Distribution Statement

Distribution A: Public Release.

The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

INVESTIGATION OF HOW TEMPERATURE AND HOLDING TIME AFFECT THE CONCENTRATION OF PERCHLOROETHYLENE IN WATER QUALITY SAMPLES

by

MAJ Roberto Sanchez Perez

Thesis submitted to the Faculty of the
Preventive Medicine and Biostatistics Graduate Program
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Masters of Science in Public Health 2020

APPROVAL FOR THE MASTER OF SCIENCE IN PUBLIC HEALTH THESIS IN THE DEPARTMENT OF PREVENTIVE MEDICINE AND BIOSTATISTICS

Title of Thesis: "INVESTIGATION OF HOW TEMPERATURE AND HOLDING TIME AFFECT THE CONCENTRATION OF PERCHLOROETHYLENE IN WATER QUALITY SAMPLES"

Name of Candidate: MAJ Roberto Sanchez Perez
Master of Science in Public Health
June 5, 2020

THESIS AND ABSTRACT APPROVED:

DATE:

19 June, 2020

MAJ Jon Sharp
DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS
Committee Chair

19 June 2020

LTC William Whitaker
DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS
Thesis Advisor

19 June 2020

Dr. Alex H. Stubner
DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS
Committee Member

ACKNOWLEDGMENTS

I want to thank the support provided by the Biostatistics Consulting Center: Dr. Cara Olsen and Ms. Sorana Raiciulescu. Your time, guidance, and counseling towards the development and completion of this work has been invaluable.

I would also like to thank all the members of my committee for their time and assistance in helping complete this program.

To my wife Brenda and my daughter Brenly, my utmost appreciation and thanks. All the hours spent next to me while I was at the computer allowed me to continue this journey, no matter how difficult the road, both motivated me to continue to move forward during times where I could not.

DEDICATION

I want to thank God for granting me the opportunity to be able to see another location in the United States (Maryland and DC area) while serving this great nation. Also, for all the strength provided during this journey towards the completion of this MSPH program.

To my beloved family: without you, I would have not been able to achieve this. We have always been supportive of each other, and I truly thank God for having him in every step of my life.

COPYRIGHT STATEMENT

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

Investigation of how Temperature and Holding Time Affect the Concentration of Perchloroethylene in Water Quality Samples

is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.

Roberto Sanchez Perez
MSPH, PMB
Uniformed Services University
06/18/2020

DISCLAIMER

The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

ABSTRACT

Title of Thesis: Investigation of how Temperature and Holding Time Affect the Concentration of Perchloroethylene in Water Quality Samples

MAJ Roberto Sanchez Perez, Master of Science in Public Health, 2020

Thesis directed by: LTC William Whitaker, Assistant Professor, Department of Preventive Medicine and Biostatistics, Occupational and Environmental Health Sciences Division

Water samples collected to determine the presence and concentration of volatile organic compounds (VOCs) are required to meet specific preservation criteria while being transported to an analytical laboratory for analysis. EPA Method 524.2 establishes that water samples collected for VOC analysis be maintained at a holding temperature of less than 4ºC for a maximum of 14 days (33). If such criteria are not met, then the samples become invalid. The purpose of this study was to determine differences in the concentration of a selected VOC, PERC, in water samples when the storage temperature and holding times outlined in EPA Method 524.2 (less than 4ºC and not more than 14 days) are modified. The EPA Method 524.2 allows for VOCs with low water solubility to be extracted from the water sample using the purge and trap (P&T) technique as the absorption of the analyte. Once removed from the water matrix via purging, the sample is analyzed using a gas chromatograph (GC) column interfaced to a mass spectrometer

(MS) (33). The current approach utilized a Solid Phase Microextraction (SPME) fiber as the absorbent process instead of the P&T and the use of a portable GC/MS such as the HAPSITE® ER. The specific type of SPME fiber and length of exposure for adsorption was pre-determined and validated to be 30 seconds as part of the pilot study's parameters identification process. A total of 160 samples were split into four groups based on storage temperature: 4ºC, 5ºC, 20ºC, and 25ºC. The holding temperatures were recorded every 45 minutes using digital thermologgers. The results of this study suggest that time and temperature both affect the concentration of PERC; however, such conclusions cannot be inferred from the study due to problems and errors encountered during sample preparation and analysis throughout the research project. The results of this study support the EPA's conjecture that time and temperature affect the stability of PERC in water samples collected for laboratory analysis. However, definitive statements or conclusions concerning the magnitude of the effect of time and temperature as evidenced by this study are difficult to reach. The challenge of working specifically with PERC in water samples was not anticipated and the researcher was unable to resolve each challenge. The data resulting from the experiment is suspect and therefore only very general conclusions were reached. Furthermore, all statistical analyses conducted used the suspect data, therefore interpretation and statements about the statistical analysis are equally suspect, but were conducted as an academic exercise.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Introduction

BACKGROUND

The United States Environmental Protection Agency (EPA) classifies a volatile organic compound (VOC) as "an organic chemical compound whose composition makes it possible for it to evaporate under normal indoor atmospheric conditions of temperature and pressure" (19). Several compounds that contain carbon are excluded from the definition such as carbon monoxide, carbon dioxide, carbonic acid, metallic carbides, and ammonium carbonate (17). The use of VOCs continues to be prominent in a variety of commercial and industrial applications. Industries such as dry cleaning, refrigeration, solvent manufacture, pest control, and plastics manufacturing heavily rely on the use of VOCs within their processes. Products containing VOCs are also used extensively in households (e.g., cosmetics, deodorants, paint, adhesives, cleaning and polishing products, degreasers, and fuels) (8). Many of the sources of contamination from VOCs are from spills or inappropriate disposal where the VOCs have been allowed to soak into the ground, percolate and contaminate groundwater sources. As part of the National Primary Drinking Water Regulations promulgated by the EPA, 53 VOCs are governed in our drinking water.

The present study selected one of these VOCs, perchloroethylene, to investigate the effect that storage temperature and holding time have on the concentration of perchloroethylene in water samples. The study aimed to analyze conditions within and beyond the determined parameters stipulated by the EPA. EPA Method 524.2 states that water samples collected for analysis need to be maintained at a holding temperature of

less than 4ºC for a maximum of 14 days (33). If such criteria are not met, then the samples are invalid.

Perchloroethylene (PERC) is a nonflammable liquid that has a sweet odor. PERC, also known as tetrachloroethylene or tetrachloroethene (13), is used as a cleaning solvent in dry cleaning and textile processing and is a chemical intermediate in the metal degreasing industry. PERC is prevalent in many consumer products such as auto brake quieters and cleaners, wood cleaners, adhesives, and spot removers.

When PERC is exposed to high temperatures, such as the ones in the welding process, PERC can be oxidized into phosgene, a compound that is highly toxic to humans, both acutely and chronically (35). Like PERC, phosgene is a colorless gas, but it differs regarding its odor, as it resembles freshly cut hay (9). Immediate effects of phosgene exposure include, but are not limited to, nausea, vomiting, blurred vision and difficulty breathing; a longer exposure to phosgene can result in the development of respiratory failure (9)

Physical and Chemical Properties

The fate and transport of PERC in the environment are partly due to its physical properties, as described in Table 1.1. When PERC is volatilized, it is released into the air and eventually degrades into other compounds, such as trichloroethylene, a gas with known serious health effects (15). If PERC is released into the water, due to its solubility, the majority will volatilize. However, if PERC is found in an enclosed environment, such as in a groundwater system, the volatilization is limited, thus allowing PERC to dissolve and be transported in water over distances. As PERC has low solubility, the remaining amount that is not solubilized will settle at the bottom of the water source (21). PERC is

classified as a dense non-aqueous phase liquid (DNAPL), which means that its density is higher than that of water. This property allows PERC to migrate from the subsurface of water and soil, and to persist in the environment (16).

Table 1.1 Physical and chemical properties of PERC (16).

Property	**Value**
Molecular formula	C_2Cl_4
Molecular weight	165.83 g/mol
Boiling point	121.3°C
Density	1.6227 g/cm3 at 20°C
Vapor pressure	18.5 mmHg at 25°C
Water solubility	206 mg/L at 25°C

Henry's law states that at a constant temperature, the quantity of a gas absorbed by water is proportional to its partial pressure in the gas phase. The proportionality factor is called Henry's law constant (HLC), and it represents the tendency of a volatile compound to volatilize from liquid to gas (44) (10). The vapor pressure of PERC is 18.5 mmHg at 25°C and its HLC is 0.0177 atm-m^3/mole at 25°C. Due to its combined physical characteristics, PERC tends to volatilize when exposed to air. When PERC is in an enclosed groundwater environment, a portion of it will dissolve, while the remaining amount will settle at the bottom. Thus, attempting to quantify the portion that will undergo each of the processes is challenging, especially during sample collection, storage, and analysis (30).

MILITARY SIGNIFICANCE

A public health incident involving PERC contamination, that lasted for over three decades occurred at Marine Corps Base Camp Lejeune in North Carolina. In 1982, the installation discovered that two of the eight water treatment plants on the base were distributing water contaminated with various volatile organic compounds, including PERC. Results from the investigation revealed that the groundwater wells serving the Tarrawa Water Treatment Plant (WTP) were contaminated with PERC from an off-base dry-cleaning facility (31). The Hadnot Point WTP also had wells contaminated with PERC during the same timeframe due to on-base spills and leaks from underground storage tanks (3). An assessment of all water wells in 1980 performed by the Agency of Toxic Substance and Disease Registry (ATSDR) identified that the wells were contaminated with PERC. By 1984, all wells identified with PERC contamination began to be monitored, and they no longer supplied water. All uncontaminated wells were also routinely monitored (31). In a health assessment conducted in 2017, the ATSDR indicated that 60% of the health risk at one of the treatment plants, Tarrawa Terrace, was due to PERC (3). It is important to note that PERC is not easily degraded in water, especially in a groundwater source due to its density and low solubility as presented in Table 1.1.

An analysis of water samples from Army bases located around the world is carried out by the United States Army Public Health Center (USAPHC). USAPHC laboratories adhere to the same method for temperature and storage time recommended by the U.S. Environmental Protection Agency (EPA) to meet accurate analytical results (45). EPA Method 524.2 describes that upon collecting a sample for VOC analysis, the

sample must be stored and maintained at a temperature of less than 4ºC. Additionally, the sample must be analyzed within 14 days, otherwise it becomes invalid (18). During military operations, particularly in remote areas where there is no nearby laboratory, shipping delays along with extreme temperatures can result in the loss of VOCs from the water sample due to evaporation, thus under-reporting chemical concentrations (22). While the USAPHC laboratory staff will still analyze the water samples even if the time and temperature limits were not met (46), the conditions under which samples are received must be noted in the report. Thus, as depicted in Figure 1.1, the sample receipt temperature varied throughout the year 2011, where the water samples were sent from the Middle East. During the winter months, most of the samples maintained a temperature of approximately 5 -10°C higher than those outlined in the regulation. During the summer months, however, the temperatures of the water sample kits received by USAPHC were the highest and exceeded the required temperature by up to 25°C (46).

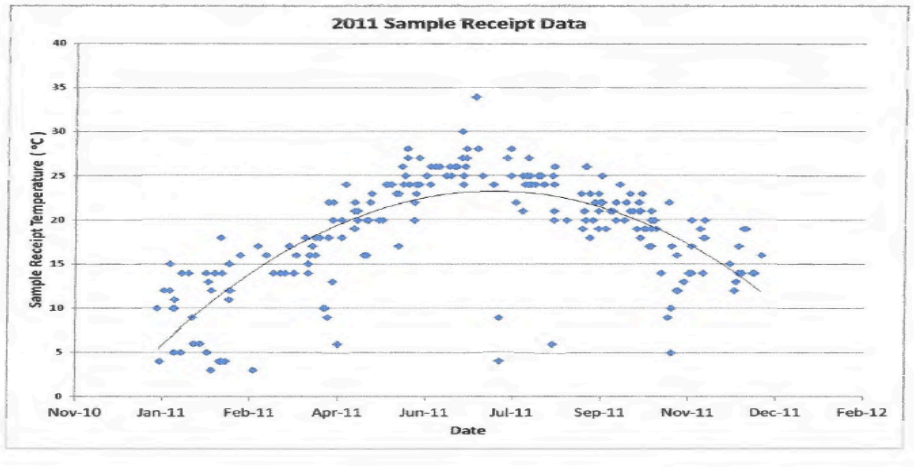

Figure 1.1: Temperature of drinking water sample kits from Operation Enduring Freedom (OEF) Jan–Dec 2011. (46)

Only 7% of the 224 samples received that year were less than the prescribed holding temperature parameters of 4°C (46). As a result, 93% of the samples taken in 2011 have a potential loss of analytical integrity due to temperature alone, and the extent of the inaccuracy is unknown. The lack of representative sample results can lead to an incorrect assessment of exposures for our deployed military and civilian forces.

PURPOSE

The intended purpose of this study was to determine and quantify differences in the concentration of PERC in water samples when varying the storage temperature or analysis timeframe that is outlined in EPA Method 524.2.

RESEARCH QUESTIONS

Is there a statistically significant difference between time and temperature in the concentration of PERC when water samples are analyzed after 14 days, compared to when they are analyzed within 14 days (the timeframe mandated by EPA Method 524.2)? How does the PERC concentration change as a function of the storage temperature?

HYPOTHESES

Hypothesis 1

Storage time of up to 27 days does not introduce a statistically significant difference in the concentration of PERC in water samples analyzed at different time points.

SA 1.1: Prepare a phosphate buffer solution with a pH of 7.2 to represent a local water source; the buffer will exclude the influence of pH fluctuations; thus, the measurements will strictly be related to the degradation profile of PERC. Water samples

with a known concentration of PERC will subsequently be prepared and stored per EPA Method 524.2.

SA 1.2: Every 3 days for 27 days, compare PERC concentration in the water samples to a known concentration of PERC.

SA 1.3: Perform appropriate statistical analyses to determine any significant differences in the concentration of PERC over time.

Hypothesis 2

There is no statistically significant difference in the concentration of PERC at each target temperature in water samples stored at temperatures above 4°C.

SA 2.1: Prepare a phosphate buffer solution with a pH of 7.2 to represent a municipal water source. The buffer will exclude the influence of pH fluctuations; thus, the measurements will strictly be related to the degradation profile of PERC. Use this solution to prepare water samples with a known concentration of PERC.

SA 2.2: Each set of water samples with a known concentration of PERC will be stored at a constant temperature in a dedicated temperature-control environment (i.e., refrigerator, low-temperature incubator) for 27 days. The analysis will be performed every 3 days.

SA 2.3: Perform appropriate statistical analyses to determine any significant differences in the concentration of PERC at each target temperature.

Hypothesis 3

Changes in PERC concentrations in water samples as a function of storage temperature or analysis timeframe are not statistically significant.

SA 3.1: Estimate a best fit curve over time for each temperature using a quadratic linear regression or with second polynomial functions of time (if needed) to identify the relationship between temperature and time (25).

SA 3.2: Controlling for storage time and temperature, determine how each of these variables affects the prediction of PERC concentration in the water samples.

SA 3.3: Determine which storage conditions (i.e., temperatures and times), result in the most accurate PERC concentrations in the water samples.

CHAPTER 2: Literature Review

INTRODUCTION

Water sampling regulations such as the Safe Drinking and Water Act (SDWA) and the Clean Water Act (CWA) have been expanded in recent years. Areas that have been modified are the maximum contaminant levels (MCLs), the number of samples needed, and the techniques used for sampling and analyzing the water samples. Since the 1990s, maintaining the established pre-analytical holding times has been dependent on currently available technology and how the EPA integrates such advances into current sampling techniques. The study performed by Maskarinec et al. (1990) showed that the holding time before the analysis of 17 VOCs, including PERC, could potentially be extended from the current holding time limit of 14 days. This study used 2 concentrations (50 and 500 ppm) with three different water matrices (distilled water, groundwater, and surface water). The results of Maskarinec's study indicates that PERC that the holding time for PERC with the 50 ppm concentration, can be extended beyond the 14 days holding time limit. Maskarinec indicated that at room temperature (25ºC), PERC had a shorter holding time that the one described in EPA Method 524.2 (30). The 500 ppm concentration could not be maintained past eleven days for any of the water matrixes. Therefore, based on those results by Maskarinec, a lower concentration is used (5 ppm) for this research to validate the results of prolonged holding time.

REGULATORY

The United States Environmental Protection Agency (EPA) is the primary entity that regulates VOCs in the air, water, and land. The SDWA was established to preserve

and protect the quality of drinking water in the United States (20). To ensure water quality, the EPA has established a limit for PERC of 5 ppb (part per billion) in water that is managed and distributed by public water systems.

The military derived its guidelines in large part from the existing Federal standards and guidelines. Those guidelines are included as part of the military exposure guidelines (MEGs), which are health-based chemical concentrations for various deployed military exposure scenarios representing levels at which no, some, or significant health effects could occur within the exposed, deployed population. It is important to note that the MEGs themselves are not “standards” or “action levels.” (45).

In 2011, the EPA embarked on a new strategy regarding the regulation of water contaminants. EPA identified 17 VOCs, including PERC, as carcinogenic volatile organic compounds (cVOCs) (38). As part of the development of the new cVOC rule, the EPA generated a list of best available technologies (BATs) that can support the effective removal or reduction of such cVOCs. One of the technologies for the treatment and removal of VOCs is packed-tower aeration (PTA), also known as air strippers. PTAs consist of a cylindrical tower, packing material, and a centrifugal blower that can remove VOCs from the water. As the water falls from the top of the tower by gravity, air is injected in the opposite direction of the water (from the bottom), which allows the VOCs to rise to the top of the system and be vented. This technique has proven to be effective in the removal of PERC found in groundwater sources (14; 38).

The categorization of cVOCs by the EPA was based on similarities in their health effects and treatment processes. While the categorization for treatment processes reduces costs by identifying BAT covering multiple VOC's, quantification of VOC concentrations

in water still presents a challenge. Eaton et al. (2012) reviewed the current analytical methods of all cVOCs and concluded that no single approach could be utilized to analyze all cVOCs (14). The study suggests that to analyze all cVOCs the use of four different analytical methods is required. One of those methods, EPA Method 524.2 (Measurement of Purgeable Organic Compounds in Water by Capillary Column GC/MS) also incorporates the use of solid-phase microextraction as an alternative method to detect VOCs in the parts per billion range (14; 33). Method 524.3 can support lower-level detection of 11 of that 17 cVOCs, including PERC. A modification to method 524.3 is needed for the analysis of 3 of that 17 cVOCs. Liquid chromatography/mass spectrometry is needed for 2 of those 17, and method 524.6 for nitrobenzene (14). Due to their physical properties, mainly vapor pressure and density, there cannot be one specific method used for detection.

SOLID-PHASE MICRO EXTRACTION (SPME)

Solid-phase microextraction (SPME) is a technique that was developed to reduce sample preparation requirements of complex and diverse contaminants. In this technique, organic compounds can be rapidly extracted from water samples (40). SPME combines two steps, sampling and preconcentration into one step. The sampling step utilizes a silica fiber coated with a sorbent material which, when exposed to the water sample, absorbs the compounds present in the sample. The second step, preconcentration, allows the sorbent material to be transferred directly into the GC/MS (40). The SPME technique and the efficiency of the SPME fibers were evaluated by Santos et al. (1996) by comparing specific VOCs that are commonly detected in industrial effluent and drinking water. The evaluation concluded that fibers coated with polydimethylsiloxane (PDMS) gave higher

recoveries of a large selection of VOCs (40). Currently, various types of SPME fibers are amiable that have properties suitable for specific VOC(s). The use of a SPME procedure for VOC analysis of water samples increases the precision and sensitivity of the GC/MS analysis (7; 40).

PERC Toxicology and Health Effects

Absorption

One of the dangers of working with PERC in an industrial setting is the exposure to its vapor form, due to its inhalation via the lungs. Gastric absorption of PERC takes place in the gut at a relatively rapid rate. Dermal absorption does take place with the vapor form. Ingestion PERC dissolves in lipids (fat-soluble compounds) and fats (a subgroup of lipids). The highest concentrations of PERC are found in body fat (2). When exposure to PERC is high, animal, and (limited) human studies have found PERC in the brain and liver (16).

Distribution

Once absorbed, PERC is distributed throughout the body from high areas of concentration (liver, lung, and kidney) to low areas of concentration (blood circulation and other tissues) via the circulatory system. Absolute tissue concentrations are directly proportional to the exposure dose and the total amount absorbed by the body. Once PERC enters the bloodstream, it can cross the blood-brain barrier, thus potentially causing damage to the nervous system (16).

Metabolism

When the body metabolizes PERC, multiple carcinogenic metabolites are generated such as trichloroacetic acid and dichloroacetic acid (35). The primary organs that are affected by the metabolic by-products of PERC are the liver, kidney, and lungs (16). Dichloroacetic acid is generated through the dechlorination of trichloroacetic acid due to increases in temperature and changes in pH (47). Previous animal studies have shown that tri-and dichloroacetic acid is responsible for liver toxicity and carcinogenicity (2).

Excretion

Unmetabolized PERC is excreted through exhalation, urine, and feces (2). However, in fat tissue, the half-life of PERC is approximately 72 hours, whereas the majority of the remaining VOCs are within minutes (1). This relatively long half-life means that PERC will spend a long time in the body, leading to prolonged short-term and long-term effects. Nonetheless, studies on these effects in humans are limited (36).

The research conducted by Aschengrau et al. (2009) examined how exposure to PERC through contaminated drinking water in pregnant women affects the development of their infants (5; 6). The source of PERC was the inner vinyl lining of asbestos that was used in the cement of water distribution pipes during the 1960s in New England (Cape Cod Area). The results of a population-based retrospective cohort study suggested that prenatal exposure to PERC increased the risk of specific congenital abnormalities in the cardiovascular and neurological system. Aschengrau et al. (2009), recommended that a follow-up investigation was needed with a larger sample size of affected children.

Aschengrau conducted a similar study in the same geographic location describing the long-term impact of early exposure to PERC and the risk of developing cancer and chronic conditions such as obesity, diabetes, hypertension, and color blindness (6). The results from this study suggested a relationship between the risk of certain types of cancer, including cervical cancer, and epilepsy among adults following PERC exposure during gestation and early childhood. No relationship was found with the development of chronic conditions (6). Although Aschengrau's study did not directly involve military forces, for planning considerations, the findings are important to consider. Understanding the current health issues within the local population where our military forces are co-located can assist with understanding the mission risk and scope, especially during humanitarian assistance and disaster relief (HADR) responses.

In addition to the above toxicological effects, PERC exposure can also affect the immune system (41). Processes affected by PERC are the production of antigen and cells responsible for mediating the immunological response. Seo et al. (2008) reported that exposure to low concentrations of PERC in drinking water stimulates the release of histamine, a molecule that supports the immune system during an allergic reaction (41; 42). The release of histamine subsequently leads to muscle contractions and capillary dilation. The increase in blood flow caused by this response allows PERC to be distributed more easily throughout the body via the circulatory system. The study utilized an animal model exposed to 1 mg/L (1 ppm) PERC in drinking water for 2 to 4 weeks. The results suggested that prolonged PERC ingestion, even at low concentrations, causes inflammation of the skin (42).

PERC Stability In Water

Sampling for PERC or any VOC of concern requires that the method for its collection and preservation be followed following the established specifications to minimize changes in sample composition. Śliwka-Kaszyńska et al. (2003) investigated the preservation of the chemical, physical, and physicochemical properties of water samples during sample storage. Chemical preservation prevents the biological activity of microorganisms by inhibiting growth. Physical preservation involves selecting a suitable container, using proper filling techniques, and maintaining and an adequate temperature. One of the separation techniques mentioned in the article that has been successfully applied is solid-phase microextraction (SPME) (39).

The temperature at the point of water sample acquisition influences the stability of PERC in water. The study by Moliner-Martinez et al. (2012) assessed the temporal variation of several VOCs, including PERC, that were present in three different water sources. The author found that the levels of VOCs are influenced by seasonal variation in temperature and precipitation (32). VOCs with a lower boiling point, equal or less than PERC (Boiling point of 121.3°C) will evaporate faster when the temperature of the water source is increased.

Careful sample preparation is essential to ensure that the analytical results are representative of the location from which the sample was taken. The temperature influences the stability of VOCs in water, as does the sample preservation measures that are used. During the execution of this research, the use of four different refrigerators temperature adjusted to maintain a specific temperature reduces the potential for volatilization.

Analyzing environmental samples requires careful selection of the methods used for determination, isolation, and enrichment. Marczak, et al. (2006) described the most widely used techniques for the analysis of VOCs in water samples; Figure 2.1 depicts the application scope isolation and enrichment techniques for VOCs in water. Depending on the VOC volatility and the water matrix composition the VOC is found in, is what will determine the level of complexity of the analysis. For example, if the matrix is simple and the VOC is a volatile one, then the direct aqueous technique is the one recommended in Marczak's study (29). The solid or stationary techniques incorporate a wider range of VOCs, and there is no need to utilize higher volumes of sample or solvents. SPME is one of the techniques discussed in the article and requires only a short analysis time. Regarding the other two techniques, although they can be applied to a wider selection of VOCs, the equipment costs and solvents needed for the analysis increase their complexity.

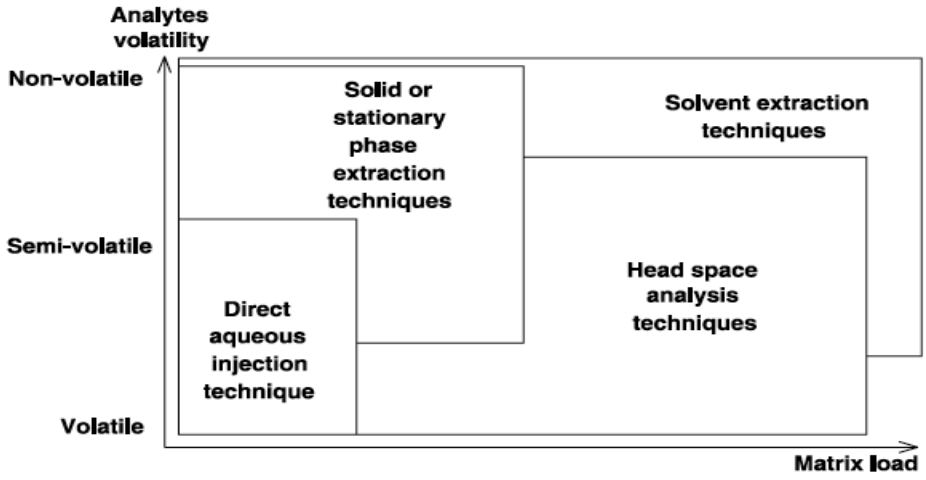

Figure 2.1. Application scope of isolation and enrichment techniques for VOCs in water depending on VOC volatility (29).

The stability of various VOCs, including PERC, in aqueous solutions, was studied by Kozlowska et al. (2006). Their objective was to establish the optimum conditions for preparing water samples for VOC analysis. The results from Kozlowska's study indicated that limiting or eliminating the headspace in the container reduces the loss of analytes from aqueous solutions, thus maintaining the original concentration of the analyte (27). Reducing the headspace minimizes the volume into which a VOC can volatilize and subsequently be lost during storage or analysis. Determining trace analytes in a water sample is increasingly sought in modern chemical analysis as the regulatory concentration limits continue to be reduced (21).

CHAPTER 3: Materials and Methods

MATERIALS

Chemicals

For the phosphate buffer solution, commercially available chemicals were purchased as follows. Monopotassium phosphate (KH_2PO_4) and dibasic potassium phosphate (K_2HPO_4) were obtained from Eisen Golden Lab (Dublin, CA). American Chemical Society (ACS)-grade disodium phosphate heptahydrate ($Na_2HPO_4.7H_2O$) was obtained from Lab Genome (Houston, TX), and lab-grade ammonium chloride (NH_4Cl) was obtained from Home Science Tools (Billings, MT). For pH adjustments, hydrochloric acid and sodium hydroxide were purchased from Sigma Aldrich (St. Louis, MO). Confirmation of the pH target concentration of 7.2 was achieved by using a pH meter from Hach (Loveland, CO). PERC was obtained from Lab Supply Outlaws (Austin, TX).

Instrumentation

The water sample analyses were performed using a portable GC/MS unit (HAPSITE® ER; Inficon, East Syracuse, NY) that contained the following accessories: service module and an SPME sampling system as depicted in Figure 3.1. The HAPSITE® ER is a portable unit capable of collecting and analyzing samples in the field as it combines two analytical techniques: gas chromatography (GC) and mass spectrometry (MS) (24). The GC subsystem separates the compounds in the sample based on their volatility as they are transported through the column using nitrogen as the carrier gas. The column is 15 meters in length and composed of a narrow bore silica tube in which

the analytes interact and are separated (24). Once separated by GC, the sample is introduced to the MS where the compounds are ionized. The ions are transported by a magnetic field and are identified based on the ratio of the radio frequency (RF) to direct current (DC) field and compared against an internal library of mass spectra (24). A 75 μm carboxen-polydimethylsiloxane (CAR/PDMS) SPME fiber (Sigma Aldrich, St. Louis, MO) was used. The selection of this type of fiber from among the currently available alternatives was based on the physical properties of PERC. A 75 μm CAR/PDMS fiber is used for polar and non-polar compounds with a low molecular weight (30 – 225 g/mol) and a boiling point of less than 220ºC; which PERC's properties are within that range as presented in Table 1.1 (43).

Figure 3.1. HAPSITE® ER configuration with the service module and the SPME sampling system.

Figure 3.2. SPME fiber assembly (left) and SPME fibers outside the box (right).

METHODS

Preparatory Work

To calibrate the experimental concentration measurements, a stock concentration of 5 ppm was first prepared. An appropriate mass of PERC was dissolved in the required volume which was calculated as follows:

Equation 1: Molarity

$$\text{Molarity (mol/L)} = \frac{(\text{PPM})(0.001)}{\text{MW}} \quad (1)$$

Where:

PPM = Target concentration
0.001 = Volume (L)
MW = Molecular weight of PERC (165.83 g/mol) (16)

Equation 2: Mass needed

$$\text{Mass needed (g)} = (Molarity)(Volume)(MW) \quad (2)$$

Where:

Molarity = Result calculated from Equation 1
Volume = Size of the container (expressed in L)
MW = Molecular weight of PERC (165.83 g/mol)

Equation 3: Volume

$$\text{Volume } (\mu L) = \frac{Mass\ Needed}{Density\ (g/L)} \text{ x } 10^6 \quad (3)$$

Where:

Mass Needed = Calculated on Equation 2
Density = 1.6227 g (1622.7 g/L)

For 1 L of a 5 ppm stock solution, the total amount of PERC used was 6.16 μL. A 10μL Hamilton microsyringe model #701 (Hamilton Co., Reno, NV) was used to measure out this amount with a precision of ±1%. The amount of PERC extracted was 6.2 μL as the syringe cannot accurately measure volumes in the hundredths. Once the buffer solution was prepared, then PERC was added from the syringe directly into the vial. A flask cover was used, to include the use of Parafilm®M 4"x250" (Oshkosh, WI) to double protect the flaks cover, and to reduce the loss of PERC due to volatilization. The flask was then placed on a magnetic stir plate. The speed of the stir plate was adjusted to 5 (out of a maximum of 10) and maintained mixing for 3.5 hours.

A total of 160 samples were prepared using 40 mL screw cap vials each equipped with a Teflon-faced silicone septum (Sigma Aldrich, St. Louis, MO). Four groups of 40 samples each were stored at 4ºC, 5ºC, 20ºC, and 25ºC. Temperatures were monitored every 45 minutes using a temperature logger (model RC-5; Elitech, Milpitas, CA). The target temperature and equipment used are described in Table 3.1.

Equipment	Model	Company and Location	Target Temperature (ºC)
Refrigerator	13-986-111	Fisher Scientific, Hampton, NH	4ºC
Refrigerator	TBF21DYD	General Electric, Boston, MA	5ºC
Incubator	2310	Precision Scientific, Chicago, IL	20ºC
Incubator	16EG	Fisher Scientific, Hampton, NH	25ºC

Table 3.1. Equipment used to control the sample storage environment.

Upon completion of the desired time points, the recorded temperatures were statistically analyzed using Statistics Analysis for Social Science (SPSS) version 25.0 (IBM, Armonk, New York). Descriptive statistics (mean, standard deviation, and variance) were calculated to describe the stability of the temperatures.

Separation Instrumentation Details

The adsorption and desorption time of the GC/MS column SPME were analyzed separately for the samples used in this study. The amount of time used for SPME insertion was 30 seconds, and the desorption time in the HAPSITE® ER was 45 seconds. The depth of insertion of the SPME fiber assembly to expose the fiber to the water sample was 2.6". The selection of these parameters is based on practice tests performed before the execution of the main research. Currently, there are no specific guidance or parameters from both manufacturers (SPME fiber nor the HAPSITE®ER) regarding the exposure time of the SPME fiber or for how long the sample needs to be desorbed. Only major recommendations/suggestions are included in the instruction manual. Therefore, the selection of the depth of insertion was determined to be representative of exposing the

fiber evenly throughout the vial. Finally, the selection of fiber exposure and desorption time was determined based on numerous sample runs to obtain the most accurate and representative sample.

The temperature profile inside the HAPSITE®ER will reach 60°C and will maintain it for 1-minute. The sample will pass through the SPME concentrator for 45 seconds at a temperature of 80°C. After the sample passes through the SPME concentrator, the pre-desorption process begins. The temperature within the system will reach 120°C. The final two steps, Foreflush, and backflush will have the equipment reach a temperature of 200 °C for a total of 11.5 minutes. This oven ramping profile provided an analysis time of 12 minutes, as depicted in Table 3.2.

Step	Duration	Temperature Profile
SPME Line Purge	1 minute	60ºC
SPME Concentrator	45 seconds	80ºC
Pre-Desorption	11 seconds	120ºC
Foreflush	9.5 minutes	200ºC
Backflush	2 minutes	200ºC

Table 3.2. HAPSITE® ER temperature profile used for PERC.

Sample Replicates Needed For Each Target Temperature

The number of replicates required was determined using ASTM Method D4841-88e; its equation is presented as follows:

Equation 4: Number of Replicates Needed (4)

$$n = \left(\frac{t\,RSD}{D}\right)^2 \qquad n = \left(\frac{(2.1318)(13.4)}{0.15}\right)^2 \qquad (4)$$

Where

n = Number of replicates required
t = Student's t (value of 2.1318 was obtained from Daniel, 2013 Table E, using 4 degrees of freedom and a 0.05 level of significance) (12)
RSD = Relative standard deviation, % (value of 13.4 was obtained from Inficon; it is a measure of the linearity of the concentration levels in the PERC calibration curve (24).
D = 15% (A constant that describes that a VOC is determined to have degraded when 85% of the initial mean concentration remains) (4)

The value obtained from Equation 4 requires a minimum of four replicates per sampling day. Each set of replicates was sampled every 3 days throughout 27 days.

Calibration

A calibration curve was prepared as part of the requirements described in Sections 3.14 and 7.8 of EPA Method 524.2 (33). The concentrations utilized to create the calibration curve were 3, 5, and 10 ppm. A calibration curve was prepared before each data collection. The calibration standards were diluted from an initial stock concentration of 100 ppm. The three calibration standards were each prepared in a total volume of 100 mL and mixed for 3.5 hours on a stir plate (model PC-620; Corning, Tewksbury, MA). The determination of the mixing time was based on a pilot study performed before the execution of the main research as there were no literature nor manuals from the manufacturers that depict the adequate mixing time based on the analyte's concentration. Equation 5 below shows how the volumes needed to prepare the calibration standards were obtained:

Equation 5: Volume Needed

$$C_1V_1 = C_2 V_2 \qquad (5)$$

Where:

C_1 = Concentration (ppm) of PERC stock solution
V_1 = Initial volume needed
C_2 = Desired concentration (ppm)
V_2 = Desired volume of calibration solution

As part of the calibration process, a blank sample was prepared and analyzed in the same manner as all the samples before initiating the calibration curve and after completing the calibration. The use of blank samples was also implemented at the beginning and end of each sampling day. During the calibration process, each of the standards was analyzed with three replicates and the average was taken and annotated. All data corresponding to the calibration curve are included in Appendix A.

Statistical Analysis

PERC Concentration and Time

A regression model with a quadratic function was used to measure the effect of time on the concentration of PERC over the course of 27 days. The term multiple linear regression, can be substituted for the term polynomial regression, according to a previous work (26). The purpose of a multiple regression is to find an equation that best predicts the dependent variable as a linear function of the independent variable (25). A multiple linear regression model describes the extent, direction, and strength of the relationship between an independent variable (time) and a continuous (dependent) variable (concentration). The polynomial model was used for this research to determine whether an estimated concentration can be improved significantly by increasing the complexity of the relationship between the dependent and independent variables via a linear, curvilinear, or additive relationship fitted to a straight-line model (26). A quadratic functions increases the approximation of the values obtained as the function incorporates

the use of a parabola (12). For linear regression models, several assumptions need to be met- linearity, normality, independence, and equal variance. These terms are defined below.

Linearity: the behavior of the dependent variable (concentration) *Y* can be explained by a linear or quadratic curvilinear additive relationship between the dependent variables (time and temperature)

Normality: errors are normally distributed.

Independence: errors are independent of each other.

Equal variance: the errors are distributed with mean zero and a constant variance σ^2 (25).

The quadratic regression is represented by the following equation:

Equation 6: Quadratic Regression

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + E \qquad (6)$$

Where:

Y = PERC concentration
β_0 = Intercept
β_1 = Initial slope
X = Sampling day = Sampling day of the study
β_2 = Change in slope over time
E = Error

After substituting the variables with the data calculated, the equation's purpose is to be able to present what is the averaged initial PERC concentration (β_0), how much will increase of decrease per day during the sampling days ($\beta_1 X$) and, upon reaching an equilibrium, what is the rate of decrease or increase per sampling day ($\beta_2 X^2$) where sampling day is the interaction term in the model in equation 6.

PERC Concentration And Temperature

One-way analysis of variance (ANOVA) was used to measure the effect of different temperatures to determine which mean amongst a set of means differ from the rest on the concentration of PERC (13). The use of a one-way ANOVA not only requires the same assumptions used for a quadratic regression but also that the dependent variable (i.e. PERC concentration) is measured continuously. The results of the analysis in SPSS after inputting the data are presented as the F statistic. The F statistic evaluates whether the means of all target temperatures are significantly different from one another. As the value of the F statistic from one-way ANOVA considers all temperatures at the same time, the use of Tukey's pairwise comparison allows for the consideration of all possible pairwise differences of means and identifies the comparisons that are statistically significant (12).

PERC Concentration With Time and Temperature Combined

A multiple linear regression with dummy variables was used to identify the effect of temperature, sampling day, and temperature and sampling day combined on the PERC concentration. The model for this regression combines the information of time and each target temperature within the model. The representation of the independent variables in the model is presented in Equation 7.

Equation 7: Linear Regression with Dummy Variables

$$\text{PERC concentration} = (\text{sampling day} + \text{day}^2) + (\text{Dummy 1} + \text{Dummy 2} + \text{Dummy 3}) + ((\text{sampling day} + \text{sampling day}^2) \times (\text{Dummy 1} + \text{Dummy 2} + \text{Dummy 3})). \quad (7)$$

Where:

PERC concentration = ppm
Sampling day = Sampling day of the study
Dummy Variable 1 = 5ºC
Dummy Variable 2 = 20ºC
Dummy Variable 3 = 25ºC

The use of a dummy variable, also known as an indicator variable, allows for identifying different categories of a qualitative variable, normally with a value of 0 or 1 (12). The use of a dummy variable allows SPSS to analyze all sampling days for each independent variable (temperature and time) individually and combined. Each target temperature is given a code (dummy variable) in SPSS. Table 3.3 below provides the coding used for the dummy variables; the use of codes allows SPSS to compare the combined effect of all temperatures and time. When the data is being inputted into SPSS, one of the temperatures needs to be designated as the "baseline".

Temperature	**Dummy Coding Variable 1**	**Dummy Coding Variable 2**	**Dummy Coding Variable 3**
4ºC	0	0	0
5ºC	1	0	0
20ºC	0	1	0
25ºC	0	0	1

Table 3.3. Dummy variable coding in SPSS.

CHAPTER 4: Results

The results of this study should not be interpreted as scientific findings due to: problems with the calibration standards, initial research sampling day 0 sample identification, to mention a few as they will be explained in Chapter 5: Discussion and Conclusion. The experiment was not redone due to insufficient funds to acquire more materials, some of the materials were in a 3-month backorder status, plus that degree program only allowed the student to be for two years, which after such timeframe, the student received orders to relocate to another state. Lastly, the interpretation of the results was also a condition of completing the thesis requirements. are difficult to interpret and have limited reliability due to experimental problems and errors which will be explained in Chapter 5: Discussion and Conclusion.

This chapter is divided into several subsections beginning with the calibration curve results and their coefficient of determination (R^2) values, followed by descriptive statistical output. The remaining subsections pertain to each specific hypothesis and the data obtained; however, the level of confidence in the results is low due to the issues encountered. The final subsection of the Results presents the data obtained during the subset analysis that determined the adequate mixing time of PERC in a phosphate buffer solution at pH 7.2. For all tables in this chapter, all p-values highlighted are indicative of significant results at the 5% level.

CALIBRATION CURVE

Each sample was analyzed 3 times in the HAPSITE® ER, and the average of the three samples was plotted on the respective calibration curve for that day (See Appendix A). Sampling was supposed to take place every 3 days; however, one sampling took place a day late (sampling day 7 instead of sampling day 6) due to complications with the equipment. Table 4.1 provides a summary of the calibration curves with their averaged area sample concentrations for all standards, the slope, intercept, and the coefficient of determination (R^2). Note that the calibration curves are identical on days 0 and 3. This was due to a recommendation to utilize day 0 as the main calibration curve and only develop another calibration curve at the end of the research.

Calibration Curves			
Sampling Day	**Slope**	**Intercept**	**R^2**
0	5,348,091	2,490,495	1.000
3	5,348,091	2,490,495	1.000
7	5,436,993	10,880,031	0.989
9	1,529,590	320,086	1.000
12	2,937,030	1,555,535	0.999
15	3,543,486	4,030,377	0.999
18	2,935,517	3,385,523	0.999
21	2,949,357	4,091,909	0.992
24	4,090,202	3,503,805	0.991
27	3,428,421	3,232,171	0.990

Table 4.1. Summary of calibration curves for all sampling days.

DESCRIPTIVE STATISTICS

The temperature loggers recorded the temperature every 45 minutes throughout the 27-day study. The results for each temperature-controlled device are presented in Table 4.2. While the mean observed temperatures were close to the targets, the temperatures varied substantially over time. Note that the minimum and maximum temperatures for the 4ºC temperature-controlling device were as low as -3.7ºC and a

maximum of 12.5ºC. During the research timeframe, although the thermologgers were recording the temperature, a "real-time" temperature can be reviewed. Throughout the sampling days of the research, a visual reading of the temperature did not resulted in observing any temperature deviation of 2ºC. Another observation is that the length of exposure to such temperatures were only within a 45 minute timeframe, plus no observed freezing occurred to the samples.

Descriptive Statistics For Temperature Loggers

Temp°C	N	Range	Minimum	Maximum	Mean
4	851	16.2	-3.7	12.5	3.011
5	849	6.4	4.0	10.4	5.261
20	850	9.0	15.2	24.2	18.831
25	858	3.1	22.3	25.4	25.135

Table 4.2. Temperature loggers descriptive statistics

Normality

Test of normality and homogeneity of variance (HOV) were conducted for the PERC concentration as part of assumptions to test prior to performing quadratic regression or one-way ANOVA (12). The results of the Shapiro-Wilk test of normality of the PERC concentration at each target temperature are provided in Table 4.3. The target temperature of 5°C failed to meet the assumption of normality for PERC concentration. An additional step in the assumption of normality was to visually present the pattern of the data in a quantile-quantile plot (Q-Q plot). A Q-Q plot is a visual representation to determine how closely the data follow a normal distribution (12). A 45 degree reference line is plotted, and if the data points (quantiles or probability plots) fall approximately along this line then the data is plausible to have been originated from a normal

distribution. Figures 4.1 thru 4.4 provide the Q-Q plots for all target temperatures throughout all sampling days. After revising all Q-Q plots, the point distribution in Figures 4.1 thru 4.4 were all determined to be close to the diagonal line. No points can be dismissed while visually assessing the plots to avoid selection bias (12). If the q-q plots do not align close to the line, then the regression analysis cannot be performed. Although Figure 4.1 for 4°C shows a single outlier (point in the observed value of 7), the rest of the data points are approximately normal, so it is appropriate to assume normality (26).

Homogeneity of Variances (HOV)

After completion of a normality test, a homogeneity of variance (HOV) test was performed as part of the assumptions needed to implement the use of ANOVA. The Levene test is used to test if the assumption of HOV for two or more group of samples is met (25). The HOV test assessed whether the variance of PERC concentration differs significantly by target temperature at each time point. The p-values were > 0.05, which indicated no significant differences; therefore, the HOV assumption was valid (25). The results of the HOV tests (Table 4.4) indicate that the HOV assumption was not violated.

Tests of Normality

		Shapiro-Wilk		
Target Temp		Statistic	df	p-value
PPM	4	0.968	40	0.310
	5	0.939	40	0.031
	20	0.946	40	0.057
	25	0.955	40	0.114

Table 4.3. Normality test results for all target temperatures.

Test of Homogeneity of Variances				
Based on Avg. PERC Concentration (PPM)				
Day	Levene Statistic	df1	df2	P-Value
0	3.092	3	12	0.068
3	2.566	3	12	0.103
7	2.809	3	12	0.085
9	2.138	3	12	0.149
12	2.907	3	12	0.078
15	1.310	3	12	0.317
18	2.179	3	12	0.144
21	1.322	3	12	0.313
24	0.834	3	12	0.501
27	1.676	3	12	0.225

Table 4.4. Results for homogeneity of variance.

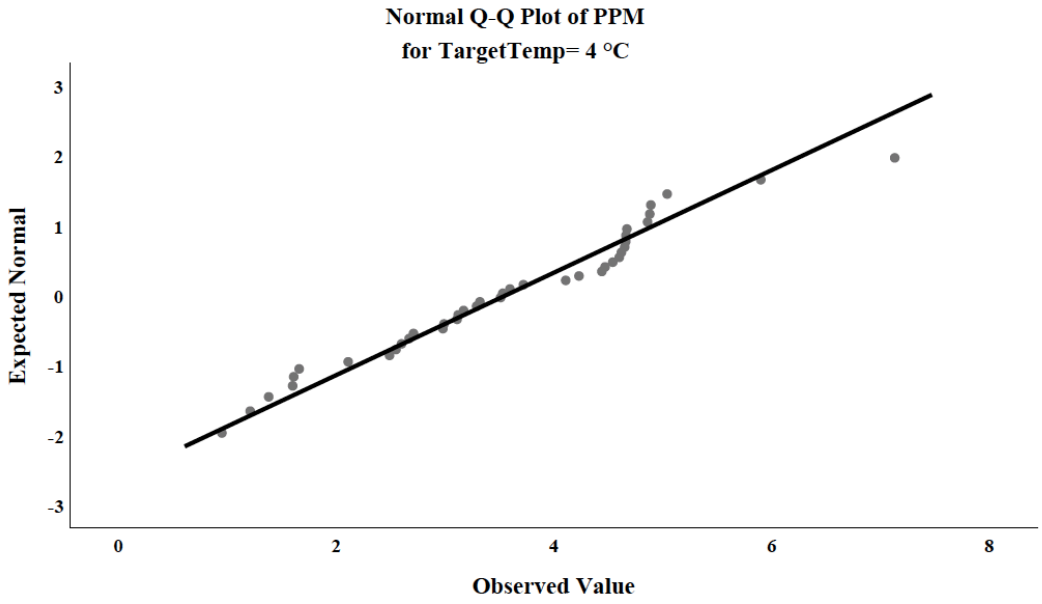

Figure 4.1. Q-Q Plot for Target Temperature of 4°C

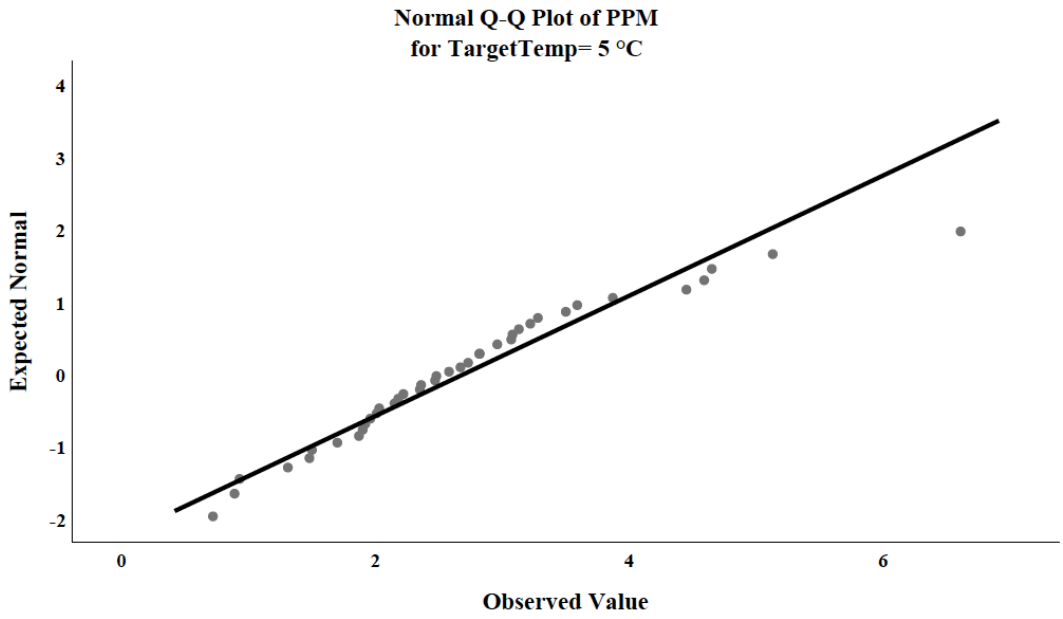

Figure 4.2. Q-Q Plot for Target Temperature of 5°C

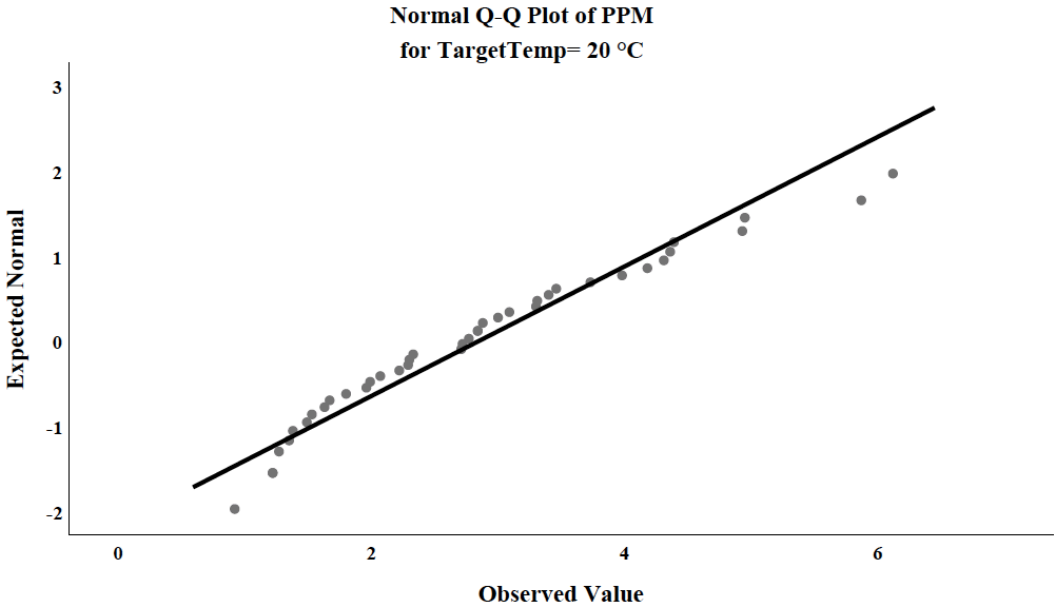

Figure 4.3. Q-Q Plot for Target Temperature of 20°C

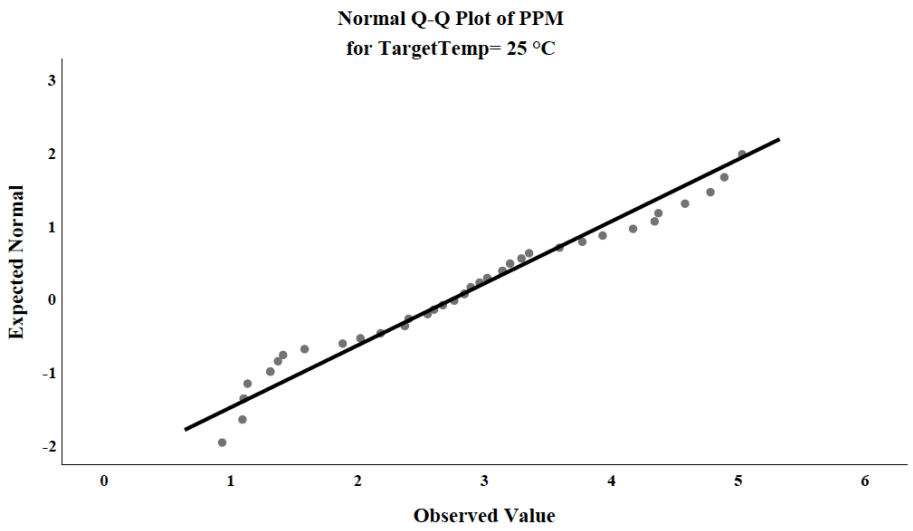

Figure 4.4. Q-Q Plot for Target Temperature of 25°C

PERC Concentration and Holding Time

The effect of the holding time on PERC concentration was assessed using a quadratic regression for each temperature. Table 4.5 shows the results of the quadratic regression models. The quadratic regression allows to use the relationship between the dependent and independent variable to obtain the regression equation that can be utilized to make estimations on what the PERC concentration will be based on the holding time and the temperature the sample was held.

Quadratic Regression for PERC at Specified Temperatures (°C)

	Model Summary		Parameter Estimates		
Temperature (°C)	R Square	p-value	Constant	Initial Slope	Change in Slope
4	0.670	0.021	1.463	0.283	-0.007
5	0.166	0.530	3.714	-0.180	0.006
20	0.404	0.163	1.378	0.261	-0.008
25	0.684	0.018	0.813	0.385	-0.013

Dependent Variable: Average PERC Concentration (PPM)

Table 4.5 Quadratic regression results for PERC concentration when stored for 27 days.

Table 4.5 presents the p-value calculated for each temperature throughout the 27 days of sampling. A p-value below 0.05 indicates that there was a statistically significant effect of holding time on the PERC concentration. Time was significant at 4°C (p-value = 0.021) and 25°C (p-values of 0.018) in predicting the PERC concentration. The other two target temperatures (5°C and 20°C) were not statistically significant (p-values 0.530 and 0.163 respectively). The first and last target temperatures yielded the highest coefficient of determination (R^2). The value of R^2 close to 1 is ideal as it can explain the relationship between the dependent and independent variable, a lower R^2 value does not make the results invalid. If the R^2 is low but the independent variables are statistically significant, important relationships can be inferred between the variables (23). R^2 represents the percent variability in the PERC concentration that is explained by the change in time, implying that a value closer to 1 would be ideal (23). Figure 4.5 provides a graphical representation of the effect of time on the PERC concentration at each target temperature. The regression equation for each graph is also included with the values of the constant, initial slope, and change in slope. For example, the equation for the target temperature of 4°C describes the intercept (constant) as 1.463 ppm (point on the y-axis through which the quadratic curve passes). The initial slope in that same equation is 0.283 ppm, which indicates that the PERC concentration is expected to increase by 0.283 ppm per day. The parameter for change in slope is -0.007 ppm per day, indicating that the rate of change in the PERC concentration decreases over time. The PERC concentration for the target temperature of 5°C begins with a decrease of 0.180 ppm per day until the PERC concentration begins to change (increase) by 0.006 ppm per day. If the data was obtained without the limitations and errors described at the beginning of this chapter, being able to

generate these regression equations allows us to ascertain at which point in time the concentration was at any particular time of the study, thus obtaining a degradation profile.

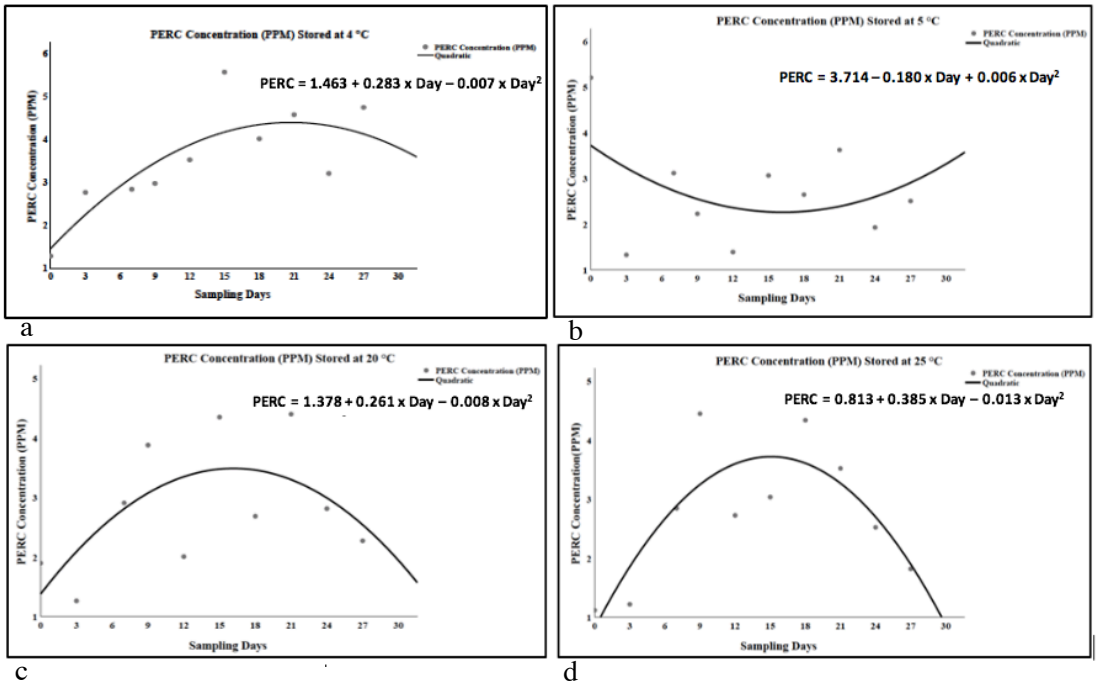

Figure 4.5. PERC concentration over time for each target temperature. The equation with the calculated values is included.

PERC CONCENTRATION AND HOLDING TEMPERATURE

The mean PERC concentration was compared between temperatures on each sampling day using one-way ANOVA followed by Tukey's post-hoc test. Prior to ANOVA, the assumptions of normality and homogeneity of variances were tested, and the results indicated that the data supported a normal distribution (normality) and that the variances were the same (homogeneity of variance) (12). Table 4.6 provides the p-value

for the comparison of PERC concentrations between target temperatures on each sampling day. The concentrations differed significantly between target temperatures on every sampling day (highlighted p-values) except on sampling days 7, 9, and 21.

One-Way ANOVA (PERC Avg. PPM)

Day	Mean Square	F	P-value
0	14.596	48.941	0.000
3	2.261	8.712	0.002
7	0.065	1.012	0.421
9	3.824	2.673	0.095
12	3.409	3.556	0.048
15	5.892	6.860	0.006
18	3.123	11.644	0.001
21	1.162	2.387	0.120
24	1.169	6.150	0.009
27	6.856	31.603	0.000

Table 4.6. One-way ANOVA for average PERC concentration between temperatures on each sampling day.

Table 4.7 presents the results of one-way ANOVA with Tukey's post-hoc comparisons. The target temperature of 5°C was statistically significantly different from 4°C, 20°C, and 25°C on sampling day 0. The target temperature of 4°C was significantly different when compared to 5°C, 20°C, and 25°C on sampling days 3 and 27. On sampling days 12 and 24, the target temperature of 4°C was significantly different when compared to 5°C. There was no statistically significant difference between the average PERC concentration and the target temperature groups on sampling days 7, 9, and 21.

Multiple Comparisons

Dependent Variable: PPM					
Tukey HSD	P-value				
Sampling Day	Target Temp	Target Temp.			
		4	5	20	25
0	4		0.000	0.428	0.970
	5	0.000		0.000	0.000
	20	0.428	0.000		0.236
	25	0.970	0.000	0.236	
3	4		0.008	0.006	0.005
	5	0.008		0.998	0.990
	20	0.006	0.998		0.999
	25	0.005	0.990	0.999	
7	4		0.471	0.986	1.000
	5	0.471		0.663	0.463
	20	0.986	0.663		0.985
	25	1.000	0.463	0.985	
9	4		0.809	0.720	0.352
	5	0.809		0.259	0.090
	20	0.720	0.259		0.906
	25	0.352	0.090	0.906	
12	4		0.040	0.178	0.658
	5	0.040		0.811	0.269
	20	0.178	0.811		0.732
	25	0.658	0.269	0.732	
15	4		0.011	0.288	0.010
	5	0.011		0.255	1.000
	20	0.288	0.255		0.240
	25	0.010	1.000	0.240	
18	4		0.012	0.015	0.830
	5	0.012		1.000	0.003
	20	0.015	1.000		0.003
	25	0.830	0.003	0.003	
21	4		0.257	0.980	0.190
	5	0.257		0.427	0.997
	20	0.980	0.427		0.329
	25	0.190	0.997	0.329	
24	4		0.006	0.577	0.166
	5	0.006		0.060	0.270
	20	0.577	0.060		0.784
	25	0.166	0.270	0.784	
27	4		0.000	0.000	0.000
	5	0.000		0.893	0.214
	20	0.000	0.893		0.537
	25	0.000	0.214	0.537	

Table 4.7. Tukey's post hoc test for average PERC concentration between temperatures on each sampling day.

PERC CONCENTRATION WITH TIME AND TEMPERATURE COMBINED

The mean PERC concentration was compared between all target temperatures, all sampling days (time), and both of them (time and temperature) simultaneously. To identify the relationship between temperature, sampling day, and concentration, multiple linear regression with dummy variables was used. Recall that the use of a dummy variable, also known as an indicator variable, allows for the identification of different categories within a qualitative variable (12).

Table 4.8 presents the results of the multiple linear regression, more specifically, the quadratic regression with the use of dummy variables. Moving from one model to the next, the change in R^2 represents how adding additional variables to the previous model improves the R^2 value. When using a quadratic regression model, R^2 measures the proportion of the variation in your dependent variable (PERC concentration) explained by your independent variables (time and temperature). The adjusted R^2 allows to provide an R^2 value as it accounts for the number of predictors in the model (25).

These models utilize 4ºC (labeled as "Constant" in table 4.8) as the reference category and sampling day 0 as the baseline. In the first model the term "R^2 change" indicates the total variation in PERC concentration that is attributed to time. Total R^2 is the cumulative variation when adding other independent variables, but for this model, as it is only presenting time, the total R^2 and R^2 change are the same. A 14% variation in PERC concentration can be attributed to time, and the p-value for this model is statistically significant ($p < 0.001$). The total R^2 of 7.1% in the second model describes the variability in PERC concentration caused by only the target temperature. When adding the individual variation from time and temperature, the overall R^2 is 21%. This

means that 21% of the variation in PERC concentration is attributed to the independent effects of time and temperature. This second model is also statistically significant ($p < 0.004$). The third model considers the combined effect of time and temperature which are represented by an R^2 value of 20.7%. When adding the individual effects of time and temperature to generate their combined effect, 41.7% of the variation in PERC concentration can be attributed to time, temperature, and their combined effect. This third model is also statistically significant ($p < 0.001$).

Model Summary

	B	Std. Error	P-value	R^2 change	Total R^2	P-value for R^2 change	
(Constant) 4°C	1.463	0.411	<0.001				
Day	0.283	0.070	<0.001				
Day²	-0.007	0.002	0.006	0.140	0.140	<0.001	Model 1
Temp 5°C	2.251	0.581	<0.001				
Temp 20°C	-0.085	0.581	0.884				
Temp 25°C	-0.650	0.581	0.265	0.210	0.071	0.004	Model 2
Day_Temp 5°C	-0.463	0.099	<0.001				
Day_Temp 20°C	-0.023	0.099	0.817				
Day_Temp 25°C	0.102	0.099	0.303				
Day²_Temp 5°C	0.012	0.003	<0.001				
Day²_Temp 20°C	-0.001	0.003	0.726				
Day²_Temp 25°C	-0.006	0.003	0.091	0.417	0.207	<0.001	Model 3

Dependent Variable: PERC Concentration (PPM)

Table 4.8. Multiple linear regression using dummy variables.

CHAPTER 5: Discussion and Conclusion

This study was initially designed to determine whether the EPA guidelines (holding time and temperature) for analyzing PERC in water samples could be extended. PERC samples were tested and maintained at a pre-determined temperatures (4ºC, 5ºC, 20ºC, and 25 ºC) over 27 days. Mixing time analysis determined the adequate amount of mixing time for water samples to achieve maximum dissolved concentration of PERC. The expected results throughout the research was to see an increase in the concentration of PERC in the early days of the analysis and to see a decrease in the later part of the study. Although the data does suggests that PERC concentration is reduced with time, it cannot be confirmed due to problems with the first day of sampling. The findings of this study cannot be interpreted as scientific results, but rather as considerations or suggestions.

CALIBRATION STANDARDS

A calibration curve was developed on the first day of the study and subsequently on every day before sampling. Three calibration standards were prepared and used to create the calibration curve per the analytical requirements in EPA Method 524.2 (33). The coefficient of determination (R^2) measures how well the model fits the calibration standards, the closer R^2 is to 1, the better the model can predict the value of the dependent variable (PERC concentration) (25). The R^2 obtained on Day 0 was 0.99969.

On sampling day 0, a calibration curve was developed and per recommendations it was intended that this calibration curve would be used for the subsequent days of analysis.

During sampling day 3, there were challenges in the reproducibility of the 1 ppm standard after 3 subsequent standard preparations, the 1 ppm standard was substituted with a 3 ppm standard. This standard was incorporated from sampling day 7 until the completion of the study, along with the 5 and 10 ppm standards. One reason for not being able to reproduce the 1 ppm standard could be the high temperature of the room on the day that calibration standards were prepared (27.7ºC), which could have accelerated the volatilization of PERC. Several attempts throughout the study were made to develop the 1 ppm standard (sampling day 9, 15, and 24), but all of them were unsuccessful. Having a higher calibration standard created concern that the data obtained from that point forward could be considered to be invalid; however, much of the raw materials were used.

The calibration curve in the first 2 iterations was prepared by adding the desired amount of PERC directly from the microsyringe to the 100mL volumetric flask. A corrective action was to first create one 100 mL stock solution of 100 ppm PERC. This solution was used to create the 3, 5, and 10 ppm solutions by diluting the required volumes into 3 separate 100mL volumetric flasks. This new plan saved materials for preparing new buffer solutions, if needed, and minimized the sources of error in comparison to the initial approach in which PERC was transferred from a microsyringe into a much larger volume (34).

TEMPERATURE LOGGERS

The temperature frequency distributions of the equipment used to hold the samples at the target temperatures were based on the statistical calculations performed using SPSS 25.0 and provided in Table 4.2. For example, the average temperature of each holding device demonstrated that each target temperature was achieved except for 20ºC,

where the average was 18.8°C. The target temperature of 4ºC ranged from -3.7°C to 12.5°C. After review of the thermologgers data, the samples were kept at the below freezing between 135-180 minutes every 8 hours. The highest temperature recorded on the thermologgers (12.5°C) only took place for 90 minutes during the fourth day of the study. All of the temperature controlling devices were tested prior to use and the temperature recorded for 96 hours and no evidence was found. These shifts in temperature could have caused changes in the solubility of PERC. All digital thermometers were set up to record data every 45 minutes. Increasing the frequency of data collection to every 15 minutes could have provided a more refined temperature profile to help determine the duration of exposure as this can affect the concentration of the samples being analyzed (27).

PERC CONCENTRATION

Holding Time Effect Analysis Using Quadratic Regression

The data presented in Table 4.5 suggest that in 2 out of the 4 target temperatures (4ºC and 25ºC) there was a statistically significant difference in PERC concentration with respect to time. Colder temperatures enable increased precipitation of PERC, whereas higher temperatures accelerate its volatilization. The temperature profile of the water is important and will influence the type of sampling approach to be implemented to better characterize the area under consideration.

When reviewing the graphs in Figure 4.5, the distribution for the target temperatures of 4ºC and 20ºC are more dispersed than for 5ºC and 25ºC. The direction of the quadratic line for 5ºC is inverse, meaning that the β_0 value from the equation $Y = \beta_0 + \beta_1 X + \beta_2 X^2$ is negative, whereas the other three values are positive since the line is

increasing (25). In Figure 4.5a, the concentration of PERC increases over time because it was dissolving until the midpoint of the study, giving an average concentration of greater than 5 ppm. Two of the samples contained air bubbles, allowing PERC to favor the gas phase rather than the liquid phase due to its tendency to volatilize. Additionally, if the fiber is exposed for longer than the 45 seconds established for this study, the increased exposure will be reflected in the results.

In Figure 4.5b the formula obtained from the regression indicates that PERC concentration will be decreasing (negative β_1 value) and is followed by a gradual increase in PERC concentration (β_2 value). This can be explained by the density property of PERC (DNAPL) and its low solubility which initially delayed the distribution of PERC throughout the sample and allowed to be volatilized faster at the 5ºC holding temperature. One important observation from the graphs is that on sampling day 0, the average PERC concentration of the samples was not close to the initial target concentration of 5 ppm.

From each 1 L batch of phosphate buffer, 24 samples were produced, and therefore two batches of phosphate buffer could produce 48 samples. However, for each target temperature only 40 samples needed to be analyzed. For this reason, some target temperatures included samples that were produced from different batches of buffer. These samples were not clearly identified to maintain randomness when choosing the 4 replicate samples used in further testing (28). For this reason, discrepancies in the data could not be attributed to one specific buffer batch.

Effect Of Temperature On PERC Concentration Using One-Way ANOVA

The use of a one-way ANOVA allows for comparison of all target temperatures against each sampling day in the study. Tukey's post-hoc test allows for the identification

of which temperature(s) has a statistically significant difference in PERC concentration. The information obtained from one-way ANOVA provided relevant information about how the temperature can affect the concentration of PERC in water samples. The results shown in Table 4.7 indicate that on all sampling days except for days 7, 9, and 21, there was a statistically significant difference in PERC concentration due to the temperature. The target temperature that had the greatest difference in PERC concentration was 4ºC. This target temperature had a higher PERC concentration on 7 of the 10 sampling days. Although not conclusive, because PERC is denser than water and the samples were held at an average temperature of 3ºC, it is probable that the combination of the temperature and the solubility of PERC at this temperature allowed it to be maintained within the sample. In summary, the 5ºC and 25ºC temperatures used in this study suggests that they both have an effect in the available PERC concentration in water samples.

Combined Effect Analysis Using Quadratic Regression With Dummy Variables

Quadratic regression with dummy variables allows a comparison of the effect that each independent variable has (individually and combined) on the PERC concentration. Interpretation of the results in Table 4.8 cannot be used as a validation tool due to discrepancies during sample preparation (discussed in the limitations section). Nevertheless, the data in Table 4.8 does provide helpful information in regards to the effect of each independent variable on the PERC concentration. The first model (day + day^2) indicates that 14% of the variation in PERC concentration is attributed to changes in time. As this is the first independent variable considered, the value of R^2 change is the same percentage as its variation. The second model (including all target temperatures), 7.1% (R^2 change) of the variation in PERC concentration is attributed to the temperature.

Regarding the R^2 value for that same row, adding the R^2 change from the first and second model, it generates the R^2 for the second model (equivalent to 21% of the variation). When considering the combined effect of both time and temperature, there is a 20.7% variation in PERC concentration. The R^2 for this part of the model considers the previous models, which indicates that for the complete model (individual independent variables and their combined influence on PERC concentration); 41.7% of the variation in PERC concentration is attributed to time and temperature combined. Because the interaction terms are highly significant ($F(6,148) = 8.744$, $p < 0.001$) it is best to evaluate the effect of time separately for each target temperature as the main effect may be misleading.

During the sample preparation process, several errors/limitations during data acquisition interfered with interpretation of results from being interpreted as scientific findings. One of these limitations was the determination of an adequate mixing time for PERC in the phosphate buffer solution prior to dispensing it into the sample vials. To address this limitation, the proper mixing time for PERC in the buffer solution was assessed, and identified.

SUBSET ANALYSIS

Mixing Time Identification

An additional investigation was performed to establish the adequate mixing time needed to thoroughly mix PERC in the water samples. The same phosphate buffer solution utilized for the sample preparation was used and added to a 250 mL volumetric flask. The pH was measured to confirm the target pH of 7.2. Hydrochloric acid and sodium hydroxide were used to adjust the pH as needed.

After filling the flask (Lurex, Type A flask, Sigma Aldrich, St. Louis, MO) to the respective fill line, the next step was to identify the volume needed to fill the bottle to the top. The objective was to minimize the headspace in the container and allow the PERC to mix with the water sample. The amount required to fill the flask to the top was 13 mL, resulting in a required sample volume of 263 mL. Once the flask was filled and a flask cover used, an additional step was to cover the top portion of the flask cover with Parafilm®M 4"x250" (Oshkosh, WI) and placed on a magnetic stir plate. The speed selected was 5 out of a maximum of 10. All subsequent tests used the same speed, and only the stirring time was varied. The stirring times analyzed were 1, 2, 3, and 4 hours. Upon completion of the mixing times, four 40 mL samples were extracted and analyzed in the HAPSITE®ER. Based on the results obtained for the first four hours, additional sampling was needed. The coefficient of variation (CV) was calculated to assess whether the PERC had been thoroughly mixed, and the mixing time was adjusted (increased or reduced) accordingly.

Mixing Time Statistical Analysis

Descriptive statistics, such as the coefficient of variation (CV) were used to compare the dispersion of the data. The coefficient of variation expresses the standard deviation as a percentage of the mean and its distribution around the mean (12). The formula is provided in Equation 8 where the calculated standard deviation of the samples (s) is divided by the average ($\overline{x}$) and multiplying by 100%.

Equation 8: Coefficient of variation

$$\text{CV} = \frac{s}{\overline{x}}(100)\% \tag{8}$$

Where:

CV = Coefficient of variation
s = Standard deviation of the samples
$\overline{x}$ = Sample average

RESULTS OF THE MIXING TIME ANALYSIS

Table 5.1 presents the results of the mixing time analysis. The first two sample runs (1 and 2 hour mixing times) yielded CVs of 23% and 10%, respectively. The next two sample runs were mixed for 3 and 4 hours giving CVs of 38% and 24%, respectively. The objective was to obtain two consecutive CVs where the difference between them was less than 5%.

A retrial for the 3 hours sample was also performed to determine the reliability of the previously obtained CV. The second iteration of the 3 hour mixing (retrial 3) produced a high percent error (26%).

The final sample runs were prepared using the 2 hour mixing time as a target. Therefore, they were mixed for 2.5 and 1.5 hours. The CV calculated for the 2.5 hour mixing time was also high (37%) when compared to the 2 hour mixing time. However, the 1.5 hour mixing time generated a CV of 14%. An additional sample test with a mixing time of 1.5 hours was prepared to confirm that the result obtained was indeed accurate. The CV of the 1.5 hour retrial was 15%.

The preliminary results of the PERC's mixing time were between 1 and 1.5 hours; however, no additional samples were performed because the materials used for the phosphate solution were exhausted. An effort was made to purchase the same products from the same manufacturers, but two of the materials were unavailable in the timeframe needed.

Subset Analysis to Determine Mixing Time					
Hour	Area (Avg)	N	Std. Deviation	% Error (CV)	Avg. Temp (℃)
1	108,255,360	4	25,206,956	23%	25.1
2	173,708,580	4	16,991,558	10%	24.3
3	42,311,838	4	16,106,401	38%	23.8
4	66,835,957	4	16,319,528	24%	22.6
Retrial 3	26,759,394	4	6,951,930	26%	18.4
2.5	49,876,892	4	18,317,331	37%	20.1
1.5	32,105,729	4	4,370,776	14%	20.9
Retrial 1.5	42,925,267	4	6,416,358	15%	20.4

Table 5.1. Coefficient of variation results for the subset analysis

MIXING TIME ANALYSIS USING CV

An additional study was included in the research as part of validating the mixing time identified in the pilot study. The pilot study determined that a mixing time of 3.5 hours was needed for 5 ppm of PERC to be mixed in 1L of buffer. Some of the items modified from the pilot study to this subset study were the removal of the headspace in the flasks and the concentration that was used. Adding 8μL of PERC to the volumetric flask yielded a concentration of 49.23 ppm. The objective of using such concentration was to be able to observe the behavior of PERC (mixing, settling on the bottom or the generation of gas) during mixing. Additionally, using the same type of flask, an agitator, a whole number for the volume of PERC (i.e., no decimals), and preparing the buffer solution the same day of the analysis were components considered to be sources of error (34). Throughout the mixing times, several observations were noted regarding the presence of PERC in the samples (related photos are provided in Appendix B.

Table 5.1 shows the averages of the four replicates and the coefficient of variation values (also known as percent error) for each sample run performed. The sample for 2 hours generated the lowest CV. The subsequent runs were carried out aiming for a CV of

less than 5%. None of the samples achieved this target, although the results in Table 5.1 demonstrate that the mixing time for PERC in a buffer solution with a pH of 7.2 is between 1.5 and 2.5 hours.

An additional aspect of this study is the differences in sample areas between the first samples analyzed (1 through 4 hour mixing times) and the last four samples (retrial runs and the 1.5 and 2.5 hour mixing times). The first four samples used a buffer solution prepared on a 1 L volumetric flask. The remaining samples were made from buffer prepared in a 2 L flask, where the initial pH was 6.6. Some of the components of the buffer preparation already contain sodium and potassium, elements that have a high affinity for water. Upon adding sodium hydroxide, the dissolved ions will bind to water molecules and potentially have an effect in the solubility of PERC. This concept is known as the "salting-out effect", although not quantified nor measured for this study, salting out could have an effect on the amount of PERC that can dissolve in the sample (37).

CONCLUSION

The following discussion identifies project gaps and recommendations for future research. In addition to the main research goals, a small study was undertaken to identify the time required to adequately mix PERC in a phosphate buffer solution.

The purpose of this study was to identify the effect on the concentration of PERC in water samples when the storage temperature and holding times outlined in EPA Method 524.2 are changed. The effect of holding time, temperature, and both parameters combined on the concentration of PERC in water samples could not be proven with any amount of certainty. In addition to time and temperature, other factors such as agitation

(mixing time), headspace in the flask, and the selection of a phosphate buffer solution could have contributed to changes in the PERC concentration. Such factors along with the physical properties of PERC (solubility, density, and vapor pressure) presented challenges in preparing the water samples. The microsyringe utilized measured in increments of 0.10μL; however, the amount of PERC that needed to be measured was 6.16μL. This meant that each sample received 6.2μL of PERC instead of 6.16μL. During the preparation of the buffer solutions, several batches required an adjustment of the pH by adding HCl, and the amount used differed among them. All of factors added variability to the study which led to unexpected changes.

The final part of the study included developing a series of test runs to more precisely identify the mixing time that the buffer solution needed before dispensing it into vials for storage and future analysis. Potential sources of error such as transferring the sample from one container to another, problems with maintaining the flask covered during mixing, and using different types of glassware were identified during the pilot phase. The effects that the buffer solution could have on the PERC concentration were not anticipated. In addition to the effects of time and temperature, the salting out effect may have impacted the distribution of PERC throughout the study, generating concentration differences (11).

Assumptions and Limitations

a) The physical properties of PERC were limiting factors recognized during the initial preparation of the study. However, consideration of the effect that a phosphate buffer solution can have on the physical properties of PERC was not anticipated (salting out).

b) The initially determined mixing time for PERC in a 1 L buffer solution was 3 to 3.5 hours. After performing the subset study for verification, the determined mixing time was reduced to 1.5 to 2 hrs. Limited availability to procure additional materials did not allowed to continue a more thorough subset study.

c) Once the buffer solutions were prepared and PERC added to the 1 L flask, the contents were then transferred to the sample vials. Each batch (1 L flask) was able to fill a total of 24 samples. Since a total of 40 samples were needed for each target temperature, more than 1 batch of buffer was required. Herein lies the source of error as no labeling system was implemented to identify the buffer batch used to prepare the samples.

Future Research

Future research is necessary to further investigate the effect of time and temperature on the concentration of PERC in water samples. Potential changes that could be implemented in future research include:

a. Combine the use of a micro-solid-phase extraction (μ-SPE) with a headspace single drop microextraction (HS-SDME) per Bagheri, et al., 2015. The combined use these techniques could prevent the loss of volatile organic compounds due to volatilization and lead to high recovery of the analytes after extraction.

b. Reduce the exposure of the sample to the air by incorporating a bottle top dispenser per Peng, et al., 1998. The positive displacement technique to transfer the liquid sample directly from the test solution bottle to the headspace vials can minimize the chemical loss due to volatilization.

c. Compare the PERC concentration in two different water matrixes, deionized water and buffer, to determine the impact of salting out on the PERC concentration.
d. If funding allows it, purchasing calibration standards rather than preparing the standards throughout the study can enhance the calibration curve and reduce errors.
e. Prepare all samples in a controlled environment. Dispensing the samples from a container that does not have any headspace could enhance the analysis to minimize PERC loss due to volatilization.

APPENDIX A

Day	Area	PPM	Date	File Number	Temp. Logger 1 Batch	pH
0	3,968,560	1.21	4-Feb-19	20190204_025	21.2	5	7.2
0	2,609,809	0.95	4-Feb-19	20190204_026	21.2	5	7.2
0	4,901,282	1.38	4-Feb-19	20190204_027	21.2	5	7.2
0	6,123,210	1.61	4-Feb-19	20190204_028	21.2	5	7.2
3	13,433,361	2.98	7-Feb-19	20190207_002	2.2 (1000)	6	7.2
3	6,088,852	1.60		20190207_003	2.9 (1055)	6	7.2
3	16,306,074	3.51		20190207_004	3.8 (1219)	6	7.2
3	13,494,026	2.99		20190207_005	5.7 (1545)	6	7.2
7	3,258,315	2.60	11-Feb-19	20190211_023	4.9 (1840)	6	7.2
7	2,632,750	2.49		20190211_024	4.6 (1900)	6	7.2
7	6,353,218	3.17		20190211_025	4.4 (1925)	6	7.2
7	6,088,064	3.12		20190211_026	4.3 (1940)	6	7.2
9	2,863,696	1.66	13-Feb-19	20190213_012	4.8 (1120)	6	7.2
9	3,553,393	2.11		20190213_013	4.6 (1440)	6	7.2
9	5,074,439	3.11		20190213_014	4.8 (1505)	6	7.2
9	8,036,232	5.04		20190213_015	6.1 (1520)	6	7.2
12	13,991,059	4.23	16-Feb-19	20190216_024	3.2 (1625)	6	7.2
12	15,272,944	4.67		20190216_025	4.5 (1640)	6	7.2
12	9,386,699	2.67		20190216_026	4.8 (1700)	6	7.2
12	9,037,880	2.55		20190216_027	5.1 (1730)	6	7.2
15	16,887,846	5.90	19-Feb-19	20190219_006	0.7 (1025)	6	7.2
15	12,437,705	4.65		20190219_007	3.2 (1340)	6	7.2
15	12,349,860	4.62		20190219_008	2.0 (1355)	6	7.2
15	21,218,614	7.13		20190219_009	3.2 (1410)	6	7.2
18	7,532,977	3.72	22-Feb-19	20190222_019	6.3 (1430)	6	7.2
18	8,673,273	4.11		20190222_021	6.4 (1510)	6	7.2
18	7,170,065	3.60		20190222_023	5.7 (1600)	6	7.2
18	10,297,566	4.66		20190222_025	5.3 (1650)	6	7.2
21	9,005,725	4.44	25-Feb-19	20190225_017	4.0 (1625)	6	7.2
21	9,095,968	4.47		20190225_018	4.8 (1655)	6	7.2
21	9,285,417	4.54		20190225_019	4.8 (1725)	6	7.2
21	10,306,436	4.88		20190225_020	3.4 (1805)	6	7.2
24	7,570,340	2.71	28-Feb-19	20190228_002	0.9 (0850)	6	7.2
24	10,943,988	3.53		20190228_003	1.5 (0915)	6	7.2
24	9,962,362	3.29		20190228_004	3.6 (0940)	6	7.2
24	10,081,978	3.32		20190228_005	4.1 (1000)	6	7.2
27	12,753,314	4.66	3-Mar-19	20190303_008	5.1 (0915)	6	7.2
27	12,541,194	4.60		20190303_009	5.2 (0925)	6	7.2
27	13,549,500	4.89		20190303_011	3.8 (0945)	6	7.2
27	13,427,898	4.86		20190303_012	3.4 (1000)	6	7.2

vial number	Vial observations prior to sampling	Observations during fiber exposure
0.1	no bubbles	some bubbles
0.2	no bubbles	some bubbles
0.3	no bubbles	one bubble
0.4	no bubbles	many bubbles
3.1	1 bubble inside the vial	1small bubble found after 5 sec
3.2	no bubbles	no bubbles
3.3	1 very small bubble; agitated for 1 min	small bubbles removed
3.4	1 medium bubble	no bubbles
6.1	no bubbles	1 small bubble quickly removed
6.2	no bubbles	1 small bubble removed
6.3	no bubbles	no bubbles
6.4	no bubbles	1 small bubble quickly removed
9.1	1 medium bubble	no bubbles
9.2	no bubbles	1 small bubble quickly removed
9.3	no bubbles	small bubbles removed
9.4	no bubbles	no bubbles
12.1	1 medium bubble	small bubbles removed
12.2	1 medium bubble	small bubbles removed
12.3	1 medium bubble	no bubbles
12.4	1 large bubble	no bubbles
15.1	no bubbles	no bubbles
15.2	no bubbles	no bubbles
15.3	large bubble	no bubbles
15.4	no bubbles	small bubbles removed
18.1	no bubbles	no bubbles
18.2	medium bubble	small bubbles removed
18.3	medium bubble	small bubbles removed
18.4	no bubbles	small bubbles removed
21.1	1 small bubble	small bubbles removed
21.2	1 small bubble	small bubbles removed
21.3	1 small bubble	small bubbles removed
21.4	no bubbles	no bubbles
24.1	small bubble	no bubbles
24.2	no bubbles	no bubbles
24.3	no bubbles	no bubbles
24.4	no bubbles	small bubbles removed
27.1	small bubble	no bubbles
27.2	no bubbles	small bubbles removed
27.3	small bubble	no bubbles
27.4	small bubble	small bubbles removed

Table A.1. Data collected for 4ºC.

Day	Area	PPM	Date	File Number	Temp. Logger 2 Batch	pH	vial number
0	24,924,802	5.13	4-Feb-19	20190204_030	23.2	4	7.2	0.1
0	21,284,118	4.45		20190204_031	23.2	4	7.2	0.2
0	22,067,790	4.59		20190204_032	23.2	4	7.2	0.3
0	32,858,218	6.61		20190204_033	23.2	4	7.2	0.4
3	2,472,411	0.93	7-Feb-19	20190207_009	5.2 (1626)	4	7.2	3.1
3	5,451,373	1.48		20190207_010	5.2 (1706)	5	7.2	3.2
3	8,283,577	2.01		20190207_011	5.3 (1723)	5	7.2	3.3
3	2,261,916	0.89		20190207_012	5.3 (1756)	5	7.2	3.4
7	4,464,930	2.82	11-Feb-19	20190211_015	4.4 (1545)	5	7.2	6.1
7	6,149,938	3.13		20190211_016	4.2 (1610)	5	7.2	6.2
7	6,959,875	3.28		20190211_017	4.0 (1635)	5	7.2	6.3
7	6,601,588	3.22		20190211_018	4.0 (1720)	5	7.2	6.4
9	3,221,856	1.90	13-Feb-19	20190213_016	4.6 (1535)	5	7.2	9.1
9	4,104,957	2.47		20190213_017	4.5 (1555)	4	7.2	9.2
9	3,907,437	2.35		20190213_018	4.6 (1610)	5	7.2	9.3
9	3,657,228	2.18		20190213_019	4.6 (1635)	5	7.2	9.4
12	5,960,695	1.50	16-Feb-19	20190216_007	5.3 (0825)	5	7.2	12.1
12	5,388,738	1.31		20190216_008	5.2 (0850)	5	7.2	12.2
12	7,514,710	2.03		20190216_009	5.8 (1225)	5	7.2	12.3
12	3,657,086	0.72		20190216_010	6.0 (1250)	5	7.2	12.4
15	4,754,937	2.48	19-Feb-19	20190219_002	5.4 (0910)	5	7.2	15.1
15	6,863,356	3.07		20190219_003	5.4 (0940)	5	7.2	15.2
15	5,975,188	2.82		20190219_004	5.6 (0955)	5	7.2	15.3
15	9,686,434	3.87		20190219_005	5.6 (1010)	5	7.2	15.4
18	3,543,340	2.36	22-Feb-19	20190222_002	5.7 (0940)	5	7.2	18.1
18	5,298,418	2.96		20190222_003	5.7 (1000)	5	7.2	18.2
18	4,453,120	2.67		20190222_004	5.8 (1020)	5	7.2	18.3
18	4,186,982	2.58		20190222_005	5.8 (1050)	5	7.2	18.4
21	6,495,539	3.59	25-Feb-19	20190225_013	5.2 (1510)	5	7.2	21.1
21	9,635,593	4.65		20190225_014	5.2 (1525)	5	7.2	21.2
21	3,967,681	2.73		20190225_016	5.4 (1550)	5	7.2	21.3
21	6,222,373	3.50		20190225_017	5.4 (1610)	5	7.2	21.4
24	3,455,246	1.70	28-Feb-19	20190228_007	5.2 (1015)	5	7.2	24.1
24	4,161,818	1.87		20190228_008	5.3 (1030)	5	7.2	24.2
24	4,334,327	1.92		20190228_009	6.4 (1050)	5	7.2	24.3
24	5,579,222	2.22		20190228_010	6.5 (1105)	5	7.2	24.4
27	7,322,204	3.08	3-Mar-19	20190303_013	5.2 (1135)	5	7.2	27.1
27	4,149,234	2.15		20190303_014	5.2 (1155)	5	7.2	27.2
27	6,435,915	2.82		20190303_015	5.1 (1205)	5	7.2	27.3
27	3,498,379	1.96		20190303_016	5.1 (1230)	5	7.2	27.4

Vial Observations prior to sampling	Observation during fiber exposure
two bubbles	two bubbles
no bubbles	no bubbles
three bubbles	three bubbles
no bubbles	no bubbles
no bubbles, agitated for 1 min (twice)	small bubbles removed quickly
medium bubble	no bubbles
1 small bubble (twice)	two bubbles removed
no bubbles, agitated for 1 min	tiny bubbles removed
no bubbles	1 bubble
no bubbles	1 bubble
no bubbles	1 bubble
1 bubble inside vial	small bubbles removed quickly
no bubbles	small bubbles removed quickly
no bubbles	small bubbles removed quickly
no bubbles	1 small bubble removed quickly
no bubbles	1 small bubble removed quickly
no bubbles	no bubbles
no bubbles	2 small bubbles removed quickly
no bubbles	1 small bubble removed quickly
no bubbles	small bubbles removed quickly
no bubbles	small bubbles removed quickly
no bubbles	no bubbles
no bubbles	small bubbles removed quickly
1 small bubble	small bubbles removed quickly
1 medium bubble	small bubbles removed quickly
1 small bubble	small bubbles removed quickly
no bubbles	small bubbles removed quickly
no bubbles	no bubbles
1 small bubble	small bubbles removed quickly
no bubbles	no bubbles
1 small bubble	no bubbles
1 small bubble	small bubbles removed quickly
1 small bubble	no bubbles
no bubbles	no bubbles
no bubbles	no bubbles
no bubbles	no bubbles
no bubbles	small bubbles removed quickly
no bubbles	no bubbles
no bubbles	small bubbles removed quickly
no bubbles	small bubbles removed quickly

Table A.2. Data collected for 5ºC.

Day	Area	PPM	Date	File Number	Temp. Logger 3	Batch	pH	vial number	Vial Observations prior to sampling	Observation during fiber exposure
0	5,496,458	1.49	4-Feb-19	20190204_034	23.5	2	7.2	0.1	some bubbles	
0	7,148,629	1.80	5-Feb-19	20190205_001	23.5	2	7.2	0.2	no bubbles	
0	8,138,188	1.99	4-Feb-19	20190205_002	23.5	2	7.2	0.3	one bubble	
0	9,807,707	2.30	4-Feb-19	20190205_003	23.5	2	7.2	0.4	one bubble	
3	5,683,422	1.53	7-Feb-19	20190207_020	18.2 (1820)	2	7.2	3.1	1 small bubble	tiny bubbles removed quickly
3	2,446,772	0.92		20190207_021	18.3 (1839)	2	7.2	3.2	1 small bubble (shaken 1 min)	no bubbles
3	4,870,771	1.38		20190207_022	18.3 (1850)	2	7.2	3.2	no bubbles (shaken 1 min)	1 small bubble removed quickly
3	4,039,372	1.22		20190207_023	18.3 (1910)	4	7.2	3.4	1 small bubble (shaken 1 min)	2 small bubbles removed quickly
7	3,835,869	2.71	11-Feb-19	20190211_019	15.4 (1730)	4	7.2	6.1	no bubbles	1 small bubble removed quickly
7	7,067,910	3.30		20190211_020	15.4 (1745)	4	7.2	6.2	no bubbles	1 bubble
7	4,575,704	2.84		20190211_021	15.3 (1800)	4	7.2	6.3	no bubbles	1 small bubble removed quickly
7	4,178,616	2.77		20190211_022	15.4 (1825)	4	7.2	6.4	no bubbles	1 small bubble remained
9	6,024,571	3.73	13-Feb-19	20190213_002	15.5 (0815)	4	7.2	9.1	no bubbles	no bubbles
9	2,871,260	1.67		20190213_003	15.7 (0850)	4	7.2	9.2	no bubbles	no bubbles
9	6,401,515	3.98		20190213_004	15.9 (0920)	4	7.2	9.3	no bubbles	no bubbles
9	9,678,935	6.12		20190213_005	16.3 (0945)	4	7.2	9.4	no bubbles	no bubbles
12	5,282,581	1.27	16-Feb-19	20190216_011	20.1 (1300)	4	7.2	12.1	no bubbles	2 small bubbles removed quickly
12	13,823,240	4.18		20190216_012	20.1 (1320)	4	7.2	12.2	no bubbles	no bubbles
12	5,148,632	1.22		20190216_013	20.1 (1400)	4	7.2	12.3	no bubbles	no bubbles
12	5,531,820	1.35		20190216_014	20.2 (1025)	4	7.2	12.4	no bubbles	no bubbles
15	16,781,518	5.87	19-Feb-19	20190219_012	18.9 (1450)	4	7.2	15.1	no bubbles	no bubbles
15	6,029,924	2.84		20190219_013	18.9 (1450)	4	7.2	15.2	no bubbles	no bubbles
15	11,410,514	4.36		20190219_014	19.0 (1540)	4	7.2	15.3	no bubbles	small bubbles removed quickly
15	11,257,042	4.31		20190219_015	19.0 (1600)	4	7.2	15.4	no bubbles	no bubbles
18	3,458,792	2.33	22-Feb-19	20190222_007	22.0 (1110)	4	7.2	18.1	no bubbles	small bubbles removed quickly
18	6,777,796	3.46		20190222_008	22.0 (1125)	4	7.2	18.2	no bubbles	no bubbles
18	4,697,681	2.75		20190222_009	22.0 (1140)	4	7.2	18.3	no bubbles	no bubbles
18	3,121,817	2.22		20190222_010	22.1 (1200)	4	7.2	18.4	no bubbles	small bubbles removed quickly
21	10,505,345	4.95	25-Feb-19	20190225_007	19.5 (1330)	4	7.2	21.1	no bubbles	small bubbles removed quickly
21	5,670,431	3.31		20190225_008	19.5 (1350)	4	7.2	21.2	no bubbles	small bubbles removed quickly
21	10,451,595	4.93		20190225_009	19.6 (1425)	4	7.2	21.3	no bubbles	small bubbles removed quickly
21	8,857,242	4.39		20190225_010	19.6 (1455)	4	7.2	21.4	no bubbles	small bubbles removed quickly
24	8,283,315	2.88	28-Feb-19	20190228_011	19.2 (1115)	4	7.2	24.1	no bubbles	small bubbles removed quickly
24	10,396,309	3.40		20190228_012	19.2 (1130)	4	7.2	24.2	no bubbles	no bubbles
24	8,759,108	3.00		20190228_013	19.2 (1145)	4	7.2	24.3	no bubbles	small bubbles removed quickly
24	4,529,759	1.96		20190228_014	19.2 (1200)	4	7.2	24.4	small bubble	no bubbles
27	3,862,352	2.07	3-Mar-19	20190303_018	18.7 (1240)	4	7.2	27.1	no bubbles	small bubbles removed quickly
27	7,355,911	3.09		20190303_019	18.7 (1300)	4	7.2	27.2	no bubbles	small bubbles removed quickly
27	2,351,600	1.63		20190303_020	18.8 (1320)	4	7.2	27.3	small bubble	small bubbles removed quickly
27	4,605,920	2.29		20190303_021	18.8 (1345)	4	7.2	27.4	no bubbles	no bubbles

Table A.3. Data collected for 20ºC.

Day	Area	PPM	Date	File Number	Temp. Logger 5	Batch	Ph	vial number	Vial Observations prior to sampling	Observation during fiber exposure
0	4,503,049	1.31	4-Feb-19	20190204_020	25.2	2	7.2	0.1	no bubbles	no bubbles
0	2,406,093	0.93		20190204_021	25.2	2	7.2	0.2	1 bubble	no bubbles
0	3,538,716	1.13		20190204_022	25.2	2	7.2	0.3	no bubbles	no bubbles
0	3,374,292	1.10		20190204_023	25.2	2	7.2	0.4	1 bubble	no bubbles
3	3,380,998	1.10	7-Feb-19	20190207_024	25.2 (1932)	2	7.2	3.1	no bubble, mixed 1 min	1 tiny bubble removed quickly
3	4,820,574	1.37		20190207_025	25.2 (1946)	2	7.2	3.2	no bubble, mixed 1 min	tiny bubbles removed quickly
3	4,516,889	1.31		20190207_026	25.1 (2004)	2	7.2	3.3	no bubble, mixed 1 min	tiny bubbles removed quickly
3	3,334,126	1.09		20190207_027	25.1 (2022)	2	7.2	3.4	no bubble, mixed 1 min	tiny bubbles removed quickly
7	3,643,522	2.67	11-Feb-19	20190211_027	24.9 (1950)	2	7.2	6.1	no bubbles	many small bubbles
7	4,570,748	2.84		20190211_031	24.9 (2010)	2	7.2	6.2	no bubbles	small bubbles
7	5,537,499	3.02		20190211_032	24.9 (2105)	2	7.2	6.3	no bubbles	small bubbles
7	4,568,850	2.84		20190211_033	25.0 (2140)	2	7.2	6.4	no bubbles	no bubbles
9	6,963,177	4.34	13-Feb-19	20190213_007	25.0 (0955)	2	7.2	9.1	no bubbles	1 small bubble removed quickly
9	6,693,630	4.17		20190213_008	25.0 (1015)	2	7.2	9.2	no bubbles	1 small bubble removed quickly
9	6,999,319	4.37		20190213_009	25.1 (1040)	2	7.2	9.3	no bubbles	2 bubbles removed quickly
9	7,799,647	4.89		20190213_010	25.1 (1100)	2	7.2	9.4	no bubbles	1 small bubble removed quickly
12	10,769,746	3.14	16-Feb-19	20190216_020	25.0 (1450)	2	7.2	12.1	no bubbles	very small bubbles attached for 4 se
12	10,960,773	3.20		20190216_021	25.1 (1540)	2	7.2	12.2	no bubbles	1 small bubble removed quickly
12	7,967,448	2.18		20190216_022	25.0 (1600)	2	7.2	12.3	no bubbles	small bubbles
12	8,521,954	2.37		20190216_023	25.0 (1620)	2	7.2	12.4	no bubbles	small bubbles
15	5,193,336	2.60	19-Feb-19	20190219_019	25.2 (1635)	2	7.2	15.1	1 small bubble	small bubbles removed quickly
15	6,215,884	2.89		20190219_020	25.1 (1700)	2	7.2	15.2	no bubbles	small bubbles removed quickly
15	7,622,375	3.29		20190219_021	25.2 (1715)	2	7.2	15.3	no bubbles	no bubbles
15	7,847,263	3.35		20190219_023	25.2 (1735)	2	7.2	15.4	1 small bubble	small bubbles removed quickly
18	7,156,075	3.59	22-Feb-19	20190222_012	25.4 (1240)	2	7.2	18.1	no bubbles	no bubbles
18	8,138,322	3.93		20190222_013	25.4 (1305)	2	7.2	18.2	no bubbles	small bubbles removed quickly
18	10,643,720	4.78		20190222_014	25.4 (1320)	2	7.2	18.3	no bubbles	small bubbles removed quickly
18	11,376,201	5.03		20190222_015	25.4 (1345)	2	7.2	18.4	no bubbles	small bubbles removed quickly
21	7,041,377	3.77	25-Feb-19	20190225_003	25.2 (1115)	2	7.2	21.1	no bubbles	one bubble removed quickly
21	9,426,302	4.58		20190225_004	25.2 (1145)	2	7.2	21.2	no bubbles	small bubbles removed quickly
21	4,651,468	2.96		20190225_005	25.2 (1300)	2	7.2	21.3	no bubbles	small bubbles removed quickly
21	4,046,427	2.76		20190225_006	25.2 (1315)	2	7.2	21.4	no bubbles	no bubbles
24	9,356,740	3.14	28-Feb-19	20190228_015	25.4 (1215)	2	7.2	24.1	no bubbles	small bubbles removed quickly
24	6,935,200	2.55		20190228_016	25.4 (1230)	2	7.2	24.2	small bubble	small bubbles removed quickly
24	6,195,026	2.37		20190228_017	25.2 (1240)	2	7.2	24.3	no bubbles	small bubbles removed quickly
24	4,754,363	2.02		20190228_018	25.4 (1345)	2	7.2	24.4	no bubbles	small bubbles removed quickly
27	1,603,061	1.41	3-Mar-19	20190303_023	25.2 (1350)	2	7.2	27.1	1 small bubble	small bubbles removed quickly
27	2,194,295	1.58		20190303_024	25.2 (1415)	2	7.2	27.2	no bubbles	small bubbles removed quickly
27	5,006,269	2.40		20190303_025	25.2 (1440)	2	7.2	27.3	no bubbles	no bubbles
27	3,201,200	1.88		20190303_026	25.3 (1455)	2	7.2	27.4	no bubbles	small bubbles removed quickly

Table A.4. Data collected for 25ºC.

Hour	Area	Date	File Number	Temp. Logger	Batch	Flask (263mL)	pH (before)	pH (after)	Vial observations prior to sampling	Observations during fiber exposure
1	104,163,760	26-Mar-19	20190326_002	4= 25.0 (1658)	16	1	7.2	7.3	no bubbles	no bubbles
1	125,171,272		20190326_003	4= 25.5 (1715)	16				no bubbles	no bubbles
1	129,420,832		20190326_004	4= 24.5 (1735)	16				no bubbles	no bubbles
1	74,265,576		20190326_005	4= 24.3 (1755)	16				no bubbles	no bubbles
2	148,230,000		20190326_007	1= 23.9 (1845)	16	2	7.3	7.4	no bubbles	no bubbles
2	181,755,984		20190326_008	1= 24.3 (1904)	16				no bubbles	no bubbles
2	182,038,560		20190326_009	1= 24.7 (1920)	16				no bubbles	no bubbles
2	182,809,776		20190326_010	1= 24.6 (1939)	16				no bubbles	no bubbles
3	27,492,258	27-Mar-19	20190327_002	1= 24.3 (1315)	16	1	7.2	7.6	no bubbles	no bubbles
3	64,650,408		20190327_003	1= 24.6 (1335)	16				no bubbles	no bubbles
3	34,593,224		20190327_004	1= 24.3 (1355)	16				no bubbles	no bubbles
3	42,511,460		20190327_005	1= 24.0 (1410)	16				1 tiny bubble	1 small bubble removed quickly
4	50,542,376		20190327_007	4= 21.0 (1555)	16	2	7.2	7.6	no bubbles	no bubbles
4	73,904,016		20190327_008	4= 21.9 (1610)	16				no bubbles	1 small bubble removed quickly
4	56,599,684		20190327_009	4= 21.5 (1625)	16				no bubbles	no bubbles
4	86,297,752		20190327_010	4= 21.4 (1640)	16				no bubbles	no bubbles
retrial 3	21,491,264	28-Mar-19	20190328_002	2= 19.6 (1310)	17	1	7.2	7.5	no bubbles	1 small bubble removed quickly
retrial 3	21,201,434		20190328_003	2= 20.4 (1325)	17				no bubbles	no bubbles
retrial 3	35,896,804		20190328_004	2= 20.8 (1340)	17				no bubbles	no bubbles
retrial 3	28,448,072		20190328_005	2= 21.0 (1355)	17				no bubbles	no bubbles
2.5	25,672,612		20190328_007	1= 19.8 (1458)	18	2	7.2	7.3	no bubbles	no bubbles
2.5	46,814,552		20190328_008	1= 22.4 (1515)	18				no bubbles	1 small bubble removed quickly
2.5	67,960,736		20190328_009	1= 22.1 (1531)	18				no bubbles	no bubbles
2.5	59,059,668		20190328_010	1= 21.8 (1550)	18				no bubbles	no bubbles
1.5	25,620,560		20190328_012	4 = 20.4 (1615)	18	1	7.2	7.0	no bubbles	no bubbles
1.5	33,362,298		20190328_013	4= 22.2 (1635)	18				no bubbles	no bubbles
1.5	34,667,380		20190328_014	4= 21.9 (1650)	18				no bubbles	no bubbles
1.5	34,772,676		20190328_015	4= 21.8 (1710)	18				no bubbles	no bubbles
retrial 1.5	52,073,648	29-Mar-19	20190329_013	2= 21.6 (1235)	18	1	7.2	7.0	no bubbles	small bubbles removed
retrial 1.5	42,392,612		20190329_014	2= 21.5 (1315)	18				no bubbles	no bubbles
retrial 1.5	39,716,716		20190329_015	2= 21.7 (1330)	18				no bubbles	1 small bubble removed quickly
retrial 1.5	37,518,092		20190329_016	2=21.3 (1410)	18				no bubbles	small bubbles removed

Table A.5. Pilot study to re-assess PERC mixing time.

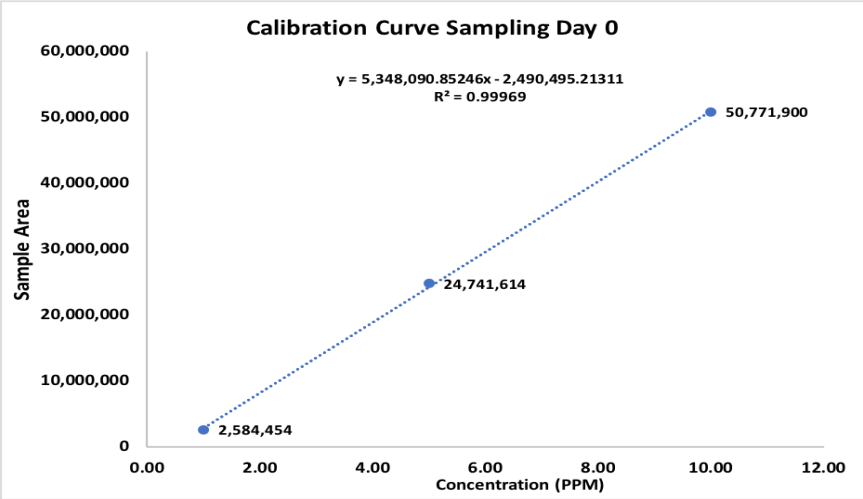

Figure A.1. Calibration Curve for Sampling Day 0.

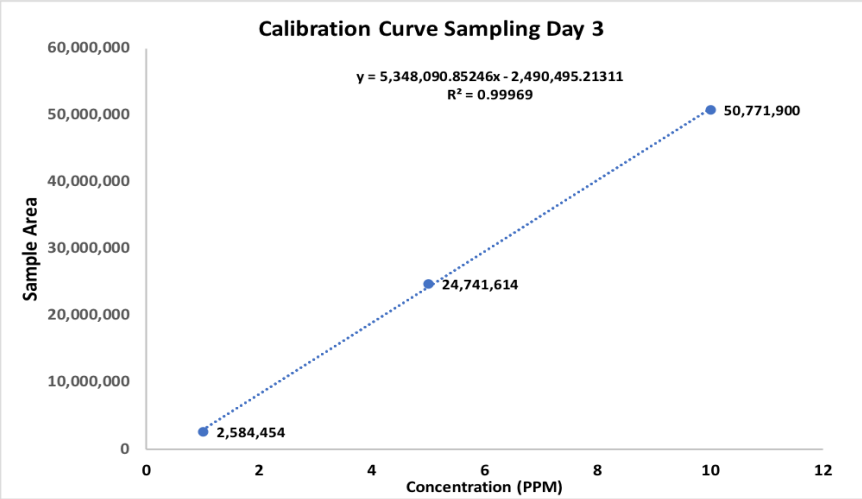

Figure A.2. Calibration Curve for Sampling Day 3.

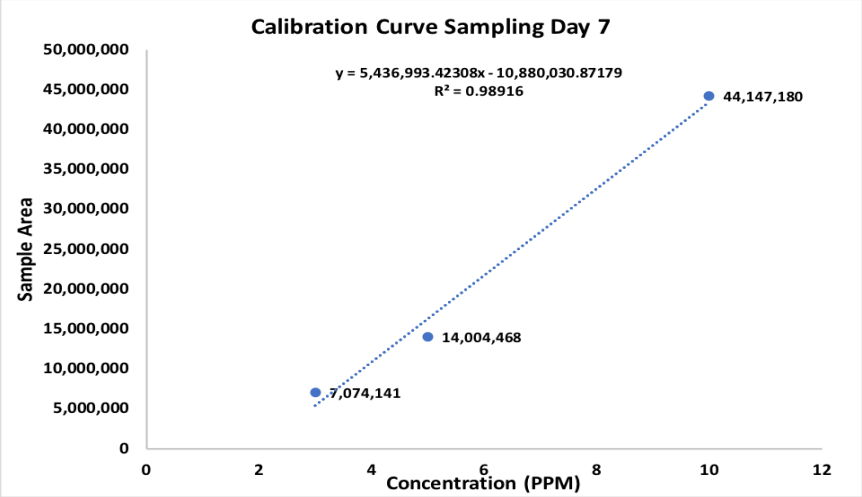

Figure A.3. Calibration Curve for Sampling Day 7.

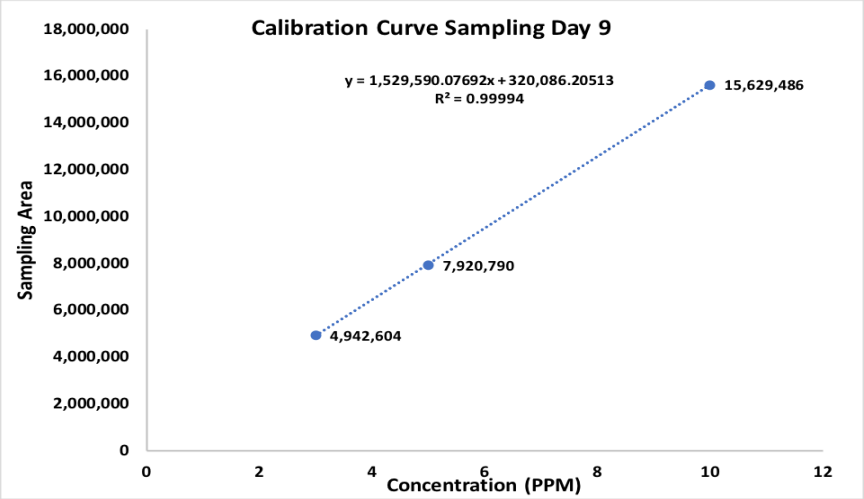

Figure A.4. Calibration Curve for Sampling Day 9.

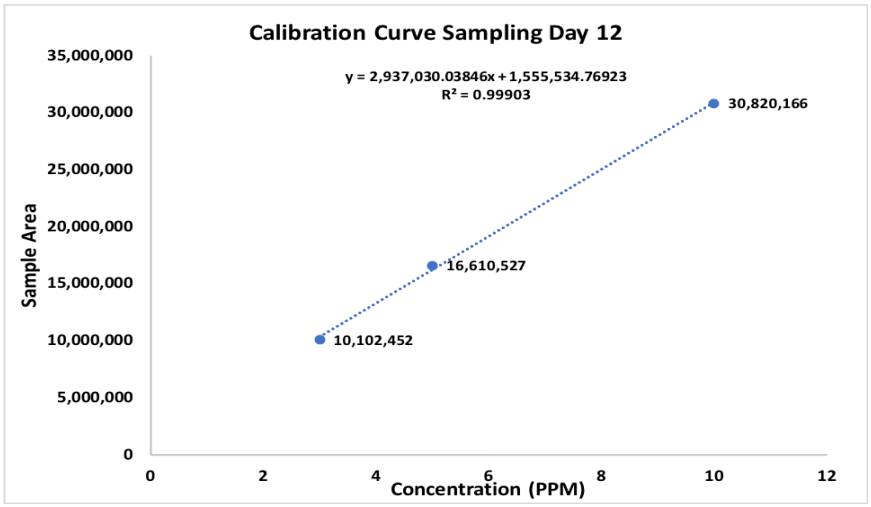

Figure A.5. Calibration Curve for Sampling Day 12.

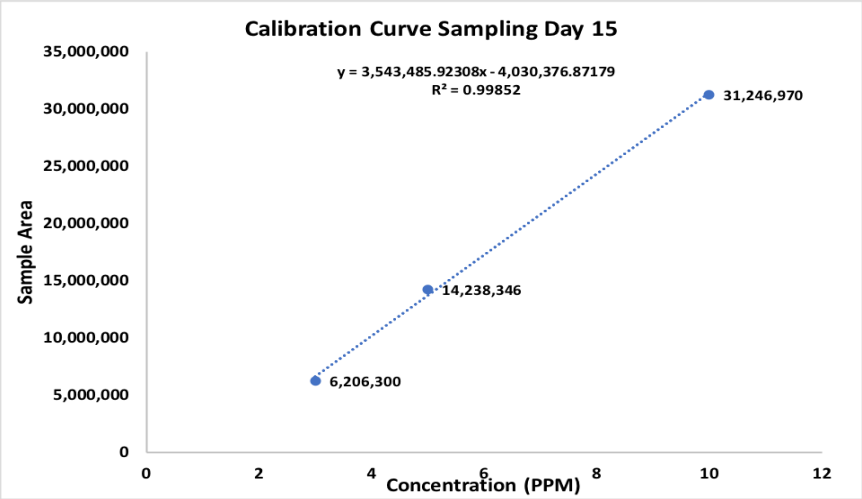

Figure A.6. Calibration Curve for Sampling Day 15.

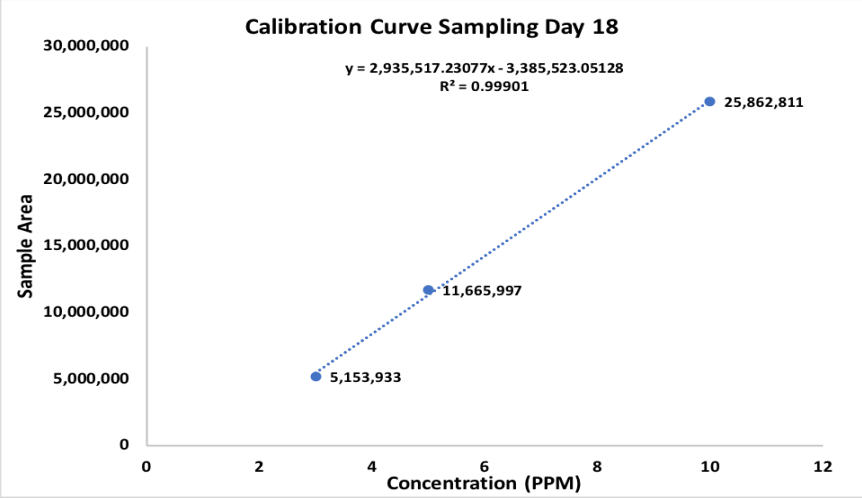

Figure A.7. Calibration Curve for Sampling Day 18.

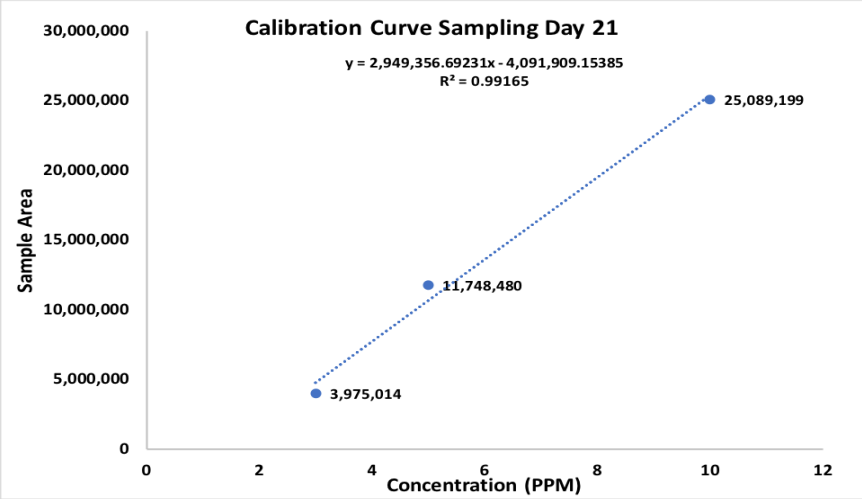

Figure A.8. Calibration Curve for Sampling Day 21.

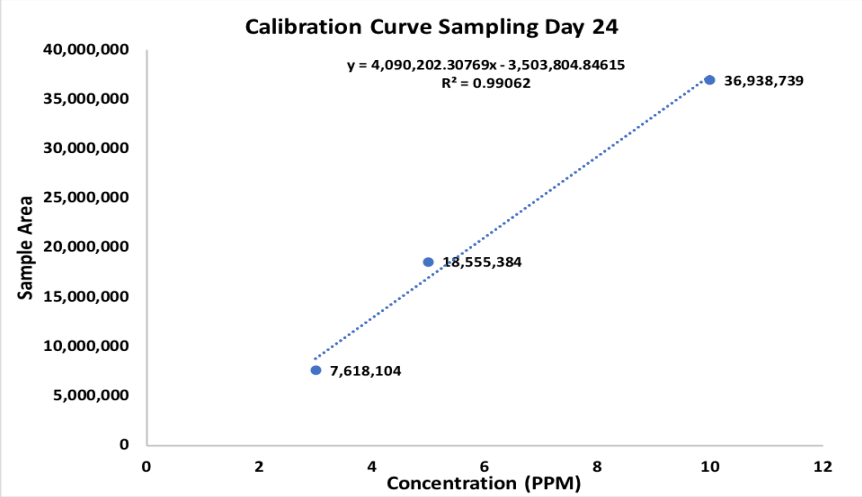

Figure A.9. Calibration Curve for Sampling Day 24.

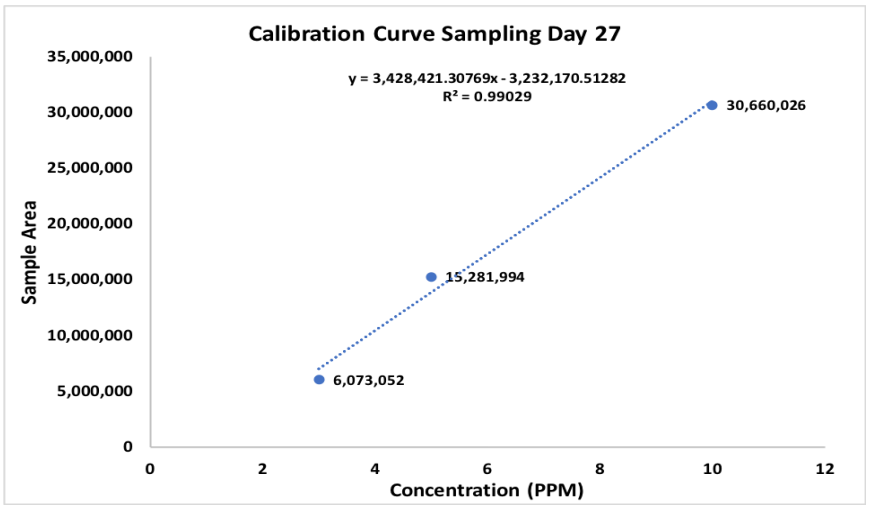

Figure A.10. Calibration Curve for Sampling Day 27.

APPENDIX B

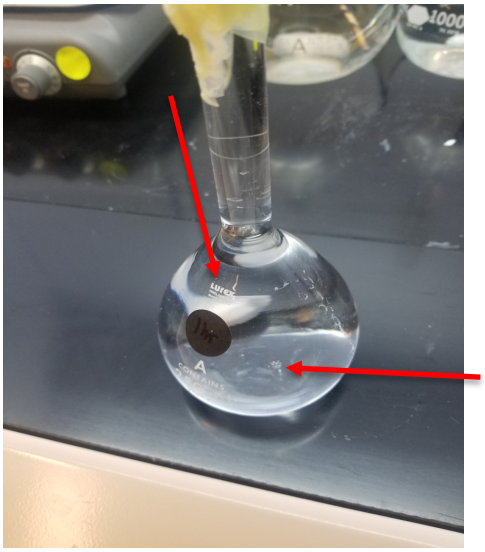

Figure B.1. Subset analysis after mixing for 1 hour showing droplet formation at the bottom of the flask and over the agitator.

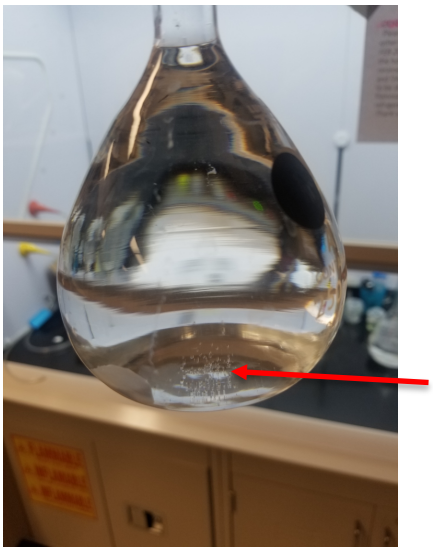

Figure B.2. Subset analysis after mixing for 2 hours. Droplets are distributed in a wide area at the bottom of the flask.

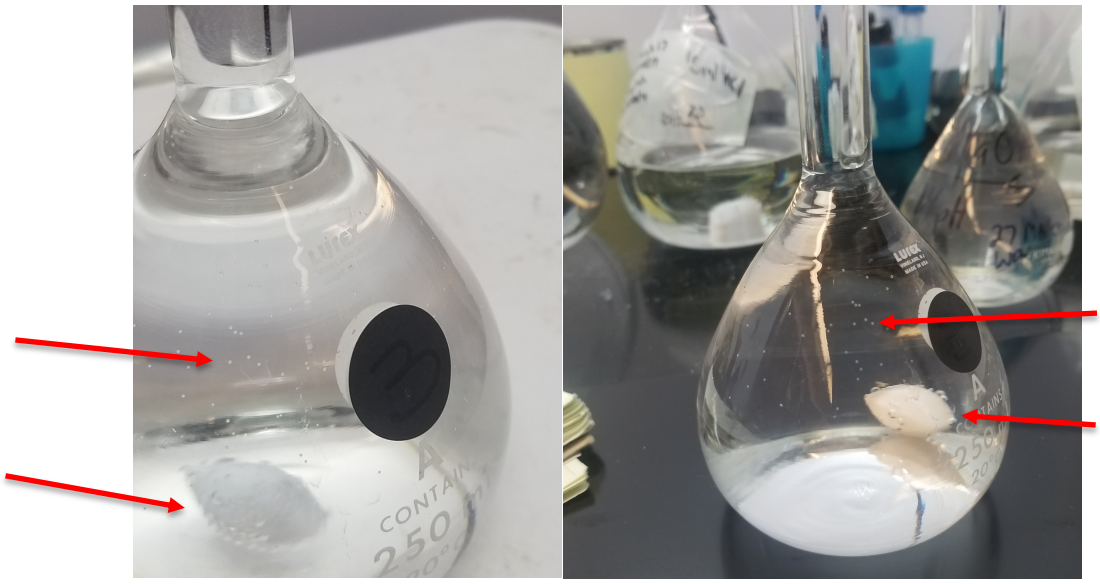

Figure B.3. Subset analysis after mixing for 3 hours. A closer look (left) and a wider view (right) at the droplets in the flask and agitator.

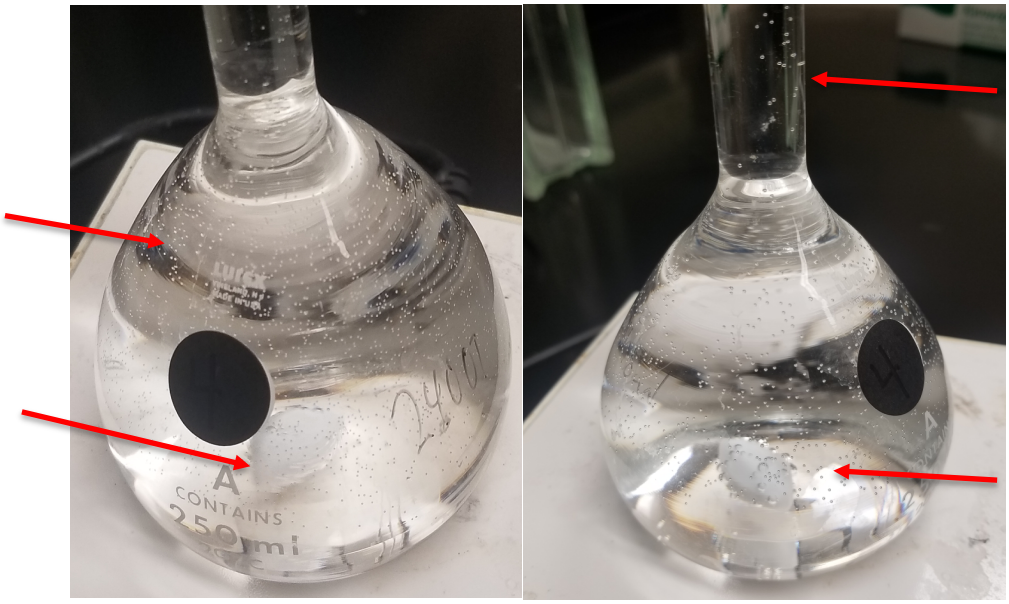

Figure B.4. Subset analysis after mixing for 4 hours. A wider distribution of droplets throughout the flask. Several droplets are moving towards the top.

REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR). 2015. *ATSDR Toxic Substances Portal, Toxicological Profile for Tetrachloroethylene (PERC)* https://www.atsdr.cdc.gov/toxprofiles/tp18.pdf
2. Agency for Toxic Substances and Disease Registry (ATSDR). 2015. *ATSDR Toxic Substances Portal, Toxicological Profile for Tetrachloroethylene (PERC)*. https://www.atsdr.cdc.gov/toxprofiles/tp18.pdf
3. Agency for Toxic Substances and Disease Registry (ATSDR). 2017. *Camp Lejune Drinking Water Public Health Assessment*. https://www.atsdr.cdc.gov/HAC/pha/MarineCorpsBaseCampLejeune/Camp_Lejeune_Drinking_Water_PHA(final)_%201-20-2017_508.pdf
4. American Society for Testing Materials (ASTM). 2013. Standard Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents.
5. Aschengrau A, Weinberg JM, Janulewicz PA, Gallagher LG, Winter MR, et al. 2009. Prenatal exposure to tetrachloroethylene-contaminated drinking water and the risk of congenital anomalies: A retrospective cohort study. *Environmental Health: A Global Access Science Source* 8
6. Aschengrau A, Winter MR, Vieira VM, Webster TF, Janulewicz PA, et al. 2015. Long-term health effects of early life exposure to tetrachloroethylene (PCE)-contaminated drinking water: A retrospective cohort study. *Environmental Health: A Global Access Science Source* 14
7. Bagheri H, Zare M, Piri-Moghadam H, Es-haghi A. 2015. A combined micro-solid phase-single drop microextraction approach for trace enrichment of volatile organic compounds. *Analytical Methods* 7:6514-9
8. Bloemen HJT, Burn J. 1993. *Chemistry and analysis of volatile organic compounds in the environment*. London; New York: Blackie Academic & Professional
9. Center for Disease Control and Prevention (CDC). 2018. *Facts About Phosgene*. https://emergency.cdc.gov/agent/phosgene/basics/facts.asp
10. Chen F, Freedman DL, Falta RW, Murdoch LC. 2012. Henry's law constants of chlorinated solvents at elevated temperatures. *Chemosphere* 86:156-65
11. Christ JA, Ramsburg CA, Abriola LM, Pennell KD, Löffler FE. 2005. Coupling Aggressive Mass Removal with Microbial Reductive Dechlorination for Remediation of DNAPL Source Zones: A Review and Assessment. *Environmental Health Perspectives* 113:465-77
12. Daniel WW, Cross CL. 2013. *Biostatistics: a foundation for analysis in the health sciences*. Hoboken, NJ: Wiley
13. Dodge DG, Goodman JE. 2015. Perchloroethylene (Tetrachloroethylene). In *Hamilton and Hardy's Industrial Toxicology: Sixth Edition*:727-32: wiley. Number of 727-32 pp.

14. Eaton A, Chowdhury Z, Shaw J, Roberson JA. 2012. The state of the science of analytical methods for cVOCs. *Journal - American Water Works Association* 104:E572-E81
15. Environmental Protection Agency (EPA). 1982. *An Exposure and Risk Assessment for Tetrachloroethylene*. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000LLOH.txt
16. Environmental Protection Agency (EPA). 2012. *Tetrachloroethylene, CASRN 127-18-4*. https://cfpub.epa.gov/ncea/iris2/chemicallanding.cfm?substance_nmbr=106
17. Environmental Protection Agency (EPA). 2014. *Report on the Enviroment, Volatile Organic Compounds Emissions*. https://cfpub.epa.gov/roe/indicators.cfm
18. Environmental Protection Agency (EPA). 2016. *Quick Guide to Drinking Water Sample Collection*. https://www.epa.gov/sites/production/files/2017-04/documents/quick-guide-drinking-water-sample-collection-2ed-update-508.pdf
19. Environmental Protection Agency (EPA). 2017. *Technical Overview of Volatile Organic Compounds*. https://www.epa.gov/indoor-air-quality-iaq/technical-overview-volatile-organic-compounds
20. Environmental Protection Agency (EPA). 2017. *Water Enforcement*. https://www.epa.gov/enforcement/water-enforcement
21. Environmental Protection Agency (EPA). 2018. *Problem Formulation of the Risk Evaluation for Perchloroethylene (PERC)*. https://www.epa.gov/sites/production/files/2018-06/documents/perc_problem_formulation_5-31-2018v3.pdf
22. Greifenstein M, White DW, Stubner A, Hout J, Whelton AJ. 2013. Impact of temperature and storage duration on the chemical and odor quality of military packaged water in polyethylene terephthalate bottles. *Science of The Total Environment* 456-457:376-83
23. Hazra A, Gogtay N. 2016. Biostatistics Series Module 6: Correlation and Linear Regression. *Indian J Dermatol* 61:593-601
24. Inficon. 2017. *HAPSITE ER, Chemical Identification System Operating Manual*. https://products.inficon.com/en-us/nav-products/product/detail/hapsite-er-identification-system/
25. Kleinbaum DG. 1998. *Applied regression analysis and other multivariable methods*. Pacific Grove: Duxbury Press
26. Kleinbaum DG, Klein M, Pryor ER. 2010. *Logistic regression: a self-learning text*. New York: Springer
27. Kozłowska K, Polkowska Ż, Namieśnik J, Przyjazny A. 2006. Investigation of stability of aqueous solutions containing trace amounts of volatile organic analytes. *TrAC Trends in Analytical Chemistry* 25:609-20
28. Lee H-K, Do S-H, Batchelor B, Jo Y-H, Kong S-H. 2009. PCE DNAPL degradation using ferrous iron solid mixture (ISM). *Chemosphere* 76:1082-7
29. Marczak M, Wolska L, Chrzanowski W, Namieśnik J. 2006. Microanalysis of Volatile Organic Compounds (VOCs) in Water Samples – Methods and Instruments. *Microchimica Acta* 155:331-48

30. Maskarinec MP, Johnson LH, Holladay SK, Moody RL, Bayne CK, Jenkins RA. 1990. Stability of volatile organic compounds in environmental water samples during transport and storage. *Environmental Science & Technology* 24:1665-70
31. Maslia ML, Aral MM, Ruckart PZ, Bove FJ. 2016. Reconstructing historical VOC concentrations in drinking water for epidemiological studies at a U.S. military base: Summary of results. *Water (Switzerland)* 8
32. Moliner-Martínez Y, Herraez-Hernandez R, Verdú-Andres J, Campíns-Falcó P, Garrido-Palanca C, et al. 2013. Study of the influence of temperature and precipitations on the levels of BTEX in natural waters. *Journal of Hazardous Materials* 263:131-8
33. Munch JW, Eichelberger JW. 1992. Evaluation of 48 Compounds for Possible Inclusion in U.S. EPA Method 524.2, Revision 3.0: Expansion of the Method Analyte List to a Total of 83 Compounds. *Journal of Chromatographic Science* 30:471-7
34. Namieśnik J. 2002. Trace analysis - Challenges and problems. *Critical Reviews in Analytical Chemistry* 32:271-300
35. National Center for Biotechnology Information (NCBI). 2005. *PubChem Compound Database, Tetrachloroethylene*. https://pubchem.ncbi.nlm.nih.gov/compound/31373#section=Top
36. National Institutes of Health (NIH). 2012. *TOXNET (Toxicological Data Network)*. https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~yHvBl0:3
37. Peng J, Wan A. 1998. Effect of ionic strength on Henry's constants of volatile organic compound. *Chemosphere* 36:2731-40
38. Roth DK, Roberson JA, Cornwell DA. 2012. The regulatory context for cVOCs. *Journal - American Water Works Association* 104:E561-E71
39. S˙liwka-Kaszyńska M, Kot-Wasik A, Namieśnik J. 2003. Preservation and Storage of Water Samples. *Critical Reviews in Environmental Science and Technology* 33:31-44
40. Santos FJ, Galceran MT, Fraisse D. 1996. Application of solid-phase microextraction to the analysis of volatile organic compounds in water. *Journal of Chromatography A* 742:181-9
41. Seo M, Kobayashi R, Okamura T, Ikeda K, Satoh M, et al. 2012. Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice. *Journal of Toxicological Sciences* 37:439-45
42. Seo M, Yamagiwa T, Kobayashi R, Ikeda K, Satoh M, et al. 2008. A small amount of tetrachloroethylene ingestion from drinking water accelerates antigen-stimulated allergic responses. *Immunobiology* 213:663-9
43. Sigma Aldrich. 2019. *Selection Guide for Supelco SPME Fibers*. https://www.sigmaaldrich.com/technical-documents/articles/analytical/selecting-spme-fibers.html
44. Smith FL, Harvey AH. 2007. Avoid common pitfalls when using Henry's law. *Chemical Engineering Progress* 103:33-9
45. U.S. Army Public Health Command. 2012. *Army Institute of Public Health - Laboratory Sciences Customer Service Manual (Technical Guide 214)*. https://phc.amedd.army.mil/PHC%20Resource%20Library/TG214_AIPH-LS_CustomerServiceManual.pdf

46. Wilson TA. 2013. *Evaluating the effect of holding time and storage temperature on the chemical stability of drinking water samples collected from military forward deployed environments*. Thesis. Uniformed Services University of the Health Sciences, Bethesda, MD
47. Yu B, Song Y, Han L, Yu H, Liu Y, Liu H. 2014. Optimizations of packed sorbent and inlet temperature for large volume-direct aqueous injection-gas chromatography to determine high boiling volatile organic compounds in water. *J Chromatogr A* 1356:221-9