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-F11**

Title: Collection, Storage, and Shipment of Blood Samples for Metal, Pesticide, PAH, VOC, and Lipid 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

1 Title of Standard Operating Procedure

NHEXAS Harvard/Emory/Johns Hopkins University Standard Operating Procedure:
F11 Collection, Storage, and Shipment of Blood Samples for Metal, Pesticide, PAH, VOC, and Lipid Analysis, Rev 1.0.

2. Overview and Purpose

This protocol describes blood sample collection, handling, and shipping requirements. Seven samples will be taken from a single puncture: two 3-mL tubes for metals, one 10-mL tube for pesticides, one 10-mL tubes for PAHs, and one 10-mL tubes for VOCs, for a total of 36 mL of blood.

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

A professional phlebotomist, licensed in the state of Maryland where sampling will take place, will be responsible for all activities associated with blood sampling. All individuals taking or handling blood samples should follow Universal Precautions as outlined by CDC. Refer to MMWR Volume 36/No. 2S.

4.1 Sampler Preparation

The phlebotomist should prepare all sampling apparatus prior to leaving the Field Coordination Center (FCC).

The 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 phlebotomist is responsible for collection of all blood samples in the field.

Pesticide fractions will be centrifuged and the serum divided at Union Memorial Hospital (UMH) laboratory. A UMH designate will be responsible.

4.4 Storage

Storage of samples in transit from the residence and prior to transport to the UMH laboratory is

the responsibility of the phlebotomist. Storage of samples at the FCC is the responsibility of the FCC clerk. Packaging of blood samples for shipment from the FCC to the CDC is the responsibility of the FCC clerk.

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

4.5 Shipment

Transport of samples from the field to the UMH laboratory is the responsibility of the Phlebotomist. Packaging and transport of serum samples for shipment from UMH to the FCC is the responsibility of the Phlebotomist. Packaging of whole blood and serum samples from the FCC to the CDC and EPA-NERL is the responsibility of the Field Coordinator.

4.6 Analysis

Analysis of blood and serum samples is the responsibility of the CDC laboratory and EPA-HERL laboratory.

5. Required Equipment and Reagents

5.1 Field Coordination Center (preparation for sampling)

All sampling equipment and supplies will be supplied by CDC. Vacutainers have expiration dates. The FCC Coordinator will log them in as specified in SOP L01 "Purchase of Consumables," keep a "to do" list of Vacutainers and other supplies that will soon reach their expiration dates, and make sure that enough fresh Vacutainers and other supplies are available for each Cycle by providing this information to designated Emory personnel.

5.2 Field Sampling (All sampling equipment and supplies will be supplied by CDC)

disposable gloves
alcohol wipes -- at least 4
sterile gauze squares
Vacutainer tubes for blood collection:

metals:	2	3-mL purple top tubes
pesticides:	1	10-mL red top tube
PAHs:	1	10-mL purple top tubes
VOCs:	1	10-mL gray-top tubes

21g 3/4" butterfly assembly with multiple sample luer adapter, sterile
23g 3/4" butterfly assembly with multiple sample luer adapter for children and difficult sticks.
21g or 22g Vacutainer multiple sample needles
10 cc plastic syringe for obtaining samples from children
tourniquet
Vacutainer holder and adapters for pediatric tubes
adhesive bandage, Band-Aid or equivalent
sharps disposal container for used needles
cooler with cold pack for transporting samples to FCC

first aid kit

5.3 Sample Tracking and Paperwork

ID Labels:

Sample Type	Sample Type ID Number	Number of Labels
blood for metals	81 (print 2 sets with different last "random" digit)	8: 2 for field logsheet 3 for chain-of-custody form 2 for sample tubes 1 spare
blood for pesticides	82	9: 2 for field logsheet 2 for laboratory logsheet 3 for field sample chain-of-custody form 1 for sample tube 1 spare
blood for PAHs	83	7: 2 for field logsheet 3 for chain-of-custody form 1 for sample tubes 1 spare
blood for VOCs	84	7: same as blood for PAHs
serum for pesticides	85	10: 2 for laboratory logsheet 3 for chain-of-custody form 3 for empty container chain-of-custody form 1 for serum bottle 1 spare
serum for lipids	86	10: same as serum for pesticides

plastic bags, resealable (Ziplok or equivalent), about 6" × 9"
Field Packet for household: logsheets, chain-of-custody forms
Field Manual (SOPs)
clipboard
pens, ballpoint

5.4 Centrifugation of Pesticide Samples

Pasteur pipette and bulb, 2 pipettes per tube of blood
5 mL Wheaton bottle (1 per tube of blood)
10 mL Wheaton bottle (1 per tube)
stoppers for Wheaton bottles:
    Teflon-lined flat-face for serum for pesticides (1 per tube)
    concave face for serum for lipids (1 per tube)
aluminum seals for Wheaton bottles (2 per tube)
crimping tool for aluminum seals
ID labels (see section 5.3)
racks for tubes

centrifuge capable of attaining a force of 1000 g (see section 6.3.3)
biological refrigerator and freezer (-20° C or below)

5.5 Shipping

shipping containers for frozen and refrigerated specimens
dry ice, cut into 1" × 7" × 10" slabs, one per "frozen" shipping container
cold packs
bubble wrap
shipping labels: address labels, "HUMAN BLOOD -- THIS SIDE UP," "DRY ICE," "REFRIGERATE -- DO NOT FREEZE"

6. Procedure

The procedures outlined here are based on CDC *Specimen Collection and Shipping Protocols*. All staff handling blood or serum 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 blood and urine samples, see page 12.

6.1 Preparation for Sampling

6.1.1 Identification Labels

At the Field Coordination Center, the FCC Clerk will:

- Make sure that the ID labels are the correct ones for the household, Cycle, and sample.
- Affix labels to tubes:
  "sample type 81" label to each 3-mL purple top metals tube (2 tubes, different last digit)
  "82" label to the 10-mL red top pesticide tube
  "83" label to each 10-mL purple top PAH tube
  "84" label to each 10-mL gray top VOC tube
- Affix five ID labels to each layer of the field logsheet: two "81" (different last digit), one "82," one "83," and one "84" to each of the three layers.
- Affix three ID labels to each layer of the laboratory logsheet: one "82," one "85," and one "86" to each of the two layers.
- Affix one ID label to each of seven chain-of-custody forms: two "81," one "82," one "83," one "84," one "85", and one "86".
- Affix one ID label to each of the Wheaton bottles: one "85" and one "86".
- Affix two ID labels to the chain-of-custody form for empty biological fraction containers: one "85" and one "86".

6.1.2 Transfer and Inspection of Equipment

On Day 2 at the Field Coordination Center, the Phlebotomist will:

- Make sure that all paperwork is present and that the ID labels are correct. Check that the collection tubes are correctly labeled.

- Pack the tote boxes to go to the field.

6.2 Location of Sampling

Sampling will be done in the target individual's home. Use a reasonably quiet area with a chair where the individual can sit and a table on which to rest his/her arm. Move or cover any family possessions that would be damaged by spilled drops of blood, such as a cloth tablecloth. A kitchen table, if available, is usually most suitable.

6.3 Sample Collection Procedure

6.3.1 Collection
(Follow Universal Precautions as outlined by CDC. Refer to MMWR Volume 36/No. 2S.)

The phlebotomist will:

- Explain the procedure to the target individual. Mention possible side effects (bruising, dizziness) and what to do if they occur. If the target individual is concerned about the amount of blood, explain that the total of the tubes is only about 1/10 of a standard blood donation.

- Locate a suitable table and chair for blood collecting and lay out blood collection supplies.

- Write your name and signature, the date, and the starting time on the field logsheet.

- Put on disposable gloves.

- Locate the puncture site. Hold an alcohol wipe with 2 fingers on one side so that only the other side touches the puncture site. Wipe the area in a circular motion beginning with a narrow radius and moving outward so as not to cross over the area already cleaned. Repeat with a second alcohol wipe.

- Locate the vein and cleanse as described above, then apply the tourniquet. If it is necessary to feel the vein again, do so; but after you feel it, cleanse with an alcohol wipe again, and dry with a sterile gauze square.

- Make sure that all the alcohol has evaporated, to prevent contamination of the specimen for VOCs. If it has not, swab the site with a dry sterile gauze square and allow the site to dry for 5-10 seconds.

- Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry into the skin and pull the skin taut.

- Approach the vein in the same direction the vein is running, holding the needle at a 15° angle with the examinee's arm.

- Grasping the butterfly wings with bevel facing up, push the needle firmly and deliberately into the vein. Activate the vacuum collection tube. If the needle is in the vein, blood will flow freely into the tube. If no blood enters the tube, probe for the vein until entry is indicated by blood flowing into the tube.

- For collection, **loosen the tourniquet immediately after blood flow is established** and release entirely as the last tube fills. Collect the samples in the following order:
  (1) 1 10-mL red top tubes for pesticides. (Do not invert. Keep upright at all times to avoid any contact with the rubber stopper while clotting.)
  (2) 2 10-mL purple top tubes for PAHs. (Invert 4-5 times to dissolve anticoagulant.)
  (3) 2 10-mL gray top tubes for VOCs. (Invert 4-5 times.)
  (4) 2 3-mL purple top tubes for metals. (Invert 4-5 times.)

- After the last tube has filled, withdraw the needle with a swift backward motion. When the needle is out of the arm, press gauze firmly on the puncture. Heavy pressure as the needle is being withdrawn should be avoided because it may cause the sharp point of the needle to cut the vein.

- Have the target individual raise his/her arm (not bend it) and continue to hold the gauze in place for several minutes. This will help prevent hematomas.

- Dispose of the needle in the sharps disposal container.

- Report to the physician any reaction experienced by the target individual during the venipuncture procedure.

- Apply a bandage to the target individual's arm.

- Place the samples in the cooler for transportation.

- Write the finishing time and any comments or problems on the field logsheet.

6.3.2 Transportation of Samples

The phlebotomist will:

- Deliver the pesticide samples (red top), fraction containers, and Pasteur pipette to UMH (if you are in the Baltimore area) or to the transfer site.

- Deliver the non-pesticide blood samples to the FCC or to the transfer site.

- Transfer custody of the samples to UMH, FCC, or transfer site staff. Both phlebotomist and recipient will sign the chain-of-custody form for each sample.

Transfer site staff will:

- Receive custody of samples from the phlebotomists.
- Keep samples refrigerated.
- Make sure that all samples for the day have been received.
- Deliver pesticide samples to the laboratory at UMH (if necessary). Pesticide samples must be delivered to UMH quickly enough to be centrifuged within 12 hours of collection.
- Deliver non-pesticide samples to the FCC (if necessary).

At UMH, staff will:

- Deliver blood samples for pesticides (red top tubes) to the laboratory for centrifugation.

At the FCC,

- Deliver the purple and gray top tubes to the laboratory shipping area. If they are not to be shipped immediately, they will be refrigerated.

6.3.3 Centrifugation of Blood Samples for Pesticides
(Follow Universal Precautions as outlined by CDC. Refer to MMWR Volume 36/No. 2S.)

The UMH technician will:

- Wear powder-free gloves and safety glasses. Work in a biological safety hood.
- Receive custody of the blood samples for pesticides (10 mL red top tubes) from the phlebotomist or the transfer site staff. Both will sign the chain-of-custody form for each sample.
- Check the field logsheets and arrange the samples in order of time of collection. Refrigerate samples until ready to centrifuge them.

Blood samples are higher priority than urine samples, but both should be processed within 12 hours of collection.

Beginning with the samples that were collected first:

- Affix ID labels to the sample containers. For each tube you will need:

Priority	Analyte	Container	Sample Size	Sample Type Number
1	pesticides	10-mL Wheaton bottle	2-4 mL	85

2	lipids	5-mL Wheaton bottle	1-2 mL	86

- Set the centrifuge to the rpm that will attain a force of 1000 g. To calculate the necessary rpm, use the formula

$$\text{rpm} = \frac{9450}{\sqrt{r}}$$

where r is the distance in centimeters from the center of rotation to the bottom of a tube when it is extended in the centrifuge head. For example:

r (cm)	rpm
12	2700
14	2500
16	2400
18	2200
20	2100
22	2000

- Load tubes into the centrifuge. Spin for 10 minutes. On the laboratory logsheet, write your name, signature, the date, the time spinning began, and any comments.

- Set the tubes in a rack. Estimate the volume of serum in the first tube. Get the two serum bottles whose labels match the label on the tube.

  - Attach a pipette bulb to a Pasteur pipette to transfer the serum as follows:

    → Transfer 2-4 mL of serum to the 10-mL bottle. Deliver the serum directly to the bottom of the bottle, taking care not to get serum around the mouth of the bottle where the Teflon stopper will be placed. This prevents the serum from contacting the stopper where contaminants from the uncoated part of the stopper may be introduced to the specimen.

    → Get a Teflon-faced stopper. The flat face is Teflon coated and very shiny. The other area of the stopper is not coated, and any exposure to this part could cause contamination of the specimen. Place the stopper on the mouth of the bottle with the coated side down. Add an aluminum seal and crimp it using the crimping tool.

    → Transfer 1-2 mL of serum into the 5-mL bottle.

    → Get a concave-faced stopper and place it on the bottle with the concave side down. Add an aluminum seal and crimp it using the crimping tool.

  - Set the bottles upright in the freezer. On the laboratory logsheet write the time they were put into the freezer, and any comments. Store the bottles in the freezer until they are shipped to the CDC.

- Discard the tubes with clots 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 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 each sampling tube, the logsheet, and the appropriate chain-of-custody form.

6.5 Preservation and Storage

While being transported to the transfer site, the FCC, or UMH, whole blood samples will be stored in a cooler provided for the storage and transport of biological samples. At the transfer site, whole blood samples will be removed from the cooler and placed in an biological refrigerator to await shipping to UMH. At the FCC, whole blood samples to be analyzed for metals, PAHs, and VOCs will be stored in a biological refrigerator to await shipping to the CDC analytical laboratory.

At UMH and the FCC, serum fractions to be analyzed for pesticides and lipids will be frozen below -20°C to await shipping to the CDC analytical laboratory. Serum samples will be transported in coolers with cold packs or dry ice at all times.

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. A copy of each chain-of-custody form will be sent to the Lisa Melnyk at EPA-NERL and to the Principal Investigator at Emory University. Samples will be packed by FCC personnel according to SOP G05 “Storage and Shipping of Samples.”

6.7 Laboratory Analysis

All laboratory analyses will be done by CDC or their designates, except for PAHs, which will be done by EPA-HERL.

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 status and location of the sample or fraction 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 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. Replicate sampling procedures will be worked out with the collaborating laboratory. 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 Quality Assurance

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		
	Cadmium	< 5 ug/L	0.1 ug/L
	Chromium		
	Lead	1-50 µg/dL	0.5 µg/dL
Pesticides	Chlordane		0.5 µg/dL
	Chlorpyrifos		
	4,4'-DDD	< 1 ppb	0.1 ppb
	4,4'-DDE	< 20 ppb	0.1 ppb
	4,4'-DDT	< 1 ppb	0.1 ppb
	Dieldrin	< 1 ppb	0.1 ppb
	Heptachlor	< 1 ppb	0.1 ppb
	Malathion		
PAHs	Anthracene		
	Benzo(a)pyrene		
	Chrysene		
	Phenanthrene		

8. References

Harvard/Emory/Johns Hopkins University Standard Operating Procedure:
G03 Identification Numbers for Samples and Forms
G04 Chain-of-Custody and Sample Tracking
G05 Storage and Shipping of Samples
L01 Purchase of Consumables

IATA Packing Instructions 650.

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: 05/01/95.)

Flow Chart for Biological Samples