Laboratory Procedure Manual

Analyte: **Volatile Organic Compounds (VOCs)**

Matrix: **Whole Blood**

Method: **Solid Phase Microextraction GCMS**

Method No: **13-OD; VO-BTHM-1.01**

Revised:

as performed by:

Emergency Response & Air Toxicants Branch
Division of Laboratory Sciences
National Center for Environmental Health

contact:

Dr. Benjamin Blount
Phone: 770-488-7894
Fax: 770-488-0181
Email: BBlount@cdc.gov

Dr. Eric J. Sampson, Director
Division of Laboratory Sciences

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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 testing the items listed in the following table

Data file name	Variable name	SAS Label
l04voc_b	LBXV4C	Blood Tetrachloroethene(ng/mL)
	LBXVBF	Blood Bromoform(pg/mL)
	LBXVBM	Blood Bromodichloromethane(pg/mL)
	LBXVBZ	Blood Benzene(ng/mL)
	LBXVCF	Blood Chloroform(pg/mL)
	LBXVCM	Blood Dibromochloromethane(pg/mL)
	LBXVCT	Blood Carbon Tetrachloride(ng/mL)
	LBXVDB	Blood 1,4-Dichlorobenzene(ng/mL)
	LBXVEB	Blood Ethylbenzene(ng/mL)
	LBXVME	Blood MTBE(pg/mL)
	LBXVOX	Blood o-Xylene(ng/mL)
	LBXVST	Blood Styrene(ng/mL)
	LBXVTC	Blood Trichloroethene(ng/mL)
	LBXV3A	Blood 1,1,1-Trichloroethene(ng/mL)
	LBXVTO	Blood Toluene(ng/mL)
	LBXVXY	Blood m-/p-Xylene(ng/mL)

1. Clinical Relevance and Summary of Test Principle

Volatile organic compounds (VOCs) are measured in specially collected whole blood samples by headspace solid phase microextraction/gas chromatography/isotope dilution mass spectrometry based on the methods described by Blount, et al.[1] and Bonin, et al[2]. The analytes are in equilibrium between the whole blood matrix and the headspace above the sample. A solid-phase microextraction fiber is inserted into the headspace and the VOCs partition into the phase on the outside of the fiber shaft. This fiber is then inserted into the heated GC inlet where the VOCs rapidly desorb because of the increased temperature. Extracted VOCs are focused at the head of the GC column using a cryogenic trap. Analytes are separated on a capillary column designed for VOC analyses and quantified using selected ion monitoring mass spectrometry. High resolution mass spectrometry was used to quantify MTBE, bromoform, bromodichloromethane, chloroform and dibromochloromethane in blood[2]. Benchtop quadrupole mass spectrometry was used to quantify the remaining 11 VOCs in the blood samples[1]. Both methods quantified target analytes by comparing relative response to isotopically-labeled internal standards with known standard concentrations. These methods are applicable to the determination of 16 VOCs in 3 mL blood with detection limits in the low parts per trillion ranges. Because 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.

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 because a number of these compounds are toxic. Wear vinyl or 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

Follow Universal Precautions. Because of the possibility of exposure to various microbiological hazards, appropriate measures should be taken to avoid any direct contact with the blood specimens. A Hepatitis B vaccination series is recommended for health care and laboratory workers who are exposed to human fluids and tissues.

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 safety 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.

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 waste

Waste materials must be disposed of in compliance with CDC laboratory, federal, state, and local regulations. Solvents and reagents should always be disposed of in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood. Disposable plastic, glass, and paper (e.g. pipette tips, vacutainers, gloves, etc.) that contact blood are placed in a biohazard autoclave bag. The biohazard autoclave bags should be kept in appropriate containers until sealed and autoclaved. Wipe down all surfaces with fresh 70% ethanol solution when work is finished. Disposable needles used to remove blood from syringes should be placed immediately into a sharps container and autoclaved when the sharps container becomes full. All syringes and other non-disposable glassware that contact blood should be decontaminated with a freshly prepared bleach solution (a 10% dilution of commercial sodium hypochlorite (bleach) or equivalent) before re-use or disposal. Commercial sodium hypochlorite solutions contain significant amounts of chloroform and bromodichloromethane that can contaminate samples; routine disinfection with bleach should therefore be isolated from preparatory areas and VOC blood samples.

Observe Universal Precautions. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.

All syringes and other non-disposable glassware that contact blood should be decontaminated with a freshly prepared bleach solution (a 10% dilution of commercial sodium hypochlorite (bleach) or equivalent) before re-use or disposal.

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 a quadrupole mass spectrometer run with the ChemStation software. Data are converted from the ChemStation software format to ThermoFinnigan's Xcaliber format for review. The reviewed data is then exported to Microsoft Excel files and entered into a 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 visual evaluation of all relevant peak integration events, proofreading all transcribed data, storage of data in multiple computer systems, and redundant data archiving.

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 such as fasting or special diets are required.

B. Sample collection

Isopropyl alcohol, which may be used to disinfect the venipuncture site, can contaminate the collected sample and cause nonspecific interferences in the analytical measurement process. Isopropyl alcohol contamination can be easily prevented by swabbing the venipuncture site with a dry gauze bandage and allowing the site to dry for 5 to 10 sec after wiping with isopropyl alcohol.

The specimen type is whole blood collected in specially prepared, glass vacutainers containing potassium oxalate and sodium fluoride. Additional information on preparation of these vacutainers can be found in Section 6.d.

C. Sample handling

The CDC-prepared vacutainers contain milligram quantities of potassium oxalate and sodium fluoride. These chemicals function to inhibit metabolism and prevent coagulation. Metabolic inhibition increases sample shelf life by minimizing metabolic impact on blood VOC levels during storage. This mixture's ability to prevent clotting of blood is not as great as many other anticoagulants. Thus, once samples have been collected, they must be mixed thoroughly to allow the complete distribution of the anticoagulant. If a blood mixer is available, samples should be placed on this mixer for at least 3 min. If a mixer is not available, the blood can be mixed by hand by inverting the tube 30 times. Because blood is perishable and VOCs are highly volatile, care must be taken to insure that samples are kept at refrigerator temperatures (i.e., 2-6ºC) 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 ensure that they will arrive at CDC on normal business days to guarantee their proper processing 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 days of collection so that they can be analyzed within 2 to 3 weeks of collection.

Specimen stability has been demonstrated for analytes measured by this method for 10 weeks at refrigerated temperatures (2-6ºC). Note that blood samples change with time of refrigerated storage so that the blood is often clotted and therefore difficult to handle after 10 weeks of storage. Because these are whole blood samples, longer storage results in samples that are harder to manipulate and produce additional analytical problems. Thus, even though analytical results may not change over this time, samples may be less amenable to analysis. Volatile organic compounds occur naturally in the body, and metabolism may alter their concentration with storage.

Whole blood samples for VOC measurement should be stored at 2-6ºC. This prevents blood cell rupture that would occur during freezing. In addition, freezing of blood can lead to breakage of vacutainers and loss of sample in some cases. Because VOCs are lost whenever the containers in which they are contained are opened, it is not appropriate to transfer the blood samples to another container, which would be more resistant to breaking.

D. Sample quantity

The optimal amount of specimen required for analysis is 10 mL; the minimum amount is 3 mL.

E. Unacceptable specimens

The criteria for unacceptable specimen are a low volume (< 3 mL), failure to maintain sample temperature between 2ºC and 6ºC for an extended period of time, suspected contamination, use of an untreated vacutainer, and significant clotting of the specimen. Clotting can occur due to the failure to properly mix the sample as described above.

Failure to obtain adequate sample volume is obvious when the samples are received. Visual inspection of the vacutainer reveals if the estimated blood volume is less than the required 3 mL. Maintenance of temperature during shipment is verified by examining the shipment temperature upon receipt. Clotting is indicated by failure of the sample to flow when the vacutainer tube is inverted. 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 (Standards), Controls, 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.

HPLC grade acetone is used for primary dilution of neat native standards and labeled analogs for improved solubility of nonpolar compounds. An acceptable HPLC grade acetone is sold by Sigma-Aldrich Co. (Milwaukee, WI). Other sources of HPLC grade acetone must first be shown to not contribute contaminants to the analytical measurement before use.

Purge and Trap grade methanol is required for secondary dilutions of native standards and labeled analogs. An acceptable purge and trap grade methanol is produced by Burdick and Jackson, and is acquired through KSE (Durham, NC). Other sources of purge and trap grade methanol must first be shown to not contribute contaminants to the analytical measurement before use.

HPLC grade water is primarily used for final dilutions. An acceptable purity is produced by Baker-Mallinckrodt and can be acquired from Lab Depot Supply Co. (Alpharetta, GA). However, 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. This water is further processed by helium sparging and distillation to further reduce VOCs before use. Directions for this procedure are given in Section 6.e and are based on previously published techniques for removing residual VOCs from reagent water (4).

2. Calibration and Control Materials

Material used for preparation of calibration standards and Quality Control materials are listed in Table 1. Material used for labeled internal standards are listed in Table 2. All chemicals are used without further purification unless otherwise noted. 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
***tert*-Butyl Methyl Ether**	$(CH_3)_3COCH_3$	99%	d,e	i
Chloroform	$CHCl_3$	99%	a,b	h
1,1,1-Trichloroethane	CH_3CCl_3	97%	a,b	h
Carbon Tetrachloride	CCl_4	99%	a,b	h
Benzene	C_6H_6	99%	a,d	h
Trichloroethylene	$CHCl=CCl_2$	99%	a,g	h
Bromodichloromethane	$CHCl_2Br$	98%	a,b	h
Toluene	$C_6H_5CH_3$	99%	b,d	h
Dibromochloromethane	$CHClBr_2$	98%	e	h
Tetrachloroethylene	$CCl_2=CCl_2$	99%	a,g	h
Ethylbenzene	$C_6H_5CH_2CH_3$	99%	d,e	h
***m*/*p*-Xylene**	$C_6H_4(CH_3)_2$	99%	d,e	h
Bromoform	$CHBr_3$	99%	a,c	h
Styrene	$C_6H_5CH=CH_2$	99%	a,d	h
***o*-Xylene**	$C_6H_4(CH_3)_2$	98%	d,e	h
1,4-Dichlorobenzene	$C_6H_4Cl_2$	99%	a,b	h

Key:

a - Cancer suspect agent
b - Toxic
c - Lachrymator
d - Flammable liquid
e - Irritant
f - Moisture sensitive
g - Mutagen
h - Sigma-Aldrich (Milwaukee, WI)
i - Chem Service (West Chester, PA)

Table 2. Internal Standard Compounds

Compound	Formula	Acceptable Grade	Safety	Source
Furan-D_4	C_4D_4O	98%	a,b,d,e	j
1,1-Dichloroethylene-D_2	$CD_2=CCl_2$	98%	c,d	h
Acrylonitrile-$^{13}C_3$	$N\equiv{}^{13}C{}^{13}CH{}^{13}CH_2$	99%	a,b,d,g,e	h
Methylene Chloride-$^{13}C_1$	$^{13}CH_2Cl_2$	99%	b,e	h
cis/trans -1,2-Dichloroethylene-D_2	CDCl=CDCl	97%	d,f	h
tert -Butyl Methyl Ether-D_{12}	$(CD_3)_3COCD_3$	99%	d,e	j
1,1-Dichloroethane-D_3	CD_3CHCl_2	98%	a,b	h
n -Hexane-$^{13}C_1$	$^{13}CH_3CH_2CH_2CH_2CH_2CH_3$	99%	b,d,e	j
Chloroform-$^{13}C_1$	$^{13}CHCl_3$	99%	a,b	h
1,2-Dichloroethane-D_4	CD_2ClCD_2Cl	98%	a,d	h
1,1,1-Trichloroethane-D_3	CD_3CCl_3	98%	a,b	h
Carbon Tetrachloride-$^{13}C_1$	$^{13}CCl_4$	99%	a,b	h
Benzene-$^{13}C_6$	$^{13}C_6H_6$	99%	a,d	h
Dibromomethane-D_2	CD_2Br_2	99%	b	h
1,2-Dichloropropane-D_5	$CD_3CDClCD_2Cl$	98%	d,e	h
Trichloroethylene-$^{13}C_1$	$^{13}CHCl=CCl_2$	99%	a,g	h
Bromodichloromethane-$^{13}C_1$	$^{13}CHCl_2Br$	98%	a,b	i
2,5-Dimethylfuran-$^{13}C_2$	$(^{13}CH_3)_2C_4H_6O$	99%	b,d	i
1,1,2-Trichloroethane-D_3	$CD_2ClCDCl_2$	99%	a,b	h
Toluene-$^{13}C_7$	$^{13}C_6H_5{}^{13}CH_3$	99%	b,d	j
Dibromochloromethane-$^{13}C_1$	$^{13}CHClBr_2$	98%	e	i
1,2-Dibromoethane-$^{13}C_1$	$Br^{13}CH_2{}^{13}CH_2Br$	99%	a,b,e	h
Tetrachloroethylene-$^{13}C_1$	$^{13}CCl_2=CCl_2$	99%	a,g	h
Chlorobenzene-$^{13}C_6$	$^{13}C_6H_5Cl$	99%	d,e	j
Ethylbenzene-$^{13}C_6$	$^{13}C_6H_5CH_2CH_3$	99%	d,e	h*
m -/*p* -Xylene-$^{13}C_6$	$^{13}C_6H_4(CH_3)_2$	99%	d,e	h*
Bromoform-$^{13}C_1$	$^{13}CHBr_3$	99%	a,c	h
Styrene-$^{13}C_6$	$^{13}C_6H_5CH=CH_2$	99%	a,d	j
1,1,2,2-Tetrachloroethane-D_4	$CDCl_2CDCl_2$	99%	a,c	h
o -Xylene-D_6	$C_6H_4(CD_3)_2$	98%	d,e	h*
1,3-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	98%	b,e	h
1,4-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	99%	a,b	h
1,2-Dichlorobenzene-$^{13}C_6$	$^{13}C_6H_4Cl_2$	99%	b,e	h
1,2-Dibromo-3-chloropropane-$^{13}C_3$	$^{13}CH_2Br^{13}CHBr^{13}CH_2Cl$	95%	b,d	h*
Hexachloroethane-$^{13}C_1$	$^{13}CCl_3CCl_3$	98%	a,e	h
Nitrobenzene-$^{13}C_6$	$^{13}C_6H_5NO_2$	99%	b,d	h
Hexachlorobutadiene-$^{13}C_4$	$Cl_2{}^{13}C={}^{13}C(Cl){}^{13}C(Cl)={}^{13}CCl_2$	99%	a,e	h

Key:

a - Cancer suspect agent
b - Toxic
c - Lachrymator
d - Flammable liquid
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f - Moisture sensitive
g - Mutagen
h - Cambridge Isotope Laboratories (Woburn, MA) - available commercially
h* - Cambridge Isotope Laboratories (Woburn, MA) - Custom Synthesis
i - Previously available from Merck, Sharp & Dohme/Isotopes (St. Louis, MO), but currently unavailable commercially
j - Sigma-Aldrich (Milw aukee, WI)

B. Preparation of glassware

All glassware used in this study is carefully cleaned to be certain to remove possible contamination. To remove these possible analytical interferences, rinse glassware (volumetric flasks, ampoules, and storage bottles) with reagent-grade methanol, and heat at 150 ± 10°C at 10 ± 5 Torr in a vacuum oven dedicated to processing only glassware with an independent vacuum source for at least 12 hr. A dedicated vacuum oven and independent vacuum source are necessary to prevent possible cross-contamination from other materials and 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. When the glassware is needed, cool it to room temperature under vacuum and restore pressure using nitrogen (UHP grade). Remove treated glassware from the oven and seal with polytetrafluoroethylene (PTFE) caps, when appropriate, prior to use.

C. Preparation of headspace vial septa

Headspace vial septa are nominally 20 ± 0.49-mm diameter, between 1 - 1.3 mm thick, and comprised of a PDMS-based polymer with a polytetrafluoroethylene (PTFE) barrier layer between 0.1 - 0.15 mm thick. These septa can be purchased from MicroLiter, Inc. precleaned to meet our minimal specifications equivalent to 17 hr at 110 ± 10ºC and either vacuum below 15 Torr (mmHg) or nitrogen purging above 100 mL/min. Prior to use, the septa are reprocessed for about 17 hr at 100 ± 10ºC under vacuum below 15 Torr to remove any residue or post-process contaminants from packaging, shipping and storage. After processing these septa are to remain <u>for all applications</u> in the oven at 70 ± 10ºC under vacuum until needed.

D. Preparation of vacutainers

Blood collection tubes (vacutainers) obtained from commercial sources contains VOC contaminants that can mask the levels of VOC analytes originally in the blood at the time of sample collection, and thus prevent accurate exposure assessment. Vacutainers are obtained commercially and specially modified by laboratory staff (DLS VOC laboratory or Battelle Volatiles laboratory) to remove measurable levels of most VOCs present. This SOP is based on our previously published research into VOC contamination from blood collection tubes[3]. It is absolutely imperative that these specially treated vacutainers be used for all VOC blood collection to ensure sample validity. Following completion of vacutainer treatment and sterilization, the tubes are labeled with a new expiration date that reflects their 1-year shelf life. These tubes are supplied by DLS Lab staff for all VOC studies.

E. 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 2500 ± 100 mL of HPLC grade water. Adjust the helium flow to produce an active flow through the sparger. Allow the helium to bubble through the raw water for at least 17 hr at 85 ± 5ºC. After 17 hr, turn on the heating mantle to bring the water to a boil. Allow the water to reflux for at least 4 hr. At the end of this period, begin collecting the finished blank water. Dispense the finished water into 100-mL glass-stoppered Pyrex bottles cleaned in accordance with Section 6.b, on glassware preparation and cap immediately. 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 5, 10 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 hermetic seal is verified by tapping the sealed end on a paper laboratory wipe lying on a hard surface and looking for a leak. The sealed ampoules are stored in the dark at room temperature.

F. Preparation of native analytical standards

1. Procedure for handling neat compounds

Most analytes are purchased as neat liquids in flame sealed ampoules. After opening an ampoule the remaining (unused) material is discarded. A few of the most expensive analytes (custom synthesis products) are aliquotted into an individual borosilicate glass ampoules and flame sealed for future use. After transferring the compounds store the ampoules in an explosion-proof -70ºC freezer. Package all neat compound containers with aluminum foil to eliminate light exposure. Store neat standards in a dedicated chemical storage refrigerator separate from blood samples, blanks and quality control materials.

2. Procedure for filling and sealing glass ampoules

Aliquot 0.5 ± 0.049 mL of the neat standard material into a chilled 1-mL borosilicate glass ampoule. Ampoules are chilled by submerging them in liquid nitrogen between 10 and 15 sec and placing them in a pre-chilled aluminum

block tray throughout the aliquoting process. Use a glass Pasteur pipette to transfer the liquid. Before using the pipette, rinse by initially filling with the neat standard and expelling to waste. (NOTE: There may not be enough neat standard material to perform this rinse step.) Make sure the liquid is placed in the bottom of the ampoule and is not adhering to the neck of the ampoule. Otherwise, during the sealing procedure, ignition of the liquid will produce a loud pop and could shatter the ampoule. Remove the ampoule from the tray and seal using a natural gas and oxygen torch. Allow the sealed ampoule to come to room temperature then invert the vial and tap the sealed end on a laboratory wipe that is lying on a counter. 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. Repeat these steps until a sufficient number of ampoules are made. Affix plastic labels and record data, contents, and initials of those involved with preparation. Place the sealed ampoules in an appropriate holder and store in a freezer below -60ºC.

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. 25-mL class A volumetric flasks 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. Final concentrations of the standards

   Standards will be prepared from low ppb to low ppt range in helium-sparged/distilled water. This involves the serial dilution of concentrated stock solutions in acetone and methanol (purge-and-trap grade), storage of a concentrated standard in flame-sealed glass ampoules, and preparation of the actual standard solution in water (helium sparged) prior to use. Typical standard concentrations are described by Blount et al[1] and Bonin et al[2].

5. Stock solutions and concentrated standards

   Primary stock solutions and intermediate standard ampoules are prepared from neat standard materials by dissolving gravimetrically confirmed amounts of standard in methanol.

6. Daily aqueous working standards

   Daily aqueous working standards are made by diluting standard material from a freshly opened ampoule (40 µL) and diluting with helium sparged/distilled water (25 mL). Following addition of internal standard, 3.0 mL of each the aqueous working standards is transferred into cleaned SPME headspace vials using a

glass/Teflon multipippettor. The vials are immediately sealed with recently cleaned caps and grouped by concentration in separate 8 oz. wide mouth specimen jars to prevent cross contamination. Furthermore, the standard set is stored in a dedicated refrigerator at 4 ± 2°C and subsequently analyzed as part of an analytical batch within 1 week.

G. Preparation of labeled analog solutions

1. Procedure for handling neat compounds

1, 1-Dichloroethene-D_2 is also known as vinylidene chloride-D_2 and is listed as a suspected carcinogen. Contact with the skin causes irritation and the vapors irritate mucous membranes. The compound volatilizes readily at room temperature and is very flammable. At temperatures above 0°C and in the presence of oxygen, explosive polymerization may occur. This deuterated analog of vinylidene chloride comes from the manufacturer with hydroquinone added to stabilize the compound and prevent polymerization. For making standards, this compound is used as received from the manufacturer. An additional issue with the deuterated analog is the container in which it is shipped. The manufacturer sends this compound in a thick walled borosilicate reaction vessel of about 500-mL volume. The container is fused shut and the only way to access the contents involves scoring the container with a file and breaking it open. This presents two problems. First, the container is pressurized because of the compound's volatility and secondly, only 1 g of material occupies this large volume. To address these problems and allow transfer of this material to smaller containers, which are more suitable for use in making standards, use the following approach. Place the glass shipping vessel into a bucket of ice and allow it to cool for about 2 hr. After 2 hr, a noticeable increase in the volume of liquid will be seen in the bottom of the glass container. Remove the container from the bucket and place it in a fume hood. Make sure you are wearing appropriate safety equipment before proceeding with the next step. Using a metal triangular file, score the glass container above the bulbous portion of the container at a location that will allow a good grip when snapping open the container. Because the glass is so thick, a very deep score will be needed. Thus, it is possible to break open the container during this filing operation. Be careful! After completing the scoring, place the container back in the ice and let it cool for about 10 min. After this time, remove the glass container from the bucket of ice. Under the fume hood, break open the container. The container should snap open with a minimum of force. If it does not snap easily, use the file to score it more deeply and try again. After the container is open, immediately, transfer the contents using a Pasteur pipette into, previously chilled, flame sealable glass ampoules. Place the ampoules back in the bucket of ice and remove what is needed to make standards. Seal the ampoules and store below -60°C.

Opening glass ampoules can result in broken glass that punctures fingers. While opening glass ampoules wear protective gloves or use a device that shields the hands from broken glass.

2. Procedure for filling and sealing glass ampoules

Aliquot the appropriate amount of primary or secondary analog solution into a 1-mL flame sealable borosilicate glass ampoule. Use a glass Pasteur pipette cleaned as described in Section 6.b to transfer the solution. 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 pre-chilled aluminum sample tray until the liquid is cooled, but not frozen. This will require 10-15 sec. Remove the chilled ampoule from the tray and seal using a natural gas and oxygen torch. Allow the sealed ampoule to cool 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. Repeat these steps until a sufficient number of ampoules are made. Place the sealed ampoules in an appropriate holder and store in a freezer at <-60^{o}C.

3. Primary analog stock solutions

Primary analog stock solutions (i.e., L-series) are made by initial dilution of neat compound into 25 mL of purge and trap grade methanol. This provides a consistent source of these compounds for further dilutions. When mixing an L-series solution, label and fill a 25-mL volumetric flask cleaned in accordance with Section 6.b and add approximately 22 mL of fresh purge and trap grade methanol. Keep the flasks sealed when not directly adding the standard. Dilute the compound to a final volume of 25 mL with methanol according to Table 6. Once the dilution is complete invert the capped flask five times and sonicate for approximately 60 sec.

Aliquot about 0.75 mL of this solution into ampoules and flame seal as described in Section 6.f. Repeat these steps until at least 25 ampoules are prepared. Label and place the sealed ampoules in an appropriate holder and store in a freezer below -60^{o}C.

Table 6. L-Series Primary Stock Solution Concentrations

Primary Stock	Compound	Neat delivery vol or wt	Density	Approx. conc. (g/mL)
L61	Furan-D_4	60	1.6200	2.3
L22	1,1-Dichloroethylene-D_2	50 µL	1.1560	2.4
L50	Acrylonitrile-$^{13}C_3$	100	0.9371	3.2
L11	Methylene Chloride-$^{13}C_1$	50 µL	2.8900	2.7
L6	1,2-*cis/trans*-Dichloroethylene-D_2	20 µL	1.4600	1.0
L51	*tert*-Butyl Methyl Ether-D_{12}	100 µL	1.2240	3.0
L4	1,1-Dichloroethane-D_3	50 µL	0.8787	2.4
L52	*n*-Hexane-$^{13}C_1$	100	0.8600	2.6
L12	Chloroform-$^{13}C_1$	25 µL	0.6590	1.5
L5	1,2-Dichloroethane-D_4	50 µL	2.5000	2.5
L23	1,1,1-Trichloroethane-D_3	200 µL	1.2880	10.7
L10	Carbon Tetrachloride-$^{13}C_1$	37.6 µL	0.8802	2.4
L25	Benzene-$^{13}C_6$	25 µL	1.2180	0.9
L20	Dibromomethane-D_2	20 µL	2.4500	2.0
L7	1,2-Dichloropropane-D_6	20 µL	0.9030	0.9
L16	Trichloroethylene-$^{13}C_1$	37.6 µL	solid	2.2
L18II	Bromodichloromethane-$^{13}C_1$	10 µL	0.8004	0.8
L40	2,5-Dimethylfuran-$^{13}C_2$	125 µL	1.4400	4.5
L15	1,1,2-Trichloroethane-D_3	25 µL	2.0930	1.4
L31	Toluene-$^{13}C_7$	100 µL	solid	3.5
L19II	Dibromochloromethane-$^{13}C_1$	10 µL	0.8669	1.0
L43	1,2-Dibromoethane-$^{13}C_1$	40	1.4800	3.5
L14	Tetrachloroethylene-$^{13}C_1$	25 µL	0.9100	1.6
L28	Chlorobenzene-$^{13}C_6$	50 µL	1.1760	2.2
L26	Ethylbenzene-$^{13}C_6$	25 µL	2.1720	0.9
L27	*m*/*p*-Xylene-$^{13}C_6$	50 µL	1.6200	1.7
L24II	Bromoform-$^{13}C_1$	25 µL	1.3300	2.9
L29	Styrene-$^{13}C_6$	25 µL	1.6000	0.9
L13	1,1,2,2-Tetrachloroethane-D_4	37.6 µL	1.3050	2.4
L2	*o*-Xylene-D_6	50 µL	1.1960	1.8
L41	1,3-Dichlorobenzene-$^{13}C_6$	50 µL	1.3390	2.6
L32	1,4-Dichlorobenzene-$^{13}C_6$	251.5 mg	1.5900	10.1
L3	1,2-Dichlorobenzene-$^{13}C_6$	40 µL	1.2350	2.1
L55	1,2-Dibromo-3-chloropropane-$^{13}C_3$	premixed	1.2840	1.0
L17	Hexachloroethane-$^{13}C_1$	51.8 mg	0.8600	2.1
L44	Nitrobenzene-$^{13}C_6$	50 µL	0.7400	2.4
L60	Hexachlorobutadiene-$^{13}C_4$	solid	1.1060	2.0

4. Secondary analog stock solutions

The secondary labeled-analog stock solution is made by initial dilution of the L-series solutions into 25 mL of purge and trap grade methanol. Label and store the sealed ampoules in a freezer below -60^{o}C.

5. Working analog stock solutions

Prepare the working internal standard stock solution for a given 2-wk period of analyses by diluting 125 µL from a fresh ampoule of secondary stock solution to a final volume of 25-mL with fresh purge and trap grade methanol.

H. Preparation of Quality Control materials

Quality control (QC) materials are prepared by fortifying fetal calf serum with two different levels of the 16 VOC analytes. Target concentrations and measured concentrations can vary between batches because of significant background levels that might not be removed during the cleaning process or diffusion loss during material preparation. The mean VOC levels are characterized in each quality control pool by at least 20 separate analyses. These characterization analyses include variables such as different analysts and instruments, and define the precision of the assay. Typical QC levels and assay precision for the assay have been published by Blount et al[1] and Bonin et al[2].

1. Preparation of bovine serum

Fetal bovine serum (Hyclone Laboratories, Logan, UT) is cleaned by extraction using cleaned 20-mm PTFE/silicone barrier septa as the absorbent. Add about 1 L of bovine serum to a 2-L volumetric flask cleaned in accordance with Section 6.b. Add about 300 20-mL-headspace-vial septa, cleaned in accordance with Section 6.c and cooled in the vacuum oven under dry nitrogen, immediately to the bovine serum. Cap the flask with a ground glass stopper and seal the top with PTFE tape. Swirl the solution gently and place it in a dedicated refrigerator that has been previously vented. Allow the extraction mixture is allowed to equilibrate for about 12 hr and during this time swirl the mixture gently from time to time no more than once per hour. Decant the extracted bovine serum into a cleaned 1-L volumetric flask, cap with a glass stopper, and wrap with PTFE tape. Bovine serum QC samples are prepared with the final concentrations given in Table 8.

2. Spiking serum for QC preparation

To formulate the low concentration QC solution, aliquot with a positive displacement pipette 105 µL of the *Secondary Stock Solution* SS0375 into approximately 1 L of clean bovine serum. Immediately cap and seal with PTFE

tape and then gently swirl for about an hour. Place the stoppered flask on ice for about 1 hr swirling about every 15 min.

Once the low concentration QC samples have been prepared and stored, prepare the high concentration QC samples in the same manner except aliquot with a positive displacement pipette approximately 51 µL of the *Secondary Stock Solution* SS0377 into about 1 L of clean bovine serum.

3. Procedure for filling and sealing glass ampoules

Using a 10-mL serological long-tip pipette, dispense 5-8 mL of spiked serum into a cleaned 10-mL borosilicate glass ampoule that has been pre-chilled. Ampoules are chilled by submerging them in liquid nitrogen between 10 and 15 sec and placing them in a pre-chilled aluminum block tray throughout the aliquoting process. Before pipetting with a new pipette, rinse three times by initially filling with the serum and expelling to waste. Make sure the serum is placed in the bottom of the ampoule and is not adhering to the neck of the ampoule. Remove the ampoule from the tray and seal using a natural gas and oxygen torch. Allow the sealed ampoule to come to room temperature then invert the vial and tap the sealed end on a laboratory wipe that is lying on a counter. 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. Repeat these steps until a sufficient number of ampoules are made. Ensure that the ampoules are labeled with the QC formulation identifier, the ampoule series number in which it was prepared, the date on which the sample was prepared, and the initials of those involved with the preparation. Place the sealed ampoules in an appropriate holder and store in a freezer below -60^{o}C.

I. Proficiency Testing (PT) Materials

Proficiency Testing materials are prepared from neat compounds in a manner similar to standard preparation. Pooled volatile reference materials are available from Sigma-Aldrich-Supelco Chemical Company. For these tests purchase the “EPA 524 VOC mix A” and “EPA 524 Rev 4 Update mix 1” as ampoules containing 0.200 mg/mL of each of the VOC assay analytes. These solutions are combined and diluted in fresh purge and trap grade methanol to within the linear range of the VOC assay. Four PT stock concentrations are prepared, aliquoted into ampoules, and flame sealed using the same preparation technique as described in Section 6.f. A quality control officer independent from the laboratory blind-codes the PT stock ampoules and administer the PT program. Assay performance is evaluated by blind analyses of aqueous proficiency testing samples prepared by dilution of PT stock ampoules (40 µL) with helium-sparged, distilled water.

J. Storage of standard solutions

Except while in use, all standard stock solutions, labeled analog stock solutions, and quality control materials are stored below -60^{o}C. The working stock solutions can be stored for up to 1 week at <3^{o}C. Once ampoules containing stock solutions have been

opened, they must be used within about 5 min. After this time these materials are discarded. The working labeled analog stock solution may be preserved and used throughout a 1-week period if carefully sealed and stored at <3°C within 8 hr of initial preparation. All stock solutions are labeled to include a reference to the preparation procedure.

K. Clean-up procedure for the 5-mL Luerlock gas-tight syringe

1. Place the spent syringes into a 600-mL beaker after use.
2. Fill the beaker with a 10% bleach solution.
3. Flush each syringe three times by completely filling them with the bleach solution and expelling.
4. Fill each syringe with the bleach solution and allowed to sit for a minimum of 15 min (20 min is preferred) for decontamination.
5. Disassemble the syringes and separate the glass barrels and plungers into different 600-mL beakers.
6. Rinse the disassembled syringes thoroughly with warm tap water, filling and emptying the beakers at least three times.
7. Fill the beakers containing the disassembled syringes with HPLC grade water and sonicate in an ultrasonic bath cleaner at 30-40ºC for about 60 min.
8. Empty the HPLC rinse water from the beakers and fill with purge and trap grade methanol and sonicate at ambient temperature for about 30 min.
9. Empty the spent methanol from the beakers disposing of the methanol in a suitable waste container.
10. Rinse the beakers with ACS grade methanol, filling the beaker completely, and immediately empty the spent methanol in a suitable waste container.
11. Allow the syringes to air dry in the hood for at least 10 min.
12. Vacuum bake the syringes in their beakers by placing them into a vacuum oven at approximately 180ºC for about 24 hr under a vacuum of <15 Torr.
13. Store the syringes under vacuum at nominally 50ºC until needed for their next use.

L. Supplies

Supplies used during the development, validation, and application of this method are listed below. Supplies procured from other sources can be used but should be equivalent to the products offered by the vendors listed below.

1. Disposable Pasteur pipettes (Fisher Scientific, www1.fishersci.com)
2. Pipette bulbs (Fisher Scientific, www1.fishersci.com)
3. 5-mL, 10-mL, and 20-mL clear pre-scored ampoules (Wheaton Scientific, Millville, NJ)
4. Portapet pipetter, 10-mL volume (Fisher Scientific, www1.fishersci.com)
5. Research-grade helium gas, 99.9999% (Airgas, www.airgas.com)
6. High-density polyethylene dewar flask (Fisher Scientific, www1.fishersci.com)
7. Glassblowing kit including torch (Fisher Scientific, www1.fishersci.com)
8. Variable or fixed positive displacement micropipettors with maximum volumes that include 20-µL, 25-µL, 40-µL, 50-µL, 100-µL, and 250-µL, (VWR, West Chester, PA)

9. Glass capillaries, 20-µL, 25-µL, 40-50-µL, 100-µL, and 250-µL (VWR, West Chester, PA)
10. Pyrex volumetric flasks with screw caps, 25-mL (Fisher Scientific, www1.fishersci.com)
11. Polytetrafluoroethylene (PTFE) cap liners, No. 22, No. 33, and No. 38 (Thomas Scientific, Swedesboro, NJ)
12. Non-powdered disposable nitrile gloves (Lab Depot Inc., Alpharetta, GA)
13. Ultrasonic cleaner with heater and timer (Fisher Scientific, www1.fishersci.com)
14. Stainless steel test tube racks for 11-mm diameter tubes (VWR, West Chester, PA)
15. Serum bottles (Wheaton Scientific, Millville, NJ)
16. Septa, flat disc, red PTFE/white silicone (Integrated Liner Technologies, Albany, NJ)
17. Hand-operated crimper (Wheaton Scientific, Millville, NJ)
18. 20-mm aluminum, magnetic headspace vial (Sun-SRi, Duluth, GA)
19. Oxygen, 99.99%, 200-300 cu. ft. (local gas supply company)
20. Sterile evacuated blood collection tubes, 10-mL draw, 16 X 100, potassium oxalate, sodium fluoride (Becton/Dickinson Vacutainer Systems, Rutherford, NJ)
21. Beveled-top standard 10-mL headspace vials, (Worldwide Glass Resources, Norma, NJ)
22. 2-mL PTFE-lined screw cap vials (Agilent, www.chem.agilent.com)
23. Gastight PTFE luerlock tip syringe, 5-mL (Hamilton, Reno, NV)
24. Sharps container (Pro Tec US Clinical Products, INC., Richardson, TX)
25. Hematology mixer (Robbins Scientific, Sunnyvale, CA)
26. Sodium hypochlorite (James Austin Co., Mars, PA)
27. 150-mm flowtube for helium 0 to 100 cc/min (Alltech Associates, Inc., Deerfield, IL)
28. Adapter 1/8” to 1/8” MPT (Alltech Associates, Inc., Deerfield, IL)
29. DB-VRX Capillary Column, 0.18-mm I.D., 40-m, 1.0-µm film thickness (J&W Scientific, Folsom, CA)
30. 75-µm Carboxen/PDMS SPME fiber assembly (Supleco, www.sigmaaldrich.com/Brands/Supelco_Home.html)
31. Standard Printer paper (local office supply)

M. Equipment

Equipment used during the development, validation, and application of this method are listed below. Equipment procured from other sources can be used, but should be equivalent to the products offered by the vendors listed below.

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-liter
    g. Powerstat, 0 - 140 volts
    h. PTFE sleeves, 0.076-mm
    i. Adapter, vacuum short stem, 14/20

j. PTFE sleeves, 0.13-mm, 14/20
k. Bottle, single neck, 14/20 joint

2. Squaroid vacuum oven, 2.3 cu. ft. (Lab-line Instruments Inc., Melrose Park, IL)
3. Vacuum pump (Fisher Scientific, www1.fishersci.com)
4. Analytical Balance (Fisher Scientific, www1.fishersci.com)
5. Ultra-low temperature freezer (Fisher Scientific, www1.fishersci.com)
6. Refrigerator (Fisher Scientific, www1.fishersci.com)
7. Standard laboratory freezer (Fisher Scientific, www1.fishersci.com)
8. Sterilized hood/biological safety cabinet (A/B3, NuAir)

The following equipment has been shown to meet the requirements of the method. Substitutes must be evaluated for their ability to meet method accuracy, sensitivity, and reproducibility.

1. LEAP Combi-Pal Prep and Load system for static headspace and direct GC injections (LEAP Technologies, Carrboro, NC)
2. Gas Chromatograph (HP 6890, Agilent Technologies, www.chem.agilent.com)
3. Mass Spectrometer (5973, Agilent Technologies, www.chem.agilent.com)
4. Distilled water purifier (Barnstead, Dubuque, Iowa)
5. Access database (Microsoft, Inc., Redmond, WA)
6. Xcalibur data analysis and processing software (ThermoFinnigan, analyze.us@thermo.com)

N. Instrumentation

SPME of the headspace sample is performed using an autosampler (Combi-Pal, Leap Technologies, Carrboro NC). Samples are queued on an autosampler tray and maintained at 15 ± 0.5^{o}C until they are analyzed. During analysis the samples are transferred to an agitating incubator set to 350 rpm and 40^{o}C as the headspace is sampled with a 75-µm Carboxen-PDMS coated SPME fiber (Supelco, Bellefonte PA) for approximately 6.0 min. The SPME fiber is then immediately transferred into the GC injection port fitted with a 1-mm id glass liner and held at 250±0.5ºC. The volatile analytes are subsequently resolved chromatographically and detected with mass spectrometry as described by described by Blount et al[1] and Bonin et al[2].

7. Calibration and Calibration Verification Procedures

All calibration standards are prepared in water because it proved to be difficult 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 result validates the use of water-based calibrators for quantifying VOCs in whole blood.

A. Creation of curve

1. Data Collection

   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 curves are constructed for each analyte from the relative response factors for each of the 7 calibrators.

2. Calculation of curve statistics

   The calibration curve is constructed from the response ratios for each analyte to its internal standard at the 7 calibration levels. Correlation coefficients should typically exceed 0.995. 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 background and standard ion contribution to the internal standard ion response. This data transformation can be performed using the ThermoFinnigan Xcalibur Quan software.

3. Evaluation of curve statistics

   The R-squared values for each analyte calibration curve must in all cases be greater than 0.95. In more than 90% of the cases the R-squared values are greater than 0.995. Linearity of the standard curves should be optimized through the use of universal transform (4) or the exclusion of any nonlinear portion of the curve. At least five concentration levels are used for curve fitting. Otherwise, a non-linear curve can be fit with a second order quadratic curve as long as no data points are quantified through extrapolation. If percent deviation from the curve varies more than 20% for the lowest standard, which is weighted the most by the 1/X treatment, it should be excluded. If any of the individual calibration curves consistently have significant y-intercepts the source of this bias should be established. Possible sources include incorrect ion ratios, contamination of water/methanol used to dilute standards, contamination of analog spiking solution, and diffusion loss.

B. Usage of curve

   The highest point on the calibration curve is above the expected range of results for non-occupationally exposed people and the lowest point is near or below the measurable detection limits. The other concentrations are distributed systematically between these two levels. The calibration curve spans three orders of magnitude.

C. Calibration verification

   Calibration is performed as part of each analytical run and a calibration curve is constructed from the seven calibration standards. Additional verification is conducted by quantifying Quality Control samples of known value against the calibration curve and statistically comparing the calculated results to known values.

8. Procedure Operation Instructions; Calculations; Interpretation

All samples and data are handled in compliance with CLIA and the Division of Laboratory Sciences Policies and Procedures manual. Further detail of procedures are described by Blount et al[1] and Bonin et al[2].

9. Reportable Range of Results

A. Linearity Limits

Blount et al[1] and Bonin et al[2] describe the reportable range of results for the analytes detectable using this method. The lower reportable limit is either the detection limit or the lowest standard whichever is lower. The upper reportable limit is the highest linear standard.

B. Analytical Sensitivity

Detection limits for these methods are listed by Blount et al[1] and Bonin et al[2] and were determined by calculating the standard deviation at each standard concentration following repeated measurements of the standards[4]. These standard deviations were then plotted versus concentration. The y-intercept of the least squares fit of this line equals S_0, with 3 S_0 being the calculated detection limit. The detection limits are generally in the low ppt range.

C. Accuracy

Because volatile organic compounds are not stable for extended periods in blood, no standard reference material is available in matrix. The accuracy basis for this method is established by determining the recovery of spiked blood samples. In order to examine the consistency of this recovery over the range of levels encountered in blood, these measurements were taken at different concentrations. The results of these measurements are described by Blount et al[1] and Bonin et al[2]. The recoveries at most individual spiking levels fall between 75 and 150%. These results are consistent over the entire range of added analyte, including many measurements that were performed close to the detection limits.

D. Precision

The results of repeated measurements on spiked blood samples are described by Blount et al[1] and Bonin et al[2]. Relative standard deviations are in most cases less than 30%. As expected, most of the exceptions were found in the low spike samples. These standard deviation results are actually higher than would be encountered in typical blood determinations because they include variation in the blood both before

and after spiking. Multiple measurements on spiked QC materials show somewhat lower standard deviation results, averaging 19.4% for all analytes combined.

E. Analytical Specificity

Analytical specificity is established by comparing the ratios of the areas of analyte ion chromatographic peaks with those of confirmation ions along with reproducible GC retention times. The combination of these two measures ensures excellent analytic specificity.

Additional steps are also critical in promoting analytical specificity by removing extraneous compounds from the sample analysis system. Interferences, which have their source in the measurement apparatus itself, are examined by measuring instrument blanks. Blank samples are measured at least twice every day for this purpose. Glassware used for standards is treated to remove possible 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 that could consistently yield water with acceptably low levels of VOCs (< 20 ppt 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 ampules. In all cases, typical blank water levels are below the detection limits given above.

10. Quality Control (QC) Procedures

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 and standards and quality control materials, which are added to each day's run sequence. At least three quality assessment samples are analyzed in each run that include a water blank prepared with the unknown blood samples (i.e., BL037) and two QC samples at different concentrations. In addition to these samples, other QC samples may be added to evaluate assay performance that include a water blank prepared with the standards and additional blank water and QC samples prepared with the unknown blood samples. Absolute labeled-internal standard response and their retention times from the first water blank are compared with that from previous runs to check method and instrument performance. All data entry errors are evaluated by the 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 quality control material are used, QC low and QC High. Different variables 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 deviations (i.e., within run, among run, and overall), and control limits are determined 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.

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 then 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. 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 human body fluids. Alteration of particular aspects of this method can result in major interferences. Care is required in order to produce non-contaminated blanks, vacutainers, and quality control materials. The range of linearity and limits of detection are given above in Sections 9.a. and 9.b., respectively.

12. 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 50% of the median of these values, this indicates that the instrumental sensitivity has fallen below tolerable limits. The following steps should be taken and the instrument sensitivity rechecked after each is performed. Once sensitivity has been reestablished further steps are not necessary.

1. Perform and Air and Water Check as described in Section 8.a.3.

2. Evaluate the instrument tuning parameters as described in Section 8.a.4.

3. Remove and clean the mass spectrometer source. Replace the filament and any ceramics that may be conducting.

4. Test the electron multiplier gain and replace this component if it has markedly decreased.

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 & trap grade methanol used for dilution of the internal standard. For further analyses use a new bottle of purge & trap grade methanol.

3. Replace all syringe clean-up materials.

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. Note that in all cases the supervisor should be

consulted for the appropriate corrective actions. No analytical results may be reported for runs not in statistical control.

13. 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 human body fluids. Alteration of particular aspects of this method can result in major interferences. Care is required to produce non-contaminated blanks, vacutainers, and quality control materials. The quantification range and limits of detection are given above in Section 9.

14. Reference Ranges (Normal Values)

Reference ranges for VOCs have been measured in a sample of 700 to 1000 persons selected from the Third National Health and Nutrition Examination Survey (NHANES III). The sample is not representative of the U.S. population but it is designed to examine the influence of age, sex, race/ethnicity, urban/rural status and region of the country on VOC levels.

Detectable analyte levels of 11 VOCs have been found in 75% or more of the samples from this reference population. For these 11 VOCs, statistical results are given in Table 16.

Table 16. Blood levels of volatile organic compounds in a reference range of the non-occupationally exposed U.S. population.

Analyte	Detection Limit (ppb)	Number	Mean (ppb)	Median (ppb)	Percentiles 5th (ppb)	Percentiles 95th (ppb)
1,1,1-Trichloroethane	0.086	574	0.34	0.13	ND*	0.8
1,4-Dichlorobenzene	0.073	1037	1.9	0.33	ND	9.2
2-Butanone	0.5	1101	7.1	5.4	1.9	16.9
Acetone	200	1062	3100	1800	640	>6000
Benzene	0.03	589	0.13	0.061	ND	0.48
Ethylbenzene	0.02	631	0.11	0.06	ND	0.25
Styrene	0.019	657	0.074	0.041	ND	0.18
Tetrachloroethene	0.03	590	0.19	0.063	ND	0.62
Toluene	0.092	604	0.52	0.28	0.11	1.5
m-/p-Xylene	0.033	649	0.37	0.19	0.074	0.78
o-Xylene	0.04	711	0.14	0.11	0.044	0.3

* Result below detection limit

Table 17 gives the percent of blood samples found above the detection limits for those analytes, which were detectable in 10 to 75% of the samples we analyzed from NHANES

III. A number of other analytes were also examined in this reference range study but were found at detectable levels in fewer than 10% of the samples examined. These analytes along with their detection limits were 1,1,2,2-tetrachloroethane, 0.008 ppb; 1,1,2-trichloroethane, 0.016 ppb; 1,1-dichloroethane, 0.009 ppb; 1,1-dichloroethene, 0.018 ppb; 1,2-dichlorobenzene, 0.044 ppb; 1,2-dichloroethane, 0.012 ppb; 1,2-dichloropropane, 0.008 ppb; 1,3-dichlorobenzene, 0.019 ppb; bromoform, 0.027 ppb; carbon tetrachloride, 0.019 ppb; cis-1,2-dichloroethene, 0.013 ppb; dibromomethane, 0.044 ppb; hexachloroethane, 0.079 ppb; methylene chloride, 0.089 ppb; and trans-1,2-dichloroethene, 0.014 ppb.

Table 17. Percent of samples with blood levels above detection limit for volatile organic compounds in a reference range of the non-occupationally exposed U.S. population.

Analyte	Detection Limit (ppb)	Number	Percent of Population Above Detection Limit
Bromodichloromethane	0.009	1072	14
Chlorobenzene	0.007	1024	21
Chloroform	0.021	979	54
Chlorodibromomethane	0.013	1035	12
Trichloroethene	0.01	677	13

15. Critical-Call Results (“Panic” Values)

The health effects resulting from exposure to low levels of VOCs is currently unclear. The method described here is designed for the measurement of low level exposure to VOCs, thus panic values will not be measured with this method.

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 can be left on a cooled sample tray at 15 $\pm$ 1^{o}C. Samples are not placed in a refrigerator that has not been recently vented. Most sample queues run for extended time periods of up to 24-hr duration. As a precaution biological samples (unknowns and QC) are racked into a chilled tray (15 $\pm$ 1^{o}C) while awaiting analysis.

17. Alternate Methods for Performing Test and Storing Specimens if Test System Fails

The analysis of VOCs in whole blood at parts-per-trillion levels is an extremely complex measurement. There are no acceptable alternative methods for this analysis. If the analytical system fails, storage of unprepared and partially prepared specimens at 2-6°C is recommended up to 24 hr.

A. Length of Time Samples may be banked

Repeat measurements of samples stored at 2-6^{o}C indicate that whole blood VOC samples may be banked for at least 7 weeks. Because these are whole blood samples, longer storage results in samples that are harder to manipulate and produce additional analytical problems. Thus, even though analytical results may not change over this time, samples may be less amenable to analysis. Volatile organic compounds occur naturally in the body, and metabolism may alter their concentration with storage.

B. Proper banking procedures

Whole blood samples for VOC measurement should be stored in the dark at 2–6^{o}C. This prevents blood cell rupture that would occur during freezing. In addition, freezing of blood can lead to breakage of vacutainers and loss of sample in some cases. Because VOCs are lost whenever the containers in which they are stored are opened, it is not appropriate to transfer the blood samples to another container that 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 because these values are not available elsewhere.

The health effects resulting from exposure to low levels of VOCs is currently unclear. Therefore no critical call levels are set.
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18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

If greater than 3 mL of sample remain after analysis, this material should be returned to storage at 2-6^{o}C.

Standard record keeping means (database, sample logs, optical disc files) are used to track specimens. 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.

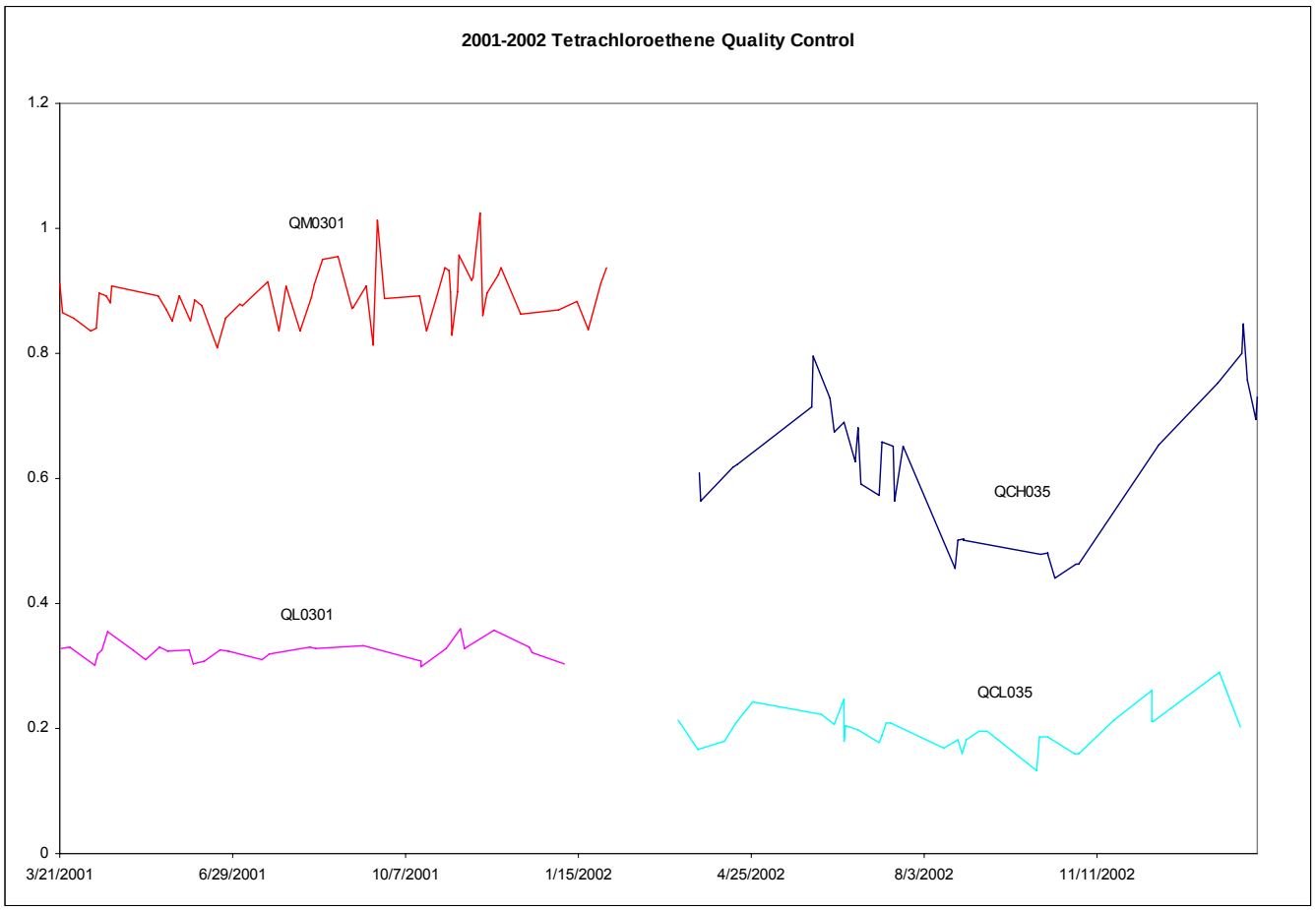
Because of the complex nature of the analyses and the unique testing capabilities of this laboratory, it is not expected that specimens will be referred to other laboratories for testing. Should such a need arise; the laboratory supervisor will consult with local subject matter experts to establish an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

19. Summary Statistics and Quality Control Graphs

A. Blood Tetrachloroethene

Summary Statistics for Tetrachloroethene by Lot

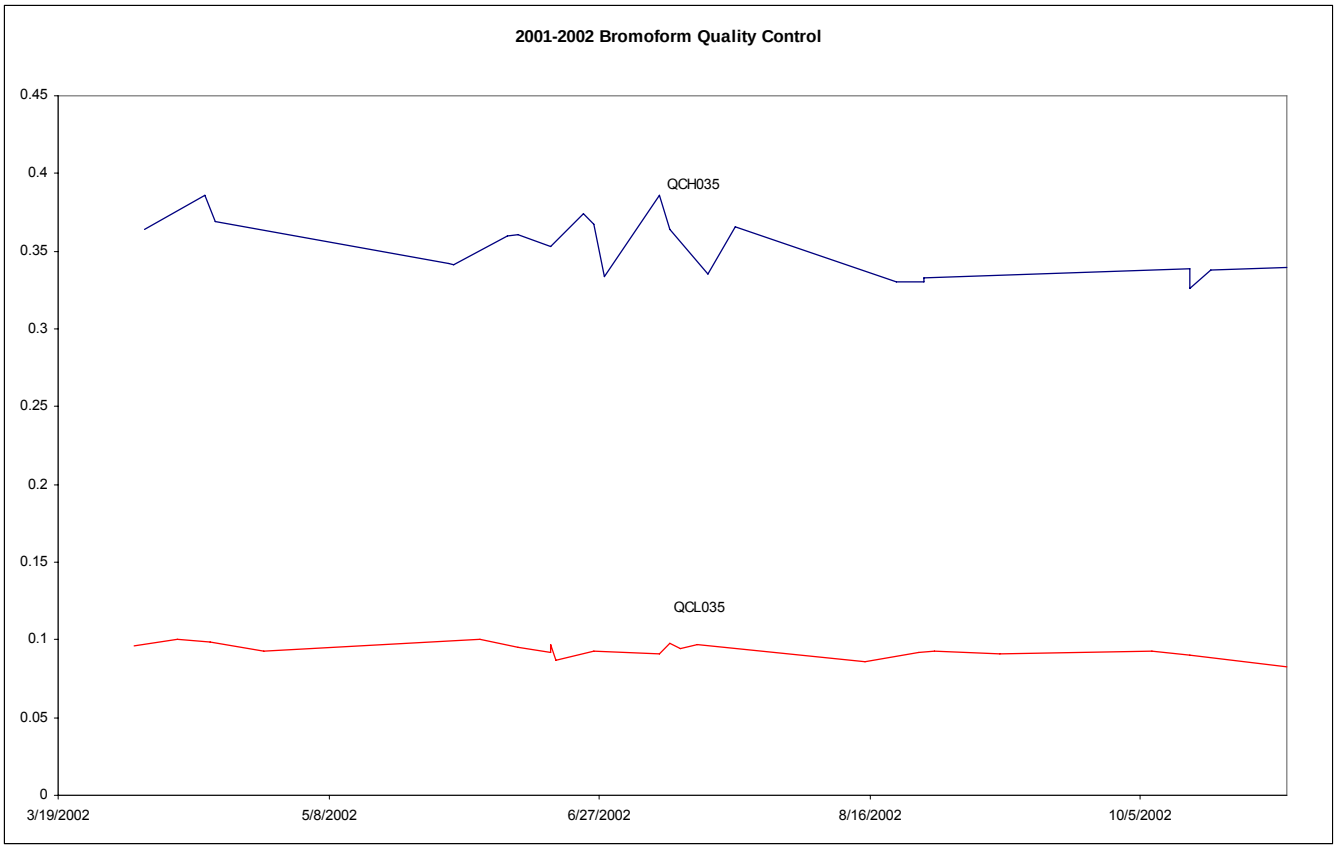
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	56	3/21/2001	1/31/2002	0.89031	0.04323	4.9
QL0301	29	3/22/2001	1/7/2002	0.32377	0.01562	4.8
QCL035	32	3/14/2002	2/2/2003	0.19841	0.03154	15.9
QCH035	34	3/26/2002	2/12/2003	0.61806	0.11331	18.3



B. Blood Bromoform

Summary Statistics for Bromoform by Lot

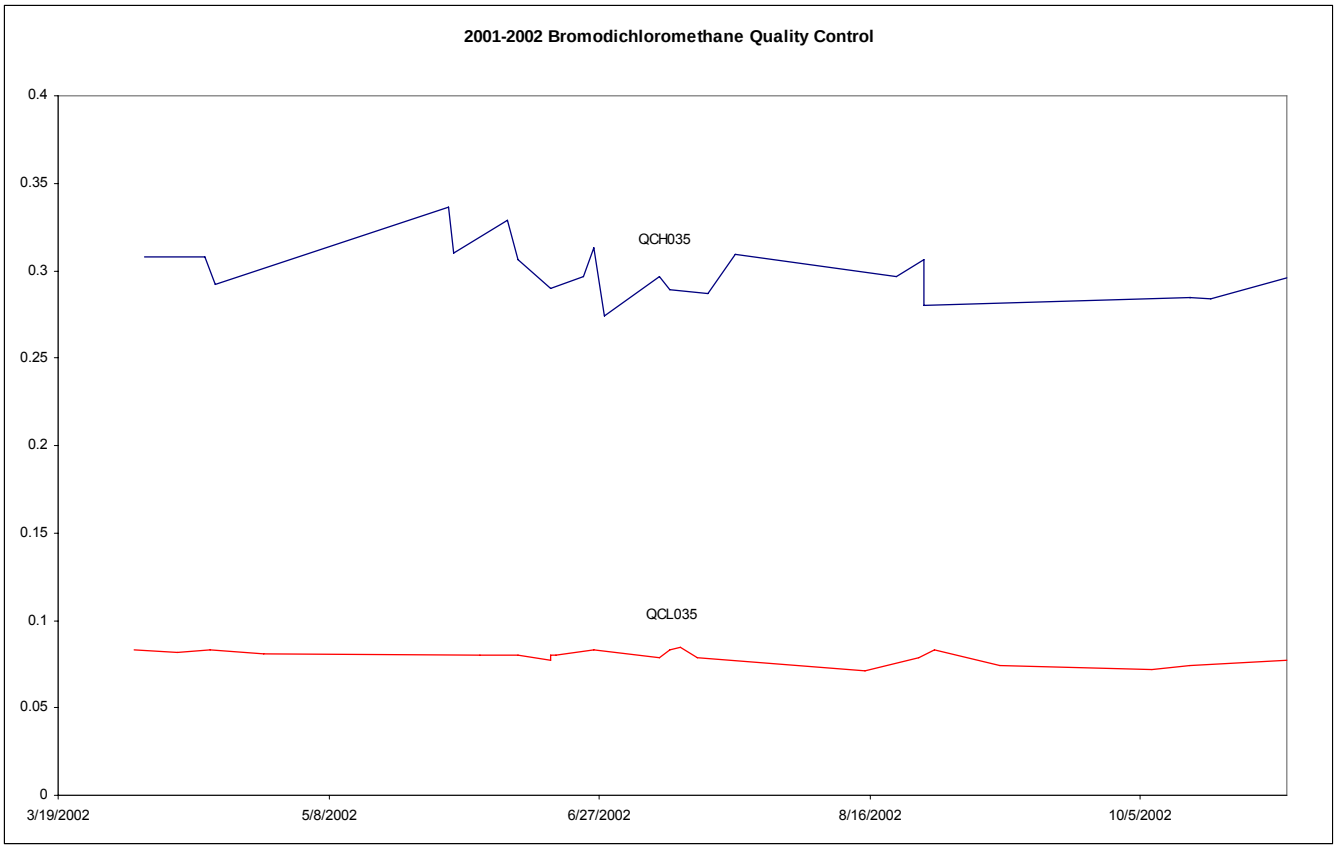
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCL035	21	4/2/2002	11/1/2002	0.09333	0.00451	4.8
QCH035	22	4/4/2002	11/1/2002	0.35173	0.01863	5.3



C. Bromodichloromethane

Summary Statistics for Bromodichloromethane by Lot

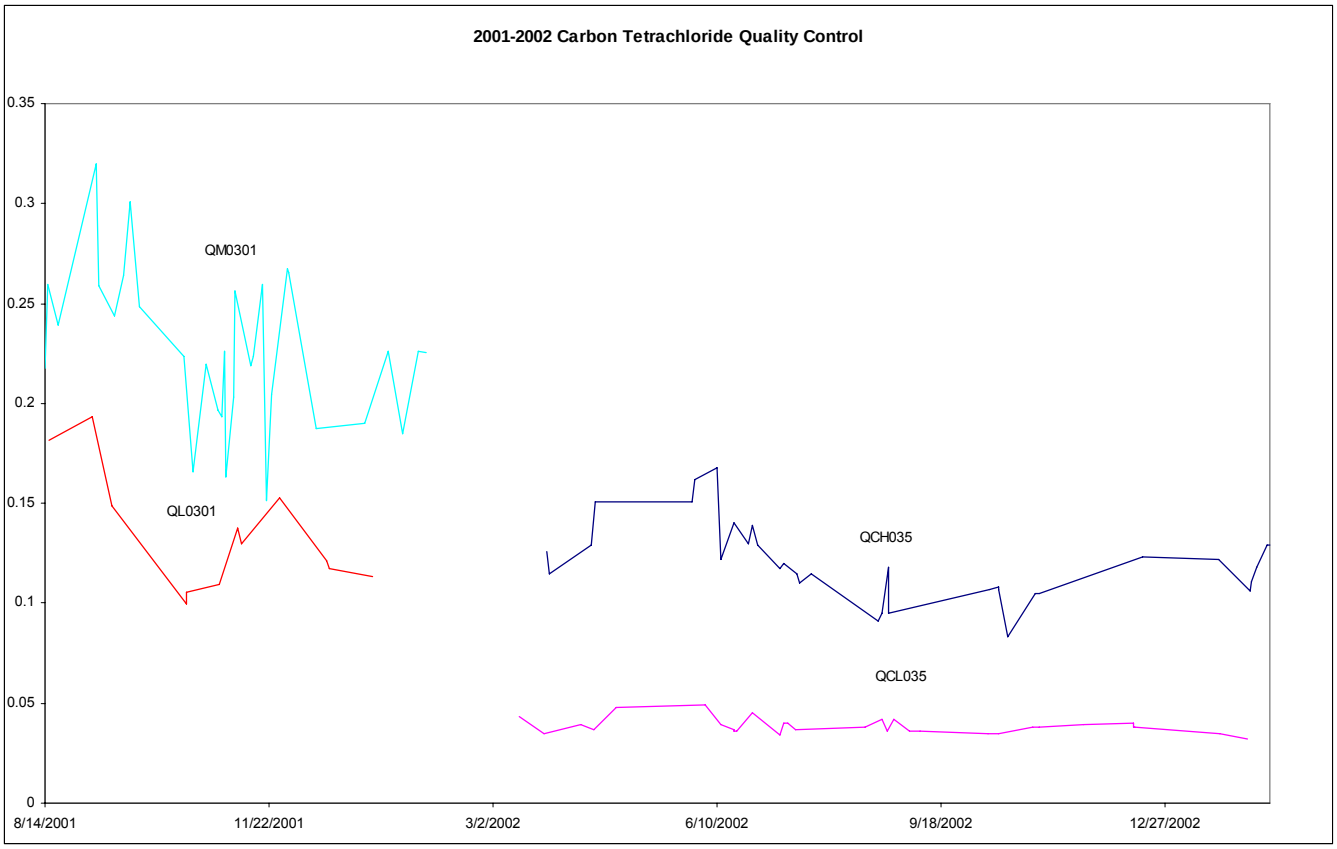
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCL035	21	4/2/2002	11/1/2002	0.07929	0.00389	4.9
QCH035	22	4/4/2002	11/1/2002	0.299	0.01534	5.1



D. Carbon Tetrachloride

Summary Statistics for Carbon Tetrachloride by Lot

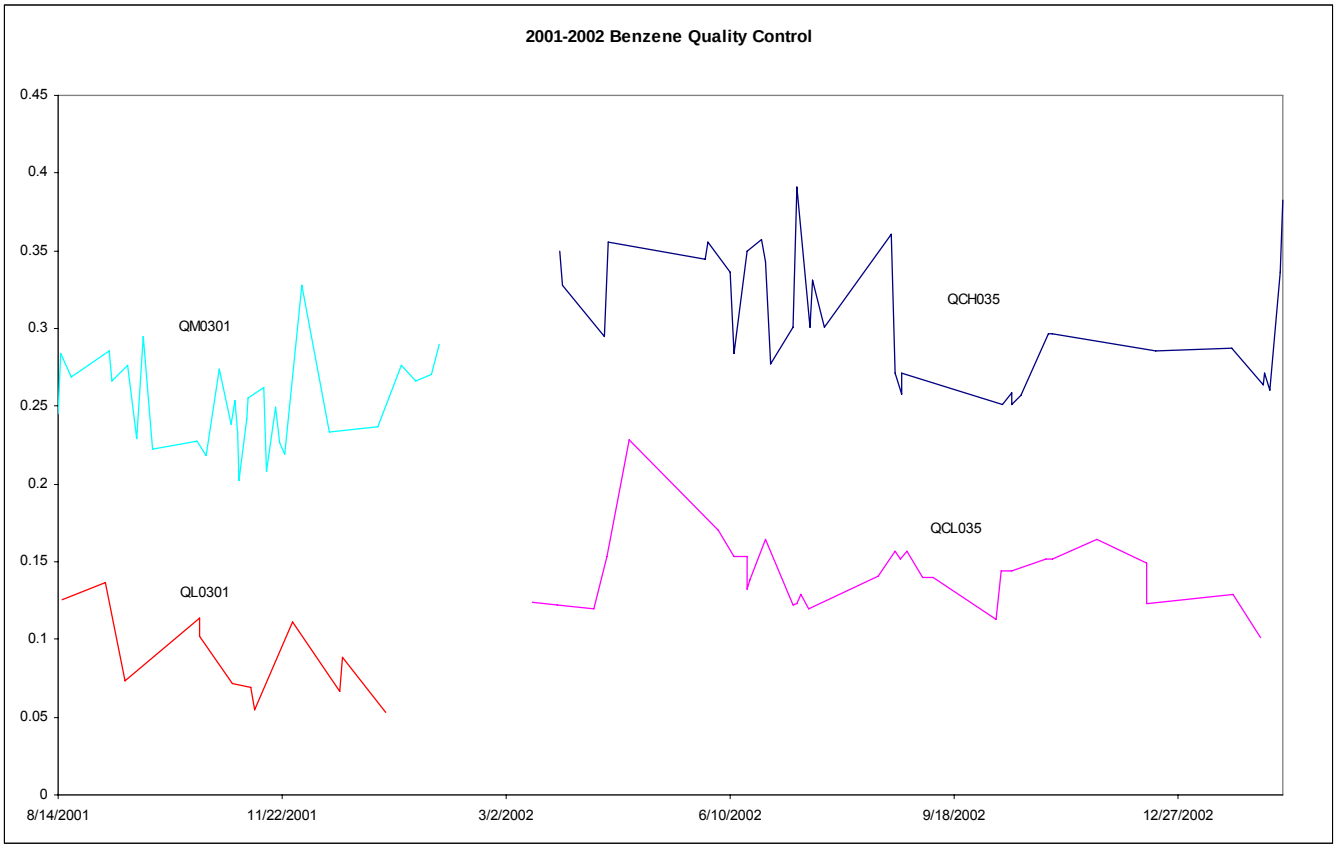
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	31	8/14/2001	1/31/2002	0.22682	0.03874	17.1
QL0301	12	8/16/2001	1/7/2002	0.13418	0.02991	22.3
QCL035	31	3/14/2002	2/2/2003	0.03848	0.00385	10.0
QCH035	34	3/26/2002	2/12/2003	0.12032	0.01914	15.9



E. Blood Benzene

Summary Statistics for Benzene by Lot

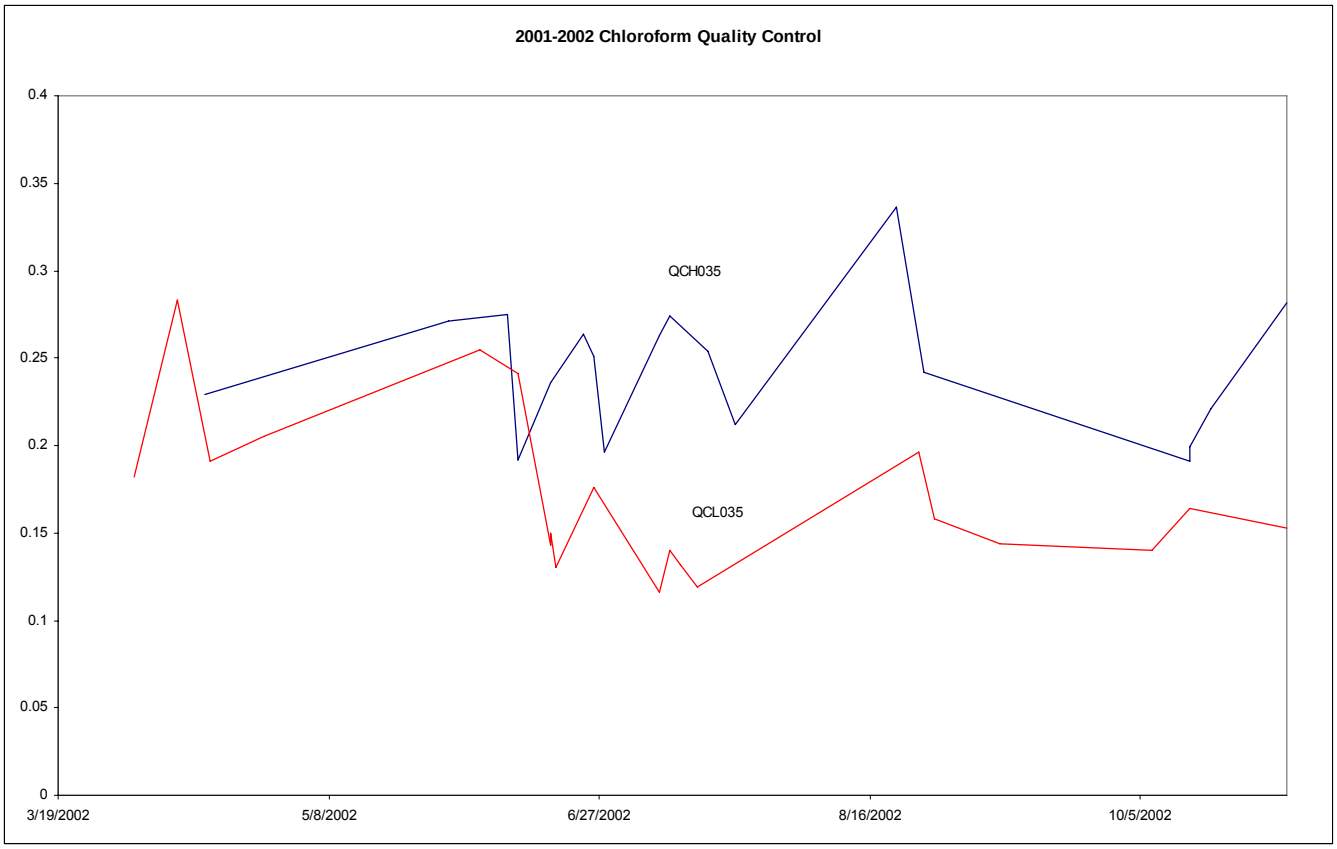
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	30	8/14/2001	1/31/2002	0.25271	0.02912	11.5
QL0301	12	8/16/2001	1/7/2002	0.08887	0.02821	31.7
QCL035	32	3/14/2002	2/2/2003	0.14163	0.02305	16.3
QCH035	34	3/26/2002	2/12/2003	0.30771	0.04131	13.4



F. Blood Chloroform

Summary Statistics for Chloroform by Lot

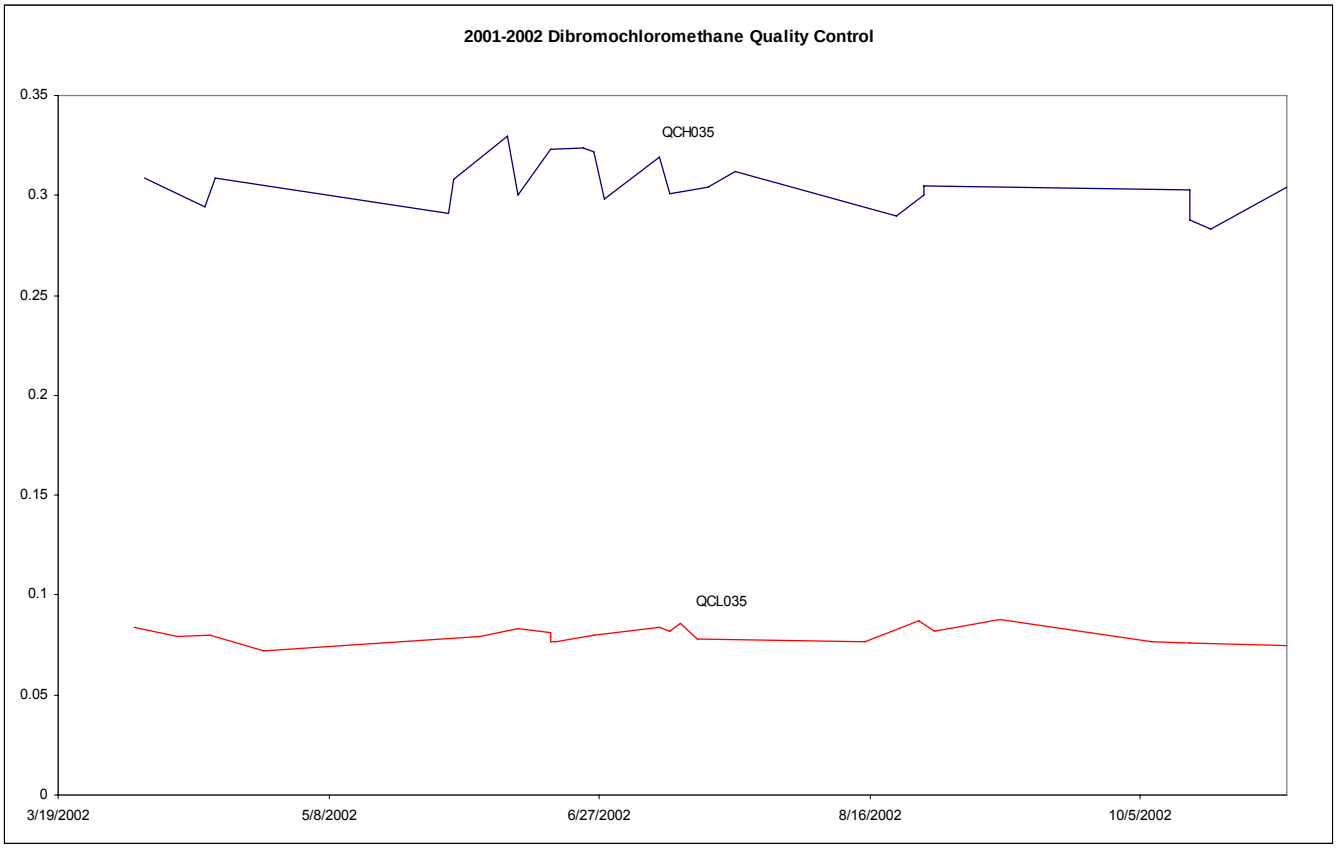
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCL035	20	4/2/2002	11/1/2002	0.17090	0.04598	26.9
QCH035	18	4/15/2002	11/1/2002	0.24378	0.03842	15.8



G. Blood Dibromochloromethane

Summary Statistics for Dibromochloromethane by Lot

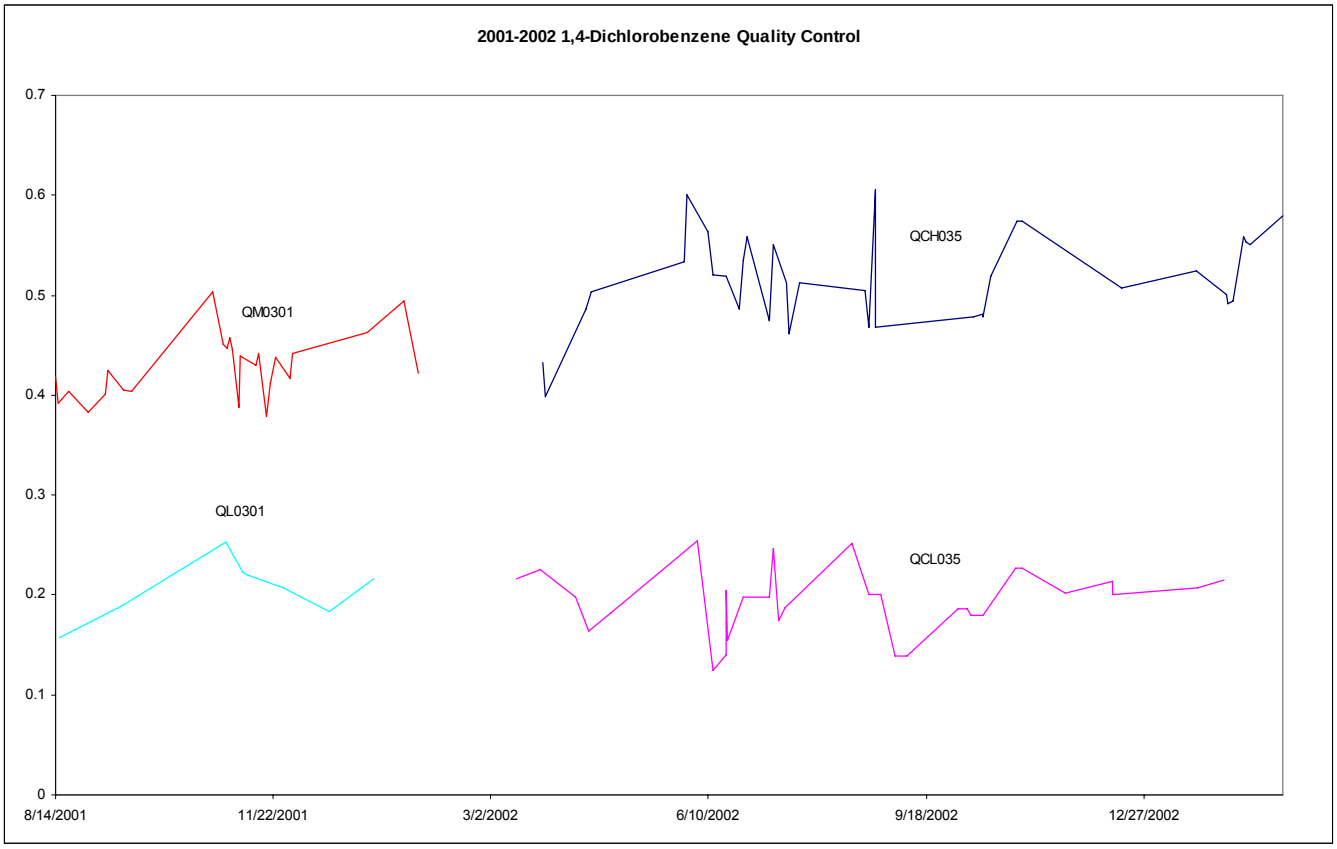
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCL035	21	4/2/2002	11/1/2002	0.08019	0.00415	5.2
QCH035	22	4/4/2002	11/1/2002	0.30532	0.01257	4.1



H. Blood 1,4-Dichlorobenzene

Summary Statistics for 1,4-Dichlorobenzene by Lot

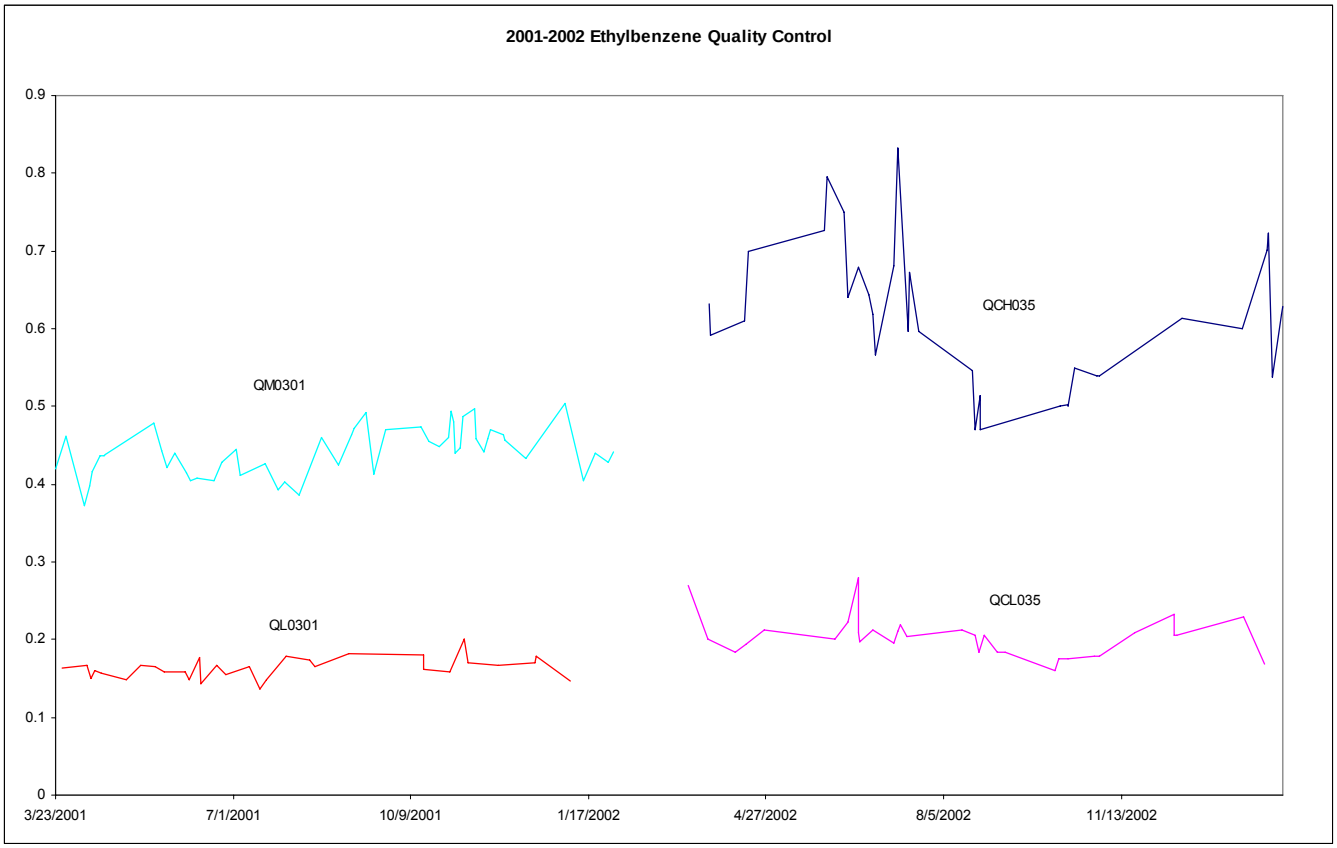
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	25	8/14/2001	1/28/2002	0.42803	0.03186	7.4
QL0301	8	8/16/2001	1/7/2002	0.20615	0.02926	14.2
QCL035	33	3/14/2002	2/2/2003	0.19473	0.03169	16.3
QCH035	36	3/26/2002	3/1/2003	0.51567	0.04596	8.9



I. Blood Ethylbenzene

Summary Statistics for Ethylbenzene by Lot

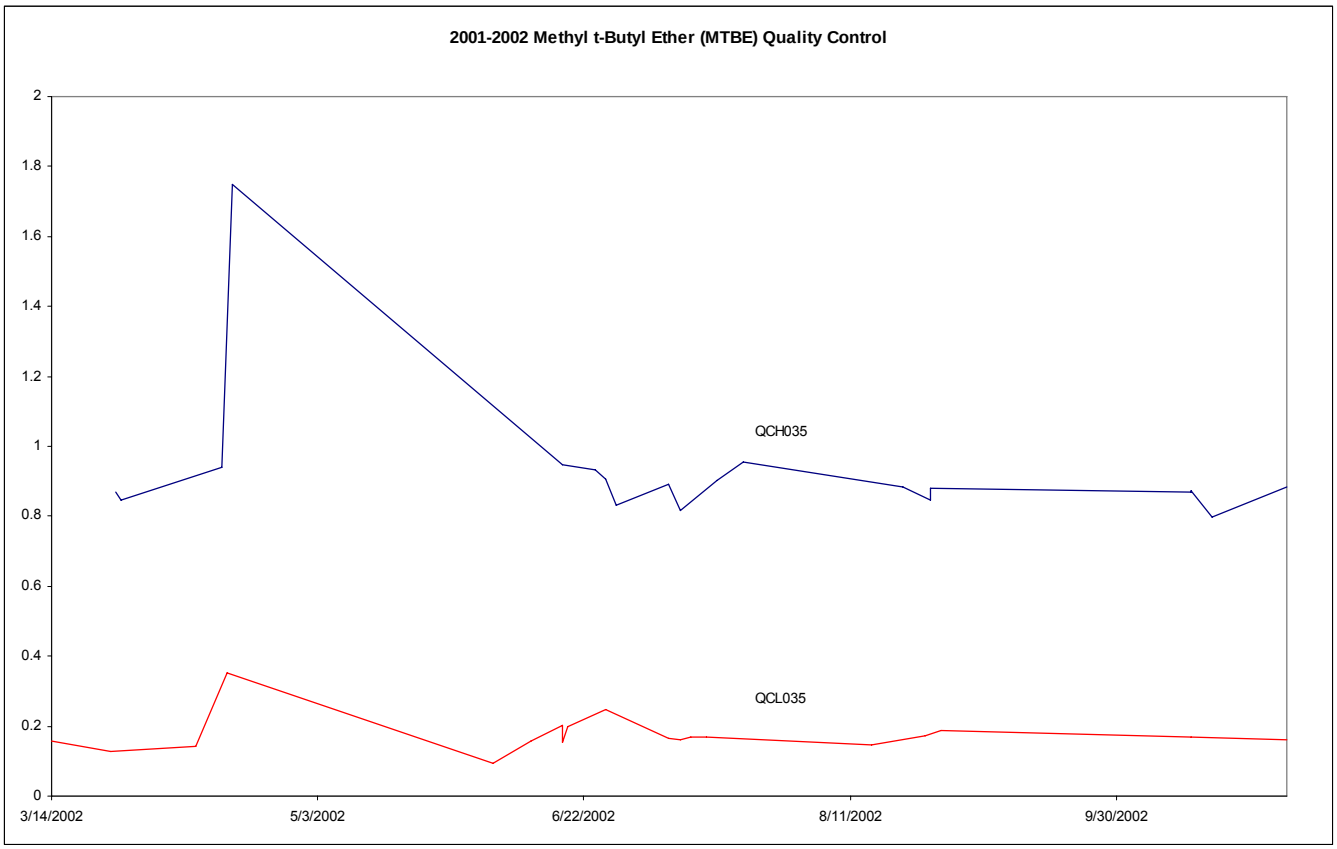
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	52	3/23/2001	1/31/2002	0.44163	0.03076	7.0
QL0301	31	3/27/2001	1/7/2002	0.16362	0.01347	8.2
QCL035	32	3/14/2002	2/2/2003	0.20344	0.02558	12.6
QCH035	34	3/26/2002	2/12/2003	0.61438	0.09054	14.7



J. Blood MTBE

Summary Statistics for Methyl t-Butyl Ether (MTBE) by Lot

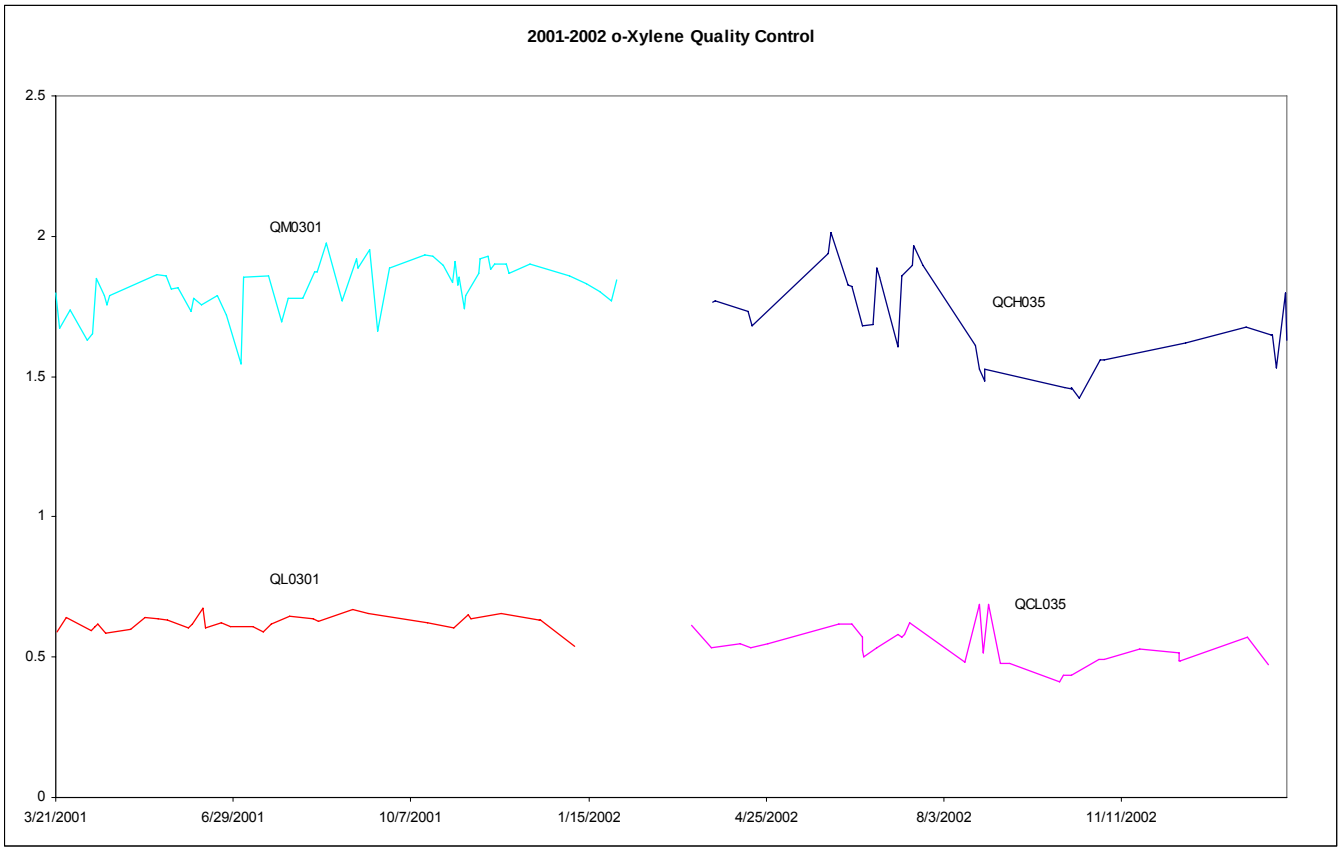
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCL035	19	3/14/2002	11/1/2002	0.17547	0.05262	30.0
QCH035	19	3/26/2002	11/1/2002	0.92747	0.20383	22.0



K. Blood o-Xylene

Summary Statistics for o-Xylene by Lot

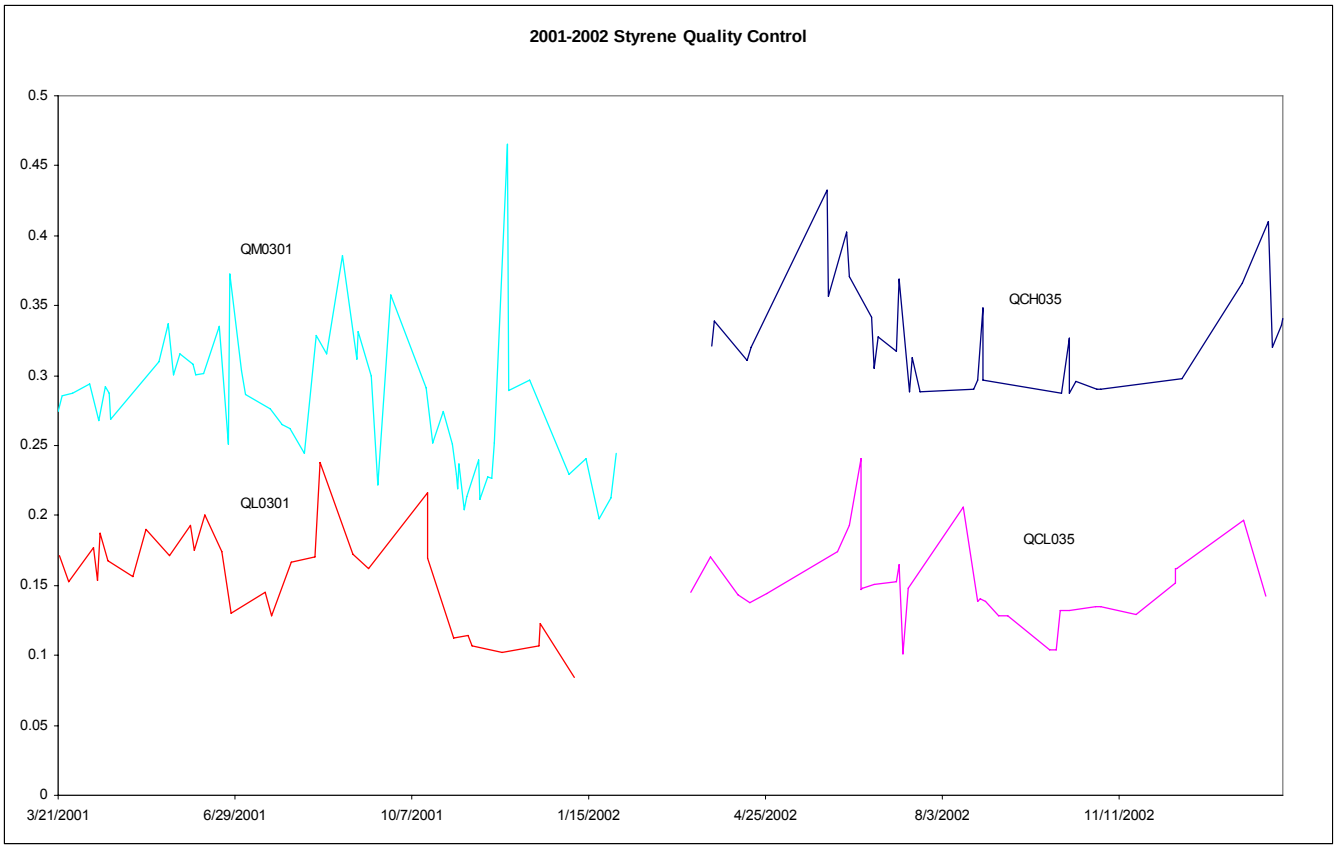
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	55	3/21/2001	1/31/2002	1.81993	0.08848	4.9
QL0301	32	3/22/2001	1/7/2002	0.62191	0.02769	4.5
QCL035	32	3/14/2002	2/2/2003	0.53563	0.06756	12.6
QCH035	34	3/26/2002	2/12/2003	1.69079	0.16498	9.8



L. Blood Styrene

Summary Statistics for Styrene by Lot

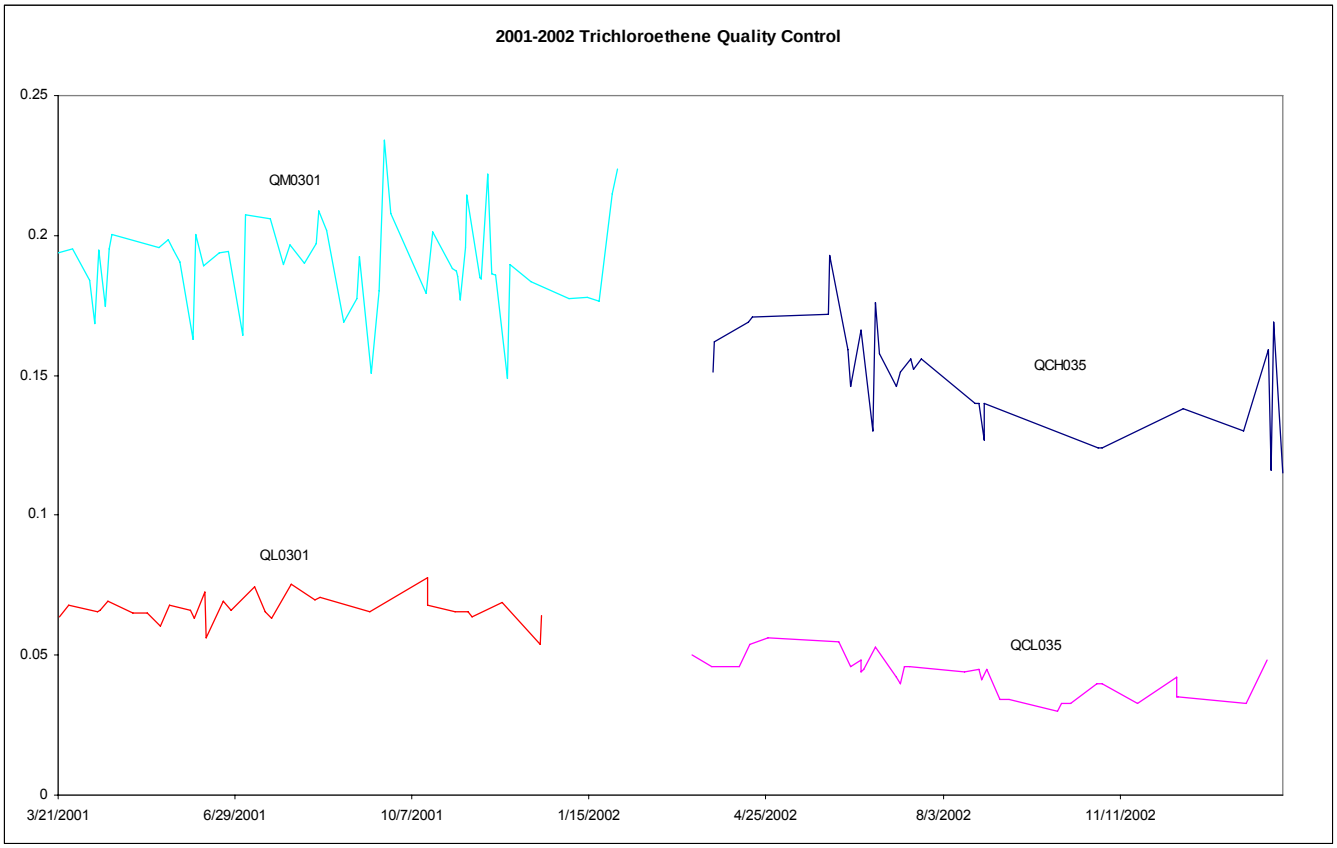
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	54	3/21/2001	1/31/2002	0.27932	0.05056	18.1
QL0301	30	3/22/2001	1/7/2002	0.15726	0.03595	22.9
QCL035	33	3/14/2002	2/2/2003	0.14930	0.02893	19.4
QCH035	32	3/26/2002	2/12/2003	0.32766	0.03861	11.8



M. Blood Trichloroethene

Summary Statistics for Trichloroethene by Lot

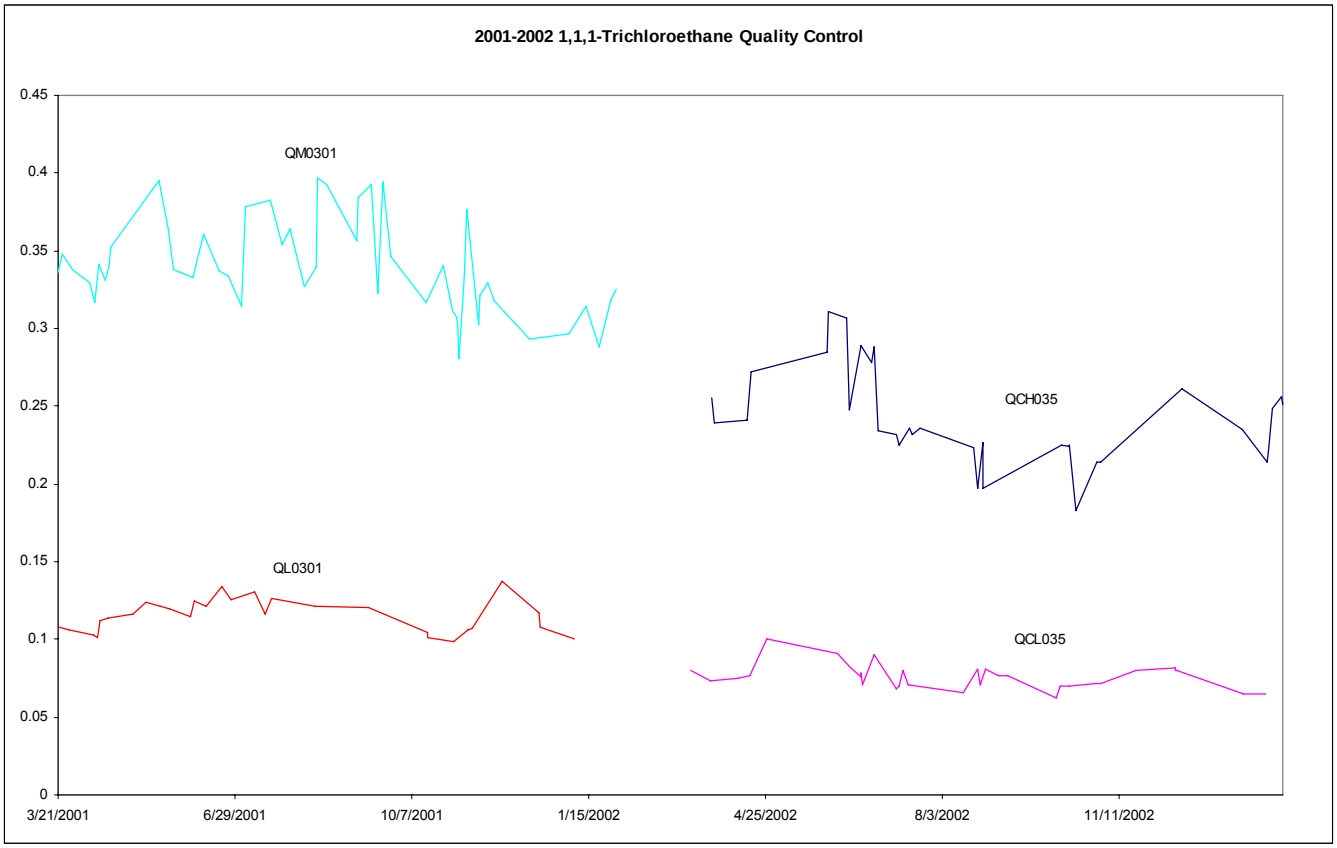
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	54	3/21/2001	1/31/2002	0.19045	0.01650	8.7
QL0301	30	3/22/2001	12/19/2001	0.06654	0.00497	7.5
QCL035	32	3/14/2002	2/2/2003	0.04256	0.00713	16.8
QCH035	29	3/26/2002	2/11/2003	0.14952	0.01928	12.9



N. Blood 1,1,1-Trichloroethane

Summary Statistics for 1,1,1-Trichloroethane by Lot

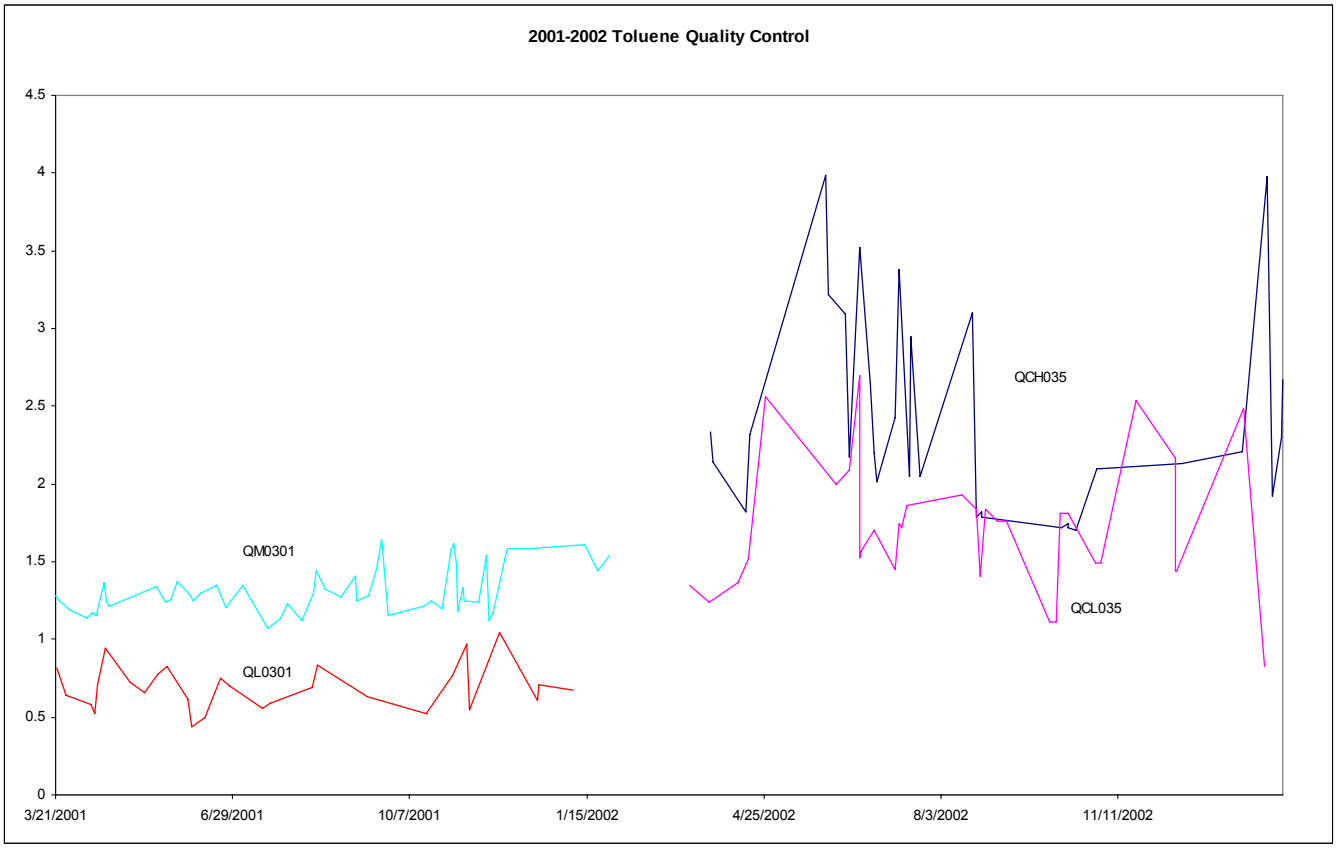
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	51	3/21/2001	1/31/2002	0.33910	0.02957	8.7
QL0301	29	3/22/2001	1/7/2002	0.11515	0.01064	9.2
QCL035	32	3/14/2002	2/2/2003	0.07606	0.00812	10.7
QCH035	34	3/26/2002	2/12/2003	0.24188	0.03052	12.6



O. Blood Toluene

Summary Statistics for Toluene by Lot

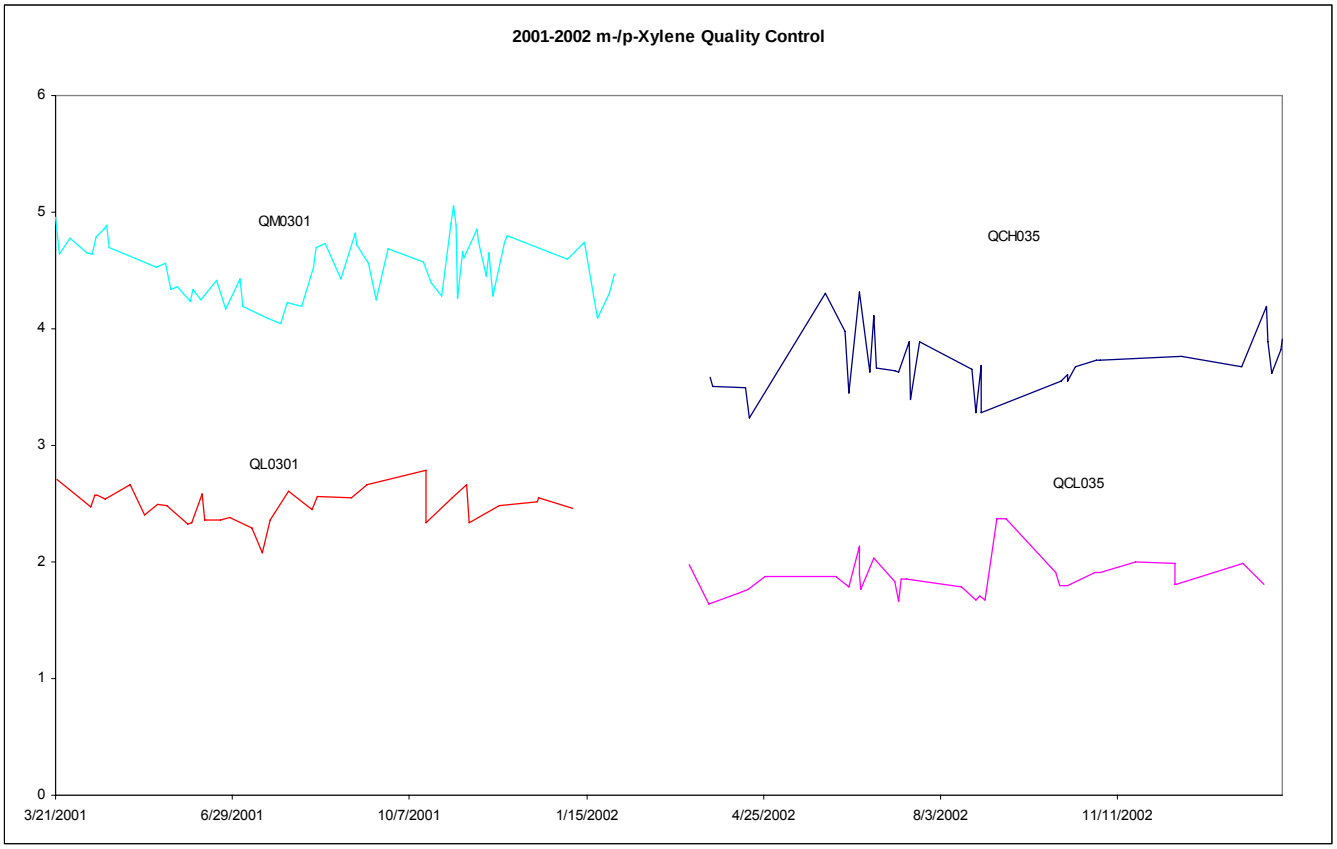
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	52	3/21/2001	1/28/2002	1.31648	0.15180	11.5
QL0301	29	3/22/2001	1/7/2002	0.68539	0.14748	21.5
QCL035	33	3/14/2002	2/2/2003	1.71694	0.43339	25.2
QCH035	34	3/26/2002	2/12/2003	2.42968	0.67098	27.6



P. Blood m-/p-Xylene

Summary Statistics for m-/p-Xylene Result by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QM0301	54	3/21/2001	1/31/2002	4.53706	0.25584	5.6
QL0301	32	3/22/2001	1/7/2002	2.48462	0.14561	5.9
QCL035	32	3/14/2002	2/2/2003	1.87503	0.17429	9.3
QCH035	33	3/26/2002	2/12/2003	3.70679	0.26807	7.2



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