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

Field Operations Protocol **CDC-Compendium**

Title: Compendium Of Method Summaries For Collection And Analysis Of Metals And VOCs In Blood And Urine

Source: Centers for Disease Control and Prevention

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

COMPENDIUM OF METHOD SUMMARIES FOR COLLECTION AND ANALYSIS OF METALS AND VOCs IN BLOOD AND URINE

TABLE OF CONTENTS

Protocol for Urine and Blood Specimen Collection and Shipping for Metals

Introduction

The proper collection, processing, storage and shipment of physiologic specimens from participants is critical to the success of the study. The following sections describe the procedures to be followed for all specimen collections. Please familiarize yourself with the study protocol and insure that you understand the concept of the study, the role of all the personnel involved, and your own role.

Note that subjects are not required to report for blood and urine collection in a fasting state although blood and urine collection should be accomplished early in the visit to avoid discomfort to the subject. Blood and urine collection must be completed and processed under carefully controlled conditions of good laboratory practice. Blood separation and processing must be accomplished promptly to avoid degradation of the specimen.

It is extremely important that all records associated with each subject be maintained in an organized and complete manner to ensure that all information is properly collected and accurate. Specimens should be labeled promptly and processed as a unit or "run" and precautions must be taken to avoid patient-specimen-label-record mix-ups. Careful planning and a well organized work area will keep such errors at a minimum. Some of the information required for the specimen label and shipping list will be collected at the time of specimen collection. Problems in blood and/or urine collection should be noted in the sample log and in the comments section of the shipping list.

URINE COLLECTION AND PROCESSING

Urine collection procedure

1. Materials needed per participant.

   - Urine collection cup (250 mL. plastic, sterile)
   - Preprinted label

2. Preparation of urine collection cup for participant.

   - Remove the collection cup with the cap in place from its plastic wrapping being careful not to dislodge the cap or touch the inside of the container or cap.
   - With the cap securely attached to the container affix the participant's preprinted label marked "URINE CONTAINER" to the urine specimen cup.

3. Instructions for urine collection.

   - The following instructions should be explained to the participant prior to urine collection:

     o Hands should be washed with soap and water.
     o The collection cup should not be opened until just before voiding.
     o The person should leave the cap turned up while voiding, then recap the filled container immediately.
     o 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.
     o The participant should deliver the capped specimen immediately to the clinic personnel.

Urine processing procedure

1. Materials and equipment needed per participant.

   - 15 mL Conical-bottom plastic tube with ORANGE cap (containing Triton X-100 and sulfamic acid as preservative for URINE MERCURY)
   - 15 mL Conical-bottom plastic tube with BLUE cap for URINE METALS
   - Powder-free lab gloves
   - Safety glasses
   - Racks
   - Deionized water (for preparing lab blanks)
   - Preprinted labels
   - Freezer (-20°C)

2. Special safety precautions.
   UNIVERSAL PRECAUTIONS (See CDC Publications for HIV-Hepatitis)

3. Processing (urine specimen)

   - Wear powder-free lab gloves, safety glasses, and work under a laboratory hood, if available.
   - Using the preprinted labels provided for each participant, add the date collected and the initials of the person preparing the aliquots and label each of the plastic tubes as follows:

Priority	Size/Type Bottle	Label
1.	15 mL ORANGE capped plastic	"URINE MERCURY"
2.	15 mL BLUE capped plastic	"URINE METALS"

- Gently swirl the specimen in the capped collection container to resuspend any solids.
- The ORANGE capped tube for "URINE MERCURY" should be filled to the 10 mL mark. THIS TUBE MUST BE EXACT (+/- 1 mL) AS TO THE AMOUNT OF URINE ADDED TO ENSURE THE PROPER RATIO OF URINE TO PRESERVATIVE.
- The BLUE capped tube for "URINE METALS" should have urine poured to the 10 mL mark. Concentrated nitric acid will be added later (at CDC) as a preservative.
- Recap and tightly seal the ORANGE capped tube and the BLUE capped tube.
- Dissolve the preservative in the ORANGE capped tube for "URINE MERCURY" by gently mixing 5-10 times on a mixer or by gently inverting the tube by hand.

4. Preparation of laboratory blanks

   - Wearing protective clothing and working under a hood, prepare one laboratory blank for "URINE MERCURY" and "URINE METALS" each day on which urine specimens are collected.
   - Select one of the 15 mL ORANGE capped plastic tubes with preservative already added and slowly add deionized water to the 10 mL line. Mix well and add a label printed for "URINE MERCURY LAB BLANK" and add the date on which it was prepared.
   - Select one of the 15 mL BLUE capped plastic tubes and add deionized water to the 10 mL line (SEE BELOW FOR INSTRUCTIONS). Cap, label with the preprinted label "URINE METALS LAB BLANK" and invert the tube five times.

5. Urine storage and shipping
   - Urine specimens should be kept frozen (-20°C) until shipment.
   - For shipment instructions, see following section: "Frozen and refrigerated specimen packing and shipping"

WHOLE BLOOD COLLECTION AND PROCESSING

NOTE: **Universal Precautions** - procedures to prevent exposure to HIV; hepatitis; etc are ASSUMED during all collection and handling of biological specimens. ALL specimens should be considered POTENTIALLY INFECTIOUS (if needed, see CDC guidelines on universal precautions).

Whole blood collection procedure

1. Materials needed per participant.

   - Gauze sponges
   - Alcohol wipe
   - Bandaid
   - 3 mL purple top tube
   - 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 children
   - Preprinted labels
   - Tourniquet
   - Vacutainer holder and adapters for pediatric tubes
   - Refrigerator
   - White storage boxes

2. Venipuncture procedure.

   - Locate a suitable table and chair for blood collecting and lay out blood collection supplies.
   - Locate the puncture site. Hold with 2 fingers on one side of the "alcohol wipe" 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 vein and cleanse in manner previously described, then apply the tourniquet. If it is necessary to feel the vein again, do so; but after you feel it, cleanse with alcohol prep again, and dry with a sterile gauze square.
   - 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 so that it is at an approximately 15^{0} angle with the examinee's arm.
   - Push the needle, with bevel facing up, 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.
- If a syringe is required to obtain the blood, attach it to the appropriate size butterfly needle and withdraw 2-3 mLs blood. After withdrawing the needle from the arm, quickly change the needle on the syringe and transfer the blood from the syringe by puncturing the top of the tube with the new needle and allowing the vacuum to draw the blood into the tube. Mix well with the anticoagulant.
- 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 examinee raise his arm (not bend it) and continue to hold the gauze in place for several minutes. This will help prevent hematomas.
- Report to the physician any reaction experienced by the participant during the venipuncture procedure.
- Label all tubes with the preprinted labels provided, and use a ballpoint pen to add the date collected and your initials to the label. The tubes should be affixed with the label showing the participant's ID number (e.g.92-0024-0001-B1).
- Place a bandaid on the subject's arm.

Whole blood processing procedure

General processing instructions

1. Determine the times 'FEDERAL EXPRESS' packages are picked up in order to connect with the best flights to Atlanta, Georgia provided the specimens are to shipped there. If Federal Express is not used as the overnight carrier, then schedules for package pick-up should be established at the outset of the study with the carrier that is selected. If a contractor is used to coordinate collection and shipment of specimens, they will provide this information.

2. Inquire about regulations in your area concerning shipment of human blood, serum, and urine specimens with dry ice and the quantity of dry ice allowed per shipper. Also, make sure the specimens will be received at CDC within 24 hours if possible.

3. For specimens that should be shipped frozen: Schedule a delivery of dry ice from a local supplier for shipping specimens. A block should be sawed at the plant into 1" slabs. Then each of these should be sawed lengthwise. A 7"x10" slab would fit easily into the shipper without having to break the slab. (Large pieces are preferable to small chunks, since they do not volatilize as rapidly.)

5. For all shipments, do not pack shippers with frozen specimens and dry ice until just before shipment.

6. Telephone the laboratory at CDC the day the shipment is mailed at (404) 488-4305. Speak with Charles Dodson.

Specimen shipping list

1. For each shipment, fill out a blank Specimen Shipping List provided by CDC. If the number of specimens in a shipment is too large to fit on one page, please use the continuation sheets provided. Please give the following information on the blank shipping lists:

   a. Page number - e.g. 1 of 4.
   b. Shipment Number- number shipments sequentially starting with 1.
   c. Number of shippers- total number of shippers containing frozen, refrigerated or unrefrigerated serum and urine specimens which are being mailed in this shipment.
   d. Type of Specimens- whole blood, serum, or urine.
   e. Number of Specimens- number of each type of specimen shipped.
   f. Name, Title, Signature, and Phone Number of person sending shipment or initials as indicated on the continuation sheets.
   g. Date shipped.
   h. Specimen ID for each participant- (e.g. 92-0024-0001). For each participant, check (X) each individual specimen type/aliquot included in this shipment.
   i. Date Collected- e.g. 01-25-92.
   j. Comments- Specify any deviations from collection, storage, and shipment protocols, and date of occurrence.

   Photocopy 2 extra copies of the completed shipping list. As will be described again later, the original will be shipped with the specimens, a copy mailed to CDC under separate cover, and a copy retained for your records.

Frozen and refrigerated specimen packing and shipping

1. Materials needed per shipper

- 1 styrofoam shipper
- 10-12 lbs dry ice FOR FROZEN SPECIMENS ONLY
- Frozen ice packs FOR REFRIGERATED SPECIMENS ONLY
- Freezer boxes
- Safety glasses or eye shield
- Strapping tape
- Gloves for handling dry ice and frozen specimens
- Sheets of bubble-pack packing material
- 'FEDERAL EXPRESS' label, preaddressed by Centers for Disease Control personnel
- DRY ICE label FOR FROZEN SPECIMENS ONLY
- KEEP REFRIGERATED label FOR REFRIGERATED SPECIMENS ONLY
- HUMAN BLOOD-THIS SIDE UP label
- CDC 'Specimen Shipping List' (completed)
- Zip-lock bag
- Whole blood, serum and urine specimen

2. Packing procedure

- When packing the shippers, use gloves to handle the dry ice to avoid burning the hands. Glasses or an eye shield should also be worn if the dry ice cakes are to be broken into small pieces. Dry ice will be used to pack the urine samples. Frozen ice packs will be used to pack the blood tubes.
- Place the specimens from each participant in the specimen boxes provided (white cardboard freezer boxes). Place each of these boxes inside one of the zip-lock bags provided.
- Pack the boxes in the bottom of the shipper. If necessary, use sheets of bubble-pack packing material to ensure the specimens vertical position. Put one layer of bubble-pack on top.
- Fill the shipper with dry ice (probably will hold 10-12 lbs) for the urine samples or with several of the frozen ice packs for the blood samples.
- Place more bubble material to even the top and place the polyfoam lid on top of the shipper.
- Insert the completed 'Specimen Shipping List' in a 12"x12" Zip-lock bag and secure to the top of the polyfoam lid with filament tape. (Remember to photocopy 2 copies of the 'Specimen Shipping List'. Keep one copy for your records and mail the other copy in a separate envelope to the following address:

  Charles Dodson Mailstop F25
  Chamblee Bldg. 17, Loading Dock
  Centers for Disease Control
  1600 Clifton Road N.E.
  Atlanta, GA 30333

- Secure the outer cardboard lid on the shipper with filament tape.

3. Shipping procedure. SEE NOTE BELOW

- Cover or remove previous address labels on all shippers.
- Label each shipper with the following:

  Charles Dodson
  Chamblee Bldg. 17, Loading Dock
  Centers for Disease Control
  4770 Buford Highway
  Chamblee, GA 30341

- HUMAN BLOOD label
- DRY ICE label for shipper with the URINE specimens - fill in the spaces with the amount of dry ice
- REFRIGERATE-DO NOT FREEZE label for the BLOOD specimens
- GENERAL DIAGNOSTIC SPECIMENS - NON-INFECTIOUS label

If 'FEDERAL EXPRESS' is being used as the carrier, call their office at 1-800-238-5355 to arrange for pick-up. Telephone the laboratory at CDC the day the shipment is mailed at (404) 488-4305 and speak with Charles Dodson.

NOTE: Any correspondence sent through the postal service will only be delivered to the Clifton Road address and forwarded from there to the Chamblee facility. Federal Express delivers to the actual physical address zip-code, therefore use the Chamblee address for any shipments by FedEx.

Pesticide metabolites in human urine

1.0 Biomarkers Measured

Pesticide metabolites can be measured in 10 mL of human urine. Table 1 lists these analytes and their corresponding parents.

Table 1. Pesticide metabolites and their parent compounds

Metabolites	Parent Compound(s)
2-isopropoxyphenol (IPP)	propoxur
2,5-dichlorophenol (25DCP)	1,4-dichlorobenzene
2,4-dichlorophenol (24DCP)	1,3-dichlorobenzene, dichlofenthion, prothiofos, phosdiphen
carbofuranphenol [2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran] (CFP)	carbofuran, benfuracarb, carbosulfan, furathiocarb
2,4,6-trichlorophenol (246TCP)	1,3,5-trichlorobenzene, hexachlorobenzene, lindane
3,5,6-trichloro-2-pyridinol (TCPY)	chlorpyrifos, chlorpyrifos-methyl
4-nitrophenol (NP)	parathion, methyl parathion, nitrobenzene, EPN
2,4,5-trichlorophenol (245TCP)	1,2,4-trichlorobenzene, fenchlorphos, trichloronate
1-naphthol (1NAP)	naphthalene, carbaryl
2-naphthol (2NAP)	naphthalene
2,4-dichlorophenoxyacetic acid (24D)	2,4-D
pentachlorophenol (PCP)	pentachlorophenol

2.0 Background and overview

Exposure to hazardous chemicals, such as pesticides or industrial waste, in water, air, or waste dump sites, continues to be a concern for residents living near industrial or contaminated sites. Pesticide applicators and users are clearly at risk of excessive exposure.

These groups would include farmers, farm workers, road workers, exterminators, gardeners, greenhouse workers, fumigators, wood workers, and others making frequent use of pesticides.

Public health officials are frequently asked to assess community and individual risk of health problems from potential exposure to these pesticides. This task is difficult because there is little information to help make decisions about these possible health risks. Measurements of environmental contaminants in air, water, food or soil provide some estimation of potential exposure; however, these multiple sources of exposure make exposure measurements by environmental measurements difficult or impossible. A better measure of an individual's exposure to these contaminants is the direct determination of those chemicals or their metabolites in human tissues or fluids such as serum or urine, a technique sometimes called biological monitoring.

As part of the Priority Toxicant Reference Range Study, we developed a reliable method for the measurement of a diverse group of urinary analytes representing exposure to pesticides. The analytes, shown in Table 1, are common pesticides or their metabolites to which many "normal" people are exposed through their food, water, or air. We had previous experience in measuring urinary phenols in low parts-per-billion concentrations [1,2], and used that experience as basis for method development, but modified the procedure to better suit our present needs.

The method was used to measure these 12 analytes in the urine of 1000 U.S. residents. The data from this study provide a baseline of "normal" or reference values for use in judging the extent of exposure among other study populations. This information can be used in making assessments of health risk from exposure to these compounds. It may also be useful in determining the relative priority of future studies.

3.0 Sample Collection

3.1 Collection procedure

Urine samples are collected in standard urine collection cups and are transferred to solvent-rinsed 60-mL glass Wheaton vials, which are sealed with Telfon®-coated stoppers. A minimum of 25 mL is requested for analysis in order to provide two 10-mL aliquots for the metabolite measurements, and a 1-mL aliquot for creatinine measurement.

3.2 Short term storage

Samples should be frozen as soon as possible after collection. The frozen samples are transported to the laboratory in shipping containers with dry ice. Samples are stored at -40 °C until analysis.

4.0 Sample Analysis

4.1 Analytical method

The method utilizes capillary gas chromatography tandem mass spectrometry (GC/MS/MS) and an isotope dilution technique with carbon-13 labelled internal standards for all analytes. It involves enzyme hydrolysis of urine, solvent extraction using laboratory

robotics, formation of a chloropropyl derivative using phase-transfer catalysis technique, chromatographic cleanup, sample concentration, and finally analysis using GC/MS/MS.

4.2 Analytical sensitivity and specificity

The limit of detection for all analytes, except 246TCP, is 1 parts per billion (ppb). The limit of detection for 246TCP is 3 ppb.

This highly specific method incorporates the use of capillary gas chromatography combined with tandem mass spectrometric determination. In conventional mass spectrometry used by many laboratories, analytes are broken apart (fragmented) into pieces (ions) and the resulting ion fragment pattern that is detected by the mass spectrometer is characteristic of that analyte. Tandem mass spectrometry, also known as mass spectrometry-mass spectrometry (MS/MS), is a more specific technique in which the ions are originally formed in the mass spectrometer are chosen and selectively broken down into smaller fragments in a second mass spectrometer. The result of tandem mass spectrometry is a very specific analysis for each compound tested. The overall combination of capillary gas chromatography and tandem mass spectrometry provides highly specific determination to assure that the resulting analyses measure the analytes of interest.

4.3 Analytical accuracy and precision

There is no standard reference material containing these pesticide metabolites in urine. The accuracy of this method is based the recovery from urine spiked with the analytes of interest. These spiked urines are used as quality control materials and are analyzed with each run of samples to insure the accuracy of each determination.

The relative standard deviations for quality control pools, spiked with the analytes and analyzed over several months, were generally about 15%.

5.0 Quality Assurance/Quality Control

A critical part of all of our analytical programs is the extensive use of quality assurance and quality control procedures. Quality control procedures adhere to standards recommended in reference 3. The performance of the mass spectrometer is evaluated and optimized before every analysis. Analytical standards are analyzed with samples and quality control samples to assure that all systems were performing according to established criteria. Quality control urine pools were developed from bulk urine spiked with analytes. The urine was sterile-filtered, dispensed into 20 mL-solvent rinsed, sterile glass Wheaton vials. These quality control samples were stored at -40 °C until analysis. Quality control samples were analyzed with each sample run and individual quality control charts were maintained to assure that the analyses were within 99% quality control limits.

6.0 Banking of samples

Quality control samples spiked with these pesticide metabolites have been stored at -40 °C for extended periods of time. Analyses of these samples have shown that the analytes are stable for at least one year.

Urine samples should be stored at -40 °C for extended periods of time. Numerous or frequent freeze-thaw cycles may lead to loss of some analytes.

7.0 Interpretation of Biomarker Measurements

We recently completed measurements of the pesticide metabolites in Table 1 in 1000 persons selected from the Third National Health and Nutrition Examination Survey (NHANES III). Although this population may not be representative of the U.S. population, it provides a good approximation of concentrations of these analytes in the urine of persons living in the U.S. Preliminary results of the analyses of these 1000 persons are shown in Table 2.

Table 2. Preliminary results of analyses of pesticide metabolites in urine of 1000 persons living in the U.S.

ANALYTE	% DETECTABLE	MEDIAN	95% PERCENTILE
IPP	7%	ND	1.6 ppb
25DCP	97%	28 ppb	760 ppb
24DCP	61%	1.7 ppb	47 ppb
CFP	1.5%	ND	ND
246TCP	12%	ND	3.6 ppb
TCPY	70%	2.1 ppb	11 ppb
NP	34%	ND	3.0 ppb
245TCP	18%	ND	3.0 ppb
1NAP	91%	4.2 ppb	37 ppb
2NAP	75%	3.0 ppb	30 ppb
24D	9.6%	ND	1.8 ppb
PCP	57%	1.3 ppb	7.7 ppb

This data can be used as a basis for comparison for populations that have potential exposure to the parent pesticides (see Table 1). For example it would be expected that most people would have concentrations of IPP (from exposure to propoxur) of 1.6 ppb [the 95% percentile] or less. Most people would not have detectable concentrations of IPP, since only 7% of the 1000 we tested had detectable concentrations.

The half lives of the parent pesticides is relatively short, usually 24 hours or less, resulting in the relatively rapid elimination of the metabolites in urine. Detection of these metabolites indicates relatively recent exposure to the parent pesticide.

References

1. RH Hill Jr, T. To, JS Holler, DM Fast, SJ Smith, LL Needham, S Binder. Residues of chlorinated phenols and phenoxy acid herbicides in the urine of Arkansas children. Arch. Environ. Contam. Toxicol., 18, 469-474 (1989).

2. JS Holler, DM Fast, RH Hill Jr, FL Cardinali, GD Todd, JM McCraw, SL Bailey, LL Needham. Quantification of selected herbicides and chlorinated phenols in urine by using gas chromatography/mass spectrometry/mass spectrometry. J. Analyt. Toxicol., 13, 152-157 (1989).

3. Taylor, JK., "Quality Assurance of Chemical Measurements", Lewis Publishers, Chelsea, MI. 1987.

Metals

1.0 Biomarkers measured

The following elements can be measured as biological markers of exposure to inorganics in either whole blood or urine.

Metal	Matrix	Analytical Method[1]	Reference
Lead	blood	GFAAS	Miller et al. **Analyst**, 112, 1701-4 (1987)
Mercury	blood	CVAAS	Greenwood et al. **J. Analyt. Tox.** , 1, pp. 265-9 (1977)
Cadmium	blood	GFAAS	Stoeppler and Brandt, **Fresenius Z. Anal. Chem.** 300, 372-80 (1980).
Arsenic	urine	GFAAS	Paschal, Kimberly, and Bailey, **ACA**, 181, 179-86 (1986).
Beryllium	urine	GFAAS	Paschal and Bailey, **At. Spectrosc.** 7, 1-3 (1986).
Cadmium	urine	GFAAS	Pruszkowska et al. **Clin. Chem.** 19, 477-80 (1983)
Chromium	urine	GFAAS	Paschal and Bailey, **At. Spectrosc.** 12, 151-4 (1991).
Lead	urine	GFAAS	Paschal and Kimberly, **At. Spectrosc.** 6, 134-6 (1985).
Mercury	urine	CVAAS	Littlejohn et al., **Clin. Chem.** 22, 1719-23 (1976).
Nickel	urine	GFAAS	Paschal and Bailey, **Sci. Tot. Env.** 89, 305-310 (1989).
Thallium	urine	GFAAS	Paschal and Bailey, **J. Analyt. Tox.** 10, 252-254 (1986).
Vanadium	urine	GFAAS	Paschal and Bailey, **At. Spectrosc.** 11, 65-69 (1990).

[1] Method Abbreviations- GFAAS- graphite furnace AAS; CVAAS- cold vapor AAS; ICP-MS - inductively coupled plasma mass spectrometry; ICAP-OES - inductively coupled argon plasma optical emission spectroscopy

2.0 Background and overview

Exposure to many inorganic toxicants can be determined by measurement of these toxicants in appropriately chosen biological fluids. A classic example is the determination of lead in whole blood, used to determine the "at risk" status of children and adults exposed to lead. EHLS has historically provided support for a wide variety of "field" studies, using well-established biomarkers to document exposure to inorganic toxicants. Among the areas of expertise of this group are modern graphite furnace AAS (GFAAS), cold vapor AAS (CVAAS), inductively coupled argon plasma optical emission spectroscopy (ICAP-OES), and inductively coupled plasma mass spectrometry (ICP-MS).

The following laboratory procedures have been found to significantly enhance quality measurements.

1) use of standards from or traceable to the National Institute of Standards and Technology (NIST);
2) use of the "best available" quality control materials in each analytical run;
3) use of methods and sample collection protocols that have proven to be rugged and reliable;
4) maintenance of a high degree of training of all analysts;
5) use of the appropriate analytical technology for measurement- not necessarily the most "high tech" approach;
6) participation (frequently as a reference laboratory) in a number of external quality control/proficiency testing programs including those from NY State, the State of Pennsylvania, the College of American Pathologists (CAP), the Center of Toxicology at Quebec, Canada (CTQ), and the CDC/Wisconsin PT program.

Most of our analytical methods are "traditional" in approach, typically one element is determined in the appropriate analytical specimen- for example, blood lead - determined by graphite furnace AAS. We have had extensive experience in developing, adapting, and troubleshooting methods of this type. Recently, we have begun to explore the usefulness of multielement techniques for either "screening" specimens for high concentrations of toxic elements, or as a truly quantitative multielement technique. Both ICAP-OES and ICP-MS lend themselves to this approach.

We have developed a "screening" method for toxic elements in urine using ICAP-OES (1), and are in the process of developing a method for ICP-MS (2). The ICP-MS methods are based on either dilution of urine (1+4) with HNO3 containing one or more internal standards; the blood analytical method is based on digestion of whole blood via microwave oven with ultrapure HNO3, followed by dilution with ultrapure water. The final dilution for blood treated this way is typically (1+19).

This general approach for blood analysis has been primarily applied to determination of lead in blood by isotope dilution mass spectrometry (3), a method we use to establish accurate target values for whole blood pools used in a national standardization program (the Blood Lead Laboratory Reference System- BLLRS). To date, we have applied the "multielement" approach in the ICAP-OES determination of multiple toxic elements in drinking water and serum (4), and to establish reference values for elements in urine (5). The

ICP-MS determination of multiple toxic elements in blood and urine, prepared as described above, has been applied to an investigation in Texas (6).

3.0 Sample Collection

Success in biological monitoring depends on a carefully developed and field tested specimen collection and shipping protocol. An extract of this protocol is attached, describing the details of collection and shipping of specimens to the laboratory. This protocol has been field tested and improved for five years, and has served as the basis of training from our staff to personnel tasked with specimen collection in a number of studies sponsored by CDC.

4.0 Sample Analysis

4.1 Method description

The analytical methods used vary from Zeeman effect background correction graphite furnace AAS to inductively coupled plasma mass spectrometry. Literature references for these methods are presented in the Introduction to this document. In many cases, the biological fluid is diluted with an appropriate matrix modifier, and vaporized thermally in a graphite furnace by electrical resistive heating. The resulting ground state neutrally charged atoms are then measured by absorbance of resonance radiation from a "line" source - either a hollow cathode lamp (HCL) or electrodeless discharge lamp (EDL). The methods based on inductively coupled argon plasma (ICP) spectroscopy use the ICP as an emission source (ICAP-OES) or radiation or as a source of positively charged ions (M+) which are subsequently measured by a quadrupole mass spectrometer (ICP-MS).

Quantification is accomplished by measurement of standards (external calibration) carried through the same analytical processes as specimens. In some cases, the same matrix (e.g. blood or urine) is spiked with the element(s) of interest and the resulting spiked material is used for calibration (matrix matched calibration).

4.2 Analytical sensitivity and specificity

Detection limits for the analytes listed are presented in Table 1. These data were calculated by estimation of the standard deviation of a "blank" or a low-concentration processed specimen, usually with ten replicate measurements. The detection limit is the concentration equal to three SD, as recommended by IUPAC.

4.3 Analytical accuracy and precision

Accuracy of these methods has been evaluated using standard reference materials (SRMs) when available. The referenced publications for each method document the accuracy, which in general is about 10% absolute. Precision of the methods is similarly referenced; generally in the 5-15% RSD range.

5.0 Quality assurance and quality control

Our system of quality control for the different methods described here has been detailed in a number of reports and publications. QC methods adhere to standard procedures described in reference 10. The basic approach is the analysis of materials (blood or urine) with known target values in duplicate with each analytical run (blanks, standards, and unknown specimens). The means and ranges of duplicate measurements are plotted on a Shewhart chart and the degree of statistical control of the measurement system is evaluated using standard statistical control criteria (9,10).

Table 1- Detection Limits for Metals

Metal	Matrix	Detection limit (µg/L or PPB)
Arsenic	urine	6
Beryllium	urine	0.6
Cadmium	urine	0.2
Cadmium	blood	0.3
Chromium	urine	0.4
Mercury	urine	0.2
Mercury	blood	0.2
Nickel	urine	1.0
Lead	urine	2.0
Lead	blood	10 (equals 1.0 µg/dL)
Thallium	urine	0.4
Vanadium	urine	1.0

6.0 Banking of samples

Recommended conditions for the storage of collected biological specimens are:

1) for whole blood: 1.5 mg/mL EDTA; -20 °C - stability at least 24 months;

2) for urine (all but mercury)- 1% v/v ultrapure nitric acid; -20 ^{o}C- stability at least 36 months;
3) for urine mercury - 20 mg sulfamic acid; 10 mg (ca. 10 uL) Triton-X-100/10 mL; -20 ^{o}C- stability at least 12 months.

These preservatives are incorporated into the collection and shipping protocol. Stability data are based on published data (D.H. Cox, **J. Analyt Tox.**, 13, pp. 367-70, 1989), and unpublished data from our laboratory (D.C. Paschal, unpublished investigation).

7.0 Interpretation of biomarker measurement

Reference intervals for concentrations of elements in "normal" or non-exposed persons is important for appropriate interpretation of biomarker levels. We have used reference ranges from human studies involving our laboratory and other studies in the literature. In general, the 95% upper limit of concentrations from "normal" subjects may be used as the upper limit of "normal" values for an element in a specific tissue. This limit should be carefully distinguished from the "lowest adverse effect" level, if known. For example, WHO published the 95% upper limit from a large population based study for blood mercury as 30 μg/L total (inorganic and methyl) mercury in whole blood (7). The "lowest adverse effect" level for total blood mercury is reported to be 200 μg/L (8). These two levels are almost one order of magnitude apart- clearly illustrating the difference in these two "limits". A table of reference intervals from selected literature is provided in Tables 2 and 3.

Table 2. Reference Ranges for Metals in Urine

Element	Method	Limit of Detection	Reference Range	Literature Reference
Ag (Sliver)	ICP-MS	0.4	9 µg/dL	1
Al (Aluminum)	ICP-MS	18.5	100 µg/dL	1
As (Arsenic)	GFAA	6	100 µg/L	1
B (Boron)	ICP-MS	180	1000 µg/dL	1
Ba (Barium)	ICP-MS	0.15	80-400 µg/L	2,3
Be (Beryllium)	ICP-MS	0.5	<20 µg/L	4
Bi (Bismuth)	ICP-MS	0.80	<20 µg/L	4
Cd (Cadmium)	GFAA	0.1	<5 µg/L	5
Ce (Cerium)	ICP-MS	0.02	-	-
Co (Cobalt)	ICP-MS	0.30	100-750 µg/L	1
Cs (Cesium)	ICP-MS	0.20	-	-
Ga (Gallium)	ICP-MS	0.05	-	-
Ge (Germanium)	ICP-MS	1.35	1400 µg/dL	1
In (Indium)	ICP-MS	0.05	-	-
Ir (Iridium)	ICP-MS	0.10	-	-
Li (Lithium)	ICP-MS	0.15	-	-
Mn (Manganese)	ICP-MS	0.75	<3 µg/L	1
Mo (Molybdenum)	ICP-MS	0.15	150 µg/dL	1
Pb (Lead)	ICP-MS	0.60	<50 µg/L	6
Pt (Platinum)	ICP-MS	0.25	-	-
Rb (Rubidium)	ICP-MS	1.55	1900 µg/dL	1
Sb (Antimony)	ICP-MS	0.45	6.2 µg/L	1
Se (Selenium)	ICP-MS	59.5	0-150 µg/L	8,9
Sn (Tin)	ICP-MS	2.05	20 µg/d L	1

Element	Method	Limit of Detection	Reference Range	Literature Reference
Sr (Strontium)	ICP-MS	0.05	390 µg/dL	7
Ta (Tantalum)	ICP-MS	0.15	5-250 µg/L	1
Th (Thorium)	ICP-MS	0.05	-	-
U (Uranium)	ICP-MS	0.05	0.05-0.5 µg/dL	1
V (Vanadium)	ICP-MS	0.65	15 µg/dL	1
W (Tungsten)	ICP-MS	0.20	-	-
Y (Yttrium)	ICP-MS	0.02	-	-
Zn (Zinc)	ICP-MS	25.1	500 µg/dL	1
Zr (Zirconium)	ICP-MS	0.05	150 µg/dL	1

Table 3. Metals in Blood - Reference Ranges

Element	Method	Limit of Detection	Reference Range	Literature Reference
Al (Aluminum)	ICP-MS	72.5	6240 µg/L	1
B (Boron)	ICP-MS	81.0	-	-
Ba (Barium)	ICP-MS	11.2	80-400 µg/L	1
Bi (Bismuth)	ICP-MS	1.80	<12 µg/L	1
Ce (Cerium)	ICP-MS	2.16	-	-
Co (Cobalt)	ICP-MS	2.88	0.5-238 µg/L	1
Cs (Cesium)	ICP-MS	2.88	-	-
Ga (Gallium)	ICP-MS	2.70	-	-
Ge (Germanium)	ICP-MS	3.06	-	-
Hg (Mercury)	CVAA	0.2	<30 µg/L	5
In (Indium)	ICP-MS	2.70	-	-
Ir (Iridium)	ICP-MS	1.62	-	-
Li (Lithium)	ICP-MS	3.96	17 µg/L	1
Pb (Lead)	GFAA	4.0	<100 µg/L	6
Pt (Platinum)	ICP-MS	2.16	-	-
Mo (Molybdenum)	ICP-MS	4.86	5-157.3 µg/L	1
Rb (Rubidium)	ICP-MS	156	2800 µg/L	1
Sb (Antimony)	ICP-MS	3.78	3 µg/L	1
Sn (Tin)	ICP-MS	7.38	7-11 µg/L	7
Sr (Strontium)	ICP-MS	4.50	<3.6 µg/L	1
Ta (Tantalum)	ICP-MS	1.80	16 µg/L	1
W (Tungsten)	ICP-MS	2.70	-	-
Y (Yttrium)	ICP-MS	173	-	-
Zr (Zirconium)	ICP-MS	3.42	10-20 µg/L	1

[1] Analytical methods: GFAA = graphite furnace atomic absorption spectroscopy; ICP-MS = inductively coupled plasma - mass spectroscopy; CVAA = cold vapor atomic absorption spectroscopy.

[2] All elements are reported in concentration units of µg/L or ppb. Det. Limit = Detection Limit; Ref. Range = Reference Range. Reference ranges for some elements are presented as the estimated total excretion in µg per day (µg/d).

1. B.L. Carson et al., **Toxicology and Biological Monitoring of Metals** in Humans, Lewis Publishers, Inc. 1986.
2. W. Gooddy, E.I. Hamilton, T.R. Williams (1975) **Brain 98**:65.
3. Y. Mauras, P. Allain (1979) **Anal. Chim. Acta 110**:271.
4. Norbert W. Tietz, **Textbook of Clinical Chemistry** W.B. Saunders (1986).
5. WHO, Special Publication 647 (1980), Geneva, Switzerland.
6. CDC, internal report.
7. Eric J. Underwood, **Trace Elements in Human and Animal Nutrition**, 4th ed., 1977.
8. R.C. Dickson, R.H. Tomlinson (1967) **Clin. Chim. Acta 16**:311.
9. B. Welz, M. Melcher, G. Schlemmer (1983) **Fresenius Z. Anal. Chem. 316**:271.

References:

1) Kimberly and Paschal, **ACA**, 174, 203-10 (1985).
2) Paschal, Caldwell, and Ting, manuscript in preparation.
3) Paschal, Caldwell, and Ting, manuscript in preparation.
4) DiPietro et al., **Sci. Tot. Env.**, 74, 249-62 (1988).
5) Paschal- private communications to Fisher Chemical Co.
6) Paschal et al. "Multi-Element Screening of Blood and Urine"- Supplemental Report August 28, 1992.
7) Goldwater et al. (cited in Mercury in the Environment, CRC Press Boca Raton FL 1977)
8) Sherlock et al. **Arch. Env. Health** 37, 271-8 (1982).
9) Hainline et al., "Selected Methods for the Small Clinical Chemistry Laboratory", AACC, Washington, D.C. 1982.
10) Taylor, JK., "Quality Assurance of Chemical Measurements", Lewis Publishers, Chelsea, MI, 1987.

Volatile Organic Compounds (VOCs)

1.0 Biomarkers measured

The following VOCs can be measured in 10 mL of human blood:

1,1,1-Trichloroethane
1,1,2,2-Tetrachloroethane
1,1,2-Trichloroethane
1,1-Dichloroethane
1,1-Dichloroethene
1,2-Dichlorobenzene
1,2-Dichloroethane
1,2-Dichloropropane
1,3-Dichlorobenzene
1,4-Dichlorobenzene
2-Butanone
Acetone
Benzene
Bromodichloromethane
Bromoform
Carbon Tetrachloride
Chlorobenzene
Chloroform
cis-1,2-Dichloroethene
Dibromochloromethane
Dibromomethane
Ethylbenzene
Hexachloroethane
m-/p-Xylene
Methylene chloride
o-Xylene
Styrene
Tetrachloroethene
Toluene
trans-1,2-Dichloroethene
Trichloroethene

2.0 Background and overview

In our society, individuals are exposed to volatile organic compounds (VOCs) from many sources. VOC exposure occurs during many normal everyday activities which involve most if not all of the citizens of developed countries. These activities include drycleaning of clothes, house painting (especially indoor), furniture refinishing, gasoline dispensing, automobile transportation (especially stop and go), showering, and tobacco smoking. In addition, many individuals are exposed at their workplaces, including chemical manufacturing industries, oil refineries, offices in close proximity to copiers or other printing machines, drycleaning establishments, oil fields, as well as many other industries.

VOCs have been linked to neurological, hematologic, and hepatic health effects [1,2]. The combination of ubiquitous exposure and possible serious health effects makes VOCs a major public health concern.

VOCs can be measured in environmental media (air, water, soil, food) and biological specimens. A discussion of the usefulness of toxicant measurements in environmental media and the usefulness of toxicant measurements in biological specimens was given earlier in: Rationale for the use of biomarkers in exposure assessment. For the purposes of this discussion, breath will be included as a biological specimen.

Attempts to determine internal dose levels of VOCs have concentrated on measuring the native compounds in blood, the native compounds in exhaled breath or metabolites of these compounds in urine. Since breath is considered to be the major pathway for removing VOCs from the body, numerous attempts have been made to relate breath levels to levels of VOCs in environmental media [3-6]. Approaches which have focused on measurement of metabolites in urine have run into difficulty with specificity since many metabolites of VOCs are also metabolites of other compounds [7]. Directly measuring VOCs in blood estimates the concentrations of these compounds that are circulating through the body and contacting the potential target site(s) of effect.

Early studies of VOCs in blood concentrated on only one or a few particular analytes [8-11], whereas, in more recent studies, the number of VOCs under investigation has expanded [12-17]. Generally, the methods used in these studies have had detection limits for VOCs in blood in the high parts per trillion (ppt) to high parts per billion (ppb) range. These detection limits are sufficient to measure VOC levels in persons with occupational or high non-occupational exposure, but are not sufficient to examine the exposures which occur commonly in individuals who encounter VOCs in their homes, automobiles, and in workplaces not normally associated with chemical exposure. To assess these exposures, a detection limit in the low ppt range is needed. Previously published methods have also 1) required large blood samples (50 mL or more), and 2) not adequately addressed sample contamination, especially from sample collection materials.

3.0 Sample collection

3.1 Collection procedure

Vacutainer tubes obtained from commercial sources contain VOC contamination which can greatly interfere with the ability to obtain analytical results which estimate the extent of exposure. We therefore modify commercially available blood collection tubes so that they no longer contain measurable levels of most VOCs. The vacuum on individual vacutainers is released and the glass tubes and rubber stoppers are both heated for 2 weeks at 70°C under vacuum. The vacutainers are reassembled, the vacuum restored and the vacutainers sterilized using a Cobalt-60 radiation source. This process produces a sampling container with substantially less contamination.

Isopropanol used to disinfect the venipuncture site has appeared as a contaminant in measuring VOCs. This contaminant is effectively eliminated by swabbing the venipuncture site with a dry gauze bandage after wiping with isopropanol and allowing the site to dry for 5 - 10 seconds.

Whole blood samples are collected by venipuncture into grey top vacutainers which have been prepared as described above. Within 15 minutes of collection, whole blood samples are placed on wet ice or stored at refrigerator temperatures. The anticoagulant used in the prepared tubes is a mixture of sodium oxalate and sodium fluoride. This anticoagulant is chiefly intended to stop metabolism so that VOC levels do not change appreciably during storage. This mixture's ability to prevent clotting of blood is not as great as many other anticoagulants. Thus, once samples have been collected, they must be mixed thoroughly to allow the complete distribution of the anticoagulant. If a blood mixer is available, samples should be placed on this mixer for at least 3 minutes. If a mixer is not available, the blood

tube should be rocked back and forth by hand at least 30 times to completely mix the anticoagulant into the blood sample.

3.2 Short term storage

Since VOCs are highly volatile, care must be taken to insure that samples are kept at refrigerator temperatures during storage and shipment. All samples should be placed on wet ice or into a refrigerator within 30 minutes of sample collection. In addition, samples should be shipped with enough wet ice or equivalent cooling material to insure that the samples will remain cool throughout the shipment process. Samples should not be frozen or stored at freezer temperatures at any time during sample collection and shipment.

4.0 Sample analysis

4.1 Analytical method

The method is a purge and trap gas chromatographic method using high resolution isotope dilution mass spectrometric detection in the full scan mode. Stable isotopically labelled analogs of the compounds of interest are added to 10 mL of blood and this entire sample is injected into a specially designed sparging vessel which is already attached to the purging apparatus via air-tight seals. Prepurified helium gas is bubbled through the blood which is heated to approximately 35°C. This process removes volatile compounds from the sample into the gas stream. The purged volatile compounds pass into and are captured by a Tenax trap.

Once the 15 minute purge cycle is complete, the Tenax trap is purged with dry helium gas for 6 minutes to remove absorbed water. The trap is then heated to 180°C for 4 minutes to desorb all volatile compounds. As the compounds are desorbed, they are trapped at the gas chromatograph injection port by a liquid nitrogen trap at -150°C. Following this period, the site is ballistically heated to 200°C injecting the compounds onto the DB-624 capillary column which is interfaced to the mass spectrometer. The mass spectrometer is operated in the full scan mode (40 - 200 amu) with one scan collected per second. Quantitation is accomplished from specific ion responses relative to those of the corresponding isotopically labelled analogs. Correction is made for the o-xylene contribution to the styrene signal. Final concentrations are calculated based on six-point calibration curves.

4.2 Analytical sensitivity and specificity

Detection limits for these analytes are given in Table I. These values were determined from the plot of the standard deviation of calculated concentration of standards versus concentration [18]. The y-intercept of the least squares fit of this line equals s_0, with $3s_0$ being the calculated detection limit [18]. The detection limits are generally in the low ppt range except for analytes in which the range of standards had to be adjusted because blood levels were much higher (e.g., acetone).

High resolution (3000 resolving power) eliminates most interferences that can have substantial effects on results. By going to high resolution measurements, the mass window

can be reduced to 0.03 mass units. The high selectivity of this small mass window combined with the GC separation provides excellent analytic specificity.

Additional steps are also critical in promoting analytical specificity by removing extraneous compounds from the sample analysis system. Interferences which have their source in the measurement apparatus itself are examined by measuring instrument blanks. For this purpose, a pure water sample remains attached to the measuring apparatus and is examined regularly to check for operational levels of instrument blanks.

Glassware used for standards is treated to remove possible interferences and contamination. All glassware is heated in a vacuum oven at 150°C for at least 8 hours to remove adsorbed volatiles. The vacuum oven used contains an independent vacuum source since cross-contamination from other laboratory operations has been determined to be a major source of contamination of laboratory glassware. This glassware is cooled to room temperature before removal from the oven and sealed to diminish exposure to volatile compounds which are present in laboratory air.

The water used for dilution of standards and as water blanks is an extremely critical potential source of interference. No commercial filtering or purification system was found which could consistently yield water with acceptably low levels of VOCs (< 20 ppt for most analytes). An acceptable source of water was discovered at a non-commercial site and all further studies make use of this source. Under some circumstances even this source of water failed to yield acceptable levels of volatile organic compounds. In this case, the water is further purified by helium refluxing. To prevent further contamination from the laboratory air, water samples are sealed in glass ampules. In all cases, typical blank water levels are below the detection limits given above.

4.3 Analytical accuracy and precision

Since volatile organic compounds are not stable for extended periods in blood, no standard reference material is available. The accuracy basis for this method is established by determining the recovery of spiked blood samples. In addition, new quality control pools are overlapped with existing pools to insure a verified stable accuracy base is maintained over time.

For spiked samples, relative standard deviations are in most cases less than 30%. As expected, most of the exceptions were found in the low spike samples. These standard deviation results are actually higher than would be encountered in typical blood determinations since they include variation in the blood both before and after spiking. Multiple measurements on spiked QC materials show somewhat lower standard deviation results, averaging 19.4% for all analytes combined.

5.0 Quality assurance/quality control

Quality assurance and quality control procedures follow standard practices [18]. Daily experimental checks are made on the stability of the analytical system and standards and quality control materials which are added to each day's run sequence. A 'pure-water' blank sample is run at the beginning of each day to check for the presence of contamination in the purge and trap system or the labelled analog solution. In addition, determination of label ion

counts for this material is used to check daily method sensitivity. Relative retention times are examined for each analyte to insure the choice of the correct chromatographic peak.

Serum quality control materials were developed from bulk serum spiked with the analytes being examined in this study. This material was sealed in ampules and frozen at -60°C to insure long term stability. Quality control materials were developed at two different analyte concentration levels. These samples have proven to be stable over at least two years. Individual quality control charts are created for each analyte examined and quality control limits are used to insure analytical stability. 99% confidence limits are used for analytical control limits.

Table I. Detection limits for VOCs in whole blood

VOC	Detection limit (ppb)
1,1,1-trichloroethane	0.045
1,1,2,2-tetrachloroethane	0.010
1,1,2-trichloroethane	0.018
1,1-dichloroethane	0.007
1,1-dichloroethene	0.010
1,2-dichlorobenzene	0.048
1,2-dichloroethane	0.016
1,2-dichloropropane	0.012
1,3-dichlorobenzene	0.041
1,4-dichlorobenzene	0.042
2-butanone	0.38
acetone	200
benzene	0.026
bromodichloromethane	0.005
bromoform	0.020
carbon tetrachloride	0.011
chlorobenzene	0.010
chloroform	0.015
cis-1,2-dichloroethene	0.032
dibromochloromethane	0.009
dibromomethane	0.032
ethylbenzene	0.025
hexachloroethane	0.035
m-/p-xylene	0.034
methylene chloride	0.081

VOC	Detection limit (ppb)
o-xylene	0.024
styrene	0.022
tetrachloroethene	0.022
toluene	0.059
trans-1,2-dichloroethene	0.012
trichloroethene	0.007

6.0 Banking of samples

Repeat measurements of samples stored at 4°C indicate that whole blood VOC samples may be banked for at least 7 weeks. Since these are whole blood samples, longer storage results in samples which are harder to manipulate and produce additional analytical problems. Thus, even though analytical results may not change over this time, samples may be less amenable to analysis. Preliminary results suggest that the 7 week storage time may not be appropriate for acetone and 2-butanone. These compounds occur naturally in the body, and metabolism may alter their concentration with storage.

Whole blood samples for VOC measurement should be stored at 4°C. This prevents blood cell rupture which would occur at lower temperatures. In addition, freezing of blood can lead to breakage of vacutainers and loss of sample in some cases. Since VOCs are lost whenever the containers in which they are contained are opened, it is not appropriate to transfer the blood samples to another container which would be more resistant to breaking.

7.0 Interpretation of biomarker measurement

Reference ranges for these VOCs have been measured in a sample of 700 to 1000 persons selected from the Third National Health and Nutrition Examination Survey (NHANES III). The sample is not representative of the U.S. population but it is designed to examine the influence of age, sex, race/ethnicity, urban/rural status and region of the country on VOC levels. At this writing, the analytical results are complete and the analysis examining the relationship of demographic variables to blood VOC levels is in progress.

Detectable analyte levels of 12 VOCs have been found in most if not all samples from this reference population. For these 12 VOCs, preliminary results (N approximately 400) are given in Table II for the 5th percentile, mean, median, and 95th percentile.

Table II. Preliminary results: Volatile Organic Compounds levels in a reference population (ppb)

Analyte	N	5th percentile	Mean	Median	95th percentile
1,1,1-trichloroethane	363	ND	0.29	0.13	0.71
1,4-dichlorobenzene	395	0.071	2.40	0.34	14.4
2-butanone	426	2.37	7.72	5.63	18.5
acetone	415	629	2960	1690	8520
benzene	398	0.030	0.14	0.063	0.53
chloroform	421	ND	0.046	0.022	0.099
ethylbenzene	397	ND	0.11	0.061	0.27
m-/p-xylene	426	0.071	0.42	0.19	0.83
o-xylene	422	0.038	0.14	0.10	0.29
styrene	425	ND	0.079	0.041	0.19
tetrachloroethene	426	ND	0.19	0.058	0.60
toluene	405	0.13	0.52	0.29	1.54

Some VOCs are found in less than half of the samples examined in the reference population. These compounds and the percent of samples found above the detection limit are bromodichloromethane, 22%; dibromochloromethane, 22%; and trichloroethene, 20%.

Sixteen of the analytes determined by our method have been found above the detection limits in less than 5% of the normal human blood samples investigated to date. The compounds are 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,1-dichloroethane, 1,1-dichloroethene, 1,2-dichlorobenzene, 1,2-dichloroethane, 1,2-dichloropropane, 1,3-dichlorobenzene, bromoform, carbon tetrachloride, chloro-benzene, cis-1,2-dichloroethene, dibromomethane, hexachloroethane, methylene chloride, and trans-1,2-dichloroethene.

Measurements carried out in our laboratory and previous reports by others [19-23] agree that the elimination of VOCs is multi-phasic with at least three components. The half lives for these components are short compared to many other organic compounds. Preliminary estimates of the three half-lives are within the range 3 - 10 min, 20 - 100 min, and 150 - 1000 min. Preliminary results suggest, at least for some VOCs, that the final long half-life component only accounts for approximately 5-10% of the total elimination following acute exposure, thus these VOCs appear to be largely eliminated from blood within a few hours after exposure has ceased. It is not clear at this time to what extent bioaccumulation occurs in persons with chronic exposure to "low-level" VOC exposures.

Comparison of blood levels of VOCs with environmental measurement is in its initial stages. Our laboratory in collaboration with the Environmental Protection Agency has made

measurements of blood levels resulting from exposure to low levels of some VOCs and compared them with chamber air levels [19]. The results of these measurements are summarized in Table III. This table gives the analyte being examined, the exposure conditions, the air concentrations during exposure, and the blood levels immediately after exposure has ended.

Table III. Volatile Organic Compounds Levels in Blood After Acute Air Exposure.

Analyte	Conditions	Air concentration (mg/m^3)	Peak blood concentration (ppb)
1,1,1-trichloroethane	2-hr exercise	2.92	7.65
1,1,1-trichloroethane	10-hr at rest	1.30	7.38
1,1,1-trichloroethane	2-hr at rest	0.95	5.68
ethylbenzene	10-hr at rest	3.30	14.4
ethylbenzene	2-hr exercise	1.30	7.82
ethylbenzene	4-hr at rest	0.825	2.16
ethylbenzene	4-hr at rest	0.412	1.18
ethylbenzene	2-hr at rest	0.400	1.84
methylene chloride	10-hr at rest	3.05	8.16
methylene chloride	2-hr exercise	3.04	14.5
methylene chloride	2-hr at rest	1.05	5.34
o-xylene	10-hr at rest	2.80	8.22
o-xylene	2-hr exercise	1.00	5.23
o-xylene	2-hr at rest	0.35	1.57
p-xylene	10-hr at rest	10.50	83.4
p-xylene	4-hr at rest	8.250	17.5
p-xylene	2-hr exercise	4.54	36.8

Analyte	Conditions	Air concentration (mg/m^3)	Peak blood concentration (ppb)
p-xylene	4-hr at rest	4.125	10.2
p-xylene	2-hr at rest	1.55	10.8
toluene	10-hr at rest	2.50	10.8
toluene	2-hr exercise	2.08	13.9
toluene	2-hr at rest	0.90	3.70

References

1. Hayden, J.W.; Comstock, E.G.; Comstock, B.S. Clin. Toxicol. **1976**, 9, 169-184.
2. Ramsey, J.D.; Flanagan, R.J. J. Chromatogr. **1982**, 240, 423-444.
3. Droz, P.O.; Krebs, Y.; Nicole, C.; Guillemin, M. Am. Ind. Hyg. Assoc. J. **1988**, 49, 319-324.
4. Berlin, M. Am. J. Ind. Med. **1985**, 7, 365-73.
5. Wallace, L.A.; Pellizzari, E.D.; Hartwell, T.D.; Sparacino, C.; Whitmore, R.; Sheldon, L.; Zelon, H.; Perritt, R. Environ. Res. **1987**, 43, 290-307.
6. Brugnone, F.; Perbellini, L.; Faccini, G.B.; Pasini, F.; Maranelli, G.; Romeo, L.; Gobbi, M.; Zedde, A. Int. Arch. Occup. Environ. Health **1989**, 61, 303-311.
7. Carlsson, A. Scand. J. Work Environ. Health **1982**, 8, 43-55.
8. Snyder, C.A.; Laskin, S.; Goldstein, B. Am. Ind. Hyg. Assoc. J. **1975**, 36, 833-836.
9. Dowty, B.; Carlisle, D.; Laseter, J.L.; Storer, J. Science **1975**, 187, 75-77.
10. Dowty, B.J.; Laseter, J.L.; Storer, J. Pediatr. Res. **1976**, 10, 696-701.
11. Peoples, A.J.; Pfaffenberger, C.D.; Shafik, T.M.; Enos, H.F. Bull. Environ. Contam. Toxicol. **1979**, 23, 244-249.
12. Pfaffenberger, C.D; Peoples, A.J.; Enos, H.F. Int. J. Environ. Anal. Chem. **1980**, 8, 55-65.
13. Barkley, J.; Bunch, J.; Bursey, J.T.; Castillo, N.; Cooper, S.D.; Davis, J.M.; Erickson, M.D.; Harris, B.S.; Kilpatrick, M.; Michael, L.C.; Parks, S.P.; Pellizzari, E.D.; Ray, M.; Smith, D.; Tomer, K.B.; Wagner, R.; Zweidinger, R.A. Biomed. Mass Spectrom. **1980**, 7, 139-47.
14. DeLeon, I.R.; Antoine, S.R. Clin. Ecol. **1985**, 2, 108-109.
15. Kroneld, R. Bull. Environ. Contam. Toxicol. **1985**, 34, 486-496.
16. Antoine, S.R.; DeLeon, I.R.; O'Dell-Smith, R.M. Bull. Environ. Contam. Toxicol. **1986**, 36, 364-371.

17. Cramer, P.H.; Boggess, K.E.; Hosenfeld, J.M.; Remmers, J.C.; Breen, J.J.; Robinson, P.E.; Stroup, C. Bull. Environ. Contam. Toxicol. **1988**, 40, 612-618.
18. Taylor, J.K. Quality Assurance of Chemical Measurements; Lewis Publishers, Inc.: Chelsea, MI, 1987.
19. Raymer, J.H.; Thomas, K.W.; Pellizzari, E.D.; Cooper, S.D.; Smith, D.J.; Kizakevich, P.; Pugh, D.. Preliminary Draft. RTI/4948-31A-02FR. **1992.**
20. Fernandez, J.; Guberan, E.; Caperos, J. Am. Ind. Hyg. Assoc. J. **1976**, 37, 143-150.
21. Monster, A.C.; Boersma, G.; Steenweg, H. Int. Arch. Occup. Environ. Health **1979**, 42, 303-309.
22. Nise, G.; Attewell, R.; Skerfving, S.; Orbek, P. Brit. J. Ind. Med. **1989**, 46, 407-411.
23. Sabourin, P.J.; Medinsky, M.A.; Bechtold, W.E.; Henderson, R.F. Adv. Mod. Environ. Toxicol. **1989**, 16, 153-176.