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Generation and Evaluation of Reference Samples as part of an Evacuated Canister Interlaboratory Study

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ABSTRACT

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Evacuated canisters offer an opportunity to expand on the way volatile organic compounds (VOCs) are measured in indoor air quality investigations, industrial hygiene assessments, and emergency response scenarios. There is a growing need for alternative sampling methods for VOCs as traditional sorbent tube sampling methods may not adequately capture the multitude of chemicals present in mixed exposure environments due to sorbent-analyte specificity.

This study is part of a larger work designed to address this need across a suite of 17 VOCs. This study assesses generation and evaluation methods for the production of reference evacuated canister samples as part of an ASTM-style interlaboratory study. The interlaboratory study was designed to assess evacuated canister method performance for the development of a National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods protocol.

The reference canister samples were generated in two concentration ranges (part-per-million and part-per-billion) and at three nominal concentration levels within the two ranges. For the PPB range, samples were generated using either a flow-based dilution or combination pressure dilution and canister-to-canister transfer technique. For the PPM range, samples were generated by either the combination pressure dilution and canister-to-canister transfer technique, or a manifold dilution method. The reference canister samples were analyzed via gas chromatography-mass spectrometry (GC-MS). The performance of three preparation methods and three analytical methods were assessed by the NIOSH 95% confidence interval on accuracy criterion.

Results indicate that method accuracy is concentration dependent with respect to certain combinations of analytical method, preparation technique, and analyte. Some sample preparation techniques were found to be better for certain groups of compounds and at certain concentration ranges. All 17 VOCs passed the NIOSH accuracy criterion for the PPM range when prepared using the pressure dilution technique and analyzed via a 1 cc loop injection into a GC-MS. For the PPB range of concentration levels, 15 VOCs passed the NIOSH accuracy criterion when prepared by the pressure dilution method and analyzed via a 250 cc, cryogenically concentrated injection into a GC-MS.

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Introduction

This study aims to investigate the accuracy and precision with which reference evacuated canister standards can be produced by analyzing a suite of volatile organic compounds (VOCs) in two concentration ranges, part-per-million (PPM) and part-per-billion (PPB). The hypothesis of this study is that the evacuated canister sample preparation and analytical methods pass the NIOSH accuracy and 95% confidence interval criterion.This research will be incorporated into a larger project designed to validate a protocol for evacuated canister sampling and subsequent analysis via gas chromatography-mass spectrometry (GC-MS) for a suite of VOCs; culminating in the incorporation of said protocol into the National Institute for Occupational Safety and Health's Manual of Analytical Methods. This method will be used in conjunction with, or in place of currently validated sorbent tube sampling methods to establish ambient air concentration levels of VOCs in occupational settings.

Chemical compounds normally found in the vapor phase at room temperature are usually referred to as volatile organic compounds. These compounds typically have a vapor pressure greater than 0.1 mmHg at 25˚C (Kelly, Mukund, Gordon, & Hays, 1994). Less volatile compounds are known as semi-volatile organic compounds (SVOCs). SVOCs are normally found as aerosols, either liquid or dust droplets, but may also be found in the vapor phase (Harper, 2000). Only specific VOCs are considered to be within the scope of this document. There are many reasons for measuring the concentrations of VOCs in ambient air. Some of these reasons include: health effects' studies, environmental pollution research, and for the determination of compliance with regulatory concentration limits (Harper, 2000). Of the VOCs evaluated in this study, 14 were selected because of their relevance to a concurrent healthcare VOC exposure characterization project (R. LeBouf *et al.*, 2014), and the remaining three (α-diketones 2,3-butanedione; 2,3-pentanedione; and 2,3-hexanedione) were chosen because of their health effects and presence in commonly used flavorings agents (Harber, Saechao, & Boomus, 2006; Hubbs *et al.*, 2008; Sahakian, Kullman, Lynch, & Kreiss, 2008). The 14 non-α-diketone VOCs were selected due to their presence in cleaning and disinfecting products, latex related materials, bioaerosols, and/or other asthmagenic substances commonly found in healthcare settings (Arif & Delclos, 2012; Dimich-Ward, Wymer, & Chan Yeung, 2004; Liss *et al.*, 2011; Mirabelli *et al.*, 2007). The compounds chosen for evaluation in this study are listed in Table 1.

Table 1. Suite of VOCs

Ethanol	Acetone	2-Propanol
Methylene Chloride	2,3-Butanedione	*n*-Hexane
Chloroform	Benzene	2,3-Pentanedione
Methyl Methacrylate	Toluene	2,3-Hexanedione
Ethylbenzene	meta-Xylene	para-Xylene
ortho-Xylene	Alpha-Pinene	*d*-Limonene

As previously mentioned, determination of compliance with regulatory limits is a reason for measuring the concentration of VOCs in ambient air in occupational settings. Legislation such as the Occupational Safety and Health Act of 1970 (OSHA) requires the evaluation and control of workers' exposure to airborne toxic chemicals. Currently validated methods for sampling ambient air for VOCs in occupational settings rely heavily on sorbent tube sampling methods (NIOSH, 2003). Occupational sorbent tube sampling methods typically use battery-powered air pumps worn on the belt of a worker connected via flexible tubing to a glass tube containing sorbent material attached to the lapel of a worker's shirt, inside the breathing zone. The pump then pulls ambient air through the sorbent tube at a fixed, predetermined and calibrated rate. The sampling flow rate of the pump is recorded during pre-and post-sampling calibration. The average of the two flow rate values is then used to calculate the volume of sampled air. The sorbent tubes are sent to laboratory for analysis, and the reported mass of analyte can then be divided by sampled air volume to generate a concentration.

During sorbent tube sampling, VOCs in the ambient air are extracted from the sampled volume by adsorption or reaction with sorbent in the tube (Harper, 2000). The sorbents found in air sampling sorbent tubes are materials designed to adsorb liquids or gases. Adsorption is the adhesion of chemical species to a surface. Adsorption differs from absorption in that adsorption is merely a surface phenomenon, while absorption relies on the absorbate being dissolved by or permeating the absorbent. Adsorption efficiency is thus heavily dependent on the available amount of adsorbent surface area (Gregg & Sing, 1982). This is a disadvantage of the technology as sorbents can only extract as much analyte from a sampled volume as they have surface area of sorbent to retain. One way to combat this shortcoming is to sample with sorbent tubes containing a "back-up section" in-line in the sample air flow path to detect breakthrough of analyte(s) from one section of sorbent to the next. This can be extended to using multiple sorbent tubes in series in the sample sampling train. There are other sorbent tube sampling methods that make use of derivatization and/or trapping by reaction, where sorbents act as

a base for chemicals which react with analytes found in the sampled air volume to generate a more stable or easily analyzed derivative (Levin & Lindahl, 1994; Otson & Fellin, 1988).

Regardless of the method of adsorption of the chemical species onto the sorbent, the analytes must be desorbed prior to analysis. Most currently validated occupational sorbent tube air sampling methods call for samples to be analyzed via gas chromatography (GC) and can be found in the U.S. National Institute for Occupational Safety and Health's Manual of Analytical Methods (NMAM). When the desired method of sample analysis is a GC, analytes trapped onto a solid sorbent are typically desorbed with a liquid solvent that is suitable for GC analysis (Harper, 2000). This process presents its own set of disadvantages as some commonly used desorption solvents, such as carbon disulfide and methylene chloride are toxic to humans (Bus, 1985; Sobue *et al.*, 2015).

Thermal desorption is the second method used for sample desorption. This method involves using high levels of heat instead of solvents to desorb trapped analytes. The primary disadvantage of thermal desorption technology is the necessity of analyzing the entirety of a sample in a single analysis. Another disadvantage of thermal desorption tubes is their lack of a back-up section that can be analyzed separately to detect breakthrough (Harper, 2000). All traditional sorbent tube air sampling methods share a distinct set of disadvantages. These disadvantages include their reliance on the use of active air movement mechanisms such as sampling pumps, which can be unreliable and bulky in a field sampling setting; their inability to collect a whole-air sample; and their collection of a relatively small sample size. Additionally, more than one sorbent type is typically required to cover all classes of a compound. Finally, whole classes of compounds, such as reduced sulfur gases (H_2S, CS_2, mercaptans) and very volatile hydrocarbons such as propylene, ethylene, methane, and ethane are simply not suitable for sorbent tube sampling. All of these compounds have been found to be amenable to evacuated canister whole-air sampling techniques (Harper, 2000).

An alternative method to sorbent tube sampling for VOCs involves the introduction of ambient air into a specially-prepared, evacuated stainless steel canister. In evacuated canister sampling, the sampling train is comprised of components designed to regulate the speed and duration of sampling into the canister vessel. Once the sample is collected, the canister is closed and sent to a laboratory for analysis. Analysis of an air sample collected in an evacuated canister involves passing a known volume through a multi-sorbent concentrator, in which the sample is dehumidified and then concentrated to a small final volume that is injected into a GC for chromatographic separation and analysis (EPA, 1999). Evacuated canister sampling technology offers advantages over traditional sorbent tube sampling

methods, both in terms of sensitivity and the number of viable compounds (Brymer, Ogle, Jones, & Lewis, 1996; N. Ochiai, Takino, Daishima, & Cardin, 2001). Evacuated canister sampling methods have other practical advantages over traditional sorbent-based sampling methods, such as collection of a whole air sample (Harper, 2000). Evacuated canister sampling is a passive sampling technique that does not rely on pumps or other powered devices, and is capable of collecting sufficient sample volumes for multiple analyses of the same sample (EPA, 1997; N. Ochiai, Daishima, & Cardin, 2003). In addition, evacuated canisters samples do not require refrigeration (R. F. LeBouf, Stefaniak, & Virji, 2012) and can be safer to analyze, as they do not require the use of toxic desorption solvents such as carbon disulfide (Bus, 1985).

Evacuated canisters have been validated for sampling suites of VOCs in ambient air since the establishment of EPA method TO-15 in 1999 (EPA, 1999). Most VOCs sampled from ambient air with evacuated canisters have been shown to be stable near their original concentration for storage times up to 30 days (EPA, 1999; Herrington, 2015). However, evacuated canisters have certain limitations as well, including sample loss due to physical adsorption of VOCs on canister walls, dissolution of VOCs in condensed water inside the canister, chemical reactions of VOCs with ozone or other gas species, and aqueous hydrolysis (EPA, 1999; Nobuo Ochiai, Tsuji, Nakamura, Daishima, & Cardin, 2002). These losses have been characterized and evaluated in other published methods, such as EPA TO-15. Some of the aforementioned avenues of sample loss in canisters can be combatted by lining the inner surfaces of stainless steel canisters with fused silica and limiting the effects of excess humidity in the sample (Nobuo Ochiai *et al.*, 2002). Lining the inner sections of the canister with fused-silica material reduces the number of active-sites available for analytes to occupy, resulting in greater sample retention. Reducing the humidity of the canister sample increases sample integrity as the likelihood of hydrolysis of compounds present in the sample is reduced.

As a testament to the value of canister sampling as an ambient air sampling technique, evacuated canisters are currently used for exposure assessment in a variety of environments, including general industry, indoor air quality surveys, healthcare settings, and even agriculture (Bari, Kindzierski, Wheeler, Héroux, & Wallace, 2015; R. LeBouf *et al.*, 2014; Rumsey, Aneja, & Lonneman, 2014). The advantages of evacuated canister sampling methods over traditional sorbent-based methods, the diversity of compounds found in the occupational setting, and the established use of evacuated canister sampling in such a variety of air sampling settings necessitate the development of a standard operating procedure for their use in the occupational exposure assessment environment. This research is part of a larger study

designed to meet the need for a validated evacuated canister sampling method for the occupational setting.

Samples collected both by traditional sorbent tube methods and evacuated canister methods must be subsequently analyzed to estimate concentrations of chemical species in the sampled air. GC-MS allows for high temporal resolution, analyte selectivity, and sensitivity in analyzing VOCs collected in evacuated canisters (EPA, 1999). The combined use of gas chromatographic retention time and the relatively unique ion fragmentation patterns of individual analytes allows for a more definitive identification procedure than the use of other, single specific detectors such as an electron capture detector, a flame ionization detector, or a photoionization detector. In addition, the identification of VOCs in the sample is further enhanced by the use of the National Institute of Standards and Technology's (NIST) Mass Spectral Library, containing the relatively unique mass spectral ion fragmentation patterns of thousands of chemical species. The use of GC-MS for sample analysis results in desirable and scientifically-defensible analyte detection scheme (EPA, 1999).

The National Institute for Occupational Safety and Health (NIOSH) has been charged with the responsibility for the development and evaluation of workplace exposure determination sampling and analytical methods since the adoption of the Occupational Safety and Health Act of 1970 (Kennedy, Fischbach, Song, Eller, & Shulman, 1995). NIOSH maintains a collection of validated methods for the sampling and analysis of workplace air, known as the NIOSH Manual of Analytical Methods or NMAM. For evacuated canisters to be validated as a viable sampling method for a suite of VOCs and incorporated into the NMAM, sampling and analytical protocols must be established and evaluated using NIOSH validation procedures for air sampling and analytical method development. The current NIOSH standard for air sampling and analytical method development is the Kennedy document (Kennedy *et al.*, 1995). However, this document is under internal revision and the current trend for air sampling and analytical method development is to align NIOSH validation protocols with ASTM standards which includes method precision estimates from an interlaboratory study (ASTM, 2012).

As individual test laboratory performance on presumably identical samples do not generally yield identical results, criteria have been established by ASTM International to interpret precision estimates for the purpose of testing analytical methods (ASTM, 2012). Conducting an ASTM-style interlaboratory study to assess method validity requires reference standards to be generated and analyzed by a reference laboratory and that analogous references samples are disseminated to several laboratories for analysis. Results from each test laboratory are then compared to theoretical target concentrations to

establish the accuracy of the analytical method. For inclusion into the NMAM, analytical methods must pass an accuracy criterion described in the Kennedy document, which includes a 25% accuracy at the 95% confidence level.

The goal of this research was to assess the accuracy, bias, and precision of reference evacuated canister sample generation techniques and analysis via GC-MS for a suite of VOCs as part of an ASTM-style interlaboratory study. This research will be incorporated into a larger study designed to develop an evacuated canister sampling and analysis method for the NMAM. The use of evacuated canister sampling methods in conjunction with, or in place of traditional sorbent tube sampling methods can lead to the collection and characterization of a more representative ambient air sample.

Methods

This section describes the preparation techniques, materials, and instrumentation used to generate reference evacuated canister samples.

Chemicals and Materials

Reference canister samples were generated from NIST-traceable (by weight) certified gas standards (Linde Inc., Braddock, PA). Gas standards included the suite of VOCs listed in Table 1. A separate gaseous standard mixture was used as a set of internal standards (Restek Corp., Bellefonte, PA), including the compounds bromochloromethane, chlorobenzene-d5, and 1,4-difluorobenzene. Ultra-high purity (UHP) nitrogen was used as a diluent for standards preparation. UHP helium (Butler Gas Products Company, McKees Rocks, PA) was used as the carrier gas for GC-MS sample analysis.

Silonite® (fused silica, Entech Instruments, Simi Valley, CA) -coated air sampling canisters of 450-cc (Model #29-MC450SQT) and 6-L (Model# 29-10622) capacity were purchased from Entech Instruments. Prior to use in this study, evacuated canisters were cleaned according to the recommendations outlined in EPA's Compendium Method TO-15 (EPA, 1999). Deionized water (18Ω), which was used for humidification of calibration and internal standards, was cleaned by a Millipore Milli-Q system (Billerica, MA).

Sample Preparation

Reference canisters were generated in two different concentration ranges, parts-per-billion (PPB) and parts-per-million (PPM), and at three nominal concentration levels per range. The nominal levels were 5, 10, and 15 ppb and 0.8, 1.3 and 1.7 ppm, respectively. Reference samples of the same concentration range (either PPB or PPM) that were generated at approximately the same time using the

same preparation technique, constituted a spike batch. At least three replicate reference canisters were produced at each nominal concentration level for each spike batch. Six replicate reference canisters were produced per nominal concentration level and spike batch when feasible and as permitted by the sample preparation technique. Table 2 displays sample counts by concentration range and preparation method.

Table 2. Sample Counts by Concentration Range and Preparation Method

Range	Spike Batch	Preparation Method	N
PPB	1	Flow	18
PPB	2	Flow	18
PPB	3	Pressure	9
PPB	4	Pressure	9
PPB	5	Flow	18
PPM	1	Pressure	9
PPM	2	Pressure	9
PPM	3	Pressure	9
PPM	4	Pressure	9
PPM	5	Pressure	9
PPM	6	Pressure	9
PPM	7	Pressure	9
PPM	8	Pressure	18
		Total	153

Reference canister samples were generated by diluting certified gas standard mixtures of the suite of 17 VOCs. The certified gas standard was diluted via flow or pressure combination with UHP nitrogen. The standard gas mixture was diluted to nominal target concentration levels using an Entech 4600 Dynamic Diluter, Entech 4700 Precision Static Diluter, and an Entech Digital Dilution System (DDS) (Figure 1a-1c).

Figure 1a. Entech 4600

Figure 1b. Entech 4700

Figure 1c. Entech DDS

Flow dilution generation of PPB-range canister samples was accomplished using the Entech 4600 Dynamic Diluter (Figure 1a). The Entech 4600 used mass flow controllers (Unit Instruments, Yorba Linda, CA) and a multi-port mixing chamber to dynamically combine source gases into a single stream that was then directed through a short, fused-silica-coated stainless steel line to an evacuated canister. Due to the concentration of the 17 VOCs in the certified gas standard (~2 PPM for each compound) and the limitations of flow ranges of the mass flow controllers in the Entech 4600, PPM-range samples could not be generated via the flow dilution method. For the purpose of this study, the two streams of gas combined by this method were the certified gas standard containing the suite of 17 VOCs and UHP nitrogen.

Pressure dilution of certified gas standard to nominal concentration levels was accomplished using either the Entech 4700 Precision Static Diluter (Figure 1b) or the Entech DDS gauge (Figure 1c). Pressure transducers in both the Entech 4700 (Ametek-PMT Products, Feasterville, PA) and Entech DDS gauge (Automation Products Group, Logan, UT) were used to measure the amount of gas transferred from one fixed volume container to another. Pressure dilution was a step-wise process. First, an evacuated canister was filled to approximately one-half atmosphere (~7 psiA) with UHP nitrogen to raise the pressure in the canister into a more reliable region of the pressure transducers range. Next, certified gas standard was introduced up to the desired pressure needed for nominal concentration target

values. Finally, the canister was diluted to a final pressure of approximately two atmospheres (~30 psiA) with UHP nitrogen. The pressure dilution process is displayed in Figure 2.

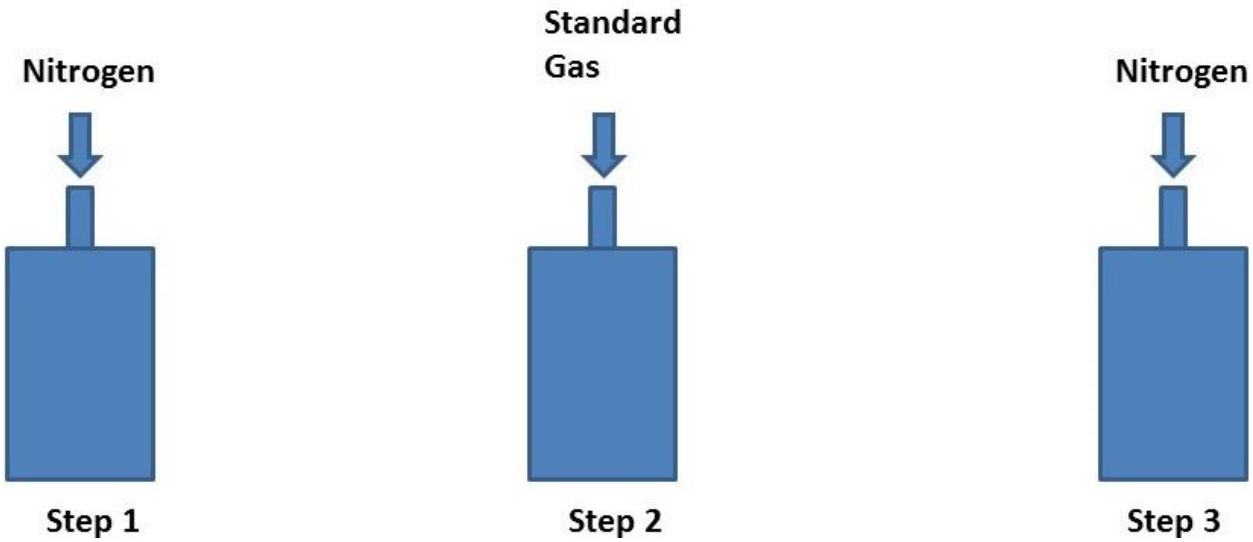

Figure 2. Pressure Dilution Process

Pressure-diluted samples were generated by one of two preparation techniques. One method involved using a nine-port manifold attached to either the Entech 4700 or DDS gauge and up to nine evacuated canisters. The lines of the manifold were stainless steel with a fused-silica coating. Reference canisters produced by this preparation method were generated in parallel, had the same target value, and constituted a spike batch. The other sample preparation technique involved using the Entech 4700 to generate a working standard at a target concentration level in a 6-L evacuated canister and subsequently using the canister-to-canister sample transfer functionality of the Entech 4700 to prepare individual 450 cc reference canister samples directly from the 6-L working standard (see Figure 3). This technique produced reference canisters in series. However, as the canisters were only filled with gas from the working standard 6-L canister, the reference canisters shared a target concentration value and also consituted a spike batch.

Figure 3. Entech 4700 6-L to 450 cc Canister Transfer

Pressure dilution was also used to dilute PPM-range reference samples to the PPB-range for analysis. During this dilution process, a desired amount of pressure from a reference canister sample was transferred via the Entech 4700 to another evacuated canister that was then pressurized with UHP nitrogen. This process was capable of up to 100x dilutions. After generation, reference canister samples were allowed to equilibrate for a period of no less than 24 hours before analysis.

Sample Analysis

This section describes the analytical methods and instrumentation used to analyze reference evacuated canister samples.

Strategy

Reference canister samples were analyzed in two ways. The nominal injection volume method involved sampling 250 cc of volume from a canister of interest, dehumidifying and cryogenically concentrating the sample to a final volume of 1 cc, then injecting the sample into a GC-MS for analysis. This method was used to analyze PPB-range and PPM-range pressure diluted reference canister samples.

The loop injection method involved sampling 1 cc from a reference canister using a 1 cc volume sampling loop, dehumidifying the sample and then directly injecting into the GC-MS without cryogenic concentration. This method was used to analyze non-pressure diluted PPM-range reference canister samples. Most PPM-range samples were analyzed via both the pressure dilution and 1 cc loop injection methods. This influenced the observational totals in the overall reference canister sample data set. The observational totals for the data set are presented in Table 3.

Table 3. Reference Canister Data Set

Concentration Range	Analysis Technique	Sample N	Analyte Observations
PPB	250 cc injection	72	1224
PPM	Diluted 250 cc injection	63	1071
PPM	1 cc Loop injection	81	1377
Total		216	3672

Analytical Methods

Reference canister samples were analyzed using a GC-MS system (7890B/5977A, Agilent Technologies, Santa Clara, CA) (see Figure 4a) coupled to a sample preconcentrator (Entech 7200) and autosampler (Entech 7032AQ) (see Figure 4b).

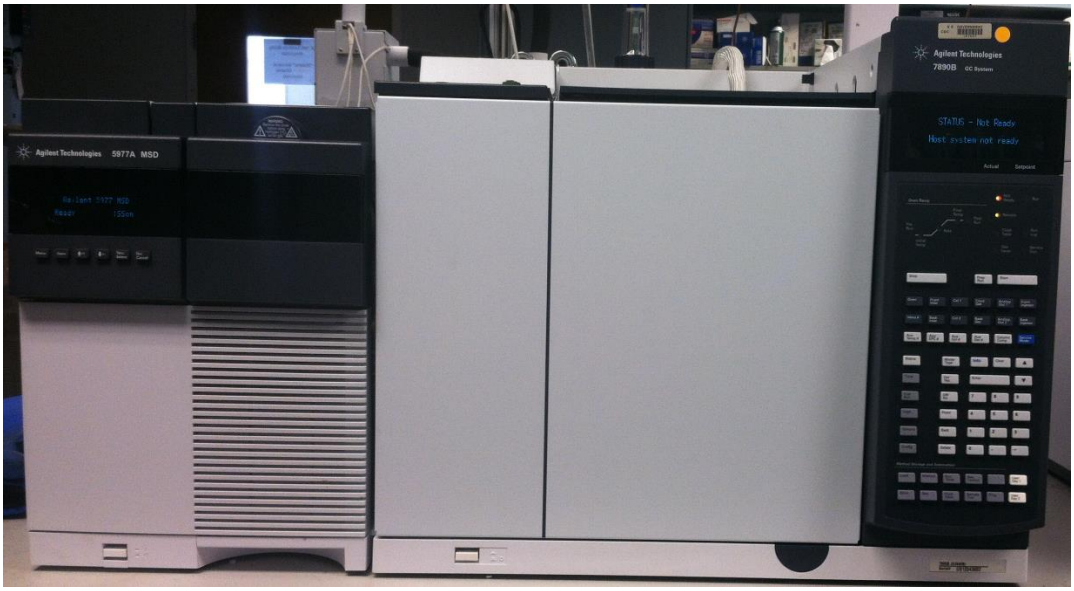

Figure 4a. Agilent GC-MS System

Figure 4b. Entech 7200 (L) and 7032AQ (R)

The Entech 7032AQ autosampler had 21 stainless steel, fused-silica coated sampling lines, each connected to a stainless steel rotary valve. Solenoids electronically directed the rotary valve to create a pathway between a singular reference sample canister and a heated, fused-silica coated, stainless steel sample transfer line coupled to the Entech 7200 preconcentrator. The desired volume of sample from the canister was then transferred to the Entech 7200 for sample dehumidification and concentration. The Entech 7200 used two traps and a cryo-focuser module for water and carbon dioxide management and sample concentration. The inner flow path of the Entech 7200 was also fused-silica coated stainless steel. The first trap in the flow path of the 7200 was empty, to allow water in the sample to freely break through beyond the trap and be more easily separated from the sample. The sorbent in the second 7200 trap was Tenax® TA, a 2,6-diphenylene oxide-based, porous polymer resin. Tenax® TA sorbent is acceptable for the trapping of volatile and semi-volatile compounds from ambient air (Harper, 2000).The VOCs in the reference sample adsorbed onto the sorbent of the trap while water and carbon dioxide were dry purged from the sample by flushing the trap with dry helium while the sorbent compounds retained possession of the VOCs. The VOCs were then thermally desorbed back into the carrier gas stream by ballistically heating the traps to 250 °C over the course of less than one minute. The final stage of sample concentration was to cryogenically cool the sample and carrier gas in the cryo-focuser module to -180 °C with liquid nitrogen. This reduced the sampled volume from 250 cc to 1 cc. The sample was then carried along another heated, fused-silica-coated, stainless steel transfer line and injected into the inlet of a gas chromatograph. A simplified diagram of the sample flow is presented in Figure 5.

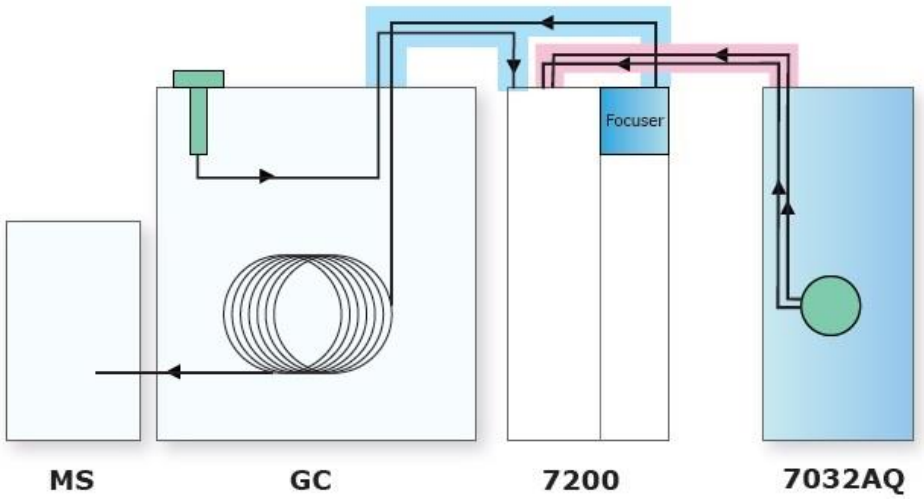

Figure 5. Simplified Sample Analysis Flow Path (Entech Instruments)

The GC column used for analysis was a fused silica Rxi®-1ms (Restek Corp) of 60 m length, 0.32 mm internal diameter, and a 1.0 µm film thickness. This nonpolar phase, crossbond dimethyl polysiloxane column was designed specifically for analysis of C6-C12 "gasoline range organics" (GRO). Many of the compounds in the suite of VOCs for this study fall into the C6-C12 GRO classification. The dimethyl polysiloxane film of the column provided varying interaction with the vastly different structures of the compounds in the suite of VOCS evaluated, allowing for effective chromatographic separation. Such chromatographic separation is a function of the film of the GC column interacting with individual chemical species during the sample's journey along the 60 meter length of the column.

The resultant time that the sample is retained on the column before reaching the detector is referred to as that compound's "retention time". While chromatographic separation was possible for most species in the suite of VOCs of interest, the geometric isomers meta- and para-xylene were so similar in shape and interaction with the column that affecting retention time separation proved to be impossible using the chromatographic parameters selected. Thus, meta- and para-xylene were treated as co-eluting compounds and reported together as is typically done by most contract laboratories. The temperature ramp program of the GC oven is depicted numerically in Table 4 and visually in Figure 6. The method conditions of the GC-MS instruments are listen in Table 5.

Table 4. GC Oven Ramp Program

	Rate (C/min)	Value (C)	Hold Time (min)	Run Time (min)
Initial		35	5	5
Ramp 1	6	95	0	15
Ramp 2	10	140	0	19.5
Ramp 3	15	220	5.17	30.003

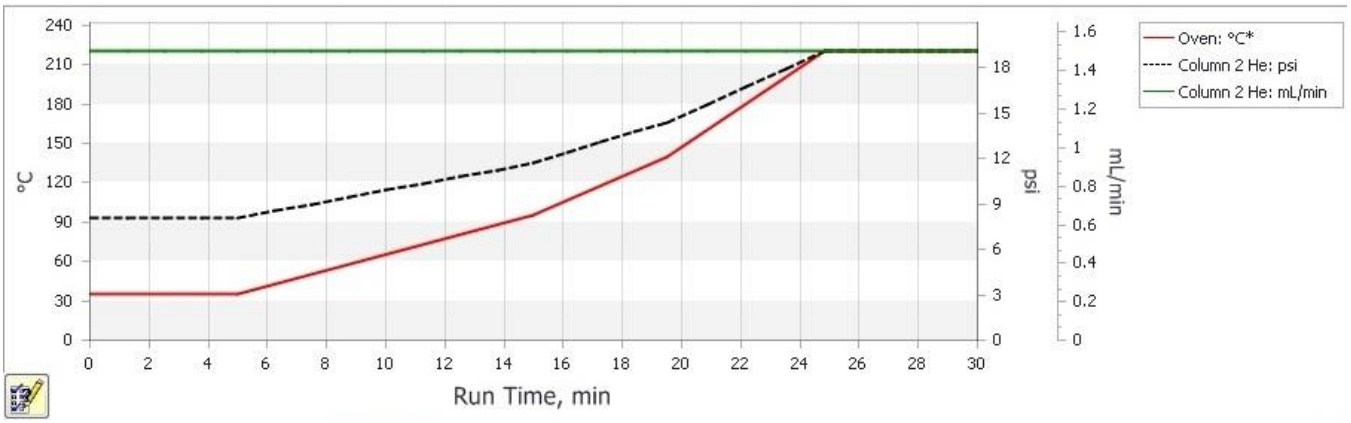

Figure 6. GC Oven Ramp Program

Table 5. GC-MS Conditions

Parameter	Setting
Column Flow	1.5 mL/min Helium
MS Transfer Line Temperature	250 °C
MS Quadrupole Temperature	150 °C
MS Source Temperature	300 °C
MS Mass Scan Range	35-350 AMU

The samples were analyzed using the linear quadrupole mass spectrometer. The mass spectrometer identified the presence of specific compounds by reacting column elution compounds (eluates) with electrons to generate ions. Ions are generated when electrons accelerated through a potential of 70 electron volts (eV) in the ion source impact molecules arriving in the MS from column eluates. The resultant ions are fragments of the original compounds. Individual compounds have characteristic fragmentation patterns when passed through a constant 70 eV ion source. For an individual compound, some fragmentation ions are generated in relatively larger numbers than others. These characteristic ion fragmentation patterns and relative ion abundances allow for identification of individual compounds found in the column eluate. After fragmentation, the resultant ions are then moved by an electromagnetic field through a parallel set of four rod-shaped electrodes arranged in a roughly square relation to one another (the quadrupole).

The electromagnetic field is generated by applying direct current and radio frequency potentials across oppositely located electrodes. The detector then records ions successfully transported through the quadrupole. For this study, the linear quadrupole mass spectrometer was operated in total ion current (TIC) and in combined TIC and selected-ion monitoring (SIM) modes. Under total ion current mode, the direct current potential is set to zero and a wide range (35-350 m/z) of mass to charge (m/z) ratios were allowed through the quadrupole. In this mode of operation, the mass spectrum was generated by scanning radiofrequency (RF) and direct current (DC) voltages using a set DC/RF ratio at a constant frequency. A simplified figure of a linear quadrupole mass spectrometer is presented in Figure 8.

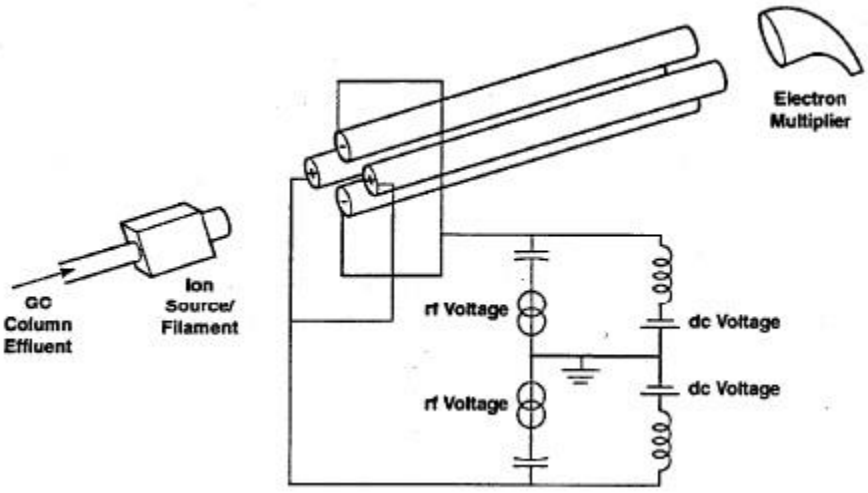

Figure 8. Linear Quadrupole (EPA, 1999)

As previously mentioned, characteristic ion fragmentation patterns and relative abundances allowed for compound identification and quantitation. For an individual compound, the fragmentation ion of largest relative abundance, and thus signal, was typically used as a quantifier ion. The quantifier ion is the ion used by the chromatography analytical software to integrate a chromatographic peak for comparison to a calibration curve and ultimately, reporting of analyte concentration. Exceptions were made for quantifier ion choice when the ion of largest relative abundance was not unique to the analyte of interest, with respect to the suite of VOCs used for this study. In such cases, the largest unique fragmentation ion was chosen as the quantifier ion. The presence of the quantifier ion alone is not enough for positive identification of a compound. Secondary, tertiary, and even quaternary fragmentation ions can be required for positive compound identification. These fragmentation ions are typically not ions of highest relative abundance, as said ions are generally used for quantitative purposes. The ions used for

compound identification are referred to as qualifier ions and should appear in relative abundance to one another as indicated by an individual compound's unique fragmentation pattern. MS parameters such as quantifier ions, qualifier ions, and retention times can be found in Table 6.

Table 6. MS TIC Compounds

Analyte	Ret. Time (min)	Quantifier Ion	Qualifier Ion(s)
Ethanol	4.969	45	46, 43
Acetone	5.420	43	58
Isopropyl Alcohol	5.671	45	43
Methylene Chloride	6.337	84	49, 86, 51
n-Hexane	8.821	57	41, 43, 56
Chloroform	8.887	83	85, 47, 87
Benzene	10.690	78	77, 52, 51
Methyl Methacrylate	12.414	69	41, 100, 39
Toluene	14.698	91	92
Ethylbenzene	17.879	91	106
m,p-Xylene	18.144	91	106, 105, 77
o-Xylene	18.783	91	106, 105, 77
α-Pinene	20.166	93	91, 92, 77
d-Limonene	22.011	68	93, 67, 79

Under the SIM mode, the quadrupole did not scan a large range of m/z ratios and instead permitted the transportation and detection of only two to three m/z ratios. The period of time during the sample run dedicated to SIM was referred to as a SIM "window". These retention time windows allowed for greater sensitivity in detecting specific ions of interest as, instead of continuously scanning a large mass range, the mass spectrometer focuses on particular m/z ratios of interest, thus increasing the

likelihood of detecting and recording the presence of said species. Three SIM windows were used in this method to enhance sensitivity for ions related to the presence of 2,3-butanedione, 2,3-pentanedione, and 2,3-hexanedione, as these compounds were of particular interest to concurrent research projects. The SIM settings of the MS are listed in Table 7.

Table 7. MS SIM Windows

Analyte	Ret. Time(min)	m/z
2,3-Butanedione	3.77	29, 43, 86
2,3-Pentanedione	8.90	43, 57, 100
2,3-Hexanedione	12.00	43, 71, 114

Calibration

PPB-range calibration standards were made in humidified 6-L canisters from a certified gas standard. Calibration standards were generated to a target concentration of approximately 20 ppb for each analyte at the nominal injection volume of 250 cc. Calibration curves were produced by analyzing seven different injection volumes of the calibration standard, from 25 cc (~2 ppb) to 250 cc. PPM-range instrument calibration was accomplished by producing and analyzing seven 450 cc canisters of various target concentrations across the range of 0.2 ppm to 2.0 ppm. Linear regressions of relative response ratios of internal standard to analyte, weighted by the inverse of concentration, was used to address possible non-linearity of the calibrated dynamic range, or heteroscedasticity in the data. Linearity of the calibration curves for each analyte was tested by plotting the relative response of the internal standard to the concentration of the analyte.

Data Structure

Due to our inability to produce PPM-range reference canister samples via flow dilution of a gas standard, the number of spike-batches for PPB-range and PPM-range samples were uneven. For each range there were three nominal concentration levels (5, 10, 15 ppb and 0.8, 1.3, 1.7 ppm) with either three or six replicates per level depending on the logistics of the batch preparation. PPM-range and PPB-range spike batches and sample counts by preparation method are displayed in Tables 8 and 9, respectively.

Table 8. PPM-range Spike Batches and Sample Counts by Preparation Method

Preparation Method	Spike Batch N	Sample N
Pressure	2	18
Manifold	6	63
Total N	8	81

Table 9. PPB-range Spike Batches and Sample Counts by Preparation Method

Preparation Method	Spike Batch N	Sample N
Flow	3	54
Pressure	2	18
Total N	5	72

As previously mentioned, PPB-range reference canisters were analyzed via a 250 cc injection. All but 18 of the 81 PPM-range reference canisters were analyzed in two ways: 1 cc loop injection, and dilution of the sample into the PPB range and a resultant 250 cc injection. These 18 PPM-range canisters were analyzed with only the 1 cc loop injection analytical method. These 18 canisters were generated late in the two-year timeline of the study via the manifold preparatory technique and it was felt that sufficient data was present to support the choice of only analyzing said canisters in one way. The observational totals for the data set are presented in Table 3.

Data Analysis

Chemstation software version F.01.00.1903 (Agilent Technologies) was used to analyze the GC-MS data. Quantification of analytes was accomplished by integrating the area under the "peak" curve to generate a "response". The response was then divided by the response of the relevant internal standard to generate a relative response. This relative response was then plotted against the calibration curve for the individual analyte of interest to produce a concentration. Concentration data was analyzed using JMP (SAS Institute, Cary, NC) for statistical analysis.

Statistical Analysis Methods

This section describes the statistical methods used to analyze reference canister concentration data.

Bias

The bias of individual observations was assessed by taking the ratio of measured to target concentration minus 1 (see Equation 1).

$$\%\ bias = \left(\frac{measured}{theoretical} - 1\right) * 100\% \qquad \text{Equation 1}$$

Biases were averaged across spike batch, nominal concentration level, and analyte with respect to grouping variables preparatory technique and analytical method for the purpose of testing homogeneity of bias distributions.

Outliers

Outliers were identified and removed by calculating Mahalanobis distances. The Mahalanobis distance of a point observation is equivalent to the number of standard deviations the point observation is away from the mean of the distribution. A Mahalanobis distance of three standard deviations away from the mean of the biases was chosen as the threshold for outlier identification and removal. In a normal distribution, 99.7% of values will lie within three standard deviations of the mean. According to Chebyshev's inequality, for any distribution, the probability that any value will lie more than X standard deviations away from the mean of the distribution is equal to $1/X^2$. Thus it can be assumed, that at the very least, 89% of observations lie within three standard deviations of the mean. It happened that for this data set, only 29 of 3,672 observations whose bias were found to be greater than three standard deviations from the mean of the distribution. This resulted in an outlier exclusion percentage of only 0.8% of the data set.

Homogeneity of Bias and Variance

Before the accuracy of sample generation and analysis of reference canisters could be calculated, determinations needed to be made concerning the homogeneity of bias and variance for distributions across groupings. This was done to determine whether the means or maximums of bias and variance, respectively, could be used in the accuracy and confidence limit calculations. Homogeneity of bias was assessed across groupings of spike batch and nominal concentration level grouped by analyte, sample preparatory technique (i.e., pressure, manifold, or flow), and analysis technique (i.e., PPM dilution, PPM

loop, and PPB). This was accomplished by applying a one-way analysis of variance (ANOVA) statistical test to the means of percent bias across the groupings. Bartlett's test (Bartlett & Kendall, 1946) was applied to the means of the standard deviation of bias across groupings to test for homogeneity of variance among the distributions.

Accuracy at the 95% Confidence Level

For a method to be considered to have acceptable performance by NIOSH, the method must pass a 25% accuracy criterion. For the method to pass, the 95% upper confidence limit on accuracy of the method must be less than 25% error. The 95% confidence interval represents the interval of accuracy in which an observation generated by this method is expected to fall with a statistical significance level of 0.05. Accuracy was calculated according to the strategy presented in the Components for Evaluation of Direct-Reading Monitors for Gases and Vapors NIOSH Technical Report (NIOSH, 2012). Accuracy was calculated as:

$$\hat{A} = A\left(\hat{B}, \hat{S}_{rT}\right) = \begin{cases} 1.96 \times \sqrt{\hat{B}^2 + \hat{S}_{rT}^2} & \text{if } \left|\hat{B}\right| < \dfrac{\hat{S}_{rT}}{1.645}, \\ \left|\hat{B}\right| + 1.645 \times \hat{S}_{rT} & \text{otherwise} \end{cases}$$

Equation 2

where $\hat{B}$ is an estimate of bias based on N data points and $\hat{S}_{rT}$ is an estimate of precision with M degrees of freedom for the evaulation of n samples from each of k concentrations,

$N = n \times k, M = k(n-1)$ (NIOSH, 2012). The confidence limit was calculated as:

$$\hat{A}_p = \begin{cases} 1.96 \times \lambda \times \sqrt{\hat{B}^2 + \hat{S}_{rT}^2} & \text{if } \left|\hat{B}\right| < \dfrac{\hat{S}_{rT}}{1.645}, \\ \left|\hat{B}\right| + 1.645(\tau)\hat{S}_{rT} & \text{otherwise} \end{cases}$$

Equation 3

where:

$$\lambda = \sqrt{M / \chi^2_{1-p,M}},$$
$$\tau = t_{p,M}(\Delta)/\Delta,$$
$$\Delta = 1.645 \times \sqrt{N}$$

and $\chi^2_{1-p,M}$ is the $(1-p)$ x 100 percentile of a chi-square distribution with M degrees of freedom, and $t_{p,M}(\Delta)$ is the p x 100 percentile of a noncentral t-distribution with M degrees of freedom and a

noncentrality parameter Δ (NIOSH, 2012). This is the accuracy and confidence interval calculation required by the NIOSH method accuracy criterion.

Results/Discussion

This section contains results and discussion of the statistical analyses used to evaluate the reference canister data.

Bias and Variance

Assessment of homogeneity of bias across spike batches, regardless of nominal concentration level resulted in 122 significances out of 306 observational groupings. This resulted in approximately 40% of accuracy calculations being forced to use the maximum mean of bias across all spike batches instead of the mean of mean bias across spike batches for the accuracy and 95% confidence interval calculations. The P-value results of the ANOVA analyses are displayed in Tables 10-12 by analysis method, preparation method, nominal concentration level, and analyte.

Table 10. Part-Per-Billion Range P-value Results of ANOVA of Percent Bias across Spike Batches by Preparation Method, Nominal Level, and Analyte

Analyte	Flow 5 PPB	Flow 10 PPB	Flow 15 PPB	Pressure 5 PPB	Pressure 10 PPB	Pressure 15 PPB
Ethanol	0.0339	0.0848	0.0701	0.1882	0.1293	0.5946
Isopropyl Alcohol	0.0001	0.4694	0.0495	0.2759	0.1099	0.3838
Acetone	<0.0001	0.0065	<0.0001	0.1744	0.4601	0.3851
2,3-Butanedione	0.9259	0.0549	0.0001	0.0756	0.3477	0.4903
2,3-Pentanedione	0.8389	0.4321	<0.0001	<0.0001	0.3214	0.4613
2,3-Hexanedione	0.0137	<0.0001	<0.0001	0.0017	0.2994	0.4125
Methylene Chloride	<0.0001	<0.0001	0.0008	0.7417	0.5246	0.6236
Chloroform	<0.0001	<0.0001	<0.0001	0.9876	0.6401	0.7039
n-Hexane	0.0002	<0.0001	0.0012	0.9082	0.3548	0.8036
Methyl Methacrylate	0.0002	0.1204	<0.0001	0.3136	0.4875	0.5294
Benzene	0.0001	<0.0001	0.0001	0.7667	0.5907	0.6866
Toluene	<0.0001	<0.0001	<0.0001	0.5191	0.4646	0.6610
Ethylbenzene	<0.0001	<0.0001	<0.0001	0.0710	0.5721	0.8561
m,*p*-Xylene	<0.0001	<0.0001	<0.0001	0.0287	0.4370	0.5856
o-Xylene	<0.0001	<0.0001	<0.0001	0.0217	0.5916	0.5866
alpha-Pinene	<0.0001	0.0002	<0.0001	0.0742	0.8072	0.9814
d-Limonene	<0.0001	<0.0001	<0.0001	0.0546	0.7446	0.4465

Table 11. Diluted Part-Per-Million Range P-value Results of ANOVA of Percent Bias across Spike Batches by Preparation Method, Nominal Level, and Analyte

Analyte	Pressure 0.8 PPM	Pressure 1.3 PPM	Pressure 1.7 PPM	Manifold 0.8 PPM	Manifold 1.3 PPM	Manifold 1.7 PPM
Ethanol	0.4024	0.3426	0.4366	0.6575	0.0586	0.3546
Isopropyl Alcohol	0.8232	0.9911	0.3992	0.2000	0.4157	0.0558
Acetone	0.4395	0.5528	0.7037	0.2723	0.0537	0.0041
2,3-Butanedione	0.4454	0.4403	0.7192	0.2422	0.0957	0.0056
2,3-Pentanedione	0.9124	0.6778	0.7137	0.1386	0.4445	0.0672
2,3-Hexanedione	0.7386	0.7445	0.6505	0.0001	0.1497	0.1112
Methylene Chloride	0.4241	0.3392	0.8191	0.2486	0.0362	0.0002
Chloroform	0.4915	0.2145	0.8255	0.0388	<0.0001	<0.0001
n-Hexane	0.9128	0.3811	0.7924	0.1767	0.0002	<0.0001
Methyl Methacrylate	0.6795	0.5149	0.9876	0.2436	0.2739	0.0131
Benzene	0.5008	0.4428	0.8660	0.5491	0.0027	<0.0001
Toluene	0.4018	0.1092	0.8363	0.5692	0.0351	0.0005
Ethylbenzene	0.5744	0.2113	0.8812	0.2938	0.0484	0.0015
m,p-Xylene	0.7161	0.2753	0.7942	0.2371	0.0903	0.0105
o-Xylene	0.5242	0.1387	0.6673	0.2375	0.0513	0.0030
alpha-Pinene	0.4093	0.0683	0.8927	0.4362	0.0111	0.0030
d-Limonene	0.1686	0.2830	0.6263	0.0566	0.3152	0.0703

Table 12. Loop Part-Per-Million Range P-value Results of ANOVA of Percent Bias across Spike Batches by Preparation Method, Nominal Level, and Analyte

Analyte	Pressure 0.8 PPM	Pressure 1.3 PPM	Pressure 1.7 PPM	Manifold 0.8 PPM	Manifold 1.3 PPM	Manifold 1.7 PPM
Ethanol	0.1370	0.8168	0.9596	0.0011	0.0301	0.0034
Isopropyl Alcohol	0.6974	0.1393	0.5510	0.0039	<0.0001	<0.0001
Acetone	0.8105	0.7454	0.5854	0.0008	<0.0001	<0.0001
2,3-Butanedione	0.6488	0.5287	0.1311	0.0027	<0.0001	<0.0001
2,3-Pentanedione	0.6911	0.4010	0.3978	0.0181	<0.0001	<0.0001
2,3-Hexanedione	0.5388	0.6050	0.0112	0.0234	<0.0001	<0.0001
Methylene Chloride	0.5134	0.8101	0.2971	0.0011	<0.0001	0.0752
Chloroform	0.5408	0.4331	0.1324	0.0022	<0.0001	<0.0001
n-Hexane	0.6217	0.4337	0.2109	0.0007	<0.0001	0.0044
Methyl Methacrylate	0.5778	0.2384	0.8839	0.0069	<0.0001	<0.0001
Benzene	0.7265	0.0602	0.0401	0.0031	<0.0001	0.0005
Toluene	0.9995	0.5803	0.1803	0.0056	<0.0001	<0.0001
Ethylbenzene	0.8861	0.5845	0.5730	0.0052	<0.0001	<0.0001
m,p-Xylene	0.7524	0.4562	0.3756	0.0121	<0.0001	<0.0001
o-Xylene	0.8145	0.6566	0.0445	0.0036	<0.0001	<0.0001
alpha-Pinene	0.4188	0.5526	0.0276	0.0037	<0.0001	<0.0001
d-Limonene	0.7375	0.0517	0.4256	0.0001	<0.0001	0.0002

For the 102 possible combinations of analytes and preparation and analytical methods, 29% of the means of bias and 35% of the means of variance were found to be significantly different from the mean across nominal level meaning a concentration dependence may exist. This resulted in the maximum means of bias and variance being used to calculate accuracy across the groupings in 29% and 35% of the respective accuracy determinations. The P-value results of the ANOVA and Bartlett's Test analyses are displayed in Tables 13-15.

Table 13. Part-Per-Billion Range P-value Results of ANOVA and Bartlett's Test on Percent Bias across all Nominal Concentration Levels by Preparation Method and Analyte

Analyte	ANOVA Flow	ANOVA Pressure	Bartlett's Test Flow	Bartlett's Test Pressure
Ethanol	0.2540	<0.0001	0.0146	0.4681
Isopropyl Alcohol	0.3455	0.7214	0.0007	0.2230
Acetone	0.0003	0.2953	0.0002	0.0005
2,3-Butanedione	0.1088	0.1263	<0.0001	0.3615
2,3-Pentanedione	0.2084	0.2410	0.0070	0.5797
2,3-Hexanedione	0.5320	0.1223	0.7468	0.3292
Methylene Chloride	0.2702	0.5033	<0.0001	0.3710
Chloroform	0.3777	0.6512	0.0241	0.8284
n-Hexane	0.9100	0.0004	0.0432	0.3805
Methyl Methacrylate	0.2103	0.0117	0.2195	0.0237
Benzene	0.3042	0.6692	0.3566	0.6343
Toluene	0.8157	0.3959	0.7108	0.5233
Ethylbenzene	0.8228	0.0838	0.1606	0.8545
m,*p*-Xylene	0.8678	0.5613	0.1570	0.6414
o-Xylene	0.8448	0.2998	0.3054	0.0945
alpha-Pinene	0.6666	0.1196	0.1088	0.0168
d-Limonene	0.0532	0.0073	0.9436	0.4360

Table 14. Diluted Part-Per-Million Range P-value Results of ANOVA and Bartlett's Test on Percent Bias across all Nominal Concentration Levels by Preparation Method and Analyte

Analyte	ANOVA Pressure	ANOVA Manifold	Bartlett's Test Pressure	Bartlett's Test Manifold
Ethanol	0.6625	<0.0001	0.4998	0.8913
Isopropyl Alcohol	0.3303	0.0053	0.9274	0.4192
Acetone	0.9371	0.0847	0.4558	0.0590
2,3-Butanedione	0.6700	0.0510	0.0001	0.0747
2,3-Pentanedione	0.3441	0.0036	0.4204	0.0040
2,3-Hexanedione	0.8566	0.0066	0.0131	0.6582
Methylene Chloride	0.5893	0.9875	0.0033	0.7709
Chloroform	0.8083	0.6779	0.0031	0.8352
n-Hexane	0.0230	0.9568	0.0352	0.9145
Methyl Methacrylate	0.5642	0.0040	0.1840	0.0303
Benzene	0.6921	0.8978	0.0011	0.8286
Toluene	0.6827	0.9127	0.0540	0.6033
Ethylbenzene	0.7539	0.9379	0.0141	0.4518
m,p-Xylene	0.5474	0.8198	0.0042	0.3025
o-Xylene	0.3577	0.6045	0.0001	0.3662
alpha-Pinene	0.0261	0.4669	0.3098	0.5536
d-Limonene	0.7431	0.6454	0.2187	0.5752

Table 15. Loop Part-Per-Million Range P-value Results of ANOVA and Bartlett's Test on Percent Bias across all Nominal Concentration Levels by Preparation Method and Analyte

Analyte	ANOVA Pressure	ANOVA Manifold	Bartlett's Test Pressure	Bartlett's Test Manifold
Ethanol	0.5973	0.5104	0.1804	0.1394
Isopropyl Alcohol	0.5692	0.0050	0.4112	0.5503
Acetone	0.6074	0.0059	0.2998	0.6234
2,3-Butanedione	0.5191	0.0037	0.0172	0.5948
2,3-Pentanedione	0.0081	0.0006	0.0016	0.1206
2,3-Hexanedione	0.0000	0.0015	0.0055	0.0440
Methylene Chloride	0.1783	0.0738	0.0004	0.0047
Chloroform	0.3051	0.0105	0.0150	0.8881
n-Hexane	0.1416	0.0283	0.0029	0.0248
Methyl Methacrylate	0.0003	0.0010	0.0182	0.6139
Benzene	0.8025	0.0782	0.1470	0.1736
Toluene	0.4383	0.0083	0.0007	0.7504
Ethylbenzene	0.3174	0.0076	0.1658	0.7471
m,p-Xylene	0.9654	0.0119	0.7333	0.5531
o-Xylene	0.8905	0.0152	0.0626	0.3889
alpha-Pinene	0.6298	0.0088	0.0107	0.3165
d-Limonene	0.0176	0.0002	0.0059	0.0280

Frequencies of significance with respect to the homogeneity of bias and variance across nominal level for preparation and analysis methods are listed in Table 16.

Table 16. Frequencies of Significance across Preparation and Analytical Methods

Analytical Method	Preparation Method	N Groupings	N Significant Bias	N Significant Variance
PPB	Flow	17	1	8
PPB	Pressure	17	4	3
PPM Dilution	Pressure	17	2	9
PPM Dilution	Manifold	17	5	2
PPM Loop	Pressure	17	4	10
PPM Loop	Manifold	17	14	4
Total N		102	30	36

Generally, a bias less than 10% and a relative standard deviation (RSD - indicator of precision) less than 5% results in a passing observation with respect to the NIOSH 25% accuracy criterion (Kennedy *et al.*, 1995). Figure 9 from the Bartley document (Bartley, 2001) illustrates the relationship between bias and precision with respect to the NIOSH accuracy calculation.

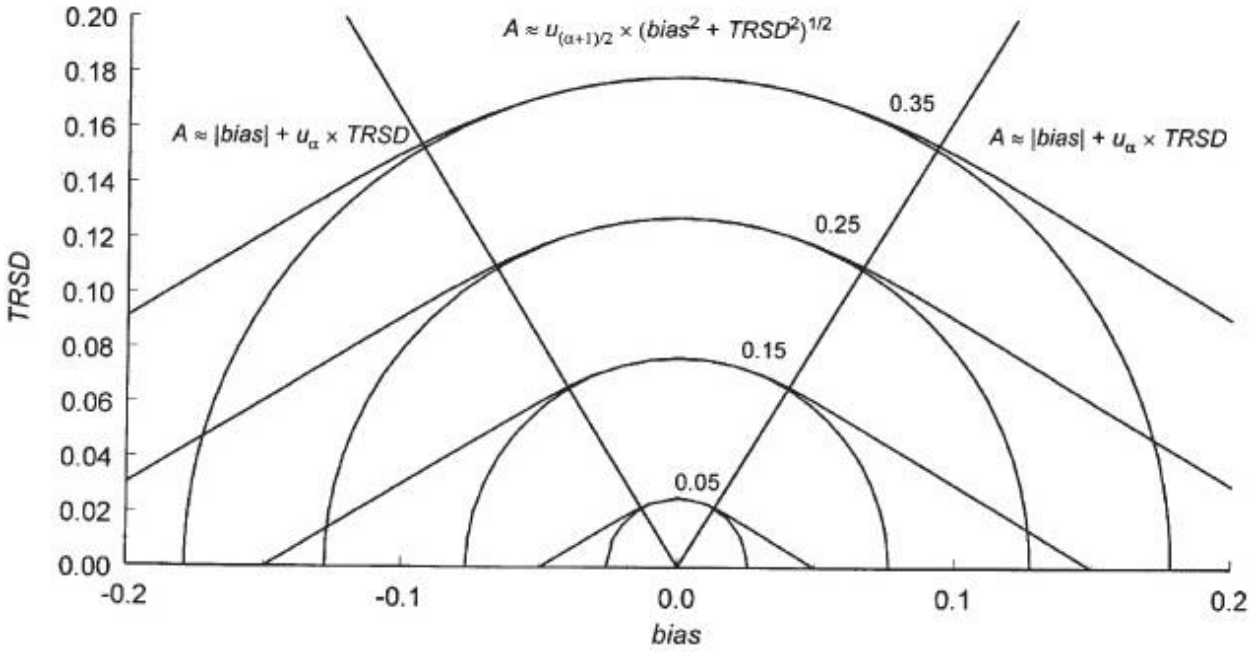

Figure 9. Curves of constant normalized root mean square error $u_{(\alpha+1)/2} \times \mathrm{MSE}^{1/2}$ (circles) in comparison to accuracy *A* (quasi-hyperbolas) at $\alpha = 0.95$. The values $u_{(\alpha+1)/2} = 1.960$ and $u_\alpha = 1.645$ are unit normal quantiles (Bartley, 2001)

It is still possible to pass the NIOSH 25% accuracy criterion in situations of high RSD and low bias, as well as situations of high bias and low RSD as depicted in Figure 9. Mean percent bias and precisions are presented by preparation technique, analytical method, and analyte in Figures 10-12. There are few results depicted in Figures 10-12 that would predict a passing set of conditions for the NIOSH accuracy calculation. General trends of bias and precision by preparation method seem to be related to analyte structure, molecular weight, and volatility. The 17 compounds in the suite of VOCs for this study are grouped by similar structure and molecular weight (MW) in Table 17.

Table 17. Compound Molecular Weights Grouped by Similar Structure

Compound	Molecular Weight (g/mol)
Ethanol	46.07
Isopropyl Alcohol	60.10
Acetone	58.08
2,3-Butanedione	86.09
2,3-Pentanedione	100.12
2,3-Hexanedione	114.14
Methylene Chloride	84.93
Chloroform	119.38
n-Hexane	86.18
Methyl Methacrylate	100.12
Benzene	78.11
Toluene	92.14
Ethylbenzene	106.17
m,p,o-Xylene	106.16
alpha-Pinene	136.24
d-Limonene	136.24

Figure 10 portrays PPB reference canister bias and precision by analyte and preparation method. For the PPB-range, there were three spike batches containing a total of 54 canisters prepared via the flow method. In contrast, there were only two spike batches of 18 total canisters prepared via the pressure method. This inequality in sample number makes comparing the two preparation methods difficult. Overall, a general trend was noticeable in which the pressure preparation method seemed to outperform the flow method for mid-to-heavy MW compounds with percent biases fluctuating around

zero and relative standard deviations (RSD) generally less than 10%. The flow dilution method fared worse with biases between 5-10% and similar percent RSDs for the same group of analytes. For lighter MW compounds, such as ethanol, isopropyl alcohol, and acetone, there is some disagreement concerning which preparation method leads to lower bias and tighter precision. Acetone and ethanol were the worst performing compounds in the PPB range. The lightest of the three compounds, ethanol, performed better in flow diluted preparation with -2.9% bias and 11.6% RSD, versus 35% bias, but a comparable 9.6% RSD for the pressure preparation method. The opposite was true for acetone. Acetone showed a 26.5% bias and 11.5% RSD for the flow method, while the pressure generated samples displayed 3.5% bias and 9.7% RSD. In terms of bias, isopropyl alcohol performed equivalently in both preparation methods, while the precision of the pressure method was better at a 6.2% RSD, versus an 11.1% RSD for the flow method. For the PPB-range, the pressure generation method appears to yield samples with overall lower bias and tighter precision than the flow generation method.

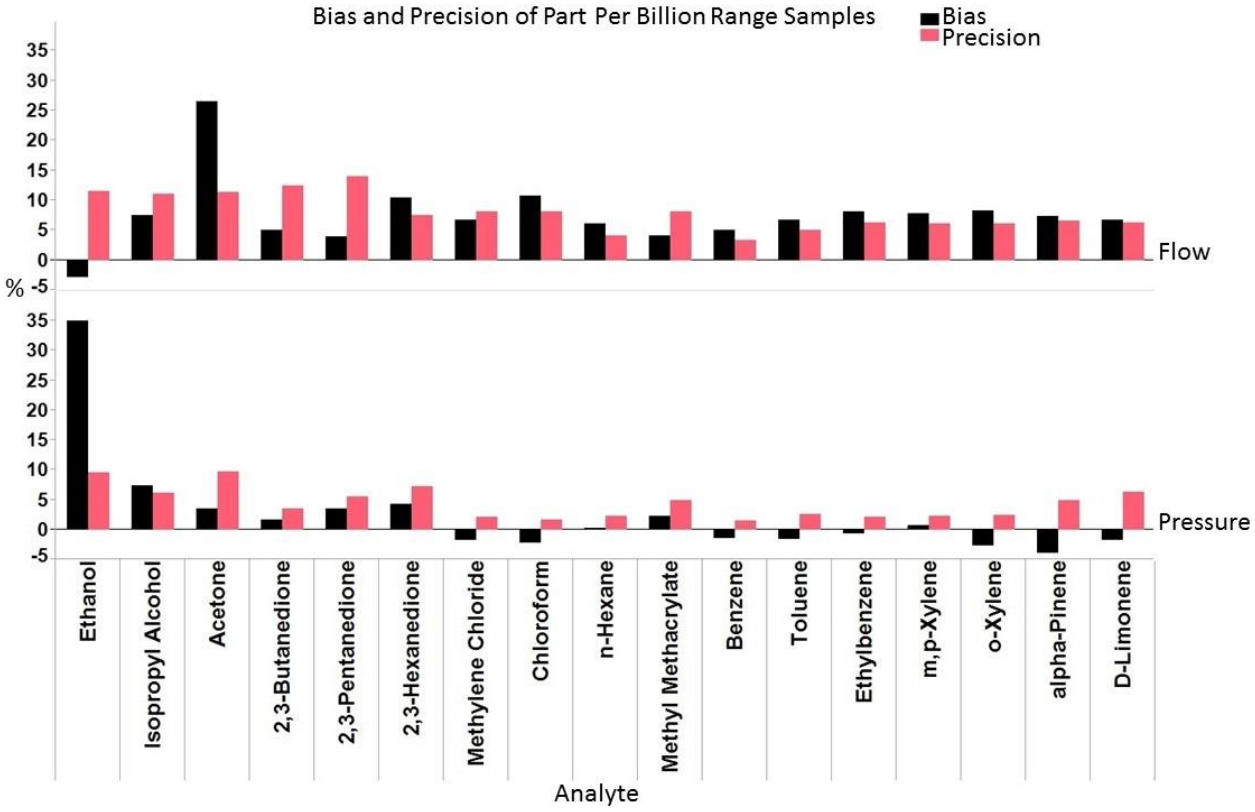

Figure 10. PPB Sample Bias and Precision by Preparation Method: Flow (top) and Pressure (bottom)

Figure 11 displays diluted PPM-range sample bias and precision for individual analytes by preparation method. The number of observations was not equal for the two preparation methods. There were five spike batches of 45 total reference canisters produced by the manifold preparation method.

Only two spike batches of 18 total reference canisters were produce by the pressure method. Neither method appears to have had a significant advantage over the other in overall measures of bias and precision. Grouping compounds by similar structure and MW allows for more clear comparisons of the preparation methods. Of the lighter MW compounds, ethanol seemed to perform better in the manifold method with a bias of -12% and RSD of 11%, compared to 22.7% and 8.5% in the pressure method. Isopropyl alcohol and acetone, performed similarly in both methods of sample preparation. The α-diketones (2,3-butanedione, 2,3-pentanedione, and 2,3-hexanedione) seemed to have consistently smaller percent bias and tighter precision when generated by the manifold preparation method. In general, for bias and precision, other mid-to-heavy MW analytes seemed to perform better when reference canisters were generated by the pressure method. *d*-Limonene, however, performed poorly in diluted PPM-range reference canisters generated by the manifold method, displaying a -26% bias.

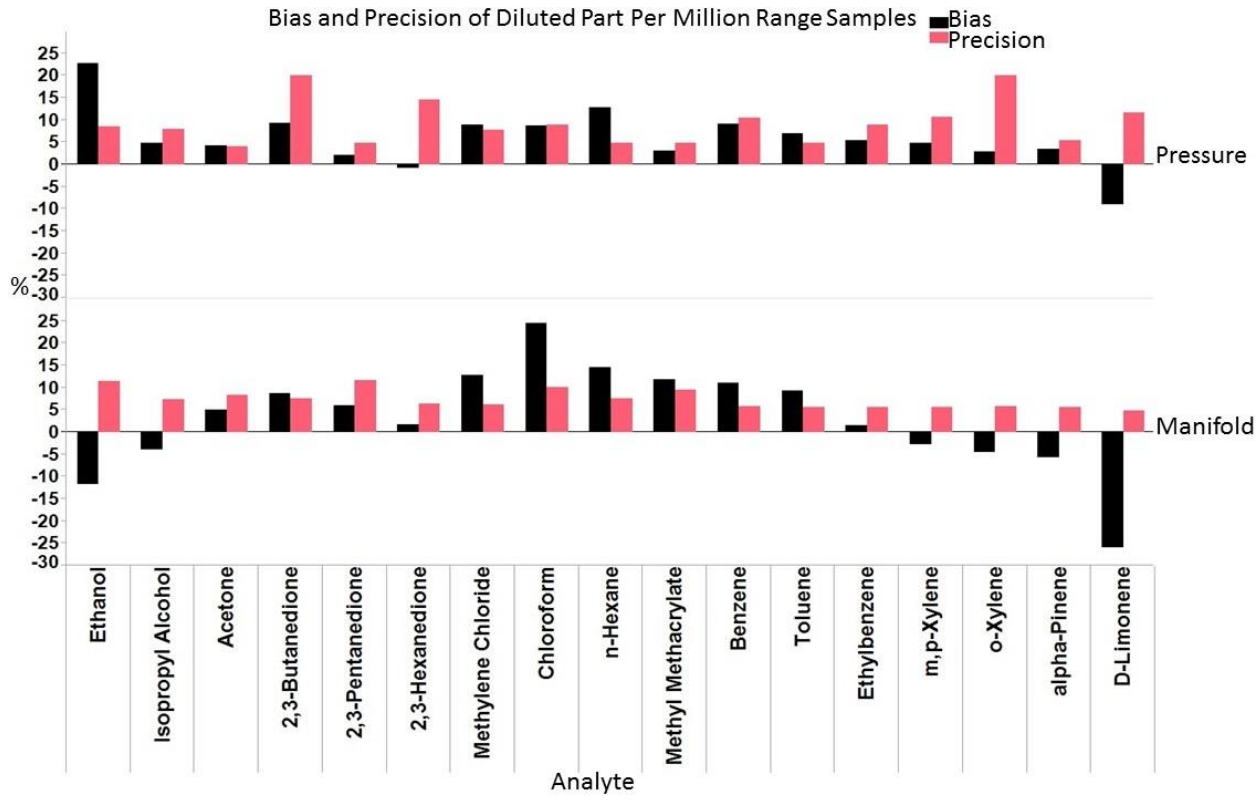

Figure 11. Diluted PPM Sample Bias and Precision by Preparation Method: Pressure (top) and Manifold (bottom)

Bias and precision results for PPM-range, loop analyzed samples are displayed in Figure 12. There were six spike batches of 63 total PPM-range samples generated by the manifold method and

analyzed via the loop method. There were two spike batches of 18 total samples generated by the pressure method and analyzed via the loop method. The overall bias and precision measurements appear to be clearly favorable for the pressure generation method. However, for the manifold method, some analytes (2,3-pentanedione, 2,3-hexanedione) at the 0.8 ppm nominal level displayed considerable (>30%) bias for individual spike batches, greatly affecting the overall bias, variance, and ultimately NIOSH accuracy and 95% CI calculation. Additionally, for the two preparation methods, the effects of uneven spike batch and sample numbers were not explored. That said, for all analytes in the PPM-range, the pressure generation method and loop analytical method seems to yield samples with smaller biases and RSDs.

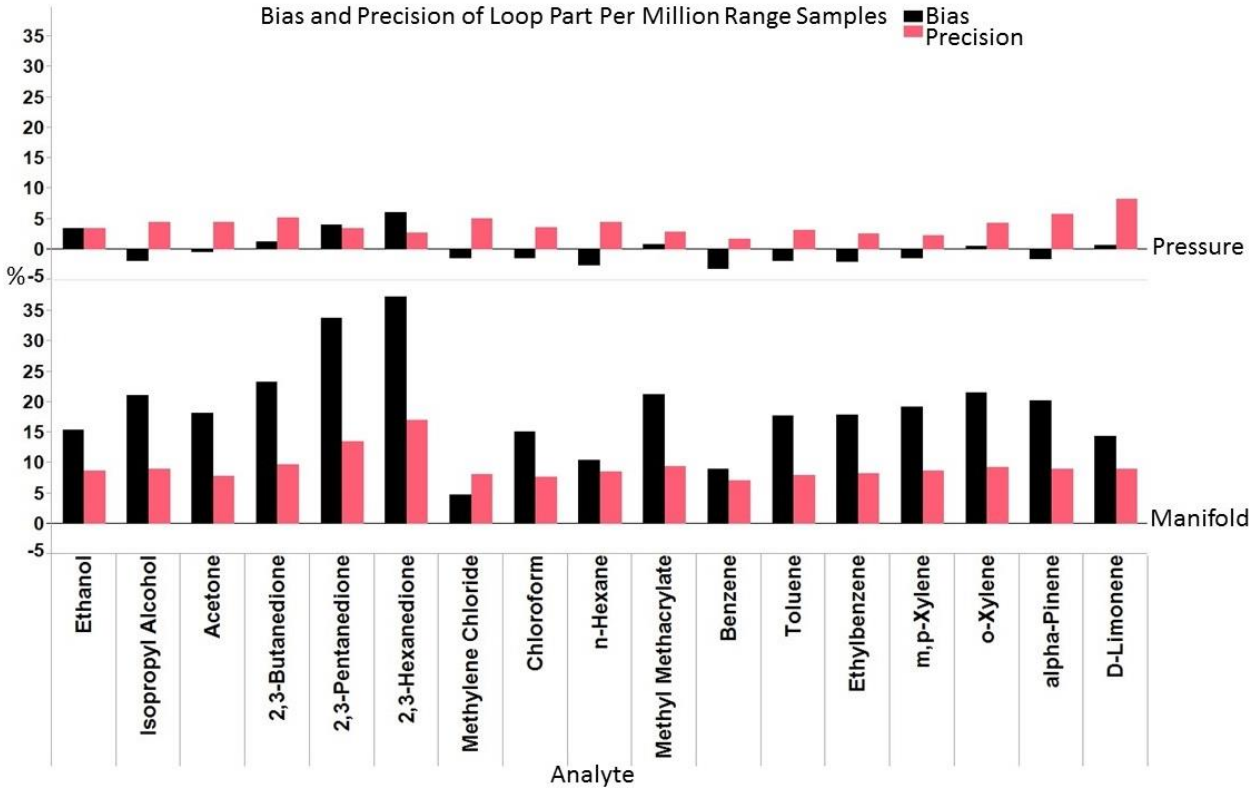

Figure 12. PPM Loop Sample Bias and Precision by preparation method: Pressure (top) and Manifold (bottom)

Accuracy at the 95% Confidence Limit

The results of the NIOSH accuracy and 95% CI calculations are displayed in Figures 13-15 and Tables 18-20, by preparation technique, analytical method, and analyte. Once again, for a method to pass the NIOSH accuracy assessment, the 95% upper confidence limit on accuracy of the method must

be less than 25% error. For the flow generation method, the NIOSH accuracy and 95% CI calculations yielded passing results for many mid-to-heavy MW compounds such as methylene chloride, benzene, and *d*-limonene across all PPB-range nominal concentration levels and spike batches (see Figure 13 and Table 18). For the flow generated samples, lighter MW compounds such as ethanol and isopropyl alcohol displayed inconclusive results, while acetone outright failed the accuracy assessment (see Figure 13 and Table 18). For the flow method, ethanol had a lower confidence limit (LCL) of 20.2% error and an upper confidence limit (UCL) of 27.8% error, yielding a confidence interval that straddles the 25% NIOSH accuracy criterion and thus, an inconclusive result. The result was also inconclusive for isopropyl alcohol for the flow method, with an LCL of 22.4% and UCL of 30.2% error. For the flow method, Acetone failed the assessment with an LCL of 41.8% error, far above the 25% error cutoff. For the PPB-range, nearly all compounds passed the NIOSH accuracy assessment expectations when spike batches were generated via the pressure dilution technique. The exceptions were ethanol and acetone. For the pressure generated samples, Ethanol failed the NIOSH accuracy assessment with an LCL value of 46% error and a UCL value of 59% error. For the pressure method, acetone displayed an inconclusive result with the LCL of 16% error and UCL of 28% error, once again straddling the 25% error NIOSH accuracy criterion.

This data suggests that the pressure dilution method is more accurate than the flow method across the PPB-range.

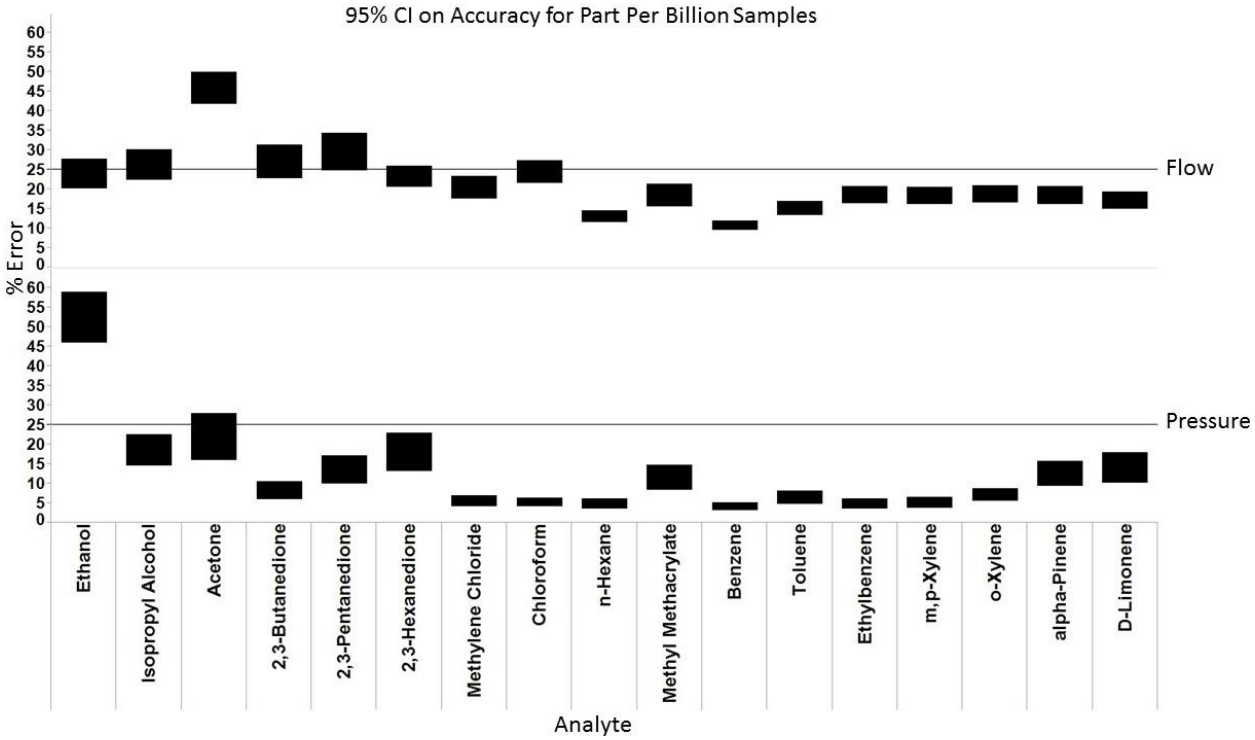

Figure 13. 95% Confidence Interval on Accuracy for PPB-range Samples by Preparation Method: Flow (top) and Pressure (bottom)

Table 18. Upper and Lower Confidence Limits on Accuracy (% Error) for PPB Samples by Preparation Method and Analyte

Analyte	Flow LCL	Flow UCL	Pressure LCL	Pressure UCL
Ethanol	20.2	27.8	46	59
Isopropyl Alcohol	22.4	30.2	14.5	22.6
Acetone	41.8	50.1	16	28
2,3-Butanedione	22.8	31.4	6.1	10.6
2,3-Pentanedione	24.8	34.3	9.9	17.1
2,3-Hexanedione	20.6	26.1	13.1	23
Methylene Chloride	17.7	23.4	4.2	7
Chloroform	21.7	27.4	4.1	6.4
n-Hexane	11.7	14.5	3.6	6.3
Methyl Methacrylate	15.6	21.4	8.4	14.8
Benzene	9.6	12	3.2	5.3
Toluene	13.4	17	4.7	8.3
Ethylbenzene	16.4	20.8	3.6	6.2
m,*p*-Xylene	16.2	20.6	3.8	6.7
o-Xylene	16.6	21	5.5	8.8
Alpha-Pinene	16.2	20.8	9.5	15.8
d-Limonene	15.1	19.5	10.3	18

For the PPM-range dilution analyzed samples, the results of the NIOSH accuracy and 95% CI calculations are displayed in Table 19 and Figure 14 by preparation method and analyte. When analyzed by dilution, there was no NIOSH accuracy trend discernible for the pressure generation method. For samples generated via the pressure method, 2,3-butanedione and *o*-xylene performed poorly with LCLs of 34% and 31.3% and UCLs of 60.6% and 55% error, respectively. In the case of manifold generated samples, aromatic compounds, such as ethylbenzene (LCL 9.9%, UCL 14.1% error) and *m,p*-xylene (LCL 10.4%, UCL 14.8% error) seemed to fare better in the NIOSH accuracy assessment than did alcohols and chlorinated compounds, such as ethanol (LCL 26.6%, UCL 35.6% error) and chloroform (LCL 37.8%, UCL 45.6% error). There were exceptions to this trend, however, as acetone met the accuracy criteria for results from both the pressure (LCL 9%, UCL 14.9% error) and manifold (LCL 16%, UCL 22.5% error) preparation techniques when analyzed via dilution. *d*-Limonene displayed an inconclusive result when generated by the pressure method (LCL 22.4%, UCL 37.5% error), and failed the assessment when generated via the manifold procedure (LCL 32.2%, UCL 36% error). Some analytes, like acetone, behaved similarly under the two preparation techniques and dilution analysis. For example, toluene and alpha-pinene passed the NIOSH accuracy assessment criteria for both of the preparatory techniques for the PPM-range when analyzed via dilution. Some compounds, such as methylene chloride and *n*-hexane yielded inconclusive results for both preparation methods. Analytes such as isopropyl alcohol, 2,3-hexanedione, benzene, ethylbenzene, and *m,p*-xylene also yielded inconclusive results when prepared via the pressure method and passed when prepared via the manifold method. The opposite was true for the compounds 2,3-pentanedione and methyl methacrylate. With such a range of determinations, it is hard to discern between the pressure and manifold PPM-range reference canister preparation techniques when diluted for analysis. However, visually, Figure 14 seems to indicate that the manifold generation technique is superior over the entire suite of VOCs.

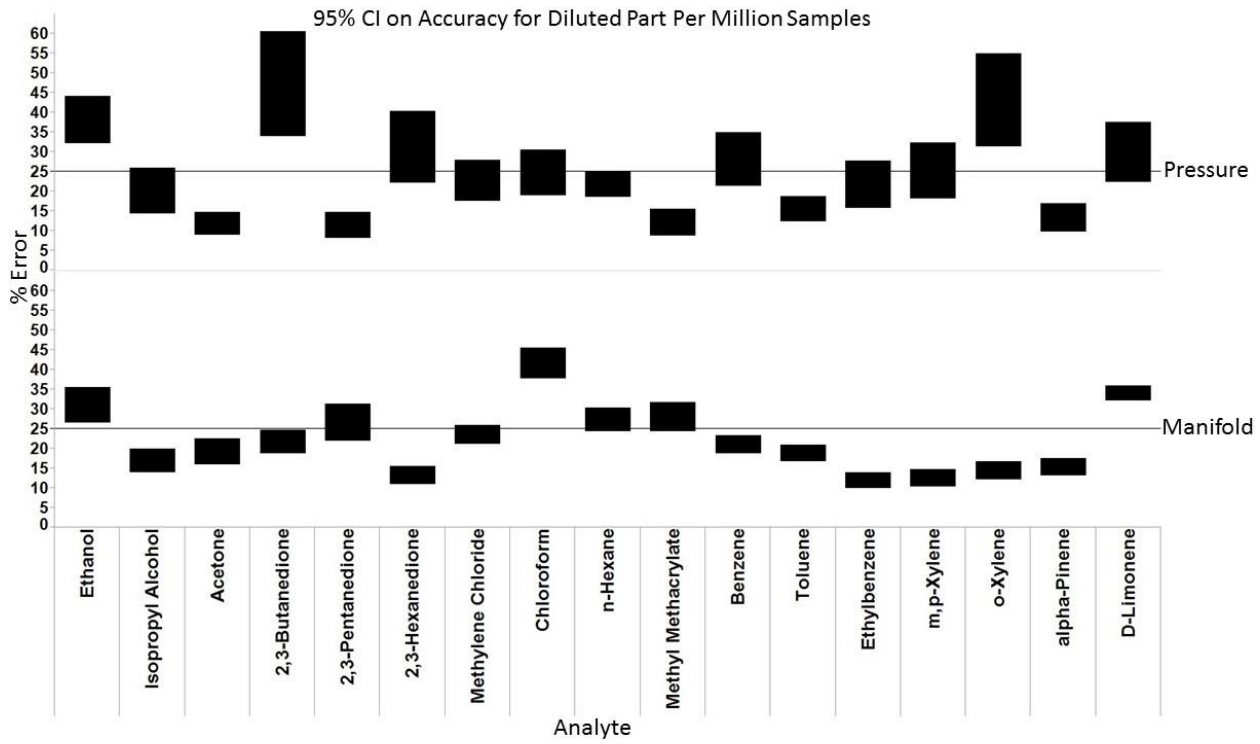

Figure 14. 95% Confidence Interval on Accuracy for Diluted PPM Samples by Preparation Method: Pressure (top) and Manifold (bottom)

Table 19. Upper and Lower Confidence Limits on Accuracy (% Error) for Diluted PPM Samples by Preparation Method and Analyte

Analyte	Pressure LCL	Pressure UCL	Manifold LCL	Manifold UCL
Ethanol	32.3	44.1	26.6	35.6
Isopropyl Alcohol	14.3	26	14.1	20.1
Acetone	9	14.9	16	22.5
2,3-Butanedione	34	60.6	18.9	24.8
2,3-Pentanedione	8.1	14.7	22.1	31.3
2,3-Hexanedione	22.2	40.4	11	15.7
Methylene Chloride	17.7	28.1	21.1	26
Chloroform	19	30.6	37.8	45.6
n-Hexane	18.5	24.9	24.5	30.4
Methyl Methacrylate	8.7	15.6	24.4	31.8
Benzene	21.3	35	18.8	23.3
Toluene	12.4	18.9	16.7	21.1
Ethylbenzene	15.7	27.7	9.9	14.1
m,*p*-Xylene	18.2	32.4	10.4	14.8
o-Xylene	31.3	55	12.2	16.7
alpha-Pinene	9.8	17	13.1	17.5
d-Limonene	22.4	37.5	32.2	36

The results of the NIOSH accuracy and 95% CI calculations for PPM-loop analyzed samples are depicted in Figure 14 and tabulated in Table 20. For these samples, the choice of superior preparation technique becomes clear. The pressure generated samples passed the NIOSH accuracy criteria for all analytes in the suite of VOCs with LCLs ranging from 4.3% (*m,p*-xylene) to 12.9% error (*d*-limonene) and UCLs ranging from 7.3% (benzene) to 22.6% error (*d*-limonene). Nearly the opposite is true for manifold generated samples. The NIOSH accuracy and 95% CI calculations yielded a failing result (LCL>25% error) for 14 of the 17 analytes, with two passing (methylene chloride and benzene) and one (*n*-hexane) displaying an inconclusive (CI encompassing 25% error) result.

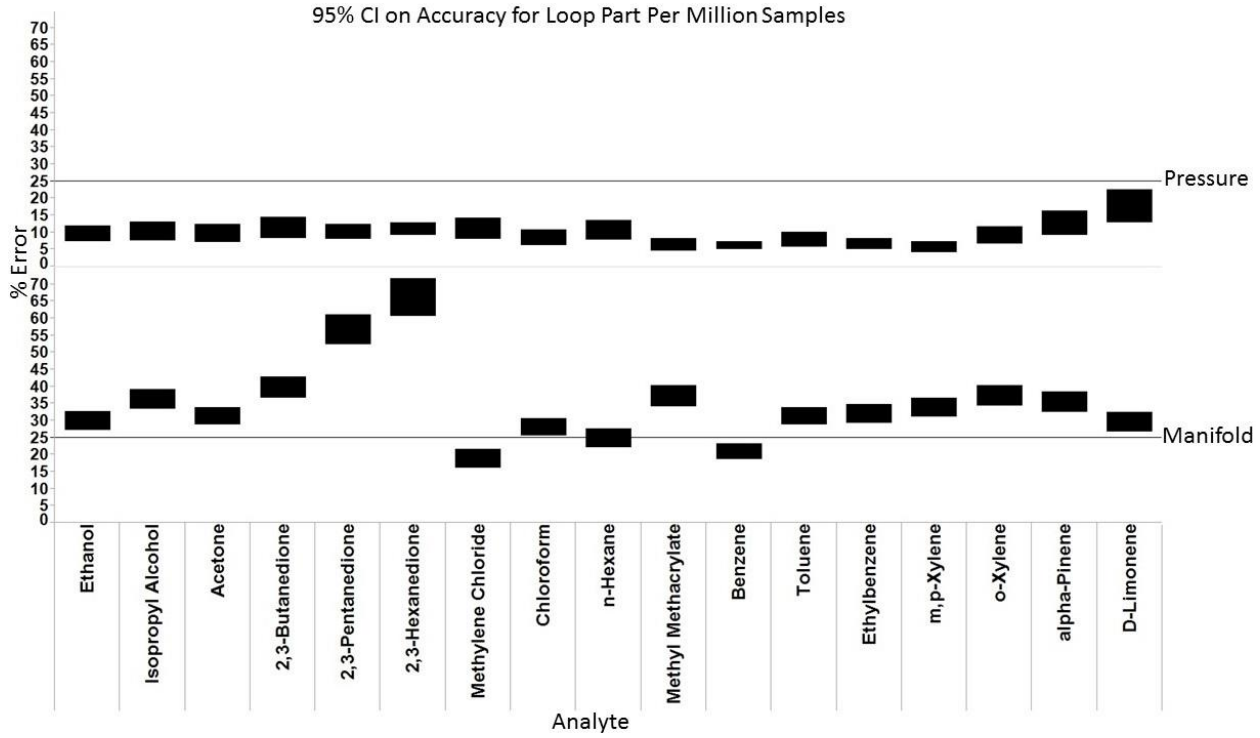

Figure 15. 95% Confidence Interval on Accuracy for Loop PPM Samples by Preparation Method: Pressure (top) and Manifold (bottom)

Table 20. Upper and Lower Confidence Limits in Percent Error for Loop PPM Samples by Preparation Method and Analyte

Analyte	Pressure LCL	Pressure UCL	Manifold LCL	Manifold UCL
Ethanol	7.5	12	27.2	32.9
Isopropyl Alcohol	7.5	13.2	33.4	39.4
Acetone	7.1	12.4	28.9	34
2,3-Butanedione	8.2	14.4	36.6	43
2,3-Pentanedione	8	12.5	52.3	61.1
2,3-Hexanedione	9.25	12.8	60.6	71.7
Methylene Chloride	8.1	14.2	16.2	21.7
Chloroform	6.1	10.7	25.7	30.7
n-Hexane	7.8	13.6	22.1	27.7
Methyl Methacrylate	4.7	8.2	34.2	40.3
Benzene	5.1	7.3	18.8	23.4
Toluene	5.7	10	28.7	34
Ethylbenzene	5	8.4	29.3	34.7
m,*p*-Xylene	4.3	7.3	31.1	36.7
o-Xylene	6.7	11.8	34.3	40.4
Alpha-Pinene	9.3	16.4	32.7	38.6
d-Limonene	12.9	22.6	26.8	32.6

The results of the NIOSH accuracy and 95% CI calculations could have been predicted with a relatively high rate of success based on the results of the bias and precision assessments with respect to the 10% bias and 5% RSD criteria outlined in the Kennedy document (Kennedy, Fischbach *et al*., 1995). While observations of high bias and low RSD or high RSD and low bias, as described by Bartley (Bartley, 2001), are theoretically capable of passing the NIOSH accuracy criterion, none such occurrences were observed to pass the assessment in this study. Accuracy results could undoubtedly be improved for individual analytes at individual concentration levels, but the aim of the larger evacuated canister NMAM method development study would be compromised. The overall goal is to create a robust sampling and analytical protocol for a wide variety of VOCs across large ranges of concentration while still maintaining analyte selectivity, resolution, and method accuracy.

Conclusions

The overall finding of this study is that passing the NIOSH method accuracy criterion was achieved for generating and analyzing reference evacuated canister samples for a suite of 17 VOCs in the PPM concentration range and 15 VOCs in the PPB range across multiple spike batches and three nominal concentration levels per range.

Specific findings include:

(1) The high rate of occurrence of significant differences in means of bias and variance across the 102 possible combinations of analytes and preparation and analytical methods strongly suggests a concentration dependence exists.

(2) The results strongly indicate that the pressure transfer method of generating reference canister samples is the preferred technique for PPM-range samples.

(3) The 1 cc loop injection analysis technique seems to be the better method for analyzing PPM-range samples.

Caveats

- The effects of unequal sample numbers with respect to preparatory methods were not explored and warrant further investigation.

- Over the two year timeline of this study, sample preparation and analytical methods were changed according to what was thought to be the best method(s) at the time of reference sample production and analysis.
- A canister tracking protocol would have been useful for avoiding errors in sample preparation and analysis.

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