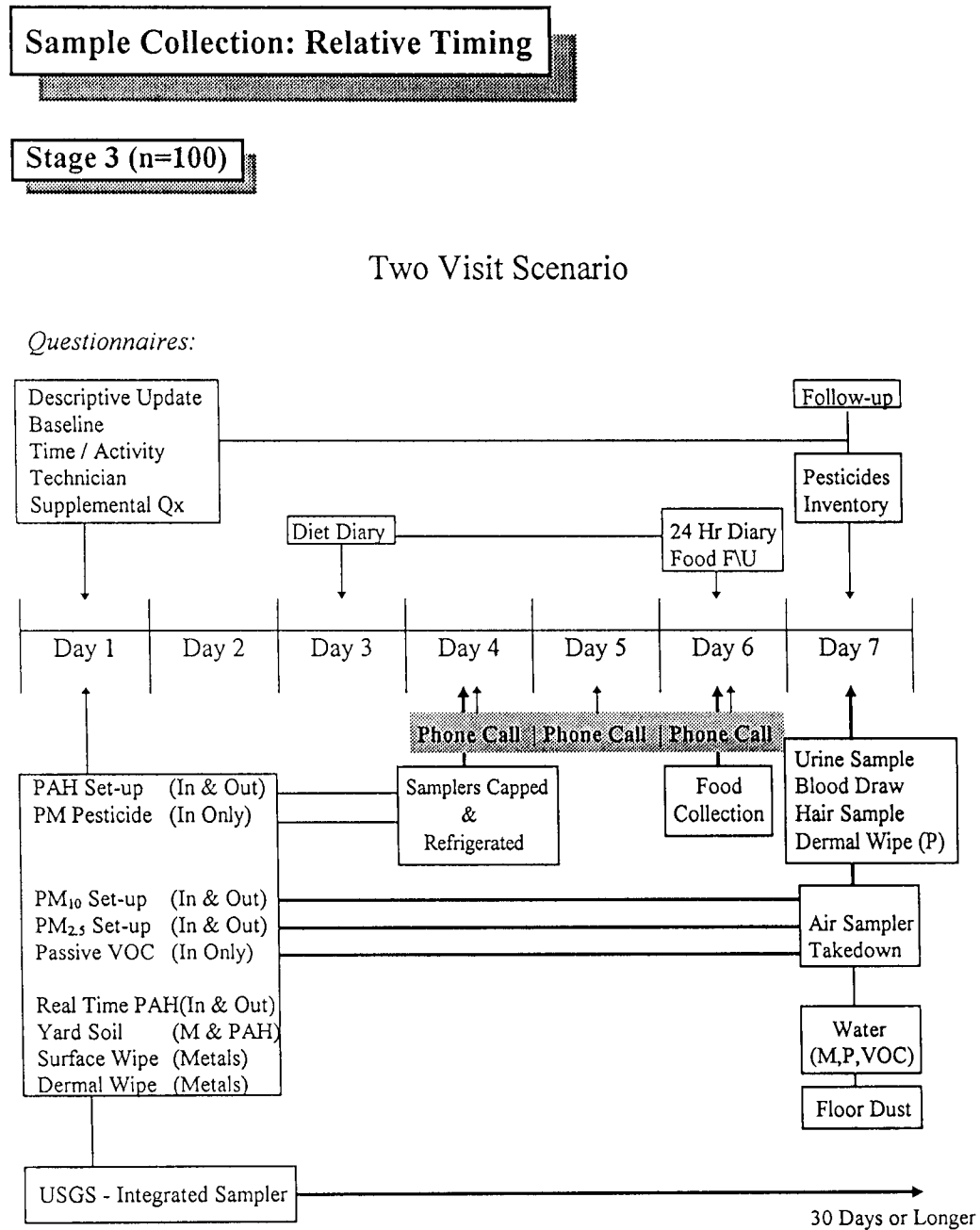
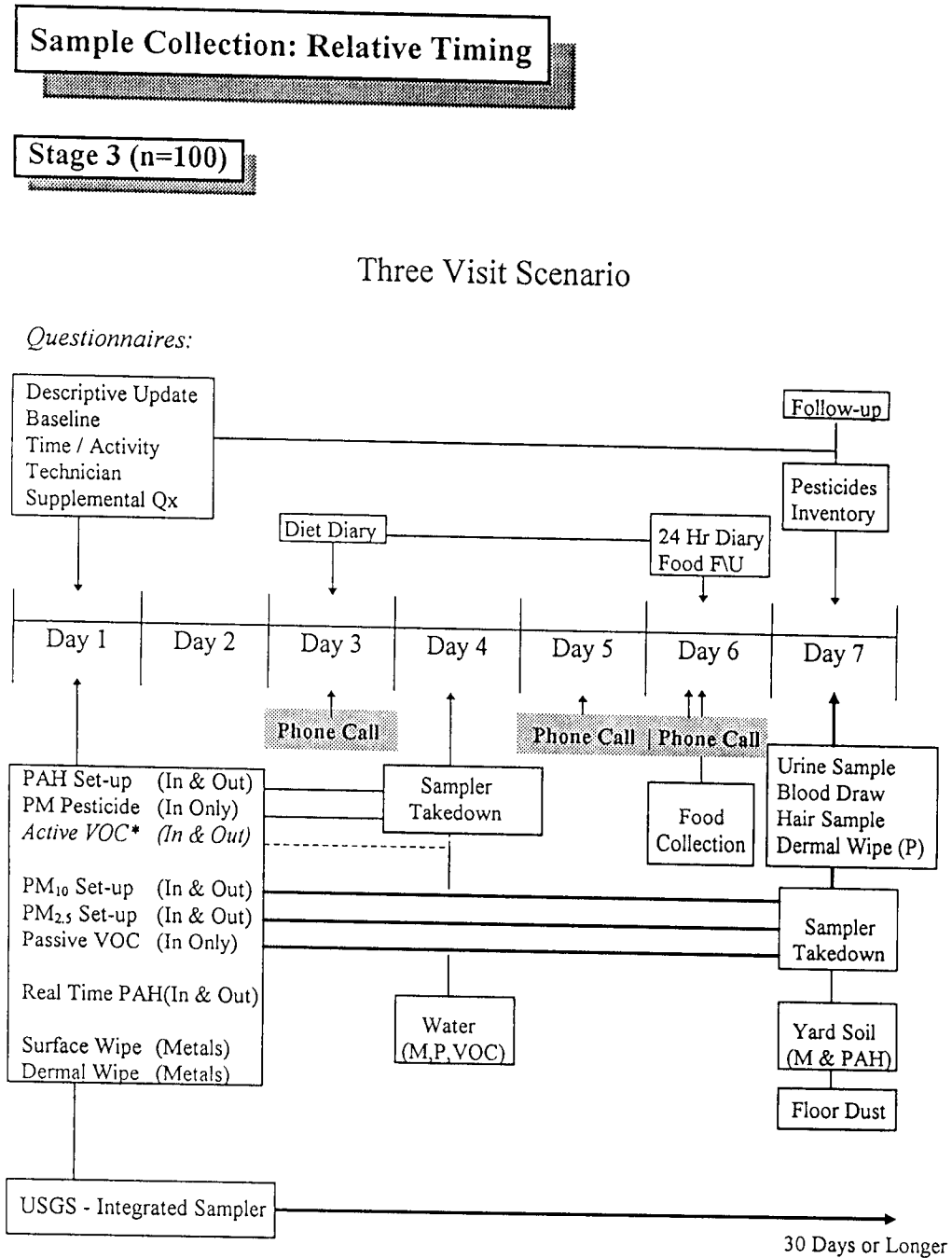


Figure 3. Relative Timing of Sample Collection (Page 2 of 3)



* Active VOC is collected in a subset of 25 homes only

Figure 3. Relative Timing of Sample Collection (Page 3 of 3)



* Active VOC is collected in a subset of 25 homes only

Figure 4. Urine Sample Collection Instructions

_____ is scheduled to collect a sample of their first urine void of the day
First Name

on _____, ____/____/____.
 day mo day yr

WHY ?

We would like to test a sample of your urine for select metals and pesticides. Certain metals are good for your health and certain metals and pesticides are hazardous to your health.

WHEN?

It is extremely important that the sample that you give to us is from your **first morning void on the day following your 24 hour duplicate diet collection**. If you are collecting your food starting on a Monday morning, for example, we want you to collect a sample of your urine from your first urination on Tuesday morning.

HOW?

- (1) Wash your hands with soap and water and towel dry.
- (2) Remove the collection cup from the plastic wrapper with the cap in place being careful not to dislodge the cap or touch the inside surfaces of the container or cap.
- (3) The collection cup should not be opened until just before you begin to urinate.
- (4) It is most important that the inside of the container and the cap not be touched or come into contact with any part of your body, clothing or other external surfaces.
- (5) Collect a sample from your urine stream. You must fill the container two thirds to three quarters full.
- (6) Try to provide a urine sample of approximately **4 fluid ounces**. The sides of the sample container are marked to help you estimate this volume.
- (7) Try not to over-fill the container since your urine will expand when you put it in the freezer.
- (8) Re-cap the container tightly and place it in the Ziploc bag. Try not to touch the inside portions of the lid or container.
- (9) Immediately place the sample inside the paper bag and put it your **freezer** in a safe place where it cannot be spilled.
- (10) Make a mental note of the time you collected the sample and record it on the paper bag.
- (11) We will return to collect the sample on:

_____, ____/____/____.
 day mo day yr

QUESTIONS?

You can call us with any questions at 1-800-238-2280.

THANK YOU!

Figure 5. Urine Sample Aliquot Chain of Custody Record

Urine Sample Aliquot Chain of Custody Record				
Original Sample ID: _____			<input type="checkbox"/> Creatinine Aliquot <input type="checkbox"/> Pesticide Aliquot	
Date Created	Time	Aliquot Sample ID	# of Containers	Remarks
____/____/____	____:____			
History of Sample Handling and Custody				
Relinquished or Received	Signature	Date mo / day / yr	Time	Action
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	
[Rel] or [Rec]		____/____/____	____:____	

note: FF = field form included with shipment and CoC = chain of custody record verified and present

[illegible]

FORM ID = UA-F20-10

Figure 7. DRY ICE WARNING / CAUTION Label

Shipper's Declaration not Required.

Part B is required

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills/airbills must have the following:

1. "Dangerous Goods - Shipper's Declaration not required".
2. **Dry Ice; 9; UN 1845; III**
3. $\frac{\text{Number}}{\text{(Number pkgs)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg 904}$

Dry Ice
_____ kg.

Shipper's name and Address

UN 1845

Consignee Name and Address

9

Logos # 106426