Laboratory Procedure Manual

Analyte: **Selected Group of Volatile Organic Compounds**

Matrix: **Air Badge**

Method: **GC/MS**

Method No.:

Revised:

as performed by: *Environmental and Occupational Health Sciences Institute (EOHSI)*

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Important Information for Users

Environmental and Occupational Health Sciences Institute (EOHSI) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
Lab21	LBXZBZ	Benzene (µg/cubic meter)
	LBXZCF	Chloroform (µg/cubic meter)
	LBXZEB	Ethylbenzene (µg/cubic meter)
	LBXZTE	Tetrachloroethene (µg/cubic meter)
	LBXZTO	Toluene (µg/cubic meter)
	LBXZTI	Trichloroethene (µg/cubic meter)
	LBXZOX	o-Xylene (µg/cubic meter)
	LBXZXY	m,p-Xylene (µg/cubic meter)
	LBXZDB	1,4-dichlorobenzene (µg/cubic meter)
	LBXZMB	MTBE (µg/cubic meter)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Analysis is performed using an HP 5890 Series II Plus GC (Gas Chromatograph) with an HP 5972 MSD (Mass Selective Detector Quadrupole Mass Spectrometer) and EnviroQuant software. We initially used a Restek RTX-1, 60 m, 0.25 mm ID column and a 1-µm film thickness (catalog #10156; Restek Corp., Bellefonte, PA) with good separation for all target analytes. More recently, we have been using a Restek RTX B624, 60 m, 0.25 mm ID with 1.4-µm thickness column (catalog # 10969), which provides even better resolution for separation of the solvent peaks and methylene chloride.

2. SAFETY PRECAUTIONS

All solvents used in this procedure are flammable. Use standard laboratory safety precautions. Carbon disulfide is toxic and an acute fire and explosion hazard (flash point = –30°C); all work with carbon disulfide must be performed in a hood.

OVM samples and extracts should be stored refrigerated. No solvent or solution containing the target compounds should be kept in the same refrigerator.

All the equipment used in the extraction procedure should be fully dedicated to that purpose and not to other analyses.

Extractions should be done in a dedicated laboratory hood, where no solvents are stored except for the extraction solvents.

The hood space should be thoroughly cleaned, and then lined with heavy-duty aluminum foil, which should be carefully wiped with acetone and allowed to dry. The foil should be replaced periodically depending on the intensity of use.

All extraction supplies should be meticulously cleaned. For example, needle nose tweezers used to manipulate the charcoal pads should be washed with acetone prior to each used and dried; gas tight syringes used to prepare extraction solvents and standards should be rinsed 5 to 10 times in the appropriate solvent before being used.

Avoid the use of polyethylene or other plastic materials that can contain significant residues of solvents such as toluene.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Raw data for all analysis is captured by the EnviroQuant software of the GC/MS instrument. Data files (raw and processed) are stored in a directory unique to the sample and instrument on the Laboratory Information Management System (LIMS) server. Data is backed up to tape storage each night by the Network Administrator, and kept on the LIMS server.

When the raw and processed data has been released by the instrument analyst peer reviewer, the data is available to the Project Manager (PM) for final calculations. Final calculations are performed by copying the processed data into a set of Excel templates that are stored in the PM's user directory on the laboratory's server. Each group of samples (shipment) is stored in a separate Excel workbook. Each sample's data is stored on a separate tabbed page in the workbook, identified by the unique NHANES number of the sample. The Excel templates are used to generate a comma delimited ASCII file that is transmitted electronically to the NHANES staff when the data is released by the PM. All data for the project is stored on the server and is backed up to tape each night by the Network Administrator.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

Samples are collected by the NHANES staff, sent by overnight delivery, and received at Environmental and Occupational Health Sciences Institute (EOHSI) by the sample receiving staff. The samples are taken out of the cooler and the identification marks on the samples are compared to the paper manifest included with the samples. If the number of samples and identification numbers match the paper manifest, the samples are logged into the LIMS. If there are discrepancies, the PM is notified and the problems are resolved by communication with the NHANES staff.

This procedure generates a unique work order number for each shipment and a unique sample number for each sample. These numbers have a one-to-one equivalency with the unique NHANES shipment number and the unique individual monitor number generated by the NHANES staff, respectively

Each sample receives a label with the numbers from the LIMS system. The group of samples from a single shipment is placed in a plastic bag and stored in the GC/MS laboratory in refrigerator 43 or 44, which are maintained at 4°C.

The data from an electronic manifest transmitted to the PM by NHANES is compared to the LIMS information generated from the paper manifest. Discrepancies (if any exist) are resolved by communication with the NHANES staff.

The monitors require no preparation.

Consult the manufacturer's recommendations for shelf life.

Badges not capped, or not labeled properly aren't tested.

Torn, perforated or stained screens are criteria for rejection.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation

HP 5890 Series II Plus GC with a 5972 MSD

B. Materials

Syringes:

25 mL SGE purge and trap syringe
500 µL gas-tight syringe
25 µL gas-tight syringe
25 mL purge and trap syringe
10 µL syringe
5 µL syringe with Chaney adapter
septum valves for syringes

Vials:

1.5 mL amber crimp seal vials
11 mm crimp seals T/S septa

2.0 mL amber screw cap vials with T/S septa
40 mL amber vial
1 mL micro-reaction vial
15 mm Mininert valve

Teflon-coated needle-nose forceps
Sonicator–
Carbon disulfide
Acetone
Argon, High Purity
Argon Enclosure Hood

C. Reagent Preparation

Preparation of Extraction Solvent

(1) The extraction solvent is prepared by mixing 2:1 v/v acetone (EM Science, OmniSolv or better) and carbon disulfide (Aldrich Chemical part number 42464-1). The acetone is transferred as needed from the 4-L container into an amber bottle wrapped in cleaned aluminum foil and fitted with a 1-10 ml bottle top dispenser (VWR 53501-048) to facilitate handling. The mixed solvent should be prepared fresh for each batch of samples to be extracted. The same mixed solvent is used for preparing working standards. The sequence of steps in the preparation of the mixed solvent for extraction is as follows:

(a) Acetone is transferred from the 1-L container to a precleaned 40-ml amber vial (VWR Scientific 15900-024) and capped with septum caps shipped with the vials (these have silicon/Teflon septa).

(b) 20 cc of acetone are withdrawn from the vial using a precleaned, calibrated 25-ml SGE purge and trap syringe (SGE International, part number 009462) and transferred to a separate 40-ml amber vial already capped. This syringe is dedicated to acetone transfer.

(c) 10 cc of carbon disulfide are withdrawn directly from the original container using a 25-ml SGE syringe also fully dedicated to carbon disulfide transfer. The 10-cc aliquot is transferred to the vial containing the acetone.

(2) Sufficient mixed solvent vials should be prepared for extraction (depending on the number of samples), preparation of standards, rinsing, etc. Mixed solvent dedicated to rinsing should be kept separate from that dedicated to extraction and that used in standard preparation.

(a) For the surrogate, a working solution of 5000 µg/ml of 4-bromofluorobenzene (BFB) is prepared by withdrawing 1 ml of a 25,000 µg/ml standard solution of BFB in methanol (Supelco, part No 4-8800) using a 1 ml gas-tight syringe (SGE) and transferring to a 5-ml precleaned, clear glass vial with a Teflon-lined septum cap. Four ml of the acetone/carbon disulfide mixed solvent is added. This surrogate standard can be kept refrigerated after exchanging the septum cap for a screw-on top (also Teflon lined) and used as necessary by transferring to 1-ml autosampler amber vials with Teflon lined septum caps.

(b) A 30-µl aliquot of the surrogate standard mix is transferred to the mixed solvent vials to be used for extraction, using a 50-µl gas-tight syringe (the final concentration of surrogate in the extraction solvent is 5 µg/ml). No surrogate is added to the mixed solvent to be used for preparing standards, only to the solvent to be used for extraction.

D. Preparation of Internal Standards

The stock internal standard is a solution of bromofluoromethane, 1,4-difluorobenzene, and chlorobenzene-d_5 in methanol at 1000 µg/ml each (Supelco, catalog No. 4-8835). A working internal

standard solution is prepared by mixing 1 ml of the stock solution with 9 ml of the acetone/carbon disulfide mixed solvent into a 25-ml glass vial with Teflon septum cap to obtain 100 µg/ml final concentration. Again, dedicated gas tight syringes are used for preparing each solution.

E. Preparation of External Standards

(1) The working standard solutions are 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 µg/ml (except for 1,3-butadiene, which is 0.5–50 µg/ml), prepared fresh for each analysis batch. Two stock standard solutions are used: 1) a custom volatile mix of benzene, carbon tetrachloride, chloroform, chloroprene, 1,4-dichlorobenzene, methylene chloride, styrene, tetrachloroethylene, and toluene in carbon disulfide at a concentration of 2000 µg/ml (AccuStandard Inc., S-2081-R3-10X; to this mix will be added methyl tertbutyl ether, mixed xylenes, trichlorothylene, and ethylbenzene at the same concentration); and 2) 1,3 butadiene in methanol (AccuStandard, part S-406A-10X) also at 2000 µg/ml. Both standards are kept in a freezer at –20°C. The working standards are prepared as follows:

(a) Stock 1: A stock standard of the mixed volatiles is prepared by withdrawing 100 µl of the concentrated solution and transferring to a 1.0-ml micro-reaction vial (Supelco, cat. # 3-3293) fitted with a Mininert valve (Supelco, 15 mm, cat. #3-3301). A 900-µl aliquot of the acetone/carbon disulfide mixed solvent is added. This working standard is stored at –20°C when not in use and discarded after 1 month if not used completely.

(b) Stock 2: a second stock standard is prepared by transferring 100 µof Stock 1 into a 2-ml mini-reactor vessel fitted with a Mininert valve, followed by 50 µl of the concentrated (2000 µg/ml) 1,3-butadiene standard, and diluted by adding 1850 µl of the acetone/carbon disulfide mixed solvent using a 1-ml gas-tight syringe. Stock 2 can be used over a 1-week period and should be stored at –20°C.

(c) Working standards are prepared fresh by using 1.5-ml amber autosampler vials fitted with Teflon/silicon/Teflon septa screw tops (Wheton, 225153-03SP) with glass inserts. The standards are prepared one at a time and capped immediately as follows:

(i) 0.1: add 10 µl of internal standard mix to the autosampler vial insert using a 500-µl gas-tight syringe held in a repeating dispenser (Supelco PB600-1, 2-0943), followed by 198 µl of the acetone/carbon disulfide mix (using 100 µl and 10 µl gas-tight syringes), and 2 µl of Stock 2 (with a 5-µl gas-tight syringe).

(ii) 0.5 to 10: the rest of the standards are prepared similarly except that 10, 20, 40, 100, and 200 µl of Stock 2 is added after the 10 µl of internal standard mix, followed by the corresponding amount of mixed solvent, so that the total volume in each case is 210 µl.

F. Preparation of Quality Control Material

Quality control materials consist of unused 3M 3520 OVMs that are either fortified with the analytes of interest, or analyzed as blank samples. The OVMs are fortified by removing the Teflon diffusion screen, placing a piece of filter paper on the spacer plate, and injecting the appropriate amount of stock standards to reach the concentration required. The plastic elution cap is then applied and the monitor is allowed to sit for 16-24 under refrigeration. These monitors are used as Laboratory Control Samples (LCS) or as positive controls.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Linearity will be demonstrated by a point calibration curve. After the establishment of linearity (either %RSD <20 over working range or 0.995 correlation coefficient), a continuing calibration check (CCC) sample are placed in the run at predetermined intervals. The CCC is one of the standards (usually the middle) from the initial point curve.

The CCC must be within 20% agreement of the initial calibration. After each CCC and before samples are analyzed, a laboratory solvent blank is analyzed. Calculations will be based on an average response

factor, and sample values will be corrected for extraction efficiencies.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Extraction Procedure

Each charcoal pad is carefully removed from the holder and transfer to labeled amber vials. The pad is removed using needle nose Teflon coated tweezers, and is carefully folded with the aid of a similar tweezers so it fits through the neck of the 1.8-ml amber vial. Then, 1 ml of the mixed solvent solution with surrogate is added (using a dedicated gas tight syringe). The vial is then tightly capped and place on a holding sample tray. Once all the pads in a batch of samples to be extracted are prepared in this manner, the tray is placed in an ultrasound bath, and enough water with a small amount of crushed ice is added to reach the solvent meniscus but not touching the bottom of the vial caps. The pads are then extracted by sonication during 40 minutes. Solvent blanks, field blanks, and laboratory blanks are similarly treated.

B. Preparation Of Analytical Samples

After extraction, 10 µl of the internal standard mix is transferred to an amber autosampler vial with glass insert (using the repetitive pipette) followed by 200 µl of each extract (using the same gas tight syringe which is rinsed with clean mixed solvent at least five times between samples) for a total volume of 210 µl. The cap (or the septum liner) of the vials containing the extracts is replaced, the extract vials tightly capped again and stored in a freezer. The vials/inserts with the analytical samples are tightly capped with Teflon lined septum caps and placed in the autosampler. The typical sequence of analysis is two solvent blanks, followed by the standards, then two solvent blanks, followed by field and laboratory blanks, then the samples with one solvent blank every ten vials, and then another set of standards. Samples are analyzed after verifying the calibration curve.

C. GC/MS Analysis Procedure

Analysis is performed using an HP 5890 Series II Plus GC (Gas Chromatograph) with an HP 5972 MSD (Mass Selective Detector Quadrupole Mass Spectrometer) and EnviroQuant software. We initially used a Restek RTX-1, 60 m, 0.25 mm ID column and a 1-µm film thickness (catalog #10156; Restek Corp., Bellefonte, PA) with good separation for all target analytes. More recently, we have been using a Restek RTX B624, 60 m, 0.25 mm ID with 1.4-µm thickness column (catalog # 10969), which provides even better resolution for separation of the solvent peaks and methylene chloride. With the last column, the GC/MS conditions are:

- scan mode from 35 to 260 amu.
- Injection splitless for 0.5 minutes and splitting 50:1 for the rest of the run.
- Helium carrier; initial pressure 3 psi for 0.5 minutes, ramp 90 psi/min to 22.5 psi; linear velocity 31.1 cm/sec.
- injection port temperature 180°C.
- detector temperature 250°C.
- temperature program: start at 40°C, hold for 12 minutes, ramp at 8°C/min to 200°C.

At these conditions and column, the solvent elutes between 1,3-butadiene and methylene chloride. The analyzer is turned off after the elution of butadiene and it is turned on again before the methylene chloride peak elution. In our system, it is turned off between 6.5 and 8.9 min.

(1) Quality Control Materials

Blank badges from the same lot as the samples and laboratory control samples (LCS) are analyzed along with each run of samples.

(2) Recording of Data

Data is recorded automatically by the EnviroQuant software of the GC/MS instrument. The data files are sequentially numbered, and the header information fields of the files contain both the LIMS generated sample numbers, and the NHANES transmitted identification numbers for each sample.

(3) Replacement and Periodic Maintenance of Key Components

Key components for the analysis that requires routine maintenance are the column liner and septum and the head of the GC column. Liners and septum are changed at the beginning of every analytical sequence and the data is recorded in the laboratory notebook. Instrument flows are checked at the same time. Other maintenance is recorded in a maintenance notebook that is kept for each instrument.

D. Preliminary Calculations: Determination Of Extraction Efficiencies

Extraction efficiencies are determined following the 3M procedure but using the range of concentrations (after extracting the spiked OVMs with 1 ml of mixed solvent) in the standards. We have found that for some compounds such as styrene there is a concentration dependence of the extraction efficiency at low concentrations.

The analyst reviews each identified compound and examines the integration performed by the instrument. The analyst also determines if the identified compound was found at a level above the linearity determined for the system. If any compound exceeds the linearity, the analyst schedules the sample to be reanalyzed with a dilution appropriate to bring the data within the linearity range.

E. Final Calculations: Concentration Calculations

For the compounds present in the blanks and or solvent, the loading MDL (i.e., the amount of target compound in µg that is present in the blank) is calculated as the t (n – 1, 99) × SD of n blanks. Typically, we run at least 20% blanks compared to the number of samples. For the purpose of calculating concentrations, the load of every charcoal pad in each sample is compared to this MDL. If the sample load is the same or smaller, that sample is assigned the concentration corresponding to the MDL using the 3M formula. When analyzing the 3520, the mass loading of the front and back charcoal pads are compared separately to MDLs calculated for each. If the back pad load is the same or lowers than the back pad MDL, the sample is considered not to have the compound present above background, and concentration is then calculated as if the sample had been obtained with a 3520 OVM. For compounds not present in blanks, the MDL is estimated based on the SD of seven repeated analysis of the lowest detectable standard using the same approach as described above (with n = 7). Currently, the only target compound present in our mixed solvent is methylene chloride. For this compound, we subtract the response ratio in the solvent from that of every standard, blank or sample before calculating mass loadings and concentrations.

Final sample calculations are performed by pasting the instrument data directly into a set of Excel templates that have been created for this project. These templates carry out the following calculations.

Front and back pads average blank loadings are subtracted from the front and back pad results respectively. All data from blank badges run with the samples are pooled and used for background subtraction. Data from the front sections of the badges are pooled separately from the backs and maintained in a separate template.

Data for the front and back pads are combined according to the formula provided by the badge manufacturer, 3M Corporation. The only modification to the calculation is that the units are in nanograms not micrograms, and the final values are in µg/m^3 not mg/m^3. The formula is:

$$\text{Conc } (\mu g/m^3) = \frac{(\text{Mass Front} + 2.2 \times \text{Mass Back}) \times A}{r \times t \text{ (minutes)}}$$

Mass Front = Mass Front – Avg. Blank Mass Front
Mass Back = Mass Back – Avg. Blank Mass Back
A = calculation constant supplied by 3M for each analyte
r = recovery coefficient
t = sampling time (exposure) in minutes

Final reported values are recovery corrected with recovery coefficients that must be determined in each laboratory performing the analysis according to the 3M sample guide.

9. REPORTABLE RANGE OF RESULTS

The eluted concentrations of the organic vapors are calculated from the area of the characteristic ion measured in the sample compared to the area of the characteristic ions measured in the initial linearity curve using an internal standard procedure. The linearity differs slightly for the different compounds. Samples that exceed the linear curve for any component are diluted sufficiently to be brought into the middle of the linear range, and then reanalyzed.

10. QUALITY CONTROL (QC) PROCEDURES

LCSs and blank badges are analyzed with each run of samples. Continuing calibration verification standards (CCVs) and solvent blanks are analyzed at predetermined intervals in the run.

Control limits have been established.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the system is declared “out-of-control” for an initial curve, the physical system is examined to determine the reason for the problem.

- The injection port on the gas chromatograph may need maintenance.
- The mass spectrometer source may need to be cleaned.
- The standards may need to be re-prepared from stocks.

No samples are run until the system is brought back into control and an initial curve passes the acceptance criteria.

If the system is declared “out of control” for a CCV, all samples analyzed after the last acceptable CCV are considered invalid and need to be re-analyzed. The same criteria performed for the initial curve out-of-control are examined.

If the results for any compound in a sample exceed the highest standard in the initial curve, the extract is diluted an appropriate amount so that it will not exceed the linearity range, and the sample is reanalyzed. The results for the compound that exceeded linearity are calculated from the diluted injection, while the results for the compounds that did not exceed linearity are calculated from the initial injection.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Interfering substances are substances found in the extraction solvents or the OVM pads that are being determined by the method. They are corrected by a blank subtraction process described in the calculation section. Compounds with retention times and characteristic ions that are similar to the compounds of

interest can also interfere. When an interference is suspected, ratios of secondary ions can be compared to the standards or full scan mass spectrometry can be employed to determine if an interferent is present.

If the material is present in the desorption solvent at levels comparable to the samples, it will appear at equal levels in the front and back pad, and will be flagged as an invalid measurement because the back pad will exceed 50% of the value of the front pad.

13. REFERENCE RANGES (NORMAL VALUES)

Not applicable.

14. CRITICAL CALL RESULTS (PANIC VALUES)

There are no critical call results for this type of testing.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

All exposed OVMs must be kept refrigerated (4°C). Standards are kept in a freezer (–10°C). Extracts are kept refrigerated (4°C). Badges are equilibrated to room temperature before extraction.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

There are no alternate methods of analysis for samples on OVMs. If the analytical system fails, the samples are stored until the system is available.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Sampling data is sent to the laboratory electronically by the NHANES laboratory. Sample identification numbers, types, and sampling times are sent in a CSV data file attached to an email notification. Sample tracking data is stored in the LIMS server.

Data is captured by the EnviroQuant software on the Gas Chromatograph/Mass Spectrometer system. The data is stored on the laboratory data server and processed on the PC by the project manager. Final results are reported to NHANES by electronic transmission of a CSV formatted data file.

Initial calibration curves are evaluated by the “Response Factor Report” generated by the system.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Records of sample tracking from the LIMS server and data file captured by the server are stored on CD-ROM for archival storage. All sample identification is numeric and is not traceable in this laboratory to any individual.