

National Human Exposure Assessment Survey (NHEXAS)

Maryland Study

Quality Systems and Implementation Plan for Human Exposure Assessment

Emory University
Atlanta, GA 30322

Cooperative Agreement CR 822038

Standard Operating Procedure

NHX/SOP-F10

Title: Collection, Storage, and Shipment of Urine Samples for
Metal, Pesticide, and Creatinine Analysis

Source: Harvard University/Johns Hopkins University

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.

1 Title of Standard Operating Procedure

NHEXAS Harvard/Emory/Johns Hopkins University Standard Operating Procedure:
**F10 Collection, Storage, and Shipment of Urine Samples for Metal, Pesticide, and
Creatinine Analysis, Rev. 1.0.**

2 Overview and Purpose

This SOP describes urine sample collection, handling, and shipping requirements. Samples will be collected on Day 2 of each Cycle. The sample will be analyzed for metals, pesticides, PAHs, and creatinine.

3 Discussion

This SOP has been developed based on CDC's *Specimen Collection and Shipping Protocol (NHEXAS)* (Prepared: 4/19/91, Revised: 05/01/95), and on the results of pretests conducted in April and May 1995.

4 Personnel Responsibilities

4.1 Sampler Preparation

Prior to the field visit, the Field Interviewer is responsible for ensuring that a 250 mL, ID-labeled plastic urine collection cups and instructions are provided for the target individual. No special preparation of the urine collection container is necessary.

The Phlebotomist is responsible for ensuring that the appropriately labeled containers of the following types and sizes are on-hand at the time the urine is picked up from the target individual: 1 15-ml blue capped plastic tube, 2 50-ml Wheaton bottles, 1 2-ml cryovial.

The Field Coordination Center (FCC) Clerk is responsible for preparing Field Packets (including printing ID labels and affixing them to forms) before sampling.

4.2 Appointments and Reminders

Making appointments and sending reminders are the responsibility of the FCC telephone interviewers.

4.3 Sample Collection

The Interviewer is responsible for supplying the target individual with information needed to take the urine sample. The target individual is responsible for supplying the urine sample. The Phlebotomist will pick up the urine sample on Day 2.

Each sample will be divided at the Union Memorial Hospital (UMH) laboratory. A UMH designate will be responsible for division.

4.4 Storage

Immediate storage of the sample prior to delivery to the transfer site or UMH is the responsibility of the Phlebotomist. Custody will be turned over to the transfer site staff for transfer to UMH or directly to UMB by the Phlebotomist.

The FCC Clerk is responsible for ordering and maintaining an adequate supply of cold packs and dry ice.

4.5 Shipment

Transportation from the residence of the target individual to the transfer site or to UMH is the responsibility of the Phlebotomist.

Transportation from the transfer site to UMH is the responsibility of the transfer site staff.

Transportation from UMH to the transfer site and to the FCC is the responsibility of the Phlebotomist or the FCC.

Shipment from the transfer site or the FCC to the analytical laboratory is the responsibility of the FCC.

4.6 Analysis

Proper laboratory procedures will be the responsibility of the analyzing laboratories, assumed to be CDC and EPA-HERL.

5 Required Equipment and Reagents

5.1 Field Coordination Center (preparation for sampling)

- 1 250-mL sterile, plastic urine collection cups, with lids
- 1 plastic bags, quart size, Ziploc or equivalent
- marker to mark bags
- Cycle 1: extra cups in bags

5.2 Field Sampling

Day 1

- 1 250-mL sterile, plastic urine collection cup with lid and label, in plastic bag
(from section 5.1)
- instruction sheet

Day 2

- cooler
- rack to hold cup in cooler
- cold pack

- 1 15-ml blue capped plastic tube
- 2 50-ml Wheaton bottles with aluminum caps
- 1 2-ml cryovial

5.3 Sample Tracking and Paperwork

ID labels:

Prepared and affixed to sample containers at FCC
7 sample ID labels with sample type 61: 2 for "Urine" logsheets, 3 for chain-of-custody form, 1 for collection cup, 1 spare 5 ID labels with each of the following sample types: 62 (metals), 63 (pesticides/PAHs), 65 (creatinine): 1 for fraction containers, 3 for chain-of-custody forms, 1 spare

- 1 plastic bag, resealable (Ziplok or equivalent), about 6" x 9"
- Field Packet for household: "Day 2" logsheet
- Field Manual (SOPs)
- clipboard
- pens, ballpoint

5.4 Division

- ID labels (see section 5.3)
- biological safety hood
- powder-free gloves
- safety glasses
- laboratory apron
- racks to hold tubes
- 15-mL conical-bottom blue capped plastic tube (for metals fraction) -- supplied by CDC
- 2 60-mL glass Wheaton bottles (for pesticide and PAH fractions) -- supplied by CDC
- 2-mL cryovial (for creatinine fraction) -- supplied by CDC
- Teflon-lined butyl rubber stoppers and aluminum seals for Wheaton bottles
- crimper for aluminum seals
- refrigerator and freezer (-20°C)
- polyethylene disposable single-use liquid droppers
- freezer tray that will hold the same number of sample containers that will fit into a shipping container
- disposal container for cups and leftover urine
- ultra-high-purity water (Milli-Q or equivalent) for blanks

5.5 Shipping

- shipping containers for frozen specimens
- dry ice, cut into 1" x 7" x 10" slabs, 1 per shipping container
- bubble wrap
- shipping labels: address labels, "DRY ICE"

6 Procedure

The procedures outlined here are based on CDC *Specimen Collection and Shipping Protocols*.

All staff handling urine will follow appropriate precautions as specified by CDC and UMH, which may include working in a biological safety fume hood and wearing gloves (double when appropriate), safety glasses, and a lab coat. For a flow chart of procedures for urine and blood samples, see page 12.

6.1 Preparation for Collection

6.1.1 Identification Labels

At the Field Coordination Center, the FCC Clerk will:

- Ø Print ID labels and inspect them to make sure that they are correct for the household, Cycle, and sample types.
- Ø Affix "sample type 61" labels to the two layers of the Urine logsheet, and one to the three layers of the chain-of-custody form. Check the "61" box on the chain-of-custody form.
- Ø Affix "62", "63", "64", and "65" labels to 4 chain-of-custody forms (one label per chain-of-custody form). Check the appropriate boxes on the forms.
- Ø Affix "62", "63", "64", and "65" labels to one chain-of-custody form (4 urine labels on one form). This chain-of-custody form should also have labels for the serum-pesticide and serum-lipid samples. Check the appropriate "6 empty container" box on the forms.
- Ø On the instruction sheet, write the date and day of the week when each sample should be collected.
- Ø Assemble the Field Packet for the household.
- Ø Remove the collection cup, with lid in place, from its plastic wrapping. Be careful not to dislodge the lid or touch the inside of the cup or lid.
- Ø Affix a "sample type 61" label to the cup. For the target individual's reference, use a marker to label a plastic bag with the day of the week and date of Day 2, when it is to be used. Place the cup in the bag and seal the bag.
- Ø Affix a sample type "62" label to 15 ml plastic tube, a sample type "63" to one of the Wheaton bottles, a sample type "64" to the other Wheaton bottle, and a sample type "65" label to the 3 ml cryovial. For the target individual's reference, use a marker to label a plastic bag with the day of the week and date of Day 2, when it is to be used. Place the cup in the bag and seal the bag.
- Ø At the beginning of Cycle 1, seal two collection cups into bags, without labels. These will be spares to be kept in the Interviewer's tote box.

6.1.2 Transfer and Inspection of Equipment

On Day 1 at the Field Coordination Center, the Interviewer will:

- Ø Make sure that all paperwork is present and that the ID labels are correct. Check that the collection cups are correctly labeled.
- Ø Pack the tote boxes to go to the field.

On Day 2 at the FCC, the Phlebotomist will:

- Ø Make sure that all paperwork is present and that the ID labels are correct. You will need the Day 2 logsheet and the chain-of-custody forms with sample types 61, 62, 63, and 65.

6.2 Location of Sampling

The sample collected on Day 2 will be the first morning void. The target individual will supply the samples in his/her residence.

6.3 Sample Collection Procedure

6.3.1 On Day 1, the Interviewer will:

- Ø Give the instruction sheet to the target individual and make sure that s/he understands the instructions:
 - Urine sample is to be taken on day 2, and will be first morning void.
 - Wash your hands with soap and water.
 - Use the collection cup labeled for the correct day.
 - The collection cup should not be opened until just before voiding.
 - Leave the lid turned up while voiding. Fill the cup at least 3/4 full if possible.
 - It is most important that the inside of the container and the cap not be touched or come into contact with any parts of the body or clothing or external surfaces. Exposure to air should be minimized.
 - Immediately put the lid securely on the filled cup.
 - Place the filled cup in the plastic bag. Keep the cup standing upright. Seal the bag.
 - Place the filled cup in the cooler that has been left for duplicate food samples, or in the household refrigerator.
- Ø Leave written instructions and the sample cup with the target individual. Suggest

keeping them in a location that will help him or her remember to take the sample. For example, the instruction sheet could be taped to the inside of the bathroom medicine cabinet.

- Ø Record your name (interviewer), the date, and any comments on the "Day 1" sections of the "Urine" logsheet.
- Ø Return the logsheets and chain-of-custody forms to the FCC to be used on Day 2.

6.3.2 On Day 2, the Phlebotomist will:

- Ø Record your name (phlebotomist) and the date on the Day 2 logsheet.
- Ø Ask the target individual for this morning's sample.
- Ø If the target individual forgot to take the sample, ask him or her to do it while you are at the residence. On the logsheet, note this and write the time the sample was actually taken.
- Ø Make sure that the cup is in a plastic bag that is properly sealed. Place the sample in the cooler with the blood sample.
- Ø Deliver the sample to UMH (if you are in the Baltimore area) or to the transfer site. See section 6.3.5.

6.3.3 Transportation from Field to UMH

The Phlebotomist will:

- Ø Deliver the sample to UMH (if you are in the Baltimore area) or to the transfer site.
- Ø Transfer custody of the samples to UMH. Both interviewer/phlebotomist and UMH designate will sign the chain-of-custody form for each sample.

Transfer site staff will:

- Ø Receive custody of samples from the Phlebotomist or Interviewer.
- Ø Keep samples refrigerated.
- Ø Make sure that all samples for the day have been received.
- Ø Deliver samples and fraction containers to the laboratory at UMH the same day the samples are collected. The UMH laboratory will need to stay open for late-hours delivery and division of samples.

6.3.6 Division

Universal Precautions will be observed. Refer to MMWR Volume 36/No. 2S.

The UMH laboratory will:

- Ø Receive custody of the sample from the transfer site staff. Both will sign the chain-of-custody form. Refrigerate samples until ready to divide them.
- Ø Write your name, signature, and the date on the logsheet.
- Ø Wear powder-free gloves and safety glasses. Work in a biological safety hood.
- Ø Assemble the containers listed in the table above. field sample label. (The field sample type is 61 or 71.)
- Ø Gently swirl the collection cup to resuspend any solids.
- Ø Open the blue capped tube labeled for metals. Pour in urine to the 12 mL line. (To reduce chances of contamination, do not use a pipet.) Recap and freeze.
- Ø Open the 2 mL cryovial labeled for creatinine. Pour in urine to the 2 mL mark. Recap and freeze.
- Ø Pour approximately 50 mL of urine into each Wheaton bottle (about 1 inch from the top of the bottle).
 - ® Place a Teflon-lined stopper (Teflon side down) and an aluminum seal on each bottle. Use the crimping tool to secure the aluminum seal. Freeze as soon as possible.
- Ø In the freezer, keep the containers for each subject together in the same tray.
- Ø Dispose of the leftover urine and the collection cup by standard methods approved by CDC and UMH.

6.4 Sample Labeling

A unique ID number will be assigned for each sample or fraction (see HSPH SOP G03 "Identification Numbers for Samples and Forms"). Printed labels will show the ID number in bar-code and human-readable format. The FCC Clerk will affix identical labels to the sample container, the logsheet, and the chain-of-custody form.

6.5 Preservation and Storage

Samples will be stored as stipulated by the CDC. Field samples will be transported in an insulated container with cold packs, and then stored in a refrigerator until they can be divided. Fractions will be frozen prior to shipping to the analytical laboratory. Dry ice will be added to the insulated shipping container to maintain the frozen state.

6.6 Handling and Shipping

Shipping will be effected in a timely basis with input from the analytical laboratory (CDC). Shipment will occur using Federal Express or similar carrier in a manner to ensure that no Saturday, Sunday, or holiday deliveries will occur. Typically, samples will be shipped on Tuesday and Thursday of week 1, and the following Monday. Acknowledgment of receipt of shipments will be in writing. Chain-of-Custody forms and CDC Specimen Shipping Lists will be shipped with all samples. Photocopies will be retained at the FCC. Samples will be packed and shipped by FCC personnel according to "Storage and Shipping of Samples."

6.7 Laboratory Analysis

Laboratory procedures have been developed by CDC. Metal analysis will be by GFAA. Pesticide analysis will be by GC/MS.

6.8 Data Workup

Field and laboratory data will be returned to Emory in both magnetic and hardcopy format. Data will be coded and checked, computer entry verified, and discrepancies resolved. Analytical results will then be merged with questionnaires and other data, using the ID number as the merge parameter.

6.9 Sample Tracking

The ID number will allow tracking of each sample or fraction. A data base management system will ensure knowledge of the status and location of any sample or fraction at any time including retrospectively.

The chain-of-custody form will accompany the sample or fraction wherever it goes. Anyone who receives, transfers, or ships the sample or fraction will sign and date it, and keep a photocopy. It must clearly contain all necessary information so that the custody of the sample can be determined at any time. Airbills, bills of lading, etc., are acceptable substitutes when a commercial or government carrier is used; copies of such bills will be attached to the chain-of-custody form.

7 Quality Assurance Procedures

7.1 Use of Laboratory and Field Blanks

Provisions for laboratory and field blanks and spikes are under development.

7.2 Duplicate Sampling

Because of the burden placed upon the respondent, no duplicate sampling will be done. Ten percent of the samples will be split into two replicate samples by Field Coordination Center

staff. (Universal Precautions will be observed. Refer to MMWR Volume 36/No. 2S.) Such replicate samples will be assigned a unique identifying number and submitted as normal samples within a batch. Laboratory personnel will have no knowledge of which samples are replicates. Analysis of replicate samples will be in accordance with the duplicate and replicate sample data analysis procedures.

7.3 Tolerance Limits, Detection Limits, and Sensitivity Limits

This section will be developed when CDC or other collaborating laboratory provides the final analysis protocol.

7.4 Tolerance Limits, Detection Limits, and Sensitivity Limits

The table below shows the expected concentrations and limits of detection given the sampling protocol.

Category	Pollutant	Expected Concentration	Expected Limit of Detection Given Sampling Protocol
Metals	Arsenic	<100 ug/L	4 ug/L
	Cadmium	< 2 ug/L	0.1 ug/L
	Chromium	< 2 ug/L	0.2 ug/L
	Lead		
Pesticides	Chlordane		
	Chlorpyrifos		0.5 µg/dL
	4,4'-DDD		
	4,4'-DDE		
	4,4'-DDT		
	Dieldrin		
	Heptachlor		
	Malathion		0.5 µg/dL

8 References

Emory University/Union Memorial Hospital Standard Operating Procedures:

G03 Identification Numbers for Samples and Forms

G04 Chain-of-Custody and Sample Tracking

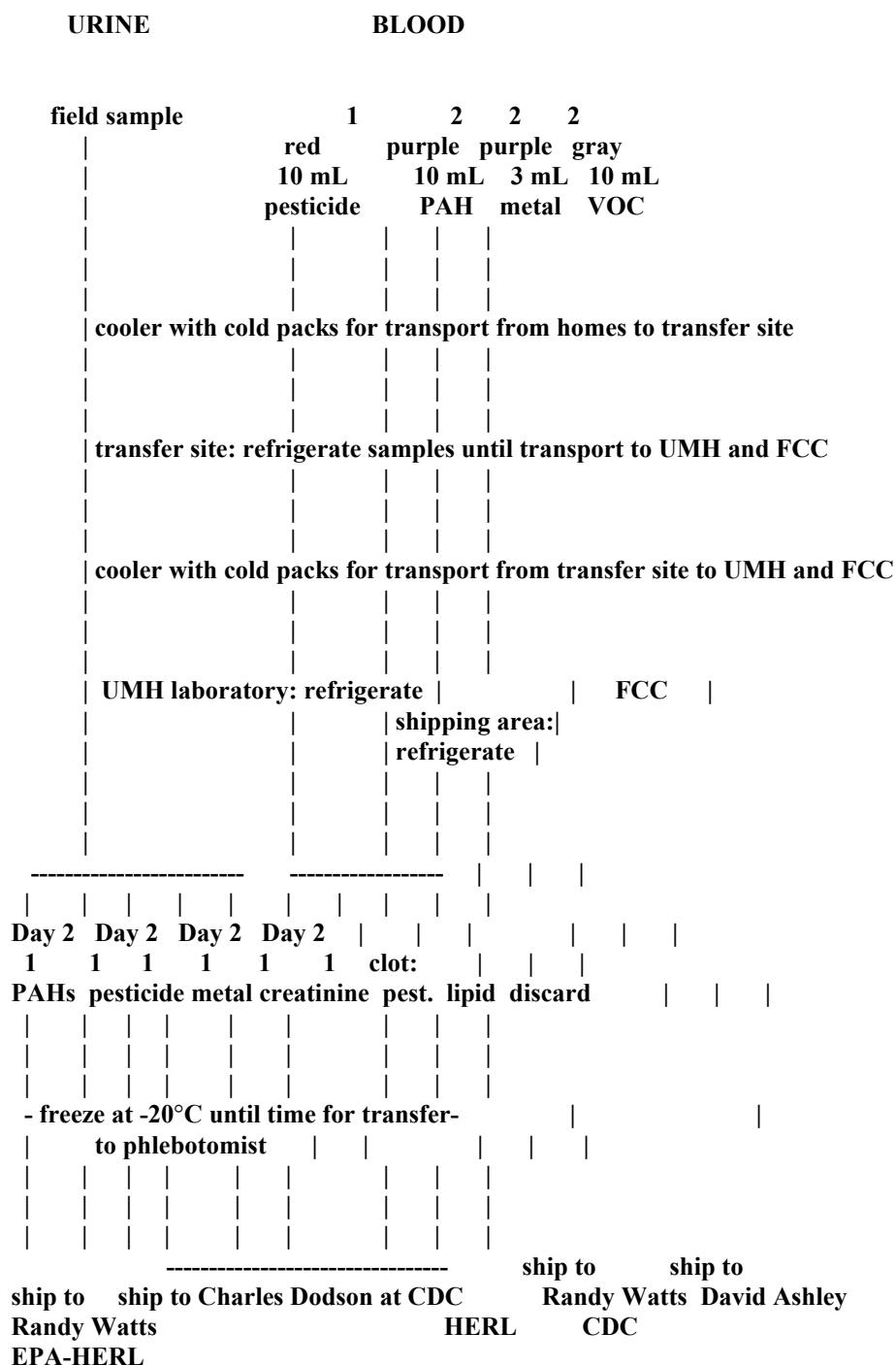
G05 Problem Management

G05 Storage and Shipping of Samples

F01 Field Sampling -- General Information

MMWR Volume 36/No. 2S.

Specimen Collection and Shipping Protocol (NHEXAS). Division of Environmental Health Laboratory. Sciences National Center for Environmental Health and Injury Control Centers for Disease Control Atlanta, Georgia 30333. Prepared: 4/19/91. Revised: 05/01/05.) Modifications to this protocol include preparation for analysis of metal species in blood and urine and pesticide metabolites only.



Urine Specimen Collection Instructions

NHEXAS Phase I Study

Day 2: date _____ day of week _____

- Urine samples are to be taken on days 2 and will be first morning void.
- Wash your hands with soap and water.
- The collection cup should not be opened until just before voiding.
- Leave the lid turned up while voiding. Fill the cup at least 3/4 full if possible.
- It is most important that the inside of the cup and the lid not be touched or come into contact with any parts of the body or clothing or external surfaces. Exposure to air should be minimized.
- Immediately put the lid securely on the filled cup.
- Place the filled cup in the plastic bag. Keep the cup standing upright. Seal the bag.
- Place the filled cup in the small refrigerator that has been left for duplicate food samples.