Laboratory Procedure Manual

Analyte: **Trihalomethanes/MTBE**

Matrix: **Water**

Method: Solid Phase Microextraction with GC Separation/ MS

Method No:

Revised:

as performed by:

Emergency Response & Air Toxicants Branch
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Important Information for Users

The Centers for Disease Control and Prevention (CDC) 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
Lab 04	LBXWBF	Water Bromoform (ng/mL)
	LBXWCF	Water Chloroform (ng/mL)
	LBXWBM	Water Bromodichloromethane(ng/mL)
	LBXWCM	Water Dibromochloromethane(ng/mL)
	LBXWME	Water MTBE(ng/mL)

1. Clinical Relevance and Summary of Test Principle

THMs and MTBE are measured in drinking water by solid phase microextraction/gas chromatography/isotope dilution mass spectrometry (SPME/GC/isotope dilution MS) based on the method described by Cardinali, et al. (1). The analytes are in equilibrium between the drinking water and the headspace above the sample. A solid-phase microextraction (SPME) fiber is inserted into the headspace and the volatile organic compounds (VOCs) partition into the phase on the outside of the fiber shaft. The SPME fiber is then inserted into the heated GC inlet where the VOCs thermally desorb into the carrier gas stream. Extracted VOCs are focused at the head of the GC column using a cryogenically cooled trap (cryo-trap). Analytes are separated on a DB-VRX capillary column and quantified using multiple ion detection (MID) mass spectrometry (using a Thermo Trace mass spectrometer). Comparison of relative response factors with known standard concentrations yields individual analyte concentrations. The method is applicable to the determination of five VOCs in 5-mL samples of drinking water with detection limits in the low parts per trillion (pptr) ranges. Since non-occupationally exposed individuals have blood VOC concentrations in this range, the method is applicable for determining these quantities and investigating cases of low-level exposure to VOCs. This method proved adequate for measuring the THMs and MTBE in most drinking water samples tested from diverse locations throughout the U.S.(1).

2. Safety Precautions

A. Reagent toxicity or carcinogenicity

ALL OF THE COMPOUNDS USED IN THIS STUDY ARE HAZARDOUS CHEMICALS! Use a high draft fume hood and lower all the sashes to recommended operating height when working with neat (undiluted) materials or highly concentrated solutions since a number of these compounds are toxic. Wear nitrile gloves when handling hazardous chemicals to prevent absorption through the skin. Some of the compounds used in this study are known or suspected carcinogens, mutagens and/or teratogens.

B. Radioactive hazards

None.

C. Microbiological hazards

None.

D. Mechanical hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Laboratorians should read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of the gas chromatograph or mass

spectrometer unless all power to the instrument is off. Generally, mechanical and electronic maintenance and repair should only be performed by qualified technicians. The autosampler and the mass spectrometer contain a number of areas which are hot enough to cause burns. Precautions should be used when working in these areas.

E. Protective equipment

Standard safety precautions should be followed when performing this procedure, including the use of a lab coat/disposable gown, safety glasses, appropriate gloves, and chemical fume hood. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

F. Training

Formal training in the use of the gas chromatograph and mass spectrometer is necessary. Users are required to read the operation manuals and should demonstrate safe techniques in performing the method. Users should be trained in use of all other potentially hazardous equipment, including centrifuges, chemical fume hoods, etc.

G. Personal hygiene

Follow Universal Precautions. Care should be taken when handling chemicals or any biological specimen. Routine use of gloves and proper hand washing should be practiced. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

H. Disposal of wastes

Waste materials must be disposed of in compliance with CDC laboratory, federal, state, and local regulations. Solvents and reagents should always be disposed in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood. Disposable needles and used glass capillary tips should be placed immediately into a sharps container and autoclaved when the sharps container becomes full.

3. Computerization; Data-System Management

A. Software and knowledge requirements

This method has been validated using the solid phase microextraction technique coupled with a gas chromatography and Thermo Trace mass spectrometer run with the Thermo Finnigan Xcalibur 1.4 software. Results are exported from Analyst software to Microsoft Excel files and entered into the ATLIS relational database.

Knowledge of and experience with these software packages (or their equivalent) are required to utilize and maintain the data management structure.

B. Sample information

Information pertaining to particular specimens is entered into the database either manually or electronically transferred. The result file is transferred electronically into the database. No personal identifiers are used, and all samples are referenced to a blind coded sample identifier.

C. Data maintenance

Integrity of specimen and analytical data generated by this method is maintained by proofreading all transcribed data, storing of data in multiple computer systems, and redundantly archiving data. Original data files contain traceable header information (date, run number, sample type and sample identification) and are stored in duplicate on two separate recordable compact disks. Once the compact disk (CD) is filled and verified for integrity, both copies are permanently archived. Thus, two copies of all data are available to be accessed at any time. One CD is stored in an office adjacent to the laboratory and the other is stored in a separate facility. Data is transferred through a CD to the shared network drive along with relevant meta-data (including peak integrations, calibration curves, blanks, and isotope corrections). Processed results files are transferred electronically into the local area network (LAN) and stored in a shared directory. Data is loaded into the Microsoft Access database system using an automated data import module. The data files are backed up onto CD and tape media.

Routine backup procedures include: 1) weekly backup of hard disks; 2) daily backup of Access database files; 3) weekly backup of database files onto CD. A separate, backup tape database is stored off site. Either the supervisor or the local area network manager should be contacted for emergency assistance

D. Information security

Information security is managed at multiple levels. The information management systems that contain the final reportable results are restricted through user ID and password security access. The computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided at multiple levels through restricted access to the individual laboratories, buildings, and site. Confidentiality of results is protected by referencing results to blind coded sample IDs (no names or personal identifiers).

4. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection

A. Special instructions

No special instructions are required.

B. Sample collection

The specimen type is tap water collected in specially prepared 12 mL borosilicate glass vials containing 125 µL of buffer-quench solution(2). Borosilicate glass vials with Teflon/silicone septa obtained from commercial sources commonly contain VOC contaminants that can mask the levels of VOC analytes originally in the tap water at the time of sample collection, and thus prevent accurate exposure assessment. Borosilicate glass vials are obtained commercially and specially modified by laboratory staff (DLS VOC laboratory) to remove measurable levels of most VOCs. The SOP is based on our previously published research into VOC contamination from borosilicate glass vials (2). It is imperative that these specially treated borosilicate glass vials be used for all tap water collection to insure sample validity. The borosilicate glass vials are supplied by DLS Lab staff for all VOC studies.

C. Sample handling

The Centers for Disease Control and Prevention (CDC)-prepared vials contain milligram quantities of potassium dihydrogen-phosphate, potassium phosphate dibasic and sodium thiosulfate. Potassium dihydrogen-phosphate and potassium phosphate dibasic are used to buffer the tap water sample to an approximate pH of 7. Sodium thiosulfate neutralizes any free chlorine that is present in the tap water samples. Since VOCs are highly volatile, care must be taken to insure that samples are kept at refrigerator temperatures, 4^{o}C, during storage and shipment. All samples should be placed on wet ice or into a refrigerator within 30 min 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 (but not frozen) throughout the shipment process. Samples should be shipped to insure that the samples will arrive at the CDC on normal business days to insure the proper processing of the samples upon arrival. Samples should not be frozen or stored at freezer temperatures at any time during sample collection and shipment. Samples should be shipped within 1 to 2 weeks of collection so that the samples can be analyzed within 9 to 10 weeks of collection. The analytes measured by this method have demonstrated stability in specimens stored for at least 52 weeks at refrigerated temperatures.

D. Sample quantity

The optimal amount of specimen required for analysis is 12 mL; the minimum amount is 5 mL.

E. Unacceptable specimens

The criteria for unacceptable specimen are a low volume (< 10 mL), failure to maintain sample temperature between 0 °C and 10 °C, suspected contamination, inverted septum, cracked or broken glass vial and use of an untreated borosilicate glass vial.

Failure to obtain adequate sample volume is obvious when the samples are received. Visual inspection of the vial reveals if the estimated tap water volume is less than the required 10 mL. Maintenance of temperature during shipment is verified by examining the shipment temperature upon receipt. Visual inspection of the glass vials reveals if they are cracked or broken vial. A description of reasons for each rejected sample is recorded in the Access database as the samples are logged into the lab.

5. Procedures for Microscopic Examinations; Criteria for Rejecting Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibration Materials, Control Materials, and All Other Materials; Equipment and Instrumentation

A. Reagents and sources

1. Solvents

Solvents used during the development, validation, and application of this method are listed below.

Purge and Trap grade methanol is required for all dilutions of native standards and labeled analogs. Other grades of methanol typically contain unacceptable levels of THM contamination. Purge and trap grade methanol is available from Burdick and Jackson (Muskegon, MI).

HPLC grade water of acceptable purity has been acquired from Baker-Mallinckrodt. Variability in the contaminant levels in this product requires the testing of product lots. Once an acceptable lot has been found, a 1-year supply of water is purchased to insure an adequate supply. The purchased water is further processed by helium sparging and distillation to reduce VOCs before use. Directions for this procedure are given below under Section 6.d, and are based on previously published techniques for removing residual VOCs from reagent water (3).

2. Calibration and Control Materials

Material used for preparation of calibration standards, labeled internal standards, and Quality Control materials are listed in Table 1 below. All chemicals are used without further purification unless otherwise noted, and final concentrations of prepared materials are calculated using the purity specified with the raw material lot. Materials procured from other sources should meet or exceed these listed requirements.

Table 1: Reagents for Calibration and Control Materials

Compound	Formula	Acceptable Grade	Safety	Source
Chloroform	$CHCl_3$	99%	a, b	f
Bromodichloromethane	$CHCl_2Br$	98%	a, b	f
Dibromochloromethane	$CHClBr_2$	98%	e	f
Bromoform	$CHBr_3$	99%	a, c	f
tert-Butyl Methyl Ether	$(CH_3)_3COCH_3$	99%	d, e	g
Chloroform-^{13}C	$^{13}CHCl_3$	99%	a, b	h
Bromodichloromethane-^{13}C	$^{13}CHCl_2Br$	99%	a, b	i
Dibromochloromethane-^{13}C	$^{13}CHClBr_2$	92%	e	i
Bromoform-^{13}C	$^{13}CHBr_3$	99%	a, c	h
tert-Butyl Methyl Ether-D_{12}	$(CD_3)_3COCD_3$	99%	d, e	j

Key: a - Cancer suspect agent
b - Toxic
c - Lachrymator
d - Flammable liquid
e - Irritant
f - Aldrich Chem. (Milwaukee, WI)
g - Chem Service (West Chester, PA)
h - Cambridge Isotope Laboratories (Woburn, MA) - available commercially

B. Preparation of glassware

All glassware used in this study is carefully cleaned to be certain to remove possible nonspecific VOC contamination**.** To remove possible analytical interferences, rinse glassware (volumetric flasks, ampoules, and storage bottles) with reagent-grade methanol, and heat at 150°C in a vacuum oven with an independent vacuum source for at least 8 hr to remove adsorbed VOCs. The independent vacuum source is necessary to prevent cross-contamination from other laboratory operations. There is the risk of changing the calibration of volumetric glassware by heating, but the error resulting from this is small compared to other sources of error in the VOC method. Cool the glassware to room temperature under vacuum and restore pressure using nitrogen (UHP grade). Remove treated glassware from the oven and store sealed with Teflon-lined caps until used in standard preparation.

C. Preparation of Glass vials

Borosilicate glass 12 mL vials are specially treated to remove VOC contamination resulting chiefly from the Teflon/silicone rubber septa. The individual glass vials and septa are both heated as described in the VOC SOP Manual - "Glass Vial Cleanup 1.0". The glass vials have 125 µL of buffer-quench solution added to them and the vials are sealed with Teflon/silicone rubber septa secured in place using a one-hole phenolic screw cap. This process produces a sampling container with substantially less contamination.

D. Preparation of Blank water

1. Apparatus

Distillation of the raw water is accomplished using a Fuchs continuous reflux apparatus which has been modified to run with helium stripping during the distillation process.

2. Procedure

a. Water distillation

Fill the 3000-mL 2-neck flask with approximately 2500 mL of HPLC grade water. Add 10 micro-porous boiling chips to the flask and seal the device. Adjust the helium flow to produce an active flow through the sparger. Allow the helium to bubble through the raw water for 17 hr. After 17 hr, turn on the heating mantle to bring the water to a boil. Allow the water to reflux for 4 hr. At the end of the reflux period, begin collecting the finished blank water. Dispense the finished water into 100-mL glass stoppered Pyrex bottles and cap immediately As described in Section 6.b. If more blank water is needed, allow the storage head to refill and repeat the process.

b. Water storage

The blank water is either used directly from the glass stoppered Pyrex bottles or stored in 2, 5 and 20-mL flame sealable Pyrex ampoules. Water is aliquoted for storage by transfer from the Pyrex bottles using a Portapet Pipetter equipped with a 10-mL long tip serum pipette. A torch (natural gas and oxygen fuel) is used to melt the ampoule neck to produce a gas-tight seal. A tight seal is checked by tapping the sealed end on a hard surface and looking for a water leak. The sealed ampoules are stored in the dark at room temperature.

E. Preparation of native analytical standards

1. Procedure for handling neat compounds

   All analytes are purchased as neat liquids in flame sealed ampoules. After opening the ampoule the remaining (unused) material is discarded. A few of the most expensive analytes (custom synthesis products) are aliquoted into an individual amber borosilicate glass container and flame sealed for future use. After transferring the compounds, flame seal the ampoule and store in an explosion-proof freezer. Cover all containers with aluminum foil to reduce light exposure to all neat compounds. Store neat standards in a separate location from tap water samples, blanks and quality control materials.

2. Procedure for filling and sealing glass ampoules

   Aliquot about 0.5 mL of the neat standard into a 1-mL borosilicate glass ampoule. Use a glass Pasteur pipette to transfer the liquid. Before using, rinse the pipette by initially filling with the neat standard and expelling to waste. (NOTE: There may not be enough neat standard to perform this rinse step.) Make sure the liquid is placed in the bottom of the ampoule and is not adhering to the neck. Otherwise, during the sealing procedure, ignition of the liquid will produce a loud pop and could shatter the ampoule. Place the ampoule in a Dewar containing liquid nitrogen until the liquid is cooled, but not frozen. The cooling of the ampoule will require approximately 5 sec. If the ampoule remains in the liquid nitrogen for too long, then oxygen and/or nitrogen will dissolve in the liquid and can cause the ampoule to shatter or develop leaks that can compromise the standard. Remove the ampoule from the Dewar. Use a natural gas and oxygen torch to seal the ampoule by melting and pulling the molten neck to affect a seal. Allow the sealed ampoule to come to room temperature then invert the vial and gently tap the sealed end on a hard surface. If the ampoule does not leak, the ampoule is ready to store. If a leak does occur, do not attempt to reseal the ampoule. Dispose of the ampoule and make a new one. Repeat the above steps until at least 12 sealed ampoules are made. Place the sealed ampoules in an appropriate holder and store in a freezer at -60 °C.

3. Transfer of liquids used in making standards

   Positive displacement pipettes are used for all transfer of liquids in the µL range. Transfers in the 5-µL to 30-µL range use a pipette with 0.1-µL increments. Transfers in the 31-µL to 100-µL range use a pipette with 0.2-µL increments. Transfers in the 101-µL to 250-µL range use a pipette with 1-µL increments. Volumetric flasks, 25 mL class A, are used to make all standards. Standard concentrations are based on the gravimetric measure of mass transferred to the volumetric flask. Standards are prepared in methanol (purge and trap grade).

4. Balance calibration

   Before sample weighing procedures for each standard set, the balance must be calibrated and checked for accuracy. With an empty pan, select the 160 g/0.1 mg weighing range. Tare and then wait for stability. Press the CAL button; the display will show 'C' and the busy symbol. When 'CC' appears, press the CAL button again, completing the calibration stage. Check the balance accuracy by weighing the 50 g weight. Record weight check results in the “Sartorius Analytical Balance Maintenance Log book”.

5. Final concentrations of the standards

   Standards are prepared in helium-sparged, distilled water at levels that are determine accurately, at the approximate concentration levels listed in Table 2 below. Preparation steps are listed in Sections 6.e.6 & 7 below.

Table 2: THM/MTBE calibration standards.

	Concentration in final 25 mL water standard (ppb)						
Analyte	**7.107**	**6.107**	**5.107**	**4.107**	**3.107**	**2.107**	**1.107**
Chloroform	48.576	15.361	4.858	1.536	0.486	0.154	0.049
Bromodichloro methane	30.096	9.517	3.010	0.952	0.301	0.095	0.030
Dibromochloro methane	121.728	38.494	12.173	3.849	1.217	0.385	0.122
Bromoform	59.088	18.685	5.909	1.869	0.591	0.187	0.059
Methyl tert-Butyl Ether	38.400	12.143	3.840	1.214	0.384	0.121	0.038

6. Stock solutions and concentrated standards

   Add about 80 mL purge and trap grade methanol to a 100-mL volumetric flask. Label this flask Stock 6. Add about 20 mL purge and trap grade methanol to eight 25-mL volumetric flask. Label one of these flasks Stock 5 and label the remaining flasks 1 through 7(Keep all flasks sealed when not directly adding standard materials).

   Weigh the flask labeled “Stock 6" accurately to 0.001 g. Set aside flasks labeled Stock 5 and flasks labeled 1 through 7. Sequentially add the following neat compounds listed in Table 3 below to the flask labeled “1" and determine the total weight, to 0.001 g, between each addition. Keep the lid tightly screwed onto the flask unless volume is being added, minimizing the loss of methanol and/or analyte due to evaporation.

Table 3: Formulation of stock solution 1.

Analyte	Volume (uL)	Density (g/mL)	Expected Wt. (mg)
Chloroform	53	1.48	76.6
Bromodichloromethane	30	1.98	60.9
Dibromochloromethane	25	2.45	60.7
Bromoform	20	2.89	55.8
Methyl tert-Butyl Ether	110	0.74	77.7

Once all of the neat standards are added to the flask labeled “Stock 6", fill the flask to the 100 mL mark with purge and trap grade methanol. Invert flask “1” 5 times and sonicate for 3 min to insure complete mixing. Stock 6 solution is now prepared and ready to be further diluted for the preparation of standards.

Use the flask labeled “Stock 5” to dilute 750 µL of “Stock 6” solution in 25 mL of purge and trap grade methanol. Invert flask “Stock 5” 5 times and sonicate for 3 min to insure complete mixing. “Stock 5” solution is now prepared and ready to be further diluted for the preparation of standards.

Prepare working standards 1 - 7 by preparing dilutions of stock solutions Stock 6 and Stock 5 as listed in Table 4 below.

Table 4: Formulation of calibration standards.

Standard number	Stock Solution Used	Volume Added (µL)	Final Volume (mL)
1	Stock 5	25	25
2	Stock 5	79	25
3	Stock 5	250	25
4	Stock 6	24	25
5	Stock 6	75	25
6	Stock 6	237	25
7	Stock 6	750	25

Mix all solutions by sonicating for 3 min. Label the ampoules with the standard level (1 - 7) and a standard batch identifier. For example "1.107005", where “1" identifies the standard made (as given in column 1 above) and “107" indicates that this is the batch number of standards produced. Dispense the standard solutions into glass ampoules and flame seal as described above in Section 6.e.2. Make at least 100 sealed ampoules of each standard, 1 - 7. Label each ampoule with a sequential 3 digit number indicative of the preparation order of that aliquot and sealed (e.g. 005 is the fifth aliquot prepared for the above standard 2. After leak checking the flame sealed ampoules as described in Section 6.e.2, place the ampoules in a 10 x 10 grid boxes and store in a freezer at approximately -60 °C.

7. Daily standard

Daily standards are made by taking particular concentrations of the above ampoules and diluting in helium sparged/distilled water. For example, standard 5.107003 is created by taking 40 µL from ampoule 5.107003 and diluting in 25 mL of helium-sparged, distilled water.

F. Preparation of labeled analog solutions

1. Procedure for handling neat compounds

The majority of the labeled compounds contains ^{13}C and is shipped in ampoules or thick walled glass containers of various sizes. It is advisable to cool these containers in ice before opening in order to minimize pressurization and volatilization problems.

2. Procedure for storing labeled analog solutions

Aliquot the appropriate amount of primary or secondary analog solution into a 1-mL flame-sealable borosilicate glass ampoule. Use a glass Pasteur pipette to transfer the solution. Before using the pipette, rinse by initially filling the pipette with the solution and expelling to waste. Make sure the solution is placed in the bottom of the ampoule and is not adhering to the neck of the ampoule. Otherwise, during the sealing procedure, the ignition of the methanol will produce a loud pop and could shatter the ampoule. Place the ampoule in a Dewar containing liquid nitrogen until the solution is cooled, but not frozen. This will require approximately 5 sec. If the ampoule remains in the liquid nitrogen for too long, then oxygen and/or nitrogen will dissolve in the liquid and can cause the ampoule to shatter or develop leaks that can compromise the standard. Remove the ampoule from the Dewar. Use a propane/oxygen torch to seal the ampoule by pulling the molten neck to create a gas-tight seal. Allow the sealed ampoule to come to room temperature then invert the vial and tap the sealed end on a hard surface. If the seal does not leak, the ampoule is ready to store. If a leak does occur, do not attempt to reseal the ampoule. Dispose of it and make a new one.

3. Final concentrations of the labeled solutions

Labeled standards are prepared at the approximate concentrations listed in Table 5 below. Preparation steps are listed in Sections 7.e.4, 5, & 6 below.

Table 5: THM/MTBE labeled internal standards.

Analyte	Concentration (ppb)
Chloroform	2.9
Bromodichloromethane	1.5
Dibromochloromethane	2.0
Bromoform	0.7
Methyl tert-Butyl Ether	1.0

4. Primary analog stock solutions

Primary analog stock solutions are made by initial dilution of the neat compound into 25 mL of purge and trap grade methanol. This provides a consistent source of these compounds for further dilutions. For each analog add approximately 20 mL purge and trap grade methanol to a 25-mL volumetric flask. Keep the flask sealed when not directly adding standards. Label the flask and dilute the compounds according to the scheme described in Table 6 below:

Table 6: THM/MTBE labeled internal stock solutions.

Standard	Compound	Volume or Weight - µL(g)	Approximate Concentration (ppm)
L12W	^{13}C-Chloroform	25 (0.0370)	1480
L18W	^{13}C-Bromodichloromethane	10 (0.0198)	792
L19W	^{13}C-Chlorodibromomethane	10 (0.0245)	980
L19W	^{13}C-Bromoform	25 (0.0723)	2890
L38W	D_{12} Methyl tert-Butyl Ether	10 (0.0195)	781

Fill the flasks to the 25-mL mark with purge and trap grade methanol. Seal approximately 0.75 mL of these solutions in ampoules as described above. Repeat these steps until as many sealed ampoules (at least 20) as possible are made. Label and place the sealed ampoules in an appropriate holder and store in a freezer at approximately -70 °C.

5. Secondary analog stock solutions

   Label and fill a 25-mL volumetric flask with approximately 20 mL of purge and trap grade methanol. Add the following solutions (L12W: 306.5 µL; L18W: 592 µL; L19W: 638 µL; L24W: 76 µL; L38W: 400 µL) to give solution L. Fill to the mark with purge and trap grade methanol and mix the solution by sonicating for 3 min. Seal approximately 250 µL of these solutions in ampoules as described above in Section 6.f.2. Repeat these steps until as many sealed ampoules as possible are made (at least 70). Label and place the sealed ampoules in an appropriate holder and store in a freezer at approximately -60 °C.

6. Working analog stock solutions

   Prepare the working stock solution for a given 4-week period of analysis by adding 250 µL from solution L to a 25-mL flask containing approximately 25 mL purge and trap grade methanol. This solution can be reused for 4 weeks if it is sealed thoroughly and stored at approximately -20 °C. To achieve the desired concentrations of internal standard, pipette 40 µL of the working analog stock solution into each sample (tap water, QC, water blank or aqueous standard).

G. Preparation of Quality Control materials

QC Materials are prepared with the final concentrations (ppb) given in Table 7 below.

1. Preparation of Low QC Material

   Add approximately 20 mL of purge and trap grade methanol to a 25 mL volumetric flask and label this flask “QL107”. Add 20 µL of “Stock 6” solution to the flask labeled “QL107” and fill to the mark with purge and trap grade methanol. Invert flask “QL107” 5 times and sonicate for 3 min to insure complete mixing.

2. Preparation of High QC Material

   Add approximately 20 mL of purge and trap grade methanol to a 25 mL volumetric flask and label this flask “QH107”. Add 186 µL of “Stock 6” solution to the flask labeled “QH107” and fill to the mark with purge and trap grade methanol. Invert flask “QH107” 5 times and sonicate for 3 min to insure complete mixing.

Table 7: Calculated concentrations of analytes for low and high QC

Analyte	Calculated concentration of QL107 (ppb)	Calculated concentration of QH107 (ppb)
Bromodichloromethane	1.295	12.047
Bromoform	0.803	7.464
Chloroform	3.246	30.189
Dibromochloromethane	1.576	14.654
MTBE	1.024	9.523

H. Storage of standard solutions

Except while in use, all standard stock solutions, labeled analog stock solutions, and quality control materials are stored at approximately -60 °C. The stock solutions can be stored for up to 4 weeks at 4 °C. Once ampoules containing stock solutions have been opened, they must be used immediately. The working analog stock solution may be preserved and used over the next 4 weeks if it is carefully sealed and stored at approximately 4 °C within 8 hr of initial preparation. All stock solutions are labeled to include a reference to the preparation procedure, batch and date.

I. Supplies

Supplies used during the development, validation, and application of this method are listed below. Supplies procured from other sources should meet or exceed these listed requirements.

1. Disposable Pasteur pipettes, (Kimble Glass, Inc., Marietta, GA)
2. Pipette bulbs (Fisher Scientific, Suwanee, GA)
3. Clear pre-scored ampoules; 2-mL, 5-mL, and 20-mL (Wheaton Scientific, Millville, NJ)
4. Clear glass vacuoles, 1-mL and 10-mL (Wheaton Scientific, Millville, NJ)
5. Fisher brand heavy glass desiccator, 250-mm I.D., with cover (Fisher Scientific, Suwanee, GA)
6. Graduated 10-mL glass pipette (Fisher Scientific, Suwanee, GA)
7. Portapet pipette, 10-mL volume (Fisher Scientific, Suwanee, GA)
8. High Vacuum grease, Dow Corning (Fisher Scientific, Suwanee, GA)
9. Stainless steel desiccator (Boekel , Philadelphia, PA)
10. Activated charcoal (Fisher Scientific, Suwanee, GA)
11. Molecular sieve (EM Science, Lab Depot, Alpharetta, GA)
12. Ultra-high purity helium gas, 99.999% (Airgas South, Chamblee, GA)
13. Nalgene high-density polyethylene Dewar flask (Fisher Scientific, Suwanee, GA)
14. VWR positive displacement Micropettors, 40-µL, 50-µL, 250-µL, (Lab Depot, Alpharetta, GA)
15. VWR replacement glass capillaries, 40-µL, 50-µL, 250-µL, (Lab Depot, Alpharetta, GA)
16. SMI positive displacement Micropettors, 20-100 µL, 50-250 µL, (Lab Depot, Alpharetta, GA)
17. SMI replacement glass capillaries, 20-100 µL, 50-250 µL, (Lab Depot, Alpharetta, GA)
18. Pyrex volumetric flasks with screw caps, 25-mL (Fisher Scientific, Suwanee, GA)
19. Wheaton caps, No. 415-18 (LabSource, Inc., Chicago, IL)
20. Nitrile gloves, non-powdered (LabSource, Inc., Chicago, IL)
21. Ultrasonic cleaner with heater and timer, Model B-221 (Thomas Scientific, Swedesboro, NJ)
22. Liquid nitrogen, 160-L, 22-psi (Airgas South, Chamblee, GA)
23. Heavy-duty, micro polypropylene test tube rack, stackable, for 11-mm diameter tubes (Cole-Palmer Instruments Co., Chicago, IL)
24. SPME Vials, 10-mL (MicroLiter, Suwanee, GA)

25. Septa, Teflon Red/White silicone, Level IV (MicroLiter, Suwanee, GA)
26. Stainless steel washers, metric size 10-cm (Hillman Fastener Company, Cincinnati, OH)
27. Hand-operated Crimper (Wheaton Scientific, Millville, NJ)
28. Open Center seals 20-mm (SUPELCO, Bellefonte, PA)
29. Oxygen, 99.99%, 200-300 cu. ft. (Airgas South, Chamblee, GA)
30. Gastight Teflon luer-lock tip syringe, 5-mL (Hamilton Company, Minneapolis, MN)
31. Sharps container (Pro Tec US Clinical Products, INC., Richardson, TX)
32. Sodium hypochlorite (James Austin Co., Mars, PA)
33. Tenax trap (Tekmar Co., Cincinnati, OH)
34. Flow tube, 150-mm, for helium 0 to 100 cc/min (Alltech Associates, Inc., Deerfield, IL)
35. Adapter 1/8" to 1/8" MPT (Alltech Associates, Inc., Deerfield, IL)
36. DB-VRX Capillary Column, 0.18-mm I.D., 20-m, 1.0-µm film thickness (J&W Scientific, Folsom, CA)
37. Standard Printer paper (local office supply)
38. HP LaserJet 1200 and 5Si microfine toner cartridges (Government Technology Services, Inc., Atlanta, GA)
39. Equipment

Equipment used during the development, validation, and application of this method are listed below. Equipment procured from other sources should meet or exceed these listed requirements.

1. Distillation Equipment (Ace Glass, Inc., Louisville, KY)
    a. Twin connecting hose adapter
    b. Column, vacuum jacketed
    c. Condenser, Allihn
    d. Head, Storage, 3000-mL
    e. Flask, two necks, 3000-mL
    f. Mantle, 3-L
    g. Powerstat, 0-140 volts
    h. Teflon sleeves, 0.076-mm
    i. Adapter, vacuum short stem, 14/20
    j. Teflon sleeves, 0.13-mm, 14/20
    k. Bottle, single neck, 14/20 joint
2. Squaroid vacuum oven, 2.3-cu. ft. (Fisher Scientific, Suwanee, GA)
3. Direct-drive vacuum pump (Fisher Scientific, Suwanee, GA)
4. Sartorius analytical balance (Fisher Scientific, Suwanee, GA)
5. Bar code scanner (Wellch-Alleyn, Skaneateles Falls, NY)
6. Datamax label matrix label printer (Computype, Inc., St. Paul, MN)
7. Brady printer labels (Brady Worldwide, Milwaukee, IL)
8. Ultra-low temperature freezer (Fisher Scientific, Suwanee, GA)
9. Refrigerator (Fisher Scientific, Suwanee, GA)
10. Standard laboratory freezer (Fisher Scientific, Suwanee, GA)
11. QC Purging equipment (Ace Glass, Inc., Louisville, KY)

a. Flask, three necks, vertical, 2000-mL, center neck 29/42, side necks, 24/40
b. Adapter, bushing, bottom inner 29/42 top outer 24/40
c. Stopper, full length 24/40
d. Stopper, medium length 14/20
e. Adapter, Claisen, 24/40
f. Adapter, Distilling trap, 24/40

12. Sterilized hood biological safety cabinet (Baker, Inc., Sanford, ME)
13. Thermo Trace Gas Chromatograph with sub ambient cooling (ThermoFinnigan, Inc. Miami, FL)
14. Trace mass spectrometer (ThermoFinnigan, Inc. Miami, FL)
15. Hewlett Packard LaserJet 5Si and 1200 Printers (Hewlett-Packard Co., Atlanta, GA)
16. Dell OPTIPLEX GX260 w/ 4.0 GB hard disk, 52 Mb RAM, flat screen monitor, and Pentium(IV) 2.4 GHz processor (Government Technology Services, Inc., Atlanta, GA)
17. Microsoft Access based database (Government Technology Services, Inc., Atlanta, GA)
18. PC SAS (Statistical Analysis System, Inc., Cary NC)

J. Instrumentation

1. Trace mass spectrometer (ThermoFinnigan) combined with a Combi-PAL autosampler (CTC Analytics) using SPME-fiber technology (Supelco) and with gas chromatographic separation (Trace GC 2000) using a 20-m DB-VRX capillary column (J&W Scientific) with 1.o-µm film thickness and 180-µm internal diameter. The carrier gas is helium.

2. Trace GC Parameters

Table 8: Event Run Table

Time (min)	Switching Valve #	Set Point	Condition Initiated
1.00	1	ON	Cryo-Trap Cools to -100°C
3.00	1	OFF	Cryo-Trap Heats to 220°C
10.00	1	ON	Cryo-Trap Cools to -100°C

Injector type: Split/splitless inlet (right inlet)

- Injection mode: Splitless
- Purge flow: 50 mL/min
- Purge time: 2.00 min
- Inlet temperature: 200 °C
- Carrier gas: Helium
- Carrier gas control mode: Constant flow at 1.50 mL/min

Front Detector: 220 °C GC/MS transfer line

GC column: DB-VRX (J&W Scientific)

- Length: 20 m
- Diameter: 180 µm
- Film thickness: 1.0 µm

GC Oven temperature program:

- Initial Temp: 20 °C for 3 min
- Ramp 1: 30 °C /min to 200 °C; 1 min
- Ramp 2: 45 °C /min to 50 °C; no hold
- Equilibrium time: 0.10 min
- Run time: 13 min

Cryogenic cooling for GC Oven:

- Liquid nitrogen at 22 psi
- Quick cooling enabled
- Ambient temp: 50 °C
- Cryo timeout: 20 min
- Shutdown enabled

3. Combi-PAL Autosampler (CTC Analytics) Parameters

PAL Method: SPME Agitated & Heated

- Carboxen-PDMS fiber 75 µm coating thickness
- Pre-incubation time: 10 sec
- Heated agitator temperature: 50 °C
- Agitator speed: 500 rpm
- Agitator on time: 5 sec
- Agitator off time: 2 sec
- Vial penetration: 22 mm
- Extraction time: 8 min
- Desorb to: GC Inj1
- Injection penetration: 54 mm
- Desorption time: 12 min
- GC run time: 13 min

Peltier Tray (CTC Analytics)

Peltier Tray uses solid state technology to cool the metal sample tray

- Temp set point: 15 °C
- Actual cooling range: 15-16 °C

4. Cryo-Trap (Scientific Instrument Services, Inc)

Cryo-Trap used to trap volatiles on head of GC column by cryogenic cooling

- Temp set point: -60 °C
- Liquid nitrogen at 22 psi

Cryo-Trap desorbs trapped volatiles by ballistic heating

- Temp set point: 220 °C
- Electric Heater

The cooling and heating cycles of the Cryo-Trap are controlled by the Agilent 6890 GC through Switching Valve Driver #7 in the RUN Table.

5. Trace Mass Spectrometer Parameters

Table 9. Mass spectrometry parameters for the analysis of trihalomethanes and methyl tert-butyl ether in household tap water.

Compound (label configuration)	**Labeled standard mass (m/z)**	**Analyte mass (m/z)**	**Confirmation mass (m/z)**	**Dwell time per mass (msec)**	**Photomultiplier Detector setting (volts)**
Methyl tert-butyl ether (2H12)	82	73	74	30	300
Chloroform (13C)	84	83	85	30	225
Bromodichloro-methane (13C)	84	83	85	50	300
Dibromochloro-methane (13C)	130	129	127	50	300
Bromoform (13C)	174	173	171	50	350

The mass spectrometer was equipped with an electron-impact source and run in the selected ion monitoring (SIM) mode (Table 1). As each analyte eluted from the GC column, the mass spectrometer measured three ions: one each for quantification, confirmation and the labeled analog. Quantification ions were selected as the most abundant ion in the mass spectrum that did not compromise the specificity of the analysis. We determined the retention time for each compound by analyzing known standards in full-scan mode and adjusting the SIM windows accordingly. Cycle time per sample was 21 minutes.

a. Quantification

Xcalibur Quan software (ThermoFinnigan) was used for peak integration, calibration and quantification. Peak integrations were performed with ICIS integrator and confirmed by visual inspection. Relative response factors were calculated on the basis of relative peak areas of analyte quantitation ion and labeled analog ion. The set of seven calibrators analyzed with each set of samples was used to generate the calibration curve for that day. These calibration curves were linear ($r^2 \geq 0.99$) and spanned three orders of magnitude. Calibration curves were adjusted for ion cross contamination between native analyte and isotopic analog according to Colby and McCaman (15). The lowest calibrators ranged from 30 to122 ng/L. The LOD was calculated as three times the standard deviation at zero concentration ($3S_0$). If $3S_0$ was less than the lowest standard, then the lowest standard served as the LOD (16).

b. Quality Assurance

Data were subjected to rigorous QC procedures using a custom laboratory information management system constructed in Microsoft Access. Before analysis of samples, the mass spectrometer was tuned against PFTBA calibration gas using the autotune function to ensure proper mass calibration. Contamination was evaluated both qualitatively and quantitatively. Laboratory air was extracted using SPME for 8 min, then desorbed into the GC-MS as described above. The resulting chromatograms were qualitatively reviewed for the presence of gross contamination of THMs or MTBE. The analysis of a water sample free of volatile organic compounds (VOC) was used to quantify any trace contamination of analyte. Following sample analysis and the visual inspection of every integrated peak, additional QC parameters were evaluated. Adequate labeled analog response was evaluated on the basis of absolute peak area signal as well as signal-to-noise ratio. We further evaluated the identity of the analyte ion by comparing the confirmation ion ratio in unknown samples with that for reference standards. Each batch of data also was judged against blind QC samples.

c. Quality Control Samples

Two QC samples were processed and analyzed with each batch of samples. We prepared these aqueous samples from concentrated standards in purge and trap grade methanol and stored them at –60°C as aliquots in flame-sealed glass ampoules. On the day of use, we further diluted these stock solutions in helium-sparged and distilled water, and the QC material sampled as though it were an unknown. Two QC pools were prepared (high and low levels) and characterized by 15 separate determinations. Blind QC samples were evaluated by an independent QC officer according to Westgard QC rules (4). If a QC sample exceeded QC limits for an analyte, then all results for that analyte on that day were rejected.

d. Blank Analysis

Volatile organic compounds such as chloroform and MTBE are ubiquitous in a typical laboratory; rigorous technique is required to minimize sample contamination from laboratory air. Potential sources of contamination include chlorinated water, common household cleaning products, laboratory solvent usage, and oxygenated fuel usage. Volatile contaminants from these sources and others easily can spread through laboratory air to samples during preparation (sample handling) or analysis (SPME fiber). Contamination was minimized by removing sources of THMs and MTBE from the laboratories where samples were prepared and analyzed. A blank water sample was used to test for contamination. Blank water was prepared by helium sparging, distillation, and flame sealed in glass ampoules. On the day of use, a water blank was removed from an ampoule, spiked with labeled analog, and run with each batch of unknowns. If the blank contained analyte levels of at least half of the LOD, then the run was flagged as contaminated. Additionally, a SPME fiber sampling of laboratory air was run to qualitatively assess airborne contaminants.

7. Calibration and Calibration Verification Procedures

A. Calibration Curve

1. A full set of 7 calibrators is analyzed with each batch of data and used for the quantification of analytes in all samples from that batch. The calibration curve is constructed from the relative response factors for each analyte at the 7 calibration levels. Correlation coefficients typically exceed 0.995. All calibration curves were performed in water because it proved impossible to consistently reduce the background VOC levels in serum or whole blood below detectable levels. Matrix spike experiments established that calibration curves in whole blood and water have the same slope. This validates the use of water-based calibrators for quantifying VOCs in whole blood.

2. The slope and intercept of this curve are determined by linear least squares of data weighted 1/X using the ThermoFinnigan Xcalibur Quan software. Some compounds require correction for internal standard contribution to the native ion. This data manipulation was also performed using the ThermoFinnigan Xcalibur Quan software.

3. The highest point on the calibration curve is above the expected range of results for non-occupationally exposed people and the lowest point is at or below the measurable detection limits. The remainder of the points in this curve is distributed evenly between these two points. The calibration curve spans three orders of magnitude. The values of these standards are given above in section 6.b.(4)(e).

4. Limits of detection are calculated as three times the standard deviation at zero concentration (3S0) as described by Taylor (4). Assay detection limits change with improvements in sensitivity, precision, and contamination control. Current detection limits are shown below:

B. Verification

1. R-squared values for daily calibration curves must in all cases be greater than 0.95; in more than 90% of the cases it is greater than 0.995. Linearity of standard curves should extend over the entire standard range, three orders of magnitude. Typically, Y-intercepts calculated from the least squares fit of the data are not significantly different from 0. Calibration curves for several analytes consistently have significant y-intercepts. When this occurs the source of this bias should be established. Possible sources include incorrect ion ratios, contamination of water/methanol used to dilute standards, and contamination of analog spiking solution.

2. Calibration verification is carried out after any alteration of the instrument which may lead to changes in instrument response. These include changing the source, the capillary column and performing any other repairs to the mass

spectrometer solid phase microextraction system. Calibration verification is carried out by measurement of a complete set of calibrators and QC. The resulting calibration curve must be linear and the calculated QC results must be within an acceptable target range determined from repeat measurements.

C. Proficiency Testing (PT) Materials

Proficiency Testing materials are prepared from neat compounds in a manner similar to standard preparation. Several levels are prepared for all analytes to cover the calibration range. The PT materials are sealed in ampoules, blind coded and stored at -70 ± 5 °C until use. PT testing is performed twice a year and following any major maintenance of the instrumentation. Proficiency testing samples are blind coded for analysis; results are evaluated by an external quality control officer.

8. Procedure Operation Instructions; Calculations; Interpretation of Results

A. Analysis of samples

Samples are transferred to the 10-mL SPME vials via 10-mL luerlock gas-tight syringes.

1. Water Sample Preparation

a. Attach a 1.5-inch needle to the syringe.
The tap water vials were removed from refrigerated storage (8-10°C) and allowed to equilibrate to room temperature before analysis. Immediately after removing the vial cap, we removed water (5.0 mL) using a precleaned gas-tight syringe (10 mL, glass, Unimetrics, Shorewood, IL) and transferred it into a SPME headspace vial. Labeled analog solution (40.0 µL) was added to the sample using a positive displacement pipettor (VWR Scientific, West Chester, PA), and the SPME vial immediately was crimp-sealed using a Teflon lined septum. Blanks, standards and QC samples were all processed in a similar manner. Samples were prepared and analyzed in daily batches of 30 unknowns, 7 calibrators, 2 QC and one blank.

B. Sample sequence set up

1. From the Xcalibur road map select the sequence that has been developed to access a sample queue. Usually an existing file can be recalled and modified.

2. Type the sample type (e.g. Blank, QC, Std bracket, or unknown).

3. For each sample, fill in the sample comments, sample ID, sample weight, dilution factor, file name (based of the Julian date), path (for data), instrument

method, process method, vial position, levels of standard or QC, and laboratory (initials of analyst).

4. For NHANES study instrument method is c:\Xcalibur\methods\AGITATOR THM1 SIM and processing method is c:\Xcalibur\methods\US_WATER.

5. Once the sequence set up is complete, samples can be run by choosing Actions menu and run this sample or sequence.

6. The last sample to be run should be the fiber bakeout procedure.

Note: Make sure the Dewar contains adequate liquid nitrogen and that the liquid access valve is open.

C. Data acquisition and initial analysis

1. Data Analysis

   a. Delete the fiber cleaning and shutdown entries from the sequence and save the file.

   b. Go to the Qual Browser from the sequence menu.

   c. Open the sequence file for the run (the run number is set according to the Julian date and year (IYYJJJ, where I is an alphabetic instrument identifier, YY is the 2 digit year identifier, and JJJ is the Julian date).

   d. For each analyte in turn, examine the chromatographic peaks to verify that the automated peak detection routine has correctly identified the peak for both the native compound and the internal standard.

   e. Create the Quan file by returning to the run sequence menu and clicking on the Batch Reprocess button on the Tool Bar. This processes the run queue using the method US_WATER.

   f. Recall the processed file by going to the Quan browser and opening the correct sequence file (the one that was just batch processed). The Quan browser will display the calibration curve and all the data parameters including the concentrations that were calculated using the calibration curve.

   g. Inspect the integration of all peaks and the baseline for all analytes; reintegrate manually where needed.

   h. Inspect the calibration curve in at the low concentration end and perform the correction to maximize the correlation coefficient. Due to the limited mass difference between native and labeled compounds, the native analytes have

some response at the mass used for the labeled isotope. Likewise, the labeled isotope often contributes some response at the mass being used for analyte quantitation. In these cases, corrections must be made for the contribution of the labeled analyte to the native ion and vice versa. In order to properly determine the relative response factors between the analyte and analog, these effects must be taken into account. Xcalibur Quan Browser allows for these adjustments using the "Isotope%" function under "Calibration Settings".

i. Save the Quan Browser file using the nomenclature IYYJJJ.XQN.

j. Create the Excel data summary sheet using the same menu "import short Excel report" and save as the same run number format.

k. Save all the data in the folder on the CD for a particular run number.

l. Make a duplicate copy of the same data on a different CD.

2. Transfer Data to the Q: Drive

a. Transfer the data and other files associated with the run to the appropriate instrument/study folder (e.g. Q:\VOC\Natasha).

b. Copy the XQN file and raw files to C:\Xcalibur\Data.

c. Inspect the data and calibration curve, Colby corrections, create the long Excel report and save (IYYJJJ.xls; ok to overwrite the short report name).

d. Copy the long Excel report to the Q folder for this data set.

3. Transferring Data to the ATLIS Data Base

a. Log onto the ATLIS data base from your PC.

b. Import the Long Excel sheet for the particular data set by selecting the proper instrument, analyst, Assay, Study reference number and Run number.

c. Inspect the data and change the dates for all runs so that they all are the same date (if your run goes over two days) and transfer the data by selecting transfer button. This is to allow for the proper logic in evaluating which unknown results correspond to a particular QC sample.

d. Finally, review the run by using the review command to make sure data are transferred.

4. Evaluation of QA/QC Data

Three QC materials are included in each analytical run (one blank water and two water QCs either low or high). Acceptability of results for that entire

analytical run is dependent upon the agreement of the results from these QC materials with established ranges.

For QC processing, examine each QC result and compare to the statistical data from the characterization runs (described in Section 10.b). If a QC result is "out-of-control," the cause of the failure must be determined and corrected. No results from the associated batch may be reported.

The following additional conditions will also necessitate elimination of results:

Low label (ion-2) counts
Possible contamination
Outside of standard curve range
Unconfirmed result
Ion Ratio out of limits
Saturated signals

9. Reportable Range of Results

A. Reportable Limits

The reportable range of results for the analytes using this method is reported in Table10A. The lower reportable limit is either the detection limit or the lowest standard, whichever is higher. The upper reportable limit is the highest linear standard.

Table 10A: Reportable range of results.

Analyte	Method LOD (ppb)	Lowest calibration standard (ppb)	Upper reportable limit (ppb)
MTBE	0.10	0.10	38.4
$CHCl_3$	0.12	0.12	121.7
BDCM	0.05	0.05	48.6
DBCM	0.10	0.10	59.1
$CHBr_3$	0.10	0.10	30.1

B. Limit of Detection

The limits of detection for these analytes are given in Table 14A above. These values were determined by calculating the standard deviation at each standard concentration following repeated measurements of the standards. These standard deviations were then plotted versus concentration (4). The y-intercept of the least squares fit of this line equals S_0, with 3 S_0 being the calculated detection limit (4). The detection limits are generally in the low ppb range.

C. Accuracy

The accuracy basis for this method was established by analyzing independently prepared and characterized proficiency testing (PT) solutions as unknowns. The PT solutions were made from neat THMs and MTBE. The actual determined accuracy values for all analytes and PT levels are in Table 10B.

Table 10B: Accuracy results for all analytes for all PT levels

Analyte	PT01		PT02	
	Conc. (ng/mL)	Accuracy (%)	Conc. (ng/mL)	Accuracy (%)
MTBE	1.0	102	9.8	103
CHCl3	3.1	94	28.3	95
BDCM	1.3	94	11.3	96
DBCM	1.5	94	13.8	96
CHBr3	0.81	95	7.1	101

D. Precision

The results of repeated measurements on spiked water samples at four different concentrations are given in Table 11A below. Relative standard deviations are in most cases less than 7%. 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 water determinations since they include variation in the water both before and after spiking. Multiple measurements on spiked QC materials show somewhat lower standard deviation results, averaging 5% for all analytes (Table 11B).

Table 11A. Reproducibility and limits of detection (LOD) for the analysis of trihalomethanes and methyl tert-butyl ether in household water.

Analyte	QC[1] pool	Analyte level (µg/L)	CV[2] (%)	LOD (µg/L)
Bromodichloromethane	High QC	11.30	4.0	0.05
Bromodichloromethane	Low QC	1.25	3.8	0.05
Bromoform	High QC	7.11	3.6	0.10
Bromoform	Low QC	0.81	4.3	0.10
Chloroform	High QC	28.31	4.0	0.12
Chloroform	Low QC	3.09	4.5	0.12
Dibromochloromethane	High QC	13.77	3.8	0.10
Dibromochloromethane	Low QC	1.52	4.4	0.10
Methyl tert-butyl ether	High QC	9.83	6.4	0.10
Methyl tert-butyl ether	Low QC	1.04	11.5	0.10

[1] - Quality control.
[2] - Coefficient of variation.

Table 11B. Theoretical concentration vs. mean concentration of QC[1] Pools

Analyte	QC pool	Analyte level (µg/L)	CV2	LOD3 (µg/L)	Recovery
Bromodichloromethane	High QC	11.30	4.0%	0.05	94%
Bromodichloromethane	Low QC	1.25	3.8%	0.05	96%
Bromoform	High QC	7.11	3.6%	0.10	95%
Bromoform	Low QC	0.81	4.3%	0.10	101%
Chloroform	High QC	28.31	4.0%	0.12	94%
Chloroform	Low QC	3.09	4.5%	0.12	95%
Dibromochloromethane	High QC	13.77	3.8%	0.10	94%
Dibromochloromethane	Low QC	1.52	4.4%	0.10	96%
Tert-butyl methyl ether	High QC	9.83	6.4%	0.10	103%
Tert-butyl methyl ether	Low QC	1.04	11.5%	0.10	102%

[1] - Quality Control.
[2] - Coefficient of variation.
[3] - Limits of detection.
[4]- Recovery of calculated theoretical concentration, based on dilution of neat standard materials.

E. Contamination

THMs and MTBE are ubiquitous in our environment. Therefore special analytical procedures are required for quantifying trace levels of these compounds. All glassware and consumables are specially treated to remove possible volatile interferences and contamination.

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 (< 1 pptr for most analytes). An acceptable commercial source of water has been identified, but this must be screened for acceptable lots. 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 yield blank water with acceptable levels of VOCs. To prevent further contamination from the laboratory air, water samples are sealed in glass ampoules. In all cases, typical blank water levels are below the detection limits given above.

10. Quality Assessment and Proficiency Testing

A. Quality Assessment

Quality assurance and quality control procedures follow standard practices (4). Daily experimental checks are made on the stability of the analytical system, standards, blanks, and quality control materials which are added to each day's run sequence. Three QC samples (1 blank and 2 water pools) are included in each day's run. A water blank containing the internal standard is run at the beginning of each day to check for the presence of contamination. 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 ensure the choice of the correct chromatographic peak. All data entry errors are evaluated by the QC Officer or supervisor and corrected only after consultation with the analyst and positive identification of the correct information.

B. Quality Control Procedures

1. Establishing QC limits

Quality control limits are established by characterizing assay precision with 20 distinct analyses of each QC pool. Two different pools of water quality control material are used, QC low and QC high. Different calibration materials are included in the analysis (e.g. different sets of standards and internal standards and 20 different sets of QC low and high) to capture realistic assay variation over time. The mean, standard deviation, coefficient of variation, and confidence limits are calculated from this QC characterization data set. Individual quality control charts for the characterization runs are created, examined, and quality control limits are used to verify assay precision and accuracy on a daily basis. Typical QC characterization statistics for MTBE and THMs are listed in Table 12.

Table 12. Quality Control Samples.

Assay ID	Analyte ID	QC ID	MEAN	STD	CV	2CV	Count	LCL3	LCL2	UCL2	UCL3
WDISBP	WBF	QH107	7.45	0.18	2.47%	4.94%	269	6.90	7.08	7.82	8.00
WDISBP	WBF	QL107	0.85	0.02	2.89%	5.77%	273	0.78	0.80	0.90	0.92
WDISBP	WBM	QH107	12.13	0.32	2.63%	5.25%	271	11.17	11.49	12.76	13.08
WDISBP	WBM	QL107	1.34	0.05	3.48%	6.95%	259	1.20	1.24	1.43	1.48
WDISBP	WCF	QH107	30.82	0.86	2.79%	5.58%	273	28.24	29.10	32.54	33.40
WDISBP	WCF	QL107	3.42	0.14	4.18%	8.36%	271	2.99	3.14	3.71	3.85
WDISBP	WCM	QH107	14.70	0.31	2.11%	4.22%	271	13.77	14.08	15.32	15.63
WDISBP	WCM	QL107	1.64	0.05	3.34%	6.69%	260	1.47	1.53	1.75	1.80
WDISBP	WME	QH107	9.72	0.53	5.40%	10.81%	270	8.14	8.67	10.77	11.29
WDISBP	WME	QL107	1.01	0.06	6.13%	12.27%	267	0.82	0.88	1.13	1.19

2. Quality Control evaluation

After the completion of a run, the quality control limits are consulted to determine if the run is "in control". The results of the analysis of the quality control samples measured at the beginning and the end of the analytical run are averaged and the quality control rules are applied to this average. The quality control results are evaluated according to Westgard (4) rules:

Standard Shewhart QC charts are maintained for this internal QC specimen. A separate QC chart is to be maintained for each QC material used for this internal QC specimen. Standard criteria for run rejection based on statistical probabilities are used to declare a run either in control or out-of-control. These rules are:

Analytical run with 2 QC results**:**
a. if both QC results within 2s limits then accept the run
b. if one of two QC results is outside the 2s limits then apply rules below and reject if any condition is met:
c. 13s – either of the two QC results is outside a 3s limit
d. 22s – both QC results in current run are outside 2s limit (same side of mean)
e. R4s sequential – the two QC results in current run are outside 2s limit on the opposite sides of the mean 10x sequential – previous 9 QC results were on same side of mean.
f. If a QC result is declared "out of control", the results for all patient samples analyzed during that run are invalid for reporting.

C. Proficiency Testing

1. Scope of PT

The proficiency testing (PT) scheme for this method is administered by an in-house Proficiency Testing Coordinator. The samples are analyzed and the results evaluated by the in-house PT coordinator.

2. Frequency of PT

Five samples of unknown PT concentrations are analyzed twice a year using the same method described for unknown samples.

3. Documentation of PT

Analytical PT results are reviewed by the analyst and laboratory supervisor, and submitted to the in-house PT Coordinator electronically. The PT results are evaluated by the PT Coordinator; the analysis passes proficiency testing if $\geq$ 80% of the results deviate $\leq$ 25% from the known value. A summary report of the PT evaluation is maintained by the laboratory supervisor. If the assay fails proficiency testing then the sample preparation and instrumentation are thoroughly examined to identify and correct the source of assay error.

Unknown specimens are not analyzed until the method successfully passes proficiency testing.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

A. Internal reference area counts

If the labeled ion counts of the blank samples fall below 20% of the median of these values, this indicates that the instrumental sensitivity has fallen below acceptable limits. The following steps should be taken and the instrument sensitivity rechecked after each is performed. Once sensitivity has been reestablished and calibration verification checked, further steps are not necessary.

1. Check for an air leak in the system.

2. Remove and clean the mass spectrometer ion volume. Replace the filament and any ceramics which may be conducting.

3. Remove and clean the mass spectrometer source.

B. Analyte in blank material

If an inordinately large amount of analyte is measured in the blank, but this is not seen in the remainder of the samples, this indicates a temporary contamination of the blank. The source of this incident should be investigated to prevent repeat occurrences but, no further action is required.

C. Analyte in all samples

If an inordinately large amount of analyte is present in all measurements for a particular day, either the labeled analog solution is contaminated or there is a continual source of contamination. The following steps should be taken until the contamination is removed.

1. Check the immediate area of the mass spectrometer and the laboratory where standards are made for use of the contaminating agent.

2. Discard the purge and trap grade methanol used for dilution of the internal standard. For further analyses use a new bottle of purge and trap grade methanol (or new lot of methanol).

3. Check all glassware which has been used.

D. QC sample outside of 99% confidence limits

If one or more of the quality control sample concentration results fall outside the 99% limits, one of the above is the most likely cause. Follow the steps outlined above to isolate and correct the problem.

In all cases the supervisor should be consulted for the appropriate corrective actions. No analytical results will be reported for runs not in statistical control. After corrective actions are carried out, calibration verification and quality control materials must be analyzed before proceeding with unknown analyses.

12. Limitations of Method; Interfering Substances and Conditions

This method is an isotope dilution mass spectrometry method, widely regarded as the definitive method for the measurement of organic toxicants in water. Alteration of particular aspects of this method can result in major interferences. Care is required in order to produce non-contaminated blanks, glass vials, and quality control materials. The range of linearity and limits of detection are given above in Sections 9.a. and 9.b., respectively.

13. Reference Ranges (Normal Values)

There are no reference ranges for VOCs in water.

14. Critical Call Results ("Panic" Values)

The health effects resulting from exposure to low levels of volatile organic compounds is currently unclear. The method described here is designed for the measurement of low level exposure to VOCs. Study participants are alerted if their water sample contains THMs or MTBE in excess of the EPA MCL value.

15. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

Since the analysis of VOC in water is inherently complex and challenging, there are no acceptable alternative methods of analysis in the NHANES laboratory. If the analytical system fails, we recommend storing the specimens at 4-8°C until the analytical system is restored to functionality.

16. Specimen Storage and Handling during Testing

Specimens may reach and maintain ambient temperature during analysis. If the measurement is delayed to the next day, samples should be refrigerated and not left at ambient temperature overnight. Most sample queues run for extended time periods of up to 24-hr duration. As a precaution water samples (unknowns and QC) are racked into a chilled tray (15 $\pm$ 1 °C) while awaiting analysis.

A. Length of Time Samples may be banked

Repeat measurements of samples stored at 4 °C indicate that water samples may be banked for at least 52 weeks.

B. Proper banking procedures

Water samples for VOC measurement should be stored in the dark at 4-10 °C. In addition, freezing of water samples will lead to breakage of glass vials and loss of sample in most cases. Since VOCs are lost whenever the containers in which they are stored are opened, it is not appropriate to transfer the water samples to another container which would be more resistant to breaking.

17. Test-Result Reporting System; Protocol for Reporting Critical Calls (if Applicable)

Results are generally reported to 2 significant digits. In addition, reports of reference range means and medians should also accompany all reports since these values are not available elsewhere.

The health effects resulting from exposure to low levels of volatile organic compounds is currently unclear. Therefore no critical call levels are set.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

If greater than 4 mL of sample remain after analysis, this material should be returned to storage at 4 °C.

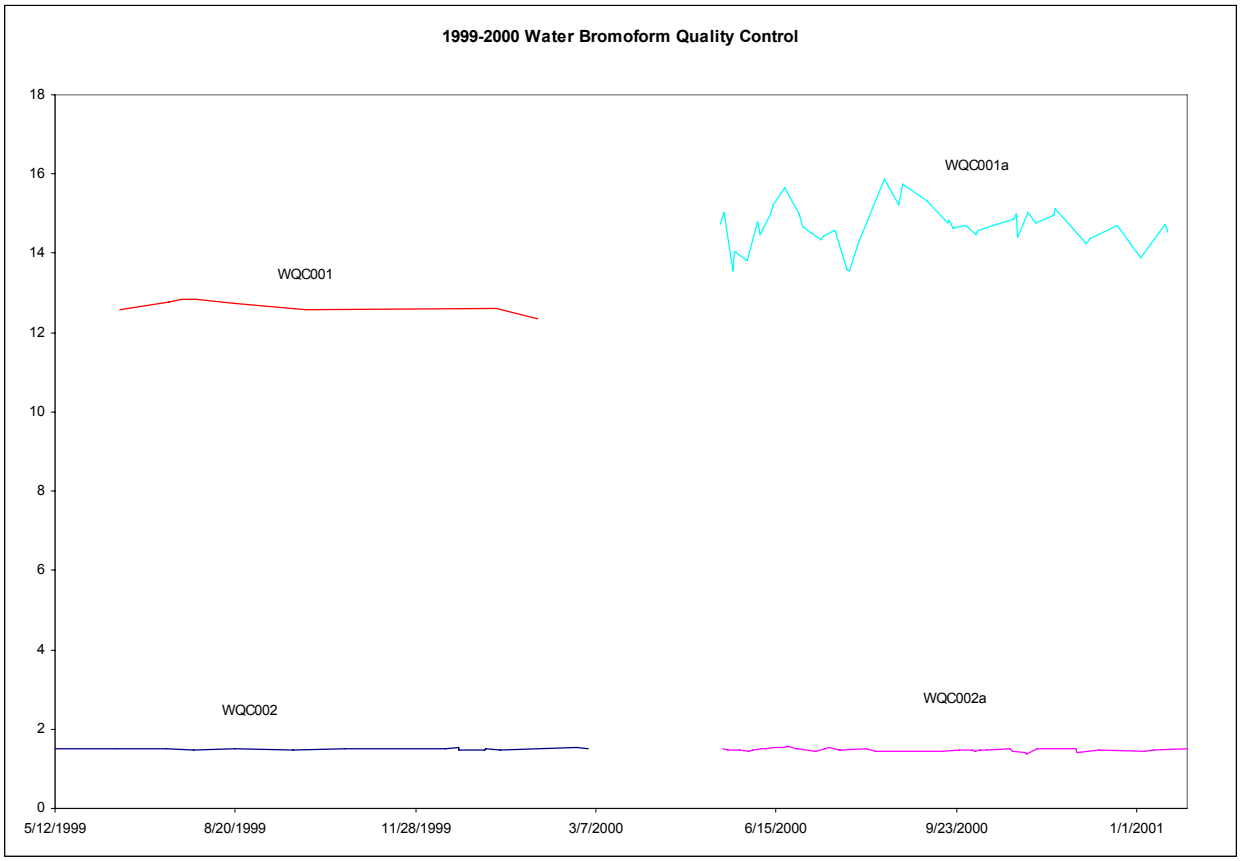
Samples are tracked using a Microsoft Access-based relational database that tracks the sample location and shelf life. It is recommended that records be maintained for 3 years, including related QA/QC data, and that duplicate records be kept off-site in electronic format. All personal identifiers should be available only to the medical supervisor to maintain confidentiality.

Due to the complex nature of the analyses and the unique testing capabilities of this laboratory, it is not expected that specimens will be referred to other laboratories for testing. Should such a need arise; the laboratory supervisor will consult with local subject matter experts to establish an appropriate mechanism and work process.

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human

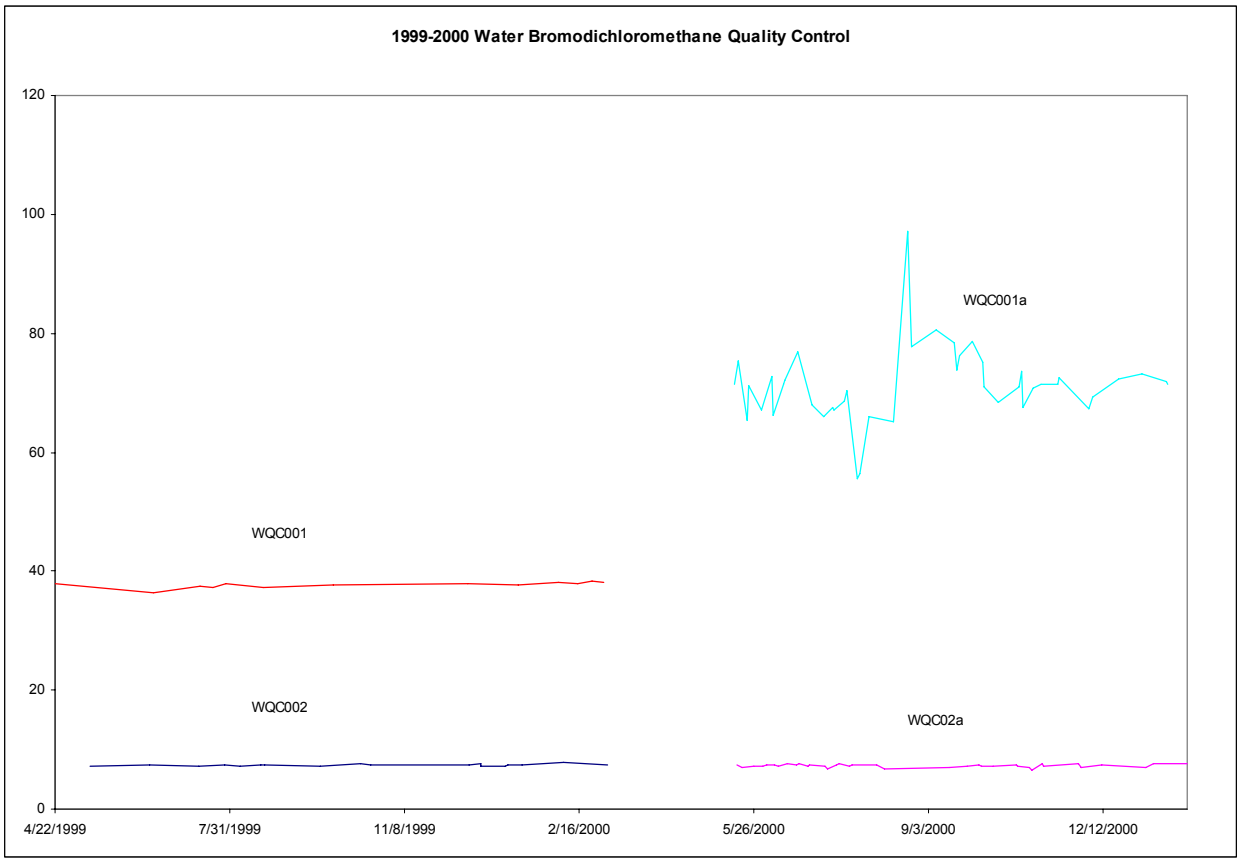
19. Services.Summary Statistics and QC Graphs
    A. Water Bromoform

Summary Statistics for Water Bromoform by Lot						
					Standard	Coefficient
Lot	N	Start Date	End Date	Mean	Deviation	of Variation
WQC002	16	5/12/1999	3/3/2000	1.49303	0.01945	1.3
WQC001	8	6/17/1999	2/4/2000	12.66533	0.17238	1.4
WQC001a	44	5/15/2000	1/18/2001	14.66678	0.52238	3.6
WQC002a	38	5/16/2000	1/29/2001	1.47453	0.03758	2.5



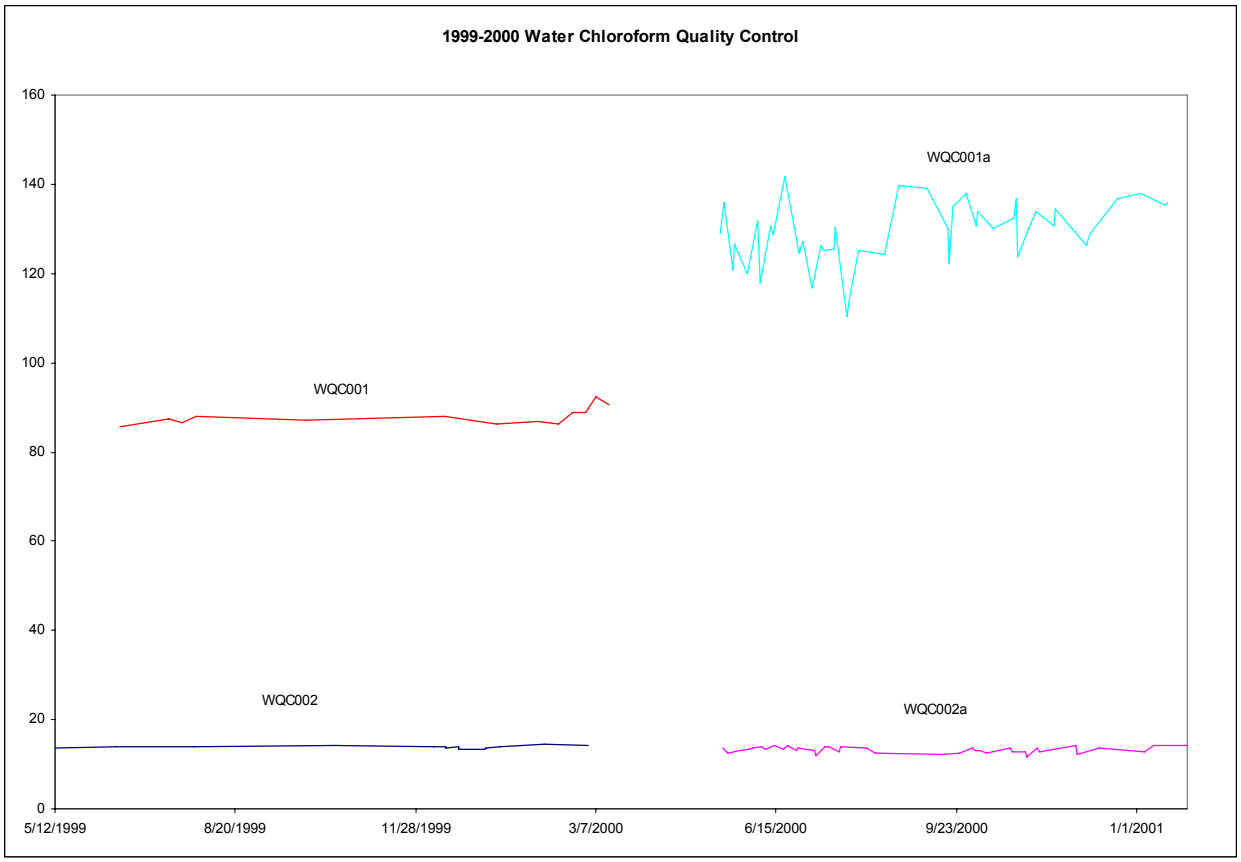
B. Water Bromodichloromethane

Summary Statistics for Water Bromodichloromethane by Lot						
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
WQC001	13	4/22/1999	3/1/2000	37.68398	0.47849	1.3
WQC002	21	5/12/1999	3/3/2000	7.36914	0.14135	1.9
WQC001a	44	5/15/2000	1/18/2001	71.08798	6.38944	9.0
WQC002a	38	5/16/2000	1/29/2001	7.26465	0.27384	3.8



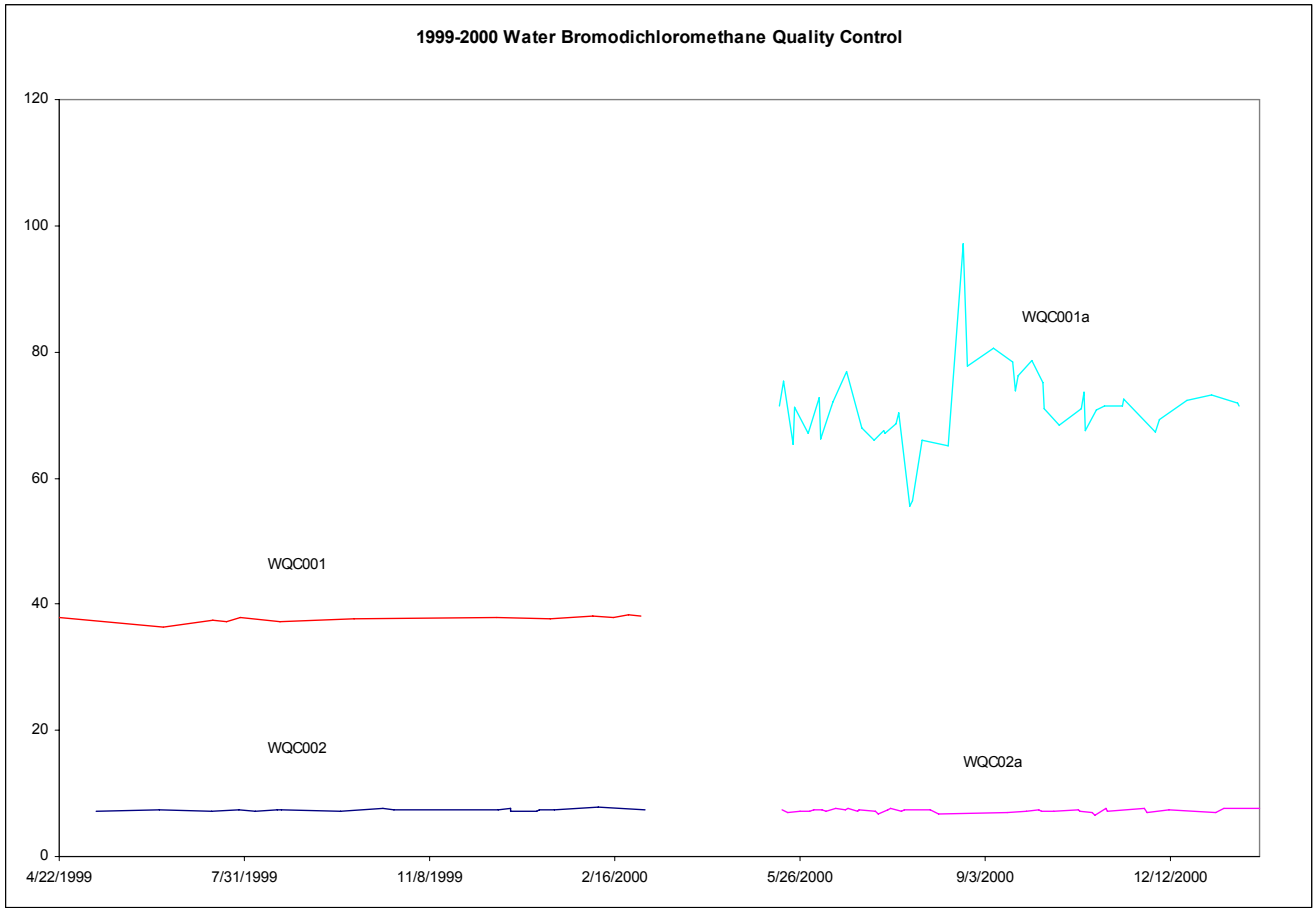
C. Water Chloroform

Summary Statistics for Water Chloroform by Lot						
					Standard	Coefficient
Lot	N	Start Date	End Date	Mean	Deviation	of Variation
WQC002	15	5/12/1999	3/3/2000	13.88617	0.30416	2.2
WQC001	13	6/17/1999	3/14/2000	87.88541	1.8675	2.1
WQC001a	44	5/15/2000	1/18/2001	129.41643	7.1781	5.5
WQC002a	38	5/16/2000	1/29/2001	13.25117	0.7028	5.3



D.-Water Dibromochloromethane

Summary Statistics for Water Dibromochloromethane by Lot						
					Standard	Coefficient
Lot	N	Start Date	End Date	Mean	Deviation	of Variation
WQC002	9	5/12/1999	1/14/2000	3.32842	0.04467	1.3
WQC001	7	6/17/1999	2/4/2000	35.38974	0.75940	2.1
WQC001a	44	5/15/2000	1/18/2001	33.83251	1.48979	4.4
WQC002a	38	5/16/2000	1/29/2001	3.39583	0.10209	3.0



Summary Statistics for Water Methyl t-Butyl Ether (MTBE) by Lot						
					Standard	Coefficient
Lot	N	Start Date	End Date	Mean	Deviation	of Variation
WQC002	22	5/12/1999	3/16/2000	1.21123	0.06741	5.6
WQC001	14	6/17/1999	3/14/2000	11.36704	0.5525	4.9
WQC001a	44	5/15/2000	1/18/2001	12.0453	0.78628	6.5
WQC002a	37	5/16/2000	1/29/2001	1.29394	0.08441	6.5